IPCS

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

WHO ILO UNEP

Environmental Health Criteria 240

Principles and Methods for the Risk Assessment of Chemicals in Food

A joint publication of the Food and Agriculture Organization of the United Nations and the World Health Organization

Food and Agriculture Organization of the United Nations

World Health Organization

Environmental Health Criteria 240

PRINCIPLES AND METHODS FOR THE RISK ASSESSMENT OF CHEMICALS IN FOOD

A joint publication of the Food and Agriculture Organization of the United Nations and the World Health Organization

Published under the joint sponsorship of the United Nations Environment Programme, the International Labour Organization and the World Health Organization, and produced within the framework of the Inter-Organization Programme for the Sound Management of Chemicals.

Food and Agriculture Organization of the United Nations

World Health Organization

The **International Programme on Chemical Safety (IPCS)**, established in 1980, is a joint venture of the United Nations Environment Programme (UNEP), the International Labour Organization (ILO) and the World Health Organization (WHO). The overall objectives of the IPCS are to establish the scientific basis for assessment of the risk to human health and the environment from exposure to chemicals, through international peer review processes, as a prerequisite for the promotion of chemical safety, and to provide technical assistance in strengthening national capacities for the sound management of chemicals.

The **Inter-Organization Programme for the Sound Management of Chemicals (IOMC)** was established in 1995 by UNEP, ILO, the Food and Agriculture Organization of the United Nations, WHO, the United Nations Industrial Development Organization, the United Nations Institute for Training and Research and the Organisation for Economic Co-operation and Development (Participating Organizations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen cooperation and increase coordination in the field of chemical safety. The purpose of the IOMC is to promote coordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

WHO Library Cataloguing-in-Publication Data

Principles and methods for the risk assessment of chemicals in food.

(Environmental health criteria ; 240)

1. Risk assessment. 2. Hazard assessment. 3. Exposure assessment. 4. Dose-response assessment. 5. Chemicals. 6. Food safety. 7. Food additives. 8. Contaminants. 9. Pesticide residues. 10. Veterinary drug residues. I.World Health Organization. II.Food and Agriculture Organization of the United Nations.

ISBN 978 92 4 157240 8 (NLM classification: WA 712)
ISSN 0250-863X

This document was technically and linguistically edited by Marla Sheffer, Ottawa, Canada.

Printed by Wissenchaftliche Verlagsgesellschaft mbH, Stuttgart, Germany.

CONTENTS

ENVIRONMENTAL HEALTH CRITERIA ON PRINCIPLES AND METHODS FOR THE RISK ASSESSMENT OF CHEMICALS IN FOOD

NOTE TO READERS OF THIS CRITERIA MONOGRAPH

The individual chapters of this monograph can largely stand alone; hence, a table of contents and reference list are included in each chapter, and some duplication may occur in the overall text. This publication will also be made available electronically, and individual chapters will be independently updated when the need arises.

Every effort has been made to present the information in this criteria monograph as accurately as possible without unduly delaying its publication. In the interest of all users of this Environmental Health Criteria monograph, readers are requested to communicate any errors that may have occurred to the Director of the Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland, in order that they may be included in corrigenda.

Environmental Health Criteria

PREAMBLE

Objectives

In 1973, the WHO Environmental Health Criteria Programme was initiated with the following objectives:

(i) to assess information on the relationship between exposure to environmental pollutants and human health, and to provide guidelines for setting exposure limits;
(ii) to identify new or potential pollutants;
(iii) to identify gaps in knowledge concerning the health effects of pollutants;
(iv) to promote the harmonization of toxicological and epidemiological methods in order to have internationally comparable results.

The first Environmental Health Criteria (EHC) monograph, on mercury, was published in 1976, and since that time an ever-increasing number of assessments of chemicals and of physical effects have been produced. In addition, many EHC monographs have been devoted to evaluating toxicological methodology, e.g. for genetic, neurotoxic, teratogenic, and nephrotoxic effects. Other publications have been concerned with epidemiological guidelines, evaluation of short-term tests for carcinogens, biomarkers, effects on the elderly, and so forth.

Since its inauguration, the EHC Programme has widened its scope, and the importance of environmental effects, in addition to health effects, has been increasingly emphasized in the total evaluation of chemicals.

The original impetus for the Programme came from World Health Assembly resolutions and the recommendations of the 1972 UN Conference on the Human Environment. Subsequently, the work became an integral part of the International Programme on Chemical Safety (IPCS), a cooperative programme of WHO, ILO, and UNEP. In

this manner, with the strong support of the new partners, the importance of occupational health and environmental effects was fully recognized. The EHC monographs have become widely established, used, and recognized throughout the world.

The recommendations of the 1992 UN Conference on Environment and Development and the subsequent establishment of the Intergovernmental Forum on Chemical Safety with the priorities for action in the six programme areas of Chapter 19, Agenda 21, all lend further weight to the need for EHC assessments of the risks of chemicals.

Scope

Two different types of EHC documents are available: 1) on specific chemicals or groups of related chemicals; and 2) on risk assessment methodologies. The criteria monographs are intended to provide critical reviews on the effect on human health and the environment of chemicals and of combinations of chemicals and physical and biological agents and risk assessment methodologies. As such, they include and review studies that are of direct relevance for evaluations. However, they do not describe *every* study carried out. Worldwide data are used and are quoted from original studies, not from abstracts or reviews. Both published and unpublished reports are considered, and it is incumbent on the authors to assess all the articles cited in the references. Preference is always given to published data. Unpublished data are used only when relevant published data are absent or when they are pivotal to the risk assessment. A detailed policy statement is available that describes the procedures used for unpublished proprietary data so that this information can be used in the evaluation without compromising its confidential nature (WHO (1990) Revised Guidelines for the Preparation of Environmental Health Criteria Monographs. PCS/90.69, Geneva, World Health Organization).

In the evaluation of human health risks, sound human data, whenever available, are preferred to animal data. Animal and in vitro studies provide support and are used mainly to supply evidence missing from human studies. It is mandatory that research on human subjects is conducted in full accord with ethical principles, including the provisions of the Declaration of Helsinki.

The EHC monographs are intended to assist national and international authorities in making risk assessments and subsequent risk management decisions. They represent a thorough evaluation of risks and are not, in any sense, recommendations for regulation or standard setting. These latter are the exclusive purview of national and regional governments.

Procedures

The procedures described below were followed in the development and publication of this EHC. A designated WHO Staff Member, Dr Sam Page and subsequently Dr A. Tritscher, served as the Responsible Officer (RO) at WHO. At the Food and Agriculture Organization of the United Nations (FAO), the ROs were Dr M. Lützow and subsequently Dr A. Wennberg. These ROs are responsible for the scientific content of the document. The editor was responsible for layout and language. A public web site was created to inform progress on the project.

FAO and WHO held a planning meeting of international experts with experience in the risk assessment activities of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) on 26–28 November 2001 at WHO Headquarters in Geneva, Switzerland, to define the scope of the project and develop a project plan. A steering group was then formed, which accompanied and guided the project until its completion.

A series of workshops were held to develop the basis for the key chapters. In addition, drafters were commissioned for certain subchapters, and these drafts were subsequently peer reviewed by the steering group and/or by invited experts. Once all chapters had been drafted, four experts familiar with the project as well as with the methods and procedures applied by JECFA and JMPR were commissioned for an overall review. Subsequently, two experts were commissioned to compile and write the first draft of the monograph based on existing chapters and taking into account comments from reviewers and the steering group. This draft monograph was then made available on the IPCS web site for external review and comment. Comments received are available on request from the WHO Secretariat. They were reviewed by an expert meeting held on 11–14 November 2008 in Seoul, Republic of

Korea, and necessary additions and revisions to the document were made.

All experts who contributed to this monograph served as individual scientists, not as representatives of any organization, government or industry. Every attempt was made to ensure that all individuals who, as authors, consultants or advisers, participated in the preparation of this EHC monograph informed the WHO Secretariat if at any time a conflict of interest, whether actual or potential, could be perceived in their work.

TASK GROUP ON ENVIRONMENTAL HEALTH CRITERIA ON PRINCIPLES AND METHODS FOR THE RISK ASSESSMENT OF CHEMICALS IN FOOD

Dr S. Page, IPCS, and Dr A. Tritscher, Department of Food Safety and Zoonoses, served as the Responsible Officers (ROs) for WHO, and Dr M. Lützow and Dr A. Wennberg served as the ROs for FAO. The ROs were responsible for the preparation of the final document and for its overall scientific content. Marla Sheffer, Ottawa, Canada, was the IPCS editor responsible for layout and language.

* * *

Risk assessment activities of IPCS are supported financially by the Department of Health, Department for Environment, Food & Rural Affairs and Food Standards Agency, United Kingdom; Environmental Protection Agency, Food and Drug Administration and National Institute of Environmental Health Sciences, United States of America (USA); European Commission; German Federal Ministry of Environment, Nature Conservation and Nuclear Safety; Health Canada; Japanese Ministry of Health, Labour and Welfare; and Swiss Agency for Environment, Forests and Landscape. Specific support for this project was received from the United Kingdom Food Standards Agency, the United States Food and Drug Administration, the Republic of Korea Food and Drug Administration and the Netherlands National Institute for Public Health and the Environment.

* * *

Steering group members

Dr D. Arnold, Federal Institute for Health Protection of Consumers & Veterinary Medicine, Berlin, Germany

Professor J. Bend, Department of Pathology, Siebens-Drake Medical Research Institute, Schulich School of Medicine & Dentistry, University of Western Ontario, London, Ontario, Canada

Dr M. Bolger, Food and Drug Administration, United States Department of Health and Human Services, College Park, Maryland, USA

Dr E. Boutrif, Food Quality and Standards Service, Food and Nutrition Division, Food and Agriculture Organization of the United Nations, Rome, Italy

Mr D. Hamilton, Department of Primary Industries, Brisbane, Australia

Dr J.-L. Jouve, Chief, Food Quality and Standards Service, Food and Nutrition Division, Food and Agriculture Organization of the United Nations, Rome, Italy (*retired*)

Dr M. Lützow, Consultant, Neuenhof, Switzerland

Dr T. Meredith, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland

Dr B. Petersen, Exponent, Inc., Washington, DC, USA

Dr G. Vaagt, Pesticide Management Group, Food and Agriculture Organization of the United Nations, Rome, Italy

Professor R. Walker, School of Biological Sciences, Guildford, Surrey, England

Dr Y. Yamada, National Food Research Institute, Tsukuba, Japan

Secretariat

Dr M. Choi, Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland

Dr S. Page, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland

Dr A. Tritscher, Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland

Dr A. Wennberg, Nutrition and Consumer Protection Division, Food and Agriculture Organization of the United Nations, Rome, Italy

Other contributors

The contributors to this monograph are listed below. Those who submitted written comments in response to a request for public comments are also acknowledged below.

Invited reviewers of the draft chapters were Dr S. Barlow, Dr A. Boobis, Dr J. Herrman and Dr J. Weatherwax.

Dr S. Barlow and Professor A. Renwick compiled and prepared the first draft of the monograph based on the draft chapters and taking into account comments from reviewers and the steering group.

Legend

1	Participant in: Workshop I, London, 9–13 December 2002: Joint FAO/WHO Project to Update Principles and Methods for the Risk Assessment of Chemicals in Food (*basis for chapter 4*)
2	Participant in: Joint FAO/WHO Expert Consultation: Dietary Exposure Assessment of Chemicals in Food, Annapolis, Maryland, USA, 2–6 May 2005 (*basis for chapter 6*)
3	Participant in: Joint FAO/WHO Expert Consultation: MRLs for Pesticides and Veterinary Drugs, Bilthoven, the Netherlands, 7–10 November 2005 (*basis for chapter 9*)
4	Participant in: Workshop on Principles for Modelling Dose–Response for the Risk Assessment of Chemicals, Geneva, 13–17 September 2004 (*basis for chapter 5*)
5	Participant in: Final Expert Meeting, 11–14 November 2008, Seoul, Republic of Korea
6	Drafter of section or chapter (chapter indicated in parentheses)

Dr A. Ambrus, Hungarian Food Safety Office, Budapest, Hungary	2, 3
Professor A. Anadón, Department of Toxicology and Pharmacology, Faculty of Veterinary Medicine, Universidad Complutense de Madrid, Madrid, Spain	1
Dr D. Arnold, Consultant, Berlin, Germany (formerly Federal Institute for Health Protection of Consumers & Veterinary Medicine, Berlin, Germany) (*3: Chair*)	1, 3, 5, 6 (chapter 3)
Dr S.A. Assimon, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA	6 (section 4.10)
Ms J. Baines, Evaluation and Surveillance, Food Standards Australia New Zealand, Canberra, Australia (*2: Rapporteur*)	2, 5
Dr S. Barlow, Consultant, Brighton, England (*5: Chair*)	5, 6 (section 4.7)
Dr L. Barraj, Exponent, Inc., Washington, DC, USA	2

Dr D. Bellinger, Harvard Medical School Children's Hospital, Boston, MA, USA	1
Dr D. Benford, Food Standards Agency, London, England	1, 5
Dr M. Bolger, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA (*4: Rapporteur*)	1, 2, 4
Professor A. Boobis, Experimental Medicine & Toxicology Division of Investigative Science, Faculty of Medicine, Imperial College, London, England (*1,4: Chair*) (*5: Rapporteur*)	1, 4, 5, 6 (sections 4.1, 4.6)
Dr A. Bruno, FAO Codex Secretariat, Food and Agriculture Organization of the United Nations, Rome, Italy	3
Professor E. Calabrese, University of Massachusetts, Amherst, MA, USA	4
Dr E. Caldas, University of Brasilia, College of Health Sciences, Pharmaceutical Sciences Department, Campus Universitàrio Darci Ribeiro, Brasilia, Brazil	2, 3
Dr C. Carrington, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA	2, 4, 5, 6 (chapter 5)
Dr C. Cerniglia, Division of Microbiology, National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR, USA	1, 6 (section 4.12)
Mrs R. Charrondiere, Nutrition Planning, Assessment and Evaluation Service, Nutrition and Consumer Protection Division, Food and Agriculture Organization of the United Nations, Rome, Italy	2
Professor B. Chen, School of Public Health, Shanghai Medical University, Shanghai, China	1
Dr M.-H. Cho, National Institute of Toxicological Research, Korean Food and Drug Administration, Seoul, Republic of Korea	5
Dr M. Choi, Korean Food and Drug Administration, Seoul, Republic of Korea (formerly Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland)	5
Dr V. Cogliano, Carcinogen Identification and Evaluation, International Agency for Research on Cancer, World Health Organization, Lyon, France	4
Dr J. Cohen, Harvard Center for Risk Analysis, Boston, MA, USA	2

Dr R. Crebelli, Unit of Genetic Toxicology, Department of Environment and Primary Prevention, Istituto Superiore di Sanita, Rome, Italy	6 (section 4.5)
Professor A. Dayan, Consultant, London, England	1, 6 (section 4.11)
Dr D. Deuk Jang, National Institute of Toxicological Research, Korean Food and Drug Administration, Seoul, Republic of Korea	5
Dr M. DiNovi, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA	2
Professor E. Dybing, Division of Environmental Medicine, Norwegian Institute of Public Health, Oslo, Norway (retired) (*4: Rapporteur*)	4
Dr L. Edler, Biostatistics Unit, German Cancer Research Center, Heidelberg, Germany	4
Ms S.K. Egan, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA (*2: Rapporteur*)	2
Dr R. Ellis, Consultant, South Carolina, USA (formerly Center for Veterinary Medicine, Food and Drug Administration, Rockville, MD, USA) (*3: Rapporteur*)	3
Professor P. Farmer, Department of Biochemistry and Department of Cancer Studies and Molecular Medicine, University of Leicester, Leicester, England	6 (section 4.5)
Professor E. Faustman, Department of Environmental and Occupational Health Sciences, School of Public Health and Community Medicine, University of Washington, Seattle, WA, USA	4
Dr C. Fisher, Consultant, Cambridge, England	1, 6 (chapter 3)
Dr S. Funk, Health Effects Division, United States Environmental Protection Agency, Washington, DC, USA	2, 3
Dr G. Gallhoff, Health and Consumer Protection Directorate-General, European Commission, Brussels, Belgium	3
Dr W. Gelderblom, Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC), Tygerberg, South Africa	1
Dr D. Germolec, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA	6 (section 4.9)
Dr K. Greenlees, Toxicology Team, Office of New Animal Drug Evaluation, Center for Veterinary Medicine, Food and Drug Administration, Rockville, MD, USA	3

Mr D. Hamilton, Consultant, Brisbane, Australia (formerly Biosecurity, Department of Primary Industries and Fisheries, Brisbane, Australia) (*3: Rapporteur*)	1, 3, 5, 6 (chapters 1, 8)
Dr C.A. Harris, Exponent International Ltd, Harrogate, England	3
Dr G.J. Harry, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC, USA	1, 6 (section 4.8)
Dr M. Healy, Food Standards Australia New Zealand, Canberra, Australia	4
Dr J. Herrman, Consultant, Placerville, CA, USA	1, 6 (chapter 1)
Dr J. Hlywka, Cantox Health Sciences International, Mississauga, Ontario, Canada	1, 6 (sections 4.10, 4.11)
Mr J. Howlett, Consultant, Surrey, England	4
Dr T. Inoue, Biological Safety Research Centre, National Institute of Health Sciences, Tokyo, Japan	1
Dr S.-H. Jeong, Toxicology Division, National Veterinary Research and Quarantine Service, Ministry of Agriculture & Forestry, Anyang City, Republic of Korea	5
Dr J.-L. Jouve, Food Quality and Standards Service, Food and Nutrition Division, Food and Agriculture Organization of the United Nations, Rome, Italy (retired)	1
Dr M. Kaethner, Bayer CropScience AG, Monheim, Germany	3
Dr R. Kavlock, United States Environmental Protection Agency, Research Triangle Park, NC, USA	1, 6 (section 4.7)
Dr J. Kleiner, European Food Safety Authority, Parma, Italy	4
Dr A. Knaap, Center for Substances and Integrated Risk Assessment, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands (retired)	4
Dr P. Kuznesof, Silver Spring, MD, USA	6 (chapter 3)
Dr J. Larsen, National Food Institute, Technical University of Denmark, Soborg, Denmark	4
Dr J.-C. Leblanc, Department for the Evaluation of Nutritional and Health Risks, French Food Safety Agency (AFSSA), Maisons Alfort, France	2
Dr C. Leclercq, Research Group on Food Safety – Exposure Analysis, National Institute for Food and Nutrition Research (INRAN), Rome, Italy	2

Dr H.M. Lee, Risk Assessment Research Team, National Institute of Toxicological Research, Korea Food and Drug Administration, Seoul, Republic of Korea	5
Professor P. Lees, The Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, England	3
Dr D. Lovell, Postgraduate Medical School, University of Surrey, Surrey, England	4
Dr R. Luebke, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC, USA	6 (section 4.9)
Dr M. Luster, National Institute for Occupational Safety and Health, Morgantown, WV, USA	6 (section 4.9)
Dr M. Lützow, Consultant, Neuenhof, Switzerland	1, 3, 5
Dr D. MacLachlan, Australian Quarantine and Inspection Service, Australian Department of Agriculture, Fisheries and Forestry, Kingston, Australia	3
Dr J.D. MacNeil, Center for Veterinary Drug Residues, Canadian Food Inspection Agency, Saskatoon, Saskatchewan, Canada	3, 6 (chapter 3)
Dr C. Madsen, Institute of Food Safety and Toxicology, Danish Veterinary and Food Administration, Copenhagen, Denmark	6 (section 4.10)
Dr A. Mattia, Division of Biotechnology and GRAS Notice Review, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA	1, 6 (section 4.3)
Dr D. McGregor, Consultant, Aberdour, Scotland	1, 6 (section 4.5)
Dr T. Meredith, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland	1
Mr D.J. Miller, United States Environmental Protection Agency, Washington, DC, USA	2
Professor E. Mitema, Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine, College of Agriculture and Veterinary Sciences, University of Nairobi, Kenya	1
Dr A. Moretto, Department of Environmental and Occupational Health, University of Milan, International Centre for Pesticides and Health Risk Prevention (ICPS), Milano, Italy	1
Dr G. Moy, Consultant, Geneva, Switzerland (previously Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland)	1, 2

Dr I. Munro, Cantox Health Sciences International, Mississauga, Ontario, Canada (*1: Rapporteur*)	1, 6 (sections 4.1, 9.1)
Dr J.L. Nappier, Pfizer Animal Health, Kalamazoo, MI, USA	3
Dr B.C. Ossendorp, Centre for Substances and Integrated Risk Assessment, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands (*3: Co-Chair*)	2, 3
Dr S. Page, Consultant, New Castle, USA (formerly International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland)	1, 2, 3, 4, 6 (chapters 1, 2)
Dr B. Petersen, Exponent, Inc., Washington, DC, USA (*2: Chair*)	1, 2
Professor R. Pieters, University of Utrecht, Utrecht, the Netherlands	6 (section 4.10)
Dr C. Portier, National Institute for Environmental Health Sciences, Research Triangle Park, NC, USA	4
Professor A. Renwick, Consultant, Ulverston, Cumbria, England	1, 4, 5, 6 (section 4.2, chapters 5, 7)
Professor F. Reyes, Department of Food Science, State University of Campinas, Campinas, Brazil	1
Ms A. Richter, Federal Institute for Risk Assessment, Berlin, Germany	3
Dr R. Roberts, AstraZeneca R&D, Safety Assessment, Alderly Park, England	6 (section 4.6)
Dr J.C. Rühl, DuPont Crop Protection, Newark, NJ, USA	2
Professor T. Sanner, Laboratory for Environmental and Occupational Cancer, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway	4
Dr J. Schefferlie, Centre for Substances and Integrated Risk Assessment, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands	3
Dr S. Scheid, Federal Office of Consumer Protection and Food Safety, Berlin, Germany	3
Dr J. Schlatter, Consumer Protection Directorate, Food Safety Division, Nutritional and Toxicological Risks Section, Swiss Federal Office of Public Health, Zürich, Switzerland	1, 4
Dr R. Setzer, Jr, Experimental Toxicology Division, Pharmacokinetics Branch, National Health and Environmental Effects Research Laboratory, United States Environmental Protection Agency, Research Triangle Park, NC, USA	4

Professor W. Slob, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands	4
Dr R. Solecki, Safety of Substances and Preparations, Coordination and Overall Assessment, Federal Institute for Risk Assessment, Berlin, Germany	4, 6 (sections 4.4, 5.2.8)
Dr A.F. Suárez, Instituto Nacional de Tecnología Agropecuaria, Centro de Agroalimentos, Buenos Aires, Argentina	3
Dr S.F. Sundlof, Center for Food Safety and Applied Nutrition (formerly Center for Veterinary Medicine), Food and Drug Administration, Rockville, MD, USA	3
Dr C. Taylor, Institute of Medicine, The National Academies, Washington, DC, USA	6 (section 9.2)
Dr A. Tejada, Pesticide Management Group, Plant Protection Service, Plant Production and Protection Division, Food and Agriculture Organization of the United Nations, Rome, Italy (retired)	1, 2
Dr A. Tritscher, Department of Food Safety and Zoonoses (formerly International Programme on Chemical Safety), World Health Organization, Geneva, Switzerland	3, 4, 5, 6 (chapters 1, 2)
Dr W. van Eck, The Food and Consumer Product Safety Authority, The Hague, the Netherlands	6 (chapter 1)
Dr R.W. Vannoort, ESR Christchurch Science Centre, Christchurch, New Zealand	2
Dr P. Verger, National Institute for Agricultural Research (INRA), Paris, France	2
Dr J. Vos, Laboratory for Pathology and Immunology, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands	1, 6 (section 4.9)
Dr A. Wadge, Food Standards Agency, London, England	4
Professor R. Walker, Consultant, Guildford, Surrey, England (formerly School of Biological Sciences, Guildford, Surrey, England)	1, 5, 6 (section 4.12, chapter 5)
Dr A. Wennberg, FAO Joint Secretary, Food Quality and Standards Service, Nutrition and Consumer Protection Division, Food and Agriculture Organization of the United Nations, Rome, Italy	3, 5, 6 (chapter 3)
Professor C.P. Wild, International Agency for Research on Cancer, World Health Organization, Lyon, France (formerly University of Leeds, Leeds, England)	1

Professor G. Williams, Environmental Pathology and Toxicology, New York Medical College, Valhalla, NY, USA	1, 4, 6 (section 4.6)
Dr Y. Yamada, Ministry of Agriculture, Forestry and Fisheries, Tokyo, Japan	1, 6 (chapter 1)
Professor J.-H. Yang, Catholic University of Taegu, Department of Pharmacology/Toxicology, School of Medicine, Namgu Taegu, Republic of Korea	5
Dr H.J. Yoon, Korean Food and Drug Administration, Seoul, Republic of Korea (formerly Department of Food Safety, Zoonoses and Foodborne Diseases, World Health Organization, Geneva, Switzerland)	3

The following individuals submitted written comments in response to the request for public comments on the final draft monograph:

B. Amzal (European Food Safety Authority)

Dr K.C. Angelova (Bulgaria)

Dr D. Benford (England)

A. Bulder (RIKILT Institute of Food Safety, Wageningen, the Netherlands)

R. Crebelli (European Food Safety Authority)

S. Fabiansson (European Food Safety Authority)

Professor P.B. Farmer (England)

D. Hamilton (Biosecurity Queensland, Australia)

J. Hopkins (Consultant, England)

O. Lindtner (Federal Institute for Risk Assessment, Germany)

C. Madsen (Denmark)

Dr H. Marvin (RIKILT Institute of Food Safety, Wageningen, the Netherlands)

D. Morris (New Zealand)

J. Nicholas (Committee for Medicinal Products for Veterinary Use)

B. Ossendorp (National Institute for Public Health and the Environment, the Netherlands)

Dr R.H. Waring (European Food Safety Authority)

PREFACE

The International Programme on Chemical Safety (IPCS) was initiated in 1980 as a collaborative programme of the United Nations Environment Programme (UNEP), the International Labour Organization (ILO), and the World Health Organization (WHO). One of the major objectives of IPCS is to improve scientific methodologies for assessing the effects of chemicals on human health and the environment. As part of this effort, IPCS publishes a series of monographs, called Environmental Health Criteria (EHC) documents, that evaluate the scientific principles underlying methodologies and strategies to assess risks from exposure to chemicals.

This EHC was prepared in response to a recommendation that the Food and Agriculture Organization of the United Nations (FAO) and WHO should consider updating and harmonizing all the common principles used by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in the toxicological evaluation of food chemicals and publish the information in a single consolidated document. It updates, harmonizes and consolidates principles and methods for the risk assessment of food additives, food contaminants, natural toxicants and residues of pesticides and veterinary drugs.

The efforts of all who helped in the preparation, review, and finalization of the monograph are gratefully acknowledged. Special thanks are due to Health Canada, the Ministry of Health of Japan, the United Kingdom Food Standards Agency and the United States National Institute of Environmental Health Sciences for their financial support of the project.

ACRONYMS AND ABBREVIATIONS

AChE	acetylcholinesterase
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
ALARA	as low as reasonably achievable
ALT	alanine aminotransferase
ARfD	acute reference dose
AST	aspartate aminotransferase
ATBC	Alpha-Tocopherol, Beta-Carotene
AUC	area under the concentration–time curve
BI	benchmark intake
BIL	lower confidence limit of the benchmark intake
BMD	benchmark dose
BMD_{10}	benchmark dose for a 10% response
BMDL	lower confidence limit of the benchmark dose
$BMDL_{10}$	lower confidence limit of the benchmark dose for a 10% response
BMR	benchmark response
BW	body weight
CAC	Codex Alimentarius Commission
CARET	Beta-Carotene and Retinol Efficacy Trial
CAS	Chemical Abstracts Service
CCCF	Codex Committee on Contaminants in Food
CCFA	Codex Committee on Food Additives
CCFAC	Codex Committee on Food Additives and Contaminants
CCMAS	Codex Committee on Methods of Analysis and Sampling
CCPR	Codex Committee on Pesticide Residues
CCRVDF	Codex Committee on Residues of Veterinary Drugs in Foods
CDF	cumulative distribution function

CERHR	Center for the Evaluation of Risks to Human Reproduction (USA)
CHO	Chinese hamster ovary
CIPAC	Collaborative International Pesticides Analytical Council
CL	clearance
C_{max}	peak concentration
CSAF	chemical-specific adjustment factor
CSFII	Continuing Survey of Food Intakes by Individuals (USA)
DBPCFC	double-blind placebo-controlled food challenge
DDT	dichlorodiphenyltrichloroethane
DNA	deoxyribonucleic acid
DRM	dose–response modelling
DTH	delayed-type hypersensitivity
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ED_{10}	effective dose for 10% of the population
EDI	estimated daily intake
EDSTAC	Endocrine Disruptor Screening and Testing Advisory Committee (USA)
EDTA	ethylenediaminetetraacetic acid
EFCOSUM	European Food Consumption Survey Method
EFSA	European Food Safety Authority
EHC	Environmental Health Criteria (WHO)
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunosorbent spot
EMDI	estimated maximum daily intake
EMEA	European Medicines Agency
EMRL	extraneous maximum residue limit
EU	European Union
EuroFIR	European Food Information Resource Network
F	bioavailability
FAO	Food and Agriculture Organization of the United Nations

FAOSTAT	Food and Agriculture Organization of the United Nations statistical database
FEMA	Flavour and Extract Manufacturers Association
FFQ	food frequency questionnaire
GAP	Good Agricultural Practice
GCP	Good Clinical Practice
GEMS/Food	Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme
GEP	Good Epidemiological Practice
GGT	gamma-glutamyl transpeptidase
GLP	Good Laboratory Practice
GM	genetically modified
GMP	Good Manufacturing Practice
GPVD	Good Practice in the Use of Veterinary Drugs
GRAS	generally recognized as safe
HBGV	health-based guidance value
HESI	Health and Environmental Sciences Institute
HLA	human leukocyte antigen
HOI	highest observed intake
hprt	hypoxanthine-guanine phosphoribosyl transferase
HR	highest level of residue in the edible portion of a commodity found in trials to estimate a maximum residue limit in the commodity
HR-P	highest residue in a processed commodity calculated by multiplying the HR of the raw commodity by the corresponding processing factor
IARC	International Agency for Research on Cancer
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IEC	International Electrochemical Commission
IEDI	international estimated daily intake
IESTI	international estimated short-term intake

Ig	immunoglobulin
ILSI	International Life Sciences Institute
INFOODS	International Network of Food Data Systems (United Nations University)
IPCS	International Programme on Chemical Safety (WHO)
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
iv	intravenous
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
JMPS	Joint FAO/WHO Meeting on Pesticide Specifications
K_{ow}	*n*-octanol–water partition coefficient
LLNA	local lymph node assay
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LOEL	lowest-observed-effect level
LOQ	limit of quantification
LP	large portion
MEST	mouse ear swelling test
MIC	minimum inhibitory concentration
MIC_{50}	minimum inhibitory concentration for 50% of strains of the most sensitive relevant organism
ML	maximum level
MOE	margin of exposure
MRL	maximum residue limit
MRLVD	maximum residue limit for veterinary drugs
mRNA	messenger ribonucleic acid
MRP	multidrug resistance associated protein
MSDI	maximum survey-derived intake
ND	not detected
NESTI	national estimated short-term intake

NHANES	National Health and Nutrition Examination Survey (USA)
NK	natural killer
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NQ	not quantified
NTE	neuropathy target esterase
NTP	National Toxicology Program (USA)
OAT	organic anion transporter
OCT	organic cation transporter
OECD	Organisation for Economic Co-operation and Development
OPAL	Operating Program for Analytical Laboratories
OPIDN	organophosphate-induced delayed neuropathy
PADI	possible average daily intake
PAH	polycyclic aromatic hydrocarbon
PBTK	physiologically based toxicokinetic
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzodioxin
PCDF	polychlorinated dibenzofuran
PMTDI	provisional maximum tolerable daily intake
POD	point of departure
POP	persistent organic pollutant
PPARα	peroxisome proliferator activated receptor of the class α
PTMI	provisional tolerable monthly intake
PTWI	provisional tolerable weekly intake
QSAR	quantitative structure–activity relationship
RAC	raw agricultural commodity
RIVM	National Institute for Public Health and the Environment (the Netherlands)
RNA	ribonucleic acid
SBPCFC	single-blind placebo-controlled food challenge
SCE	sister chromatid exchange

SCF	Scientific Committee on Food (European Commission)
SGOT	serum glutamate–oxaloacetate transaminase
SGPT	serum glutamate–pyruvate transaminase
SIGHT	Summary Information on Global Health Trends
SML	specific migration limit
SOP	standard operating procedure
SPET	single portion exposure technique
SPS Agreement	World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures
STMR	supervised trials median residue
STMR-P	supervised trials median residue in a processed commodity calculated by multiplying the STMR of the raw commodity by the corresponding processing factor
$t_{½}$	half-life
TAMDI	theoretical added maximum daily intake
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TD_{50}	tumorigenic dose for 50% of test species
TDAR	T cell–dependent antibody response
TDI	tolerable daily intake
TDS	total diet study
TEF	toxic equivalency factor
TI	tolerable intake
tk	thymidine kinase
T_{max}	time to peak concentration
TMDI	theoretical maximum daily intake
TOS	total organic solids
TTC	threshold of toxicological concern
UF	uncertainty factor
UL	upper level of intake
USA	United States of America
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency

SUMMARY

The Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) follow the same general principles and methods for chemical risk assessments, which are published in the reports of both committees. In response to recommendations made by JECFA and JMPR in the 1980s to review the validity of the evaluation procedures then in place, the International Programme on Chemical Safety (IPCS) sponsored the preparation of Environmental Health Criteria monographs (EHCs) on Principles for the Safety Assessment of Food Additives and Contaminants in Food (EHC 70) and Principles for the Toxicological Assessment of Pesticide Residues in Food (EHC 104). These monographs and the principles laid out in subsequent reports have served as the basis for the assessments that have been performed by JECFA and JMPR.

Although much of the guidance set out in EHC 70 and EHC 104 remains valid, there have been significant advances in chemical analysis, toxicology, dietary exposure assessment and risk assessment approaches for chemicals in food since these monographs were prepared. Accordingly, FAO and WHO initiated a project to update, harmonize and consolidate principles and methods used by JECFA and JMPR for the risk assessment of food additives, food contaminants, natural toxicants and residues of pesticides and veterinary drugs. This monograph is the outcome of that project.

The purpose of this monograph is 2-fold: 1) to provide descriptive guidance for JECFA and JMPR to ensure the continuation of transparent and sound expert evaluations of scientific data for risk assessments of chemicals in food; and 2) to be informative for users of the outputs from JECFA and JMPR, such as risk managers and other risk assessment bodies in Member countries and authorities.

The monograph addresses the key issues considered by JECFA and JMPR in their food chemical risk assessments, as summarized below.

Risk assessment and its role in risk analysis

Risk analysis consists of three components: risk assessment, risk management and risk communication. Risk assessment is the central component of risk analysis and provides a scientific basis for risk management decisions on measures that may be needed to protect human health. It takes into account all available relevant scientific data and identifies any uncertainties in the knowledge base. Risk assessment comprises the four steps of hazard identification, hazard characterization (including dose–response assessment), exposure assessment and risk characterization. It is a conceptual framework that, in the context of food chemical safety, provides a mechanism for the structured review of information relevant to assessing possible health outcomes in relation to exposures to chemicals present in food.

Risk assessment of chemical substances present in or on food forms the core work of JECFA and JMPR. Based on the advice from these two committees, food safety measures are taken in the risk management executed by countries nationally and by the Codex Alimentarius Commission (CAC) internationally. Whereas JECFA and JMPR base their evaluations on scientific principles and ensure necessary consistency in their risk assessment determinations, CAC and its respective committees that deal with chemicals in food are responsible, as risk managers, for the final decisions on establishing maximum limits for pesticide residues, veterinary drug residues, contaminants and additives in food and adopting other related measures.

Although it is desirable to separate the functional activities of risk assessment from those of risk management in order to ensure scientific independence, it is acknowledged that risk managers should communicate and interact with risk assessors during the process to establish the scope of the analysis, particularly during problem formulation. Thus, the relationship between risk assessment and risk management is an interactive, often iterative, process.

Chemical characterization, analytical methods and the development of specifications

This section of the monograph describes the chemical information that is required for risk assessment. Such information is also a prerequisite for surveillance and control of chemical substances in food.

Proposed analytical methods are reviewed by JECFA and JMPR for their suitability for international use. Analytical methods are necessary, for example, for the speciation of contaminants, for determination of the concentrations of a chemical and its metabolites in pharmacokinetic, toxicokinetic and residue depletion studies, and for the reliable determination of the concentrations of contaminants and of incurred residues of veterinary drugs and pesticides in foods. The monograph describes the key features of suitable analytical methods and the validation criteria for such methods.

Food additive specifications

Specifications of identity and purity are necessary products of JECFA safety assessments for food additives. Evaluations of food additives by JECFA depend on studies performed with a chemical substance or product of defined identity, purity and physical form. The safety assessment is valid only for products that do not differ significantly in identity and quality profile from the material used to generate the data used in the evaluation.

Pesticides

The Joint FAO/WHO Meeting on Pesticide Specifications (JMPS) establishes specifications for technical-grade material and formulations. JMPR takes the JMPS specifications into account during the safety assessment. JMPR evaluates the analytical methods used for generation of residue data to check that the methods are suitable for the relevant analytes and sample types. JMPR also reports information on methods that are suitable for enforcement of maximum residue limits (MRLs) and whether particular compounds are suitable for analysis by multiresidue methods.

Veterinary drug residues

JECFA must be assured that any veterinary drug it evaluates is well characterized, with details of its chemical and physical properties and the identity and concentrations of any major impurities. In addition, the manufacturing process should be described and the consistency and quality of the final products demonstrated.

The form and the distribution of the residues that result from each authorized mode of application in each species should be determined,

and the depletion of the residues from edible tissues or animal-derived foods should be studied. A marker residue should be identified, which is usually the form of the drug (parent compound or metabolite) that is found at the highest concentration for the longest period. The relationship of this marker residue to the total residue of the drug is determined.

Contaminants

The data required for the characterization of a contaminant should include its concentrations in foods and the total diet from as many countries as possible. Data should be formatted using the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) to facilitate the collation and quality control of the data. The data should be accompanied by additional details on sampling plans and analytical methods used to generate the data.

Substances consumed in large amounts

Thorough chemical analyses should be performed on high-consumption substances, such as bulk additives, to identify potential impurities and to provide information on nutritional adequacy, especially when such substances replace traditional food. Because exposure to undesirable impurities (e.g. heavy metals) concomitant with the intake of high-consumption materials is potentially high, special effort should be made to identify and quantify such impurities.

Hazard identification and characterization: toxicological and human studies

Scope and choice of test methods

Toxicological studies may be broadly divided into 1) in vitro studies, using cultured organisms or cells or tissue preparations from laboratory animals or humans; and 2) in vivo studies in laboratory animals or humans. Such studies serve a number of purposes, including the identification of potential adverse effects (hazard identification), definition of the exposure conditions necessary to produce the effects and the assessment of dose–response relationships for the adverse effects (hazard characterization). JECFA and JMPR consider data from both types of study in their risk assessments.

It is widely accepted that animal testing should be reduced, refined or replaced as far as is practicable, and this has led to an increased use of alternative approaches and to improved study designs. It is equally important that scientifically sound methods and approaches are used for the safety testing of food chemicals. Hence, although advances are being made in the development of in silico and in vitro approaches, at the present time these do not permit the replacement of animal testing for most end-points of concern. Although no experimental species is an ideal model for humans, there is evidence that studies in animals generally provide an effective means for evaluating the potential toxicity of substances in food, provided that the data are interpreted critically.

Several internationally recognized organizations, such as the Organisation for Economic Co-operation and Development (OECD), provide guidance on minimum standards for the design and conduct of toxicological studies. All studies used in the risk assessment of a substance in food are assessed for adequacy of design and conduct and should preferably be conducted according to the principles of Good Laboratory Practice. The monograph also discusses promising recent developments in testing protocols that have not yet been formally accepted by OECD.

Study of the absorption, distribution, metabolism and excretion (ADME) of a substance at an early stage of testing is important in aiding the selection of appropriate test species and test doses for toxicity studies. Where possible, investigation of any qualitative or quantitative differences in ADME between the test species and humans will provide important information for characterization of the hazard.

The extent of toxicological testing required depends on the nature and use of the substance under consideration. Not all of the tests discussed in the monograph will necessarily need to be conducted in order to reach a conclusion on the risk assessment for a particular substance. Tiered testing approaches are also discussed in which screening tests or a limited number of standard toxicity studies are conducted, which may be sufficient for risk assessment or may trigger necessary further investigations.

Short-term and long-term tests for general systemic toxicity are usually conducted. These identify target organs for toxicity and may

indicate the need for additional or more specific testing (e.g. for neurotoxicity or immunotoxicity). The effects of the test substance on a wide range of end-points indicative of toxicity, including observational, functional, biochemical and pathological end-points, are examined. Studies are typically conducted in two species, either a rodent and a non-rodent species or two rodent species, and in both sexes, to maximize the opportunity to find any effects (hazard identification). Long-term testing often also includes carcinogenicity testing in two rodent species. The use of an alternative method in place of one rodent species may be acceptable on a case-by-case basis; a variety of alternative tests for carcinogenicity have been introduced in which tumorigenic responses are enhanced and the duration of bioassays is thereby reduced, including initiation/promotion models, the neonatal mouse model and transgenic mouse models.

Testing should be conducted in a manner that best relates to human exposure scenarios. Dose selection should take into account the anticipated human exposure, the frequency of exposure and the duration of exposure. For substances present in foods, administration of the substance in repeated-dose animal studies is usually by diet, gavage or drinking-water. Ideally, the dose levels selected are such that toxic effects, but not death or severe suffering, are produced at the highest dose level, with lower dose levels producing graded responses and no adverse effects at the lowest dose level. The study design should be adequate to determine a reference point for hazard characterization, also known as a point of departure (POD), such as a no-observed-adverse-effect level (NOAEL) or a benchmark dose (BMD), which is a dose producing a low but measurable adverse response.

For all study designs, careful consideration needs to be given to dose spacing and number of study groups, maximum dose utilized, number of animals per sex in each dose group, choice of controls, dosing regimen, confirmation of dose administered compared with nominal dose, and dose ingested (e.g. palatability, wastage of food).

In addition to tests for general systemic toxicity, the potential genotoxicity of a substance should be evaluated using a range of appropriate in vitro and, if necessary, in vivo tests. For comprehensive coverage of the potential genotoxicity of a substance, information on

the ability to induce gene mutations, structural chromosomal aberrations and aneuploidy is required. A small number of well-validated in vitro assays are usually selected to cover the different genetic endpoints. Commonly used test batteries include a gene mutation test in bacteria (i.e. the *Salmonella*/microsome assay) and one or two tests in mammalian cells detecting point mutations or chromosome damage (clastogenicity/aneugenicity).

Effects of the substance on reproductive performance of both males and females and on the prenatal and postnatal development of offspring are also usually determined. The purpose of reproductive and developmental toxicity studies is to assess 1) possible effects that may be expressed through reduced fertility or fecundity in either the parents or offspring as a result of morphological, biochemical, genetic or physiological disturbances and 2) whether there is normal growth and development of the offspring. However, tests for reproductive and developmental toxicity do not necessarily cover the full range of effects that might be induced by chemicals that interfere with the endocrine system. Development of a battery of screening tests that can evaluate chemicals that interact with the estrogen, androgen and thyroid signalling pathways is still ongoing at the time of the publication of this monograph.

There should also be consideration of the need for acute toxicity testing. Some substances (e.g. certain metals, mycotoxins, veterinary drug residues, pesticide residues) could give rise to acute health effects in relation to short periods of intake. JECFA includes in its evaluations an assessment of acute effects and, where appropriate, the possibility of acute effects in sensitive individuals. JMPR also now routinely considers the need to set an acute reference dose (ARfD) for all pesticides it evaluates. JMPR has developed guidance for a single-dose study in experimental animals, with the aim of enabling more accurate derivation of ARfDs; this guidance serves as the basis for an OECD test guideline currently under development.

Additional testing may also be required for nutritional effects, neurotoxicity, including neurobehavioural effects both in adults and during development, and immunotoxicity. The need for such additional testing may be evident from the results of the standard tests described above. Specific studies on mechanism of toxicity or mode of action may provide additional useful data for the evaluation.

Interpretation of findings

Critical evaluation of study designs and their findings and interpretation of the results are the most important steps in risk assessment. The findings from treated groups are usually compared with those from concurrent controls. Comparison of test data with data from historical controls, particularly in the case of carcinogenicity and developmental toxicity, may also be necessary to understand the significance of a particular finding.

For the assessment of many toxicological end-points, a weight of evidence approach is necessary, utilizing the data from all the available studies in which the same or functionally related fluids, cells, tissues or organs have been studied. Similar findings across different studies and evidence of dose–response relationships give added weight to the hazard characterization.

Determination of whether or not a compound is genotoxic should be based on an overall assessment of the available data. Completely negative results in an in vitro test battery are normally considered sufficient to conclude that a substance is devoid of genotoxic potential, unless there are reasons for special concern (e.g. high or sustained human exposure, structural considerations). Conversely, one or more positive in vitro tests normally require follow-up by in vivo genotoxicity testing. The outcome of the genotoxicity tests may then be considered alongside experimental results from rodent carcinogenicity bioassays, as the results of short-term tests alone do not provide a reliable prediction of whether or not a chemical is a carcinogen in rodents. Positive genotoxicity studies do provide knowledge about mode of action for substances that are carcinogenic and influence the approach used in the subsequent risk characterization. Positive findings in rodent cancer bioassays require careful interpretation in relation to mode of action, possible interspecies differences in background incidence and in response, and the issue of high dose to low dose extrapolation. IPCS has developed a conceptual framework on the evaluation of the mode of action for chemical carcinogenesis in animal test species, which was subsequently extended to address the issue of human relevance of animal cancer data. Mechanisms relevant to humans include deoxyribonucleic acid reactivity or genotoxicity. Some mechanisms were identified not to be relevant to humans,

including α2u-microglobulin-induced rat nephropathy and peroxisome proliferation.

In interpreting data from reproductive and developmental toxicity studies, it is important to look for biologically related patterns of response and the relationship of outcomes across end-points and to relate any findings to the toxicological data available from other studies. As standard study designs require that the top dose exerts some minimal indication of maternal toxicity, it may be difficult to assess whether a developmental effect seen at such a dose is a direct result of the action of the chemical on the embryo or fetus or an indirect result of altered maternal homeostasis. Although there have been several examples of the latter, it is important not to infer causation from an association of developmental toxicity with maternal toxicity without additional testing and evaluation.

Food allergy and other food hypersensitivities

Food allergies are a consequence of the undesired or uncontrolled immune response to a food antigen in susceptible individuals. They are based on the body's aberrant interpretation of certain dietary proteins as "foreign", which leads to a heightened response of the immune system. Allergy develops through the process of sensitization. During the sensitization phase, exposure to the food allergen stimulates production of antigen-specific immunoglobulin E.

Food allergy risk assessment is a relatively new discipline, and there is no general consensus on how it should be conducted, although several approaches have been suggested. For example, there is no current consensus regarding a threshold dose below which sensitization to food allergens would not occur. To predict the potential allergenicity of novel food proteins, such as in genetically modified foods, decision tree strategy approaches have been described.

General principles of studies in humans

Data from human studies are of potential importance in identifying and characterizing hazards and evaluating the risks of food additives, contaminants and residues of veterinary drugs and pesticides. The information may come from controlled experiments in human

volunteers, surveillance studies, epidemiological studies (e.g. ecological studies, case–control studies, cohort studies, analytical or intervention studies) of populations with different levels of exposure, experimental or epidemiological studies in specific subgroups of people, or clinical reports (e.g. poisoning) or case-studies of individuals. End-points may include examination of safety or tolerance, nutritional and functional effects of foods or food components, the metabolism and toxicokinetics of the substance, mode of action, possibly using biomarkers for effects identified in animal studies, and adverse health effects from unintentional exposures (e.g. to a contaminant).

Critical issues for any experimental study in humans are the ethical, professional and basic legal controls that govern whether a study in humans is necessary and the circumstances under which it may be properly performed. The numbers of subjects entered into a study should be sufficient to realize the aims of the investigation. Consideration needs to be given to when the use of human tissues ex vivo or in vitro might be sufficient. Experiments on human cells or tissues or using other preparations containing or expressing human enzymes, receptors and other subcellular factors in vitro are fundamentally different from studies in people, because they do not take account of absorption, distribution, aspects of integrated metabolism and excretion. However, an advantage is that they permit mechanistic studies under controlled conditions not feasible in the clinic, and these techniques are of considerable value in suggesting metabolic pathways and response mechanisms that may be important in humans and may be worth studying as biomarkers of exposure or effect.

Gastrointestinal tract considerations, including effects on the gut microflora

Interactions that may occur between chemicals in food and the bacterial flora of the gastrointestinal tract should be considered in terms of both the effects of the gut microflora on the chemical and the effects of the chemical on the gut microflora.

In vivo methods for studying the role of the gut microflora in the metabolism of a substance include 1) parenteral administration of the compound, which should result in decreased microbial metabolism of poorly absorbed polar compounds, compared with oral dosing; 2) studies on animals in which the bacterial flora are reduced by the use

of antibiotics; and 3) studies on germ-free animals and on (formerly) germ-free animals inoculated with known strains of bacteria (gnotobiotic animals). A number of factors may influence the metabolic activation of foreign chemicals by the host microflora, including host species, diet, medication and metabolic adaptation. In addition, various in vitro and in vivo methods exist to test the potential of a substance to induce resistance in the gut microflora as a result of ingesting substances or residues with antimicrobial properties.

Dose–response assessment

Dose–response assessment is a major part of the hazard characterization within the risk assessment paradigm. Dose–response assessment is used to develop risk assessment advice and to derive health-based guidance values.

Approaches generally take one of two forms: 1) analyses that provide a quantitative or qualitative estimation of risk; and 2) analyses that establish health-based guidance values, such as an acceptable daily intake (ADI) or tolerable daily intake (TDI), which are levels of human exposure considered to be "without appreciable health risk". The TDI is used for contaminants, whereas the ADI is used in cases where exposure can be controlled, such as for food additives and residues of pesticides and veterinary drugs in foods. The approaches to dose–response assessment applied to data from studies in animals have been discussed in EHC 239 on Principles for Modelling Dose–Response for the Risk Assessment of Chemicals.

One of the primary components of a risk assessment is determination of the presence or absence of a cause–effect relationship. If there is sufficient plausibility for the presence of such a relationship, then dose–response data are essential. Dose–response data may be derived from in vivo studies in laboratory animals or humans, which usually provide the basis for risk characterization. In each case, interpretation of the data on effects usually requires recognition of the levels of exposure that do not produce a measurable effect and the relationship between the increase in incidence, severity or nature of the effect with increase in exposure.

Dose–response modelling can be described by six basic steps. The first four steps (data selection, model selection, statistical linkage and

parameter estimation) relate to the analysis of the dose–response data. In this analysis, the observed dose–response data are modelled in a way that allows prediction of the likely magnitude of the response at a given dose, either within or outside the observed dose–response range, or prediction of the likely dose causing a given magnitude of response. The last two steps deal with implementation and evaluation of the results of the analysis.

Extrapolation is a necessary part of all risk assessments. In most cases considered by JECFA and JMPR, the data used for dose–response assessment come from experiments in laboratory animals administered doses significantly exceeding the potential human exposure. For such dose–response analyses, there are two issues of extrapolation: 1) extrapolating from the test species to humans; and 2) allowing for possible human differences in response. The methods employed for these extrapolation issues are discussed in the monograph and are varied, ranging from the use of uncertainty factors to more complicated modelling schemes based upon differences in toxicokinetics and toxicodynamics between humans and experimental animals and variability between different human individuals.

Derivation of health-based guidance values

The setting of health-based guidance values provides quantitative information from risk assessment, enabling risk managers to make decisions concerning the protection of human health. Health-based guidance values are derived from the dose–response assessment for the most relevant end-point in the most relevant species. The first approach, which is the one still most commonly used by JECFA and JMPR to derive health-based guidance values in order to protect against effects considered to have a threshold, is to define the NOAEL or sometimes a lowest-observed-adverse-effect level (LOAEL) as the POD. The other approaches that have been used by JECFA and JMPR are to use the lower one-sided confidence limit of the BMD (the BMDL) as the POD for the derivation of a health-based guidance value or for calculation of a margin of exposure (MOE). Dose–response assessment is occasionally used to define the dose associated with a negligible (e.g. 1 in a million) increased response over background.

For food additives and for residues of pesticides and veterinary drugs in food, the health-based guidance value is termed the ADI. JECFA and

JMPR determine ADIs based on all the known facts at the time of the evaluation. JECFA generally sets ADIs on the basis of the lowest relevant NOAEL in the most sensitive species. The ADI is expressed in amount (e.g. mg) per kilogram of body weight, usually as a range from 0 to an upper limit. ADIs are normally expressed numerically using only one significant figure. When appropriate, JMPR and JECFA develop ARfDs, an estimate of the amount of a substance in food and/or drinking-water, normally expressed on a body weight basis, that can be ingested in a period of 24 h or less, without appreciable health risk to the consumer, on the basis of all the known facts at the time of the evaluation.

For food contaminants that are generally unavoidable, JECFA has used the term "tolerable" for health-based guidance values, as it signifies permissibility for the intake of contaminants associated with the consumption of otherwise wholesome and nutritious food. Principles in deriving tolerable intake levels are the same as for ADIs: either the NOAEL or BMD approaches can be used as the POD to set health-based guidance values for contaminants. Food contaminants include heavy metals, environmental contaminants such as dioxins and mycotoxins, impurities arising in food additives, solvents used in food processing, other substances arising from food processes such as heating, substances migrating from food contact materials and residues arising from the use of animal feed additives or the non-active components of veterinary drug formulations. Guidance values may be expressed as a TDI, provisional maximum tolerable daily intake (PMTDI), provisional tolerable weekly intake (PTWI) or provisional tolerable monthly intake (PTMI). The use of the term "provisional" expresses the tentative nature of the evaluation, when there is a paucity of reliable data on the consequences of human exposure at levels approaching those with which JECFA is concerned. PMTDIs are established for food contaminants that are known not to accumulate in the body. For contaminants that may accumulate within the body over a period of time, JECFA has used the PTWI and PTMI.

The critical steps in the NOAEL approach to deriving health-based guidance values are selection of the appropriate data and determination of the NOAEL. In calculating the health-based guidance value, a safety or uncertainty factor is applied to the NOAEL to provide a conservative margin of safety because of the inherent uncertainties in extrapolating toxicity data from experimental animals to potential

effects in humans as well as variation within the human species. The terms "safety factor" and "uncertainty factor" are often used interchangeably, "safety factor" having been used historically, but the preference now is to use "uncertainty factor". The concept of chemical-specific adjustment factors has been introduced to allow the use of specific data on species differences or human variability in either toxicokinetics or toxicodynamics to derive data-driven uncertainty factors instead of the use of default factors, where possible.

The BMD approach has been introduced as an alternative to the NOAEL approach. This method defines a level of exposure producing a low but measurable effect size or level of response as the POD for risk assessment. The BMD method has a number of advantages, including the use of the full dose–response data in the statistical analysis, which allows quantification of the uncertainty in the data. Higher uncertainty in the data—for example, due to small group sizes or high variation within a group—would be reflected in lower health-based guidance values.

There are occasions when JECFA and JMPR consider the setting of an ADI in numerical terms not to be appropriate, such as when the estimated consumption of the additive is expected to be well below any numerical value that would ordinarily be assigned to it. Under such circumstances, the term ADI "not specified" is used.

There may be situations where either the body of available data on a substance is limited on some aspects or the safety of a chemical for which JECFA or JMPR had previously assigned an ADI was brought into question by new data. When JECFA or JMPR feels confident that the use of the substance is safe over the relatively short period of time required to generate and evaluate further safety data, but is not confident that its use is safe over a lifetime, it often establishes a "temporary" ADI, pending the submission of appropriate data to resolve the safety issue within a defined time-line.

For veterinary drugs and pesticides, the ADI is used to confirm the safety of proposed MRLs when the substances are applied in accordance with good practices. In establishing the ADI for a veterinary drug or a pesticide residue, the toxicities of the parent drug and of its main metabolites are considered, and the ADI is based on the toxicological end-point of the compound of most concern.

If a veterinary drug can affect the human gut microflora at exposures lower than those causing toxicological effects, then this end-point is used as the basis for establishing the ADI. An internationally harmonized decision tree approach, for which the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) has developed a guideline, is used to determine the need to establish a microbiological ADI. The first three steps consider whether 1) residues of the drug and/or its metabolites are microbiologically active against representatives of the human intestinal flora, 2) residues enter the human colon and 3) the residues entering the human colon remain microbiologically active. If the answer is "no" to any of the first three steps, then no microbiological ADI is necessary. However, should such residues be present, then two end-points of public health concern are considered: 1) disruption of the colonization barrier and 2) increase of the populations of resistant bacteria.

If several substances that produce similar toxic effects or share a common toxic metabolite are to be considered for use as food additives, pesticides or veterinary drugs or occur as contaminants, it may be appropriate in establishing a health-based guidance value to consider the substances as a group in order to limit their overall intake. For this procedure to be feasible, the substances should have a similar mode of action and a similar range of toxic potency.

It is preferable to set health-based guidance values that will cover the whole population. These values are normally established to protect the most sensitive subpopulation, based on the most sensitive critical health outcome. However, it is recognized that the most sensitive critical health outcome may not always be relevant to some population subgroups. For example, it is particularly important to ensure that any health-based guidance value is adequate to protect the embryo or fetus from possible effects in utero. Thus, in some situations in which a developmental or other subpopulation-specific end-point determines the health-based guidance value for a substance exhibiting no other toxicity, advice regarding a second (higher) value based on another end-point relevant to the rest of the population may be provided.

Dietary exposure assessment of chemicals in food

In the assessment of dietary exposure to chemicals, food consumption data are combined with data on the concentration of chemicals in

food. The resulting dietary exposure estimate may then be compared with the relevant health-based guidance value or with the toxicological POD (NOAEL; BMDL) for the food chemical of concern as part of the risk characterization. Assessments may be undertaken for acute or chronic exposures. Dietary exposure assessments should cover the general population, as well as critical groups that are vulnerable or are expected to have exposures that are significantly different from those of the general population (e.g. infants, children, pregnant women, elderly, vegetarians).

In principle, dietary exposure assessments need to be performed for all identified chemicals present in the diet that are subject to risk assessment. Similar methods are appropriate for contaminants, pesticide and veterinary drug residues, food additives (including flavourings), processing aids and other chemicals in foods. A stepwise approach is recommended, in which screening methods can be applied to identify, among the large number of chemicals that may be present, those of no safety concern, using minimal resources in the shortest possible time. A refined exposure assessment is not needed for such substances. Further steps to allow the refinement of the dietary exposure assessment should be designed in such a way that potential high dietary exposure to a specific chemical is not underestimated.

Sources of information on concentrations of chemicals in food include proposed maximum levels (MLs) or MRLs, proposed manufacturers' use levels, monitoring and surveillance data, total diet studies (TDSs), the GEMS/Food database, veterinary drug residue depletion studies, highest and mean residues from supervised trials for pesticides, and the scientific literature. The most accurate data are obtained from the measurement of chemical concentrations in foods as consumed. Programmes to generate data on concentrations of chemicals in food require validated sampling plans and analytical methods. There are two main approaches to analysing foods when generating analytical data from surveys: 1) analysis of food group composites; and 2) analysis of individual foods (either as single samples or as composites).

Food consumption information can be obtained from food balance sheet data, which include the amounts of foods available for human consumption derived from national statistics on food production,

disappearance or utilization. They are generally available for most countries. The GEMS/Food consumption cluster diets developed by WHO are based on selected FAO food balance sheets and represent average per capita food consumption. The consumption cluster diets replace the five regional diets previously developed by WHO.

Food consumption data should be available in a format that allows matching of the consumption data with the concentration data used in the dietary exposure assessment. Data collected using population-based methods are generally compiled and reported for raw or semi-processed agricultural commodities, and they represent the total annual amount of a commodity available for domestic consumption per year. Data from individual food consumption surveys are often not publicly available in raw format (i.e. at the individual respondent level), and risk assessors have to rely on published summary statistics. Market share corrections can be applied to food consumption data for processed foods or percentage of treated crops. The approach is used mainly when the substance being evaluated has been deliberately added to the food.

The available methods for estimating dietary exposure have been divided into those that provide single (point) estimates and those that characterize the full distribution of consumer exposures. Point estimates include 1) screening methods, 2) exposure methods that rely on crude estimates of consumption, such as the theoretical added maximum daily intake (TAMDI) and other model diets, and 3) more refined exposure methods based on actual consumption data and chemical concentration data, such as TDSs, selective studies of individual foods and duplicate portion diets. A deterministic or point estimate of dietary exposure is simply a single value that describes some parameter of consumer exposure (e.g. the average exposure of a population). Characterizing the full distribution of consumer exposures is the most resource-intensive assessment, as data are required that characterize the range of food consumption practices as well as the range of chemical concentrations in the foods that are eaten. The extent to which estimates of dietary exposure need to be refined will depend, in part, on the nature of the substance and the toxicity profile.

Screening methods overestimate dietary exposure of high consumers using conservative assumptions in terms of food consumption and

chemical concentrations. Their aim is not to assess true dietary exposure but to identify food chemicals for which a more comprehensive dietary exposure assessment is necessary. Screening methods include poundage data (for food additives, including flavours), the budget method (which has been used to assess the theoretical maximum daily dietary exposure to some food additives) and model diets (which are constructed from available information on food consumption and are designed to represent a typical diet for the population whose exposure is to be considered).

Point estimate modelling may also be appropriate as a second step in a tiered approach. The model selected can be more or less conservative, depending upon the purpose and the available information. Model diets for high consumers can be developed on the basis of published data from food consumption surveys as an alternative to the budget method or as an additional step in the screening process. Food consumption amounts and dietary exposures for high consumers can also be derived from distributional data. The tendency of consumers to repeatedly purchase and consume the same food products, sometimes termed consumer loyalty, may need to be considered and a range of concentrations may need to be used to generate dietary exposure estimates to cover various scenarios of consumer behaviour.

For substances requiring further refinement beyond screening methods or point estimates of exposure, a probabilistic analysis of exposure variability can be conducted. Approaches to developing probabilistic models for dietary exposure assessments include simple empirical distribution estimate, developing probabilistic models from data sets, stratified sampling, random sampling (Monte Carlo simulation) and Latin hypercube.

For a probabilistic exposure assessment, the readily available distributions of food consumption data are from short-term studies and are not representative of true long-term consumption. Approaches that have been used to estimate long-term consumption have included methods combining food frequency data with information on amounts consumed and statistical models that use the correlations among the days of consumption to estimate the “usual” intake of the substance under consideration.

Exposures to food chemicals through other routes may occur, and exposures to chemicals or drugs sharing the same mechanism of action (toxicity) may also be encountered. Consideration of combined exposures to a single chemical across multiple routes (oral, dermal, inhalation) and across multiple pathways (food, drinking-water, residential) is known as aggregate exposure. Consideration should also be given to the assessment of risks from exposure to multiple pesticide residues that have a common mechanism of toxicity, and the exposure estimate for that situation is termed cumulative exposure. Guidance for estimating aggregate exposure has been issued.

Risk characterization

Risk characterization is the fourth step of the risk assessment process, integrating information from the hazard characterization and the exposure assessment to produce scientific advice for risk managers. Historically, different approaches have been used for the risk characterization of toxic effects considered to have a threshold for the observed adverse effect and those considered to have no threshold. Health-based guidance values are set by JECFA and JMPR for substances that produce effects exhibiting a threshold. In the risk characterization for these types of substances, the health-based guidance values are compared with estimated or measured human exposure.

In cases where exposures exceed health-based guidance values, the values themselves do not provide risk managers with advice on the possible extent of the risk to those exposed to these higher amounts. A first consideration should take into account the fact that health-based guidance values themselves incorporate safety or uncertainty factors. A small or occasional dietary exposure in excess of a health-based guidance value based on a subchronic or chronic study does not necessarily imply that adverse health effects will occur in humans.

In circumstances where the data are not sufficient to propose a health-based guidance value for a substance or the mode of action cannot be assumed to exhibit a threshold, JECFA and JMPR may comment on the MOE between the doses at which effects are seen in animals and the estimated human dietary exposure.

Risk characterization should include consideration and description of uncertainty and variability. Uncertainty refers to limitations

in the knowledge of the risk assessor about the data and models used. Variability reflects the inherent biological heterogeneity, either in exposure or in response. Thus, although both uncertainty and variability can be characterized using probability distributions, they are different concepts. Uncertainty can be decreased as the quantity or quality of the information available improves. Modelling variability is an exercise in descriptive statistics that results in a model of a population rather than an individual. Characterization of the variability in dietary exposure in the population, as an example, can be improved by better information, but the variability cannot be eliminated. The risk characterization should include a narrative evaluation of uncertainty for both exposure and health effects. Sensitivity analysis refers to quantitative techniques that may be used to identify those aspects of the inputs (e.g. concentration or food consumption data) that contribute the greatest extent to the uncertainty.

There is an increasing awareness by those involved in risk assessment of the need to consider any risks associated with combined exposure to mixtures of substances. There are four types of combined effect or interaction: dose addition, response addition, synergism and antagonism. Evaluations of mixtures have been undertaken by JECFA and JMPR for some food additives, pesticides and veterinary drugs that are produced and tested as mixtures and some co-occurring mixtures of certain contaminants. For pesticides and veterinary drugs that are mixtures, JMPR and JECFA, respectively, base the ADI for the residues on the mixture as tested. In some cases, a group ADI has been allocated. JECFA has also used the group ADI for certain food additives that are metabolized to a common potentially toxic metabolite and a group TDI for closely related contaminants that occur as mixtures. An approach that takes account of dose additivity is the toxic equivalency factor (TEF) approach, which scales the exposure for each component of a mixture relative to the potency of an index chemical (e.g. for dioxins and dioxin-like chemicals).

For substances that are genotoxic and carcinogenic, the traditional assumption is that there may not be a threshold dose and that some degree of risk may exist at any level of exposure. Thus, health-based guidance values have not been developed by JECFA for substances

that are known to be both genotoxic and carcinogenic. Some chemicals, however, induce cancer in experimental animals by non-genotoxic mechanisms that have a threshold, and for these, health-based guidance values can be established.

Substances that are both genotoxic and carcinogenic would generally not be considered acceptable for use as food additives, pesticides or veterinary drugs. JECFA has considered a number of contaminants that have been demonstrated to be both genotoxic and carcinogenic and has discussed possible approaches to the formulation of advice that would better inform risk managers about the possible magnitude of health concerns at different levels of intake in humans. Exposure (intake) assessment for a compound that is both genotoxic and carcinogenic is no different from that for other types of contaminants. Risk characterization can take different forms: 1) calculation of the MOE between the dose causing a low but defined incidence of cancer (usually in animal bioassays) and estimated human exposure; 2) dose–response analysis outside the observed dose range of animal bioassays to calculate the incidence of cancer that is theoretically associated with the estimated exposure for humans or the exposure associated with a predetermined incidence of cancer (e.g. an increased risk of cancer over a lifetime of 1 in a million); and 3) linear low-dose extrapolation from a POD such as the BMDL. Of these three options, the MOE and linear low-dose extrapolation from a POD are the most pragmatic and usable at the present time. JECFA has decided that advice on compounds that are both genotoxic and carcinogenic should be based on estimated MOEs. The monograph emphasizes that strengths and weaknesses inherent in the data used to calculate MOEs should be described in the advice to risk managers, together with advice on interpretation of the MOEs.

Maximum residue limits for pesticides and veterinary drugs

MRLs for pesticide residues and residues of veterinary drugs are the maximum concentrations of residues to be permitted in or on a food. International standards on MRLs are adopted by CAC on recommendation by the respective Codex committees, the Codex Committee on Pesticide Residues (CCPR) and the Codex Committee on Residues

of Veterinary Drugs in Foods (CCRVDF). These recommendations are based on advice provided by JMPR and JECFA. Both JECFA and JMPR have similar requirements for the identification and characterization of a substance that is under review for the establishment of an ADI, ARfD and MRLs.

JMPR evaluates pesticide residue data resulting from pesticide use according to Good Agricultural Practice (GAP) to estimate maximum residue levels in food and feed commodities. JMPR evaluates animal (livestock) and crop metabolism studies as the prime determinants of the residue definition in food and feed commodities. The recommended maximum residue levels in various crops depend mainly on the data from supervised residue trials conducted in line with maximum registered uses within GAP. The trials should cover the range of conditions expected to occur in practice, including application methods, seasons, cultural practices and crop varieties. If residue levels in the processed commodity exceed the residue levels in the raw agricultural commodity by a margin sufficient to require an MRL higher than the raw agricultural commodity MRL, it is necessary for JMPR to estimate a maximum residue level for the processed commodity. The pesticide residue dietary burden for livestock is derived from supervised residue trials for feed commodities multiplied by standard animal diets based on OECD livestock feed tables. Estimated maximum residue levels as well as highest residues (HRs) found in the supervised trials and supervised trial median residues (STMRs) derived from external animal treatments are compared with those derived from exposure through the feed. The recommended maximum residue levels, HRs and STMRs are based on whichever values are higher from this comparison. Estimates of chronic exposure are based on the STMRs from the supervised trials and food processing studies and long-term food consumption. For short-term exposure assessment, estimates of high intake of pesticide residue on a single day are based on the HRs from the supervised trials.

For veterinary drugs, JECFA evaluates residue depletion studies with radiolabelled parent drug as well as additional studies with unlabelled parent drug in intended target animal species for recommending MRLs in raw commodities of animal origin. Data from the studies using radiolabelled substance are used to estimate the time course of the concentration of the total residue of concern and to determine a marker residue. The derived MRLs are defined on the basis of the

marker residue. The marker residue may be the parent drug, a major metabolite, a sum of parent drug and metabolites, or a reaction product formed from the drug residues during analysis. It is not necessarily a residue of toxicological or microbiological concern, but is useful for monitoring purposes. Data from the studies using unlabelled substance are used to estimate the time course of the concentration of the marker residue in raw commodities of animal origin under approved practical conditions of use (i.e. Good Practice in the Use of Veterinary Drugs or GPVD). The relationship between the marker residue and total residues is used for the conversion of concentrations of the marker residue into concentrations of total residues of concern for the purpose of estimation of dietary exposure.

MRLs are generally recommended for several edible tissues and products, as appropriate for the intended use—for example, for muscle, liver, kidney and fat of slaughter animals, for fat and skin of poultry (and, where appropriate, of pigs) in natural proportions, for muscle and skin of fish in natural proportions, as well as for milk, eggs and honey.

For veterinary drugs, JECFA now develops recommendations for MRLs based on chronic intake estimates calculated from the median residue levels and a theoretical food basket (consisting of 300 g muscle, 100 g liver, 50 g kidney, 50 g fat, 1500 g milk, 100 g eggs and 20 g honey), to estimate a conservative daily intake of residues, known as the estimated daily intake (EDI). The formerly used theoretical maximum daily intake (TMDI) utilized the MRL per se as the point estimate, which is a single value representing the upper limit of a high percentile of the distribution of residues. JECFA concluded that this method was not realistic and that all concentrations in the distribution of residues should be considered in the estimation of chronic intake. In cases where the quality of the data is not sufficiently robust to estimate a median residue level or intake, the TMDI may be used to provide a conservative intake estimate.

JECFA may make full recommendations for MRLs of a veterinary drug in appropriate food animal species and tissues on the basis of an ADI and adequate residue data. Temporary MRLs may be recommended either when there is an ADI but adequate residue or analytical method performance data are lacking or when the ADI is temporary. The Committee may recommend MRLs “not specified”

or "unnecessary" when there is a very wide margin of safety between estimated consumption of residues and the ADI.

Principles related to specific groups of substances

Many of the substances evaluated by JECFA are present in food at low concentrations. Examples include flavouring substances, processing aids, extraction solvents and enzymes used in food production. For the evaluation of such substances, it may be more appropriate to use the approaches described in this section of the monograph.

One such approach is the threshold of toxicological concern (TTC) concept. The knowledge that toxicity is a function of both chemical structure and the extent of exposure is the basis of the TTC concept. The TTC concept allows risk assessors to provide science-based advice when there is a high probability of negligible harm based on low dietary exposure and chemical structure alone. It is not intended to replace established risk assessment procedures used by JECFA and JMPR for substances on which extensive toxicity data are available.

The TTC approach, as applied by JECFA, utilizes human exposure threshold values (TTC values) for three structural classes of chemicals, below which there is a very low probability of any appreciable risk to human health. These TTC values have been derived from existing toxicity data on chemicals that have been classified into one of three structural classes. The TTC values for structural classes I, II and III are 1800, 540 and 90 μg/person per day, respectively. As the human exposure threshold values are compared with known or anticipated exposure, the TTC approach requires sound estimates of human exposure.

A decision tree approach (the Procedure for the Safety Evaluation of Flavouring Agents) has been developed by JECFA for the application of the TTC concept to flavouring substances. When the Procedure was first adopted, JECFA decided that a practical and realistic approach to derive estimated dietary exposures for consumers of flavouring agents was to use annual production volume data for different regions. This estimate, termed the maximum survey-derived intake (MSDI), was derived from figures for the total annual production of flavouring agents, adjusting for the fact that not all the chemical produced

would be reported and assuming that the flavouring agent would be consumed by only 10% of the population considered.

JECFA noted that use of the MSDI might result in an underestimation of dietary exposure to a flavouring agent for regular consumers of certain foods containing that flavouring agent. An additional new method of estimating dietary exposure for flavouring agents was therefore elaborated, termed the single portion exposure technique (SPET). The SPET estimate assumes a daily consumption of a single portion of food containing the flavouring agent, based on added use levels provided by the industry. The SPET identifies all food categories likely to contain the flavouring agent, assigns an added use level to a single "standard" portion of each of these categories and then identifies the single food category that is likely to contribute the highest dietary exposure. The standard portion is taken to represent the mean food consumption amount for consumers of that food category, assuming daily consumption over a long period of time. The standard portion does not reflect high food consumption amounts reported in national dietary surveys for the food category and is therefore a more realistic prediction of long-term consumption patterns. JECFA has concluded that the MSDI and SPET dietary exposure estimates provide different and complementary information. The higher value of the two dietary exposure estimates (MSDI or SPET) will be used within the Procedure.

JECFA has considered applying the TTC approach for the risk characterization of not only flavouring substances, but also other substances present in the diet in small amounts. For further application of the TTC approach, the Committee noted that it should be used in conjunction with conservative estimates of dietary exposure and that additional data on the toxicity of structurally related substances might be required. It further recommended that guidance be drawn up on application of the approach with regard to substances present in the diet in small amounts, such as certain residues of processing aids, packaging materials and contaminants, to provide advice on the risk assessment of substances for which full toxicological data sets are not available or are unnecessary.

The safety assessment of food packaging materials presents special problems because of the very large number of them in use and

the anticipated low level of migration of substances from food contact materials and consequent low dietary exposure. In principle, two alternatives exist for performing safety assessments on food contact materials. One is to require toxicological data regardless of the level of potential dietary exposure so that a safety assessment can be performed. A second option is to apply a tiered approach in which the number of toxicological data required is related to the extent of anticipated exposure as measured by migration studies.

Processing aids are composed of diverse substances, including, but not limited to, carrier or extraction solvents and enzymes used in food processing. JECFA has elaborated and periodically updated principles and procedures for the safety assessment of enzyme preparations.

The safety assessment of substances that are consumed in relatively large amounts, such as bulk sweeteners, modified starches, nutrients and related substances, and non-traditional whole foods, presents a number of special problems. The safety assessment of such substances differs from that for other food additives because of high dietary exposure, and minor constituents and processing impurities may assume greater than usual significance.

The increased use of fortified foods, dietary or food supplements, specially formulated foods and so-called "functional foods" has increased the intake of nutrient substances around the world. JECFA evaluates only the safety of these ingredients in accordance with the principles and methods in this monograph and has expressed the view that the evaluations should not be interpreted as an endorsement of the use of these substances for their claimed nutritional or health benefits.

Nutrient substances are biologically essential or have a demonstrated favourable impact on health at specified levels of intake. This consideration influences approaches applied to adjust for uncertainty associated with the data used to estimate a health-based guidance value and necessitates that the homeostatic mechanisms specific to essential nutrient substances be taken into account. Therefore, modifications to the classic non-nutrient risk assessment approach are needed. Internationally, guidance for risk assessment of nutrients and related substances recommended the use of the upper level of intake (UL),

in addition to a minimum intake for various strata of the population necessary to avoid nutritional deficiencies. The UL is the estimate of the highest level of regular intake that carries no appreciable risk of adverse health effects. The UL can be derived for nutrients using the principles of risk assessment similar to those that have been developed for biological and chemical agents.

Foods from novel sources include traditional and non-traditional foods, novel foods and foods for special dietary uses. Specifications are necessary to ensure that levels of potentially hazardous contaminants, such as mycotoxins and heavy metals, are kept to a minimum. The influence of the introduction of the new substance on the nutrient composition of the diet as a whole should be identified, particularly with respect to groups such as children, the elderly and "captive populations" (e.g. hospital patients and schoolchildren). The nutritional value of the novel food should be assessed initially from its chemical composition with respect to both macronutrients and micronutrients, taking into account the effects of any further processing and storage. Depending on the nature and intended uses of the novel food, studies in laboratory animals may be needed to supplement the chemical studies. Human studies on novel foods need to be designed on a case-by-case basis. Human experience is an essential part of the data collection in the history of use. For novel foods, exposure will need to be estimated from proposed uses. For the risk characterization of novel foods, the MOE approach may be suitable.

1. INTRODUCTION

1.1 The need for updated guidance on risk assessment

The Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) have a long history of collaboration in the safety evaluation of chemicals in food. This activity began in 1956, when the first meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) was convened by the two organizations, and was strengthened in the early 1960s, when the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) first met.

JECFA and JMPR follow the same general principles and methods for chemical risk assessments, which have been published in the reports of both committees. In response to recommendations made by JECFA and JMPR in the early to mid 1980s to review the validity of the evaluation procedures then in place, the International

---

For acronyms and abbreviations used in the text, the reader may refer to the list of acronyms and abbreviations at the front of this monograph. Definitions of select terms may be found in the glossary at the end of the monograph.

Programme on Chemical Safety (IPCS) sponsored the preparation of Environmental Health Criteria monographs (EHCs) on Principles for the Safety Assessment of Food Additives and Contaminants in Food, EHC 70 (IPCS, 1987), and Principles for the Toxicological Assessment of Pesticide Residues in Food, EHC 104 (IPCS, 1990). These monographs and the principles laid out in subsequent reports have served as the basis for the assessments that have been performed by JECFA and JMPR, respectively, since they were published.

Although much of the guidance set out in EHC 70 and EHC 104 remains valid today, considerable development has taken place in the procedures for and complexity of assessments of chemicals in food since these monographs were prepared. There have been significant advances in chemical analysis, toxicological assessment and risk assessment procedures. JECFA and JMPR have developed many new general principles, and other international organizations and national governments have developed or are developing food safety risk assessment approaches and criteria. In addition, since the publication of these monographs, JECFA has also been charged with the evaluation of the safety of veterinary drug residues.

A conference on international food trade that was held in Melbourne in 1999 (FAO, 2000) recognized these developments and the fact that the evaluations performed by JECFA and JMPR serve as the scientific foundation for international food standards, which are of increasing importance within the Codex Alimentarius Commission (CAC) and the World Trade Organization. The conference recommended that WHO should consider updating and harmonizing all the common principles used by JECFA and JMPR in the toxicological evaluation of food chemicals and publish the information in a single consolidated document.

Following this recommendation, FAO and WHO initiated a project to update, harmonize and consolidate principles and methods for the risk assessment of food additives, food contaminants, natural toxicants and residues of pesticides and veterinary drugs. This monograph is the outcome of that project.

1.2 Development of the monograph

To develop this monograph, the principles and procedures used by JECFA and JMPR, including those in EHC 70 (IPCS, 1987) and

EHC 104 (IPCS, 1990) and those subsequently adopted by meetings of JECFA and JMPR, were reviewed. Those principles and methods that remain valid in view of current scientific knowledge have been reaffirmed. In addition, where possible, risk assessment procedures for different classes of chemicals in food (e.g. additives, contaminants, pesticide residues, veterinary drug residues and natural toxicants) have been harmonized. For those aspects that could not be harmonized, the reasons for the differences are elaborated.

FAO, WHO and other organizations have recognized the importance of the harmonization of risk assessment procedures to enhance the quality of risk assessments, achieve greater consistency when evaluating the risks from different sources of exposure, improve the transparency of the risk assessment process and facilitate risk communication. Therefore, approaches to risk assessment by other scientific groups (including national, regional, other public health and environmental organizations) were reviewed for these harmonization efforts. In particular, the outcomes of the IPCS Harmonization Project (http://www.who.int/ipcs/methods/harmonization/en/) and the Food Safety in Europe project of the European Commission (Barlow et al., 2002; Renwick et al., 2003) have been used in the development of this monograph.

1.3 Purpose, scope and outline of the monograph

1.3.1 Purpose

The primary purpose of this monograph is to provide descriptive guidance for JECFA and JMPR to ensure the continuation of transparent and sound expert evaluations of scientific data for risk assessments of chemicals in food. The principles and methods described are focused on meeting the needs of JECFA and JMPR for their provision of scientific advice to FAO and WHO, particularly in the context of CAC. This monograph is also intended to be informative for users of the outputs from JECFA and JMPR, such as risk managers and other risk assessment bodies in Member countries and regional authorities.

Another purpose of this document is to facilitate the incorporation of new scientific tools, approaches and knowledge in the implementation of risk assessment of food chemicals, as discussed in section 1.5

below. In order to allow rapid incorporation of useful new information and guidance, this monograph will be available via the Internet, with each chapter published as a "stand-alone module".

The principles and methods in this document are presented as descriptive guidance. In the final analysis, expert risk assessment bodies, including JECFA and JMPR, must decide on the most appropriate approaches for the available scientific data in order to address the risk assessment and risk management questions that have been formulated for each food chemical considered.

1.3.2 Scope

This document describes general principles and methods for the risk assessment of additives, contaminants, pesticide residues, veterinary drug residues and natural constituents in foods. It also includes general guidance on the risk assessment of novel and non-traditional whole foods.

For some food and food ingredient terms, such as "novel", "foods for special dietary uses" and "nutrient", there are differences in the definitions used by national and regional authorities. In this document, the definitions given are those developed by JECFA and JMPR or CAC.

Some general guidance is also given on risk assessment related to upper levels for nutrients and other potentially beneficial food components (see also FAO/WHO, 2006a). Nutrient requirements and the determination of the efficacy of potentially beneficial dietary components are not addressed.

1.3.3 Outline

This document is organized to support risk assessment in the framework of the risk analysis paradigm, with considerations of risk profiling and problem formulation and the necessary interactions between the risk assessors and risk managers. The risk analysis paradigm is only briefly reviewed, as other publications have covered that topic in more detail (see, for example, FAO/WHO, 2006b).

Chapter 2 describes the role of risk assessment in risk analysis.

Chapter 3 describes the importance of and varying requirements for chemical characterization and analytical methods in risk assessment and risk management.

Chapter 4 covers the general principles of toxicological testing methods and studies required for hazard identification and characterization. These areas were covered extensively in EHC 70 and EHC 104.

Chapter 5 on dose–response assessment continues the theme of hazard characterization. It discusses the derivation of health-based guidance values and dose–response modelling.

Chapter 6 provides a summary of approaches to estimating dietary exposure (intake), with consideration of the concentration and food consumption data sets that may be used to derive these estimates. Dietary exposure assessments were not covered extensively in either EHC 70 or EHC 104. Subsequently, guidance was developed at several consultations, and EHC 214 (IPCS, 2000) was devoted to the topic of human exposure assessment.

Chapter 7 describes the considerations for risk characterization, including the provision of advice to risk managers and for risk communication.

Chapter 8 reviews the JMPR and JECFA approaches to maximum residue limit (MRL) recommendations for pesticides and veterinary drug residues. Historically, the approaches for the determination of MRLs for pesticides and veterinary drug residues have differed in a number of respects, and this chapter presents those for which harmonization has been agreed and explains those for which harmonization is not currently possible.

Chapter 9 describes some principles of risk assessment related to specific groups of substances consumed in small amounts, such as flavouring agents, substances used in food contact materials and residues of products used in the processing of foods; and substances consumed in large amounts, such as nutrients and novel foods. It is recognized that different national and regional regulatory authorities may have differing regulatory definitions of and requirements related to some of these substance groups. The terms used in this document are those used by JECFA and JMPR.

Finally, the glossary includes definitions of terms used in this report.

1.4 Historical background to the work of JECFA and JMPR

1.4.1 JECFA

JECFA was established following recommendations made to the Directors-General of FAO and WHO by the Joint FAO/WHO Expert Committee on Nutrition at its fourth session (FAO/WHO, 1955), and the subsequent first Joint FAO/WHO Conference on Food Additives was held in September 1955 (FAO/WHO, 1956). The first meeting of JECFA (FAO/WHO, 1957) was held in 1956, and acceptable daily intakes (ADIs) for some food additives were first established at the sixth meeting in 1961 (FAO/WHO, 1962a). The terms of reference of the earlier meetings of JECFA related to the formulation of general principles governing the use of food additives and consideration of suitable uniform methods for evaluating their safety. For these purposes, food additives were defined by the Conference as "non-nutritive substances added intentionally to food, generally in small quantities, to improve its appearance, flavour, texture, or storage properties" (FAO/WHO, 1955). From a practical standpoint, the "food additive" definition has been expanded since then, because a variety of compounds, including nutritive substances, have applications as food additives.

Following recommendations of the third Joint FAO/WHO Conference on Food Additives and Contaminants (FAO/WHO, 1974) and requests from Codex committees, these terms of reference were broadened to include substances unintentionally introduced into human food, such as veterinary drug residues, components of packaging materials, solvents used in food processing, aerosol propellants, enzymes used in food processing, contaminants, including metals in foods, and naturally occurring toxicants. Compounds that may be incorporated into foods as ingredients, at levels higher than those previously envisaged for food additives, have also been evaluated.

The first (FAO/WHO, 1957), second (FAO/WHO, 1958) and fifth (FAO/WHO, 1961) meetings of JECFA established principles for the use of food additives and made recommendations on methods for establishing their safety in use and for the evaluation of carcinogenic

hazards. From the outset, the Committee recognized that "no single pattern of tests could cover adequately, but not wastefully, the testing of substances so diverse in structure and function as food additives" and that "the establishment of a uniform set of experimental procedures that would be standardized and obligatory is therefore undesirable" (FAO/WHO, 1958).

The Committee at its second meeting (FAO/WHO, 1958) concluded that "it was only possible to formulate general recommendations with regard to testing procedures". Subsequent meetings of JECFA have consistently avoided the adoption of rigid protocols for the testing and evaluation of food additives. This allows the Committee to respond to new problems as they arise and to encompass non-routine and ad hoc studies in the safety evaluation.

In recognition of the fact that many features of toxicity testing and evaluation are relevant to both JECFA and JMPR, the twenty-fifth meeting of JECFA (FAO/WHO, 1981) recommended that a group of experts should be convened to study the application of advances in methodology to evaluation of food additives and contaminants, and also of pesticide residues. The urgency of the need to implement this recommendation was stressed by the twenty-sixth (FAO/WHO, 1982) and twenty-seventh (FAO/WHO, 1983) meetings of JECFA.

In response to the Committee's repeated recommendations, IPCS sponsored a project to formulate specific recommendations in order to bring up to date:

- the principles set out in earlier reports of JECFA concerning safety evaluation in relation to specific toxicological problems or specific chemical entities or groups;
- the test methods used in the toxicological evaluation of chemicals in food; and
- the assessment procedures adopted by JECFA in determining quantitative end-points, including the use of "safety factors" for extrapolating animal data to humans and to allow for variability within the human population.

A unified document on these issues was drafted and reviewed at the twenty-eighth (FAO/WHO, 1984), twenty-ninth (FAO/WHO, 1986a)

and thirtieth (FAO/WHO, 1987a) meetings of the Committee. The final monograph was published as EHC 70 (IPCS, 1987).

JECFA meetings on food additives and contaminants provide an evaluation of food additives, novel foods and nutrients used as food additives to the Codex Committee on Food Additives (CCFA) and an evaluation of contaminants and natural toxicants to the Codex Committee on Contaminants in Food (CCCF) for risk management decisions by these committees. Prior to 2007, these two committees were joined as the Codex Committee on Food Additives and Contaminants (CCFAC). JECFA does not recommend maximum levels (MLs) for food additives and contaminants to these Codex committees. In contrast, MRLs for veterinary drugs are recommended by JECFA meetings on veterinary drugs, but their final recommendation and adoption as Codex MRLs are risk management decisions taken by the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) and CAC.

1.4.2 JMPR

The concept of JMPR was first proposed in 1959, when an FAO Panel of Experts on the Use of Pesticides in Agriculture (FAO, 1959) recommended that FAO and WHO should jointly study:

- the hazard to consumers arising from pesticide residues in and on food and feedstuffs;
- the establishment of principles governing the setting up of pesticide tolerances; and
- the feasibility of preparing an international code for toxicological and residue data required in achieving the safe use of a pesticide.

Consequently, in 1961, a Joint Meeting of the FAO Panel of Experts on the Use of Pesticides in Agriculture and the WHO Expert Committee on Pesticide Residues was convened. The report of the 1961 meeting (FAO/WHO, 1962b) recommended that "toxicological and other pertinent data … on those pesticides known to leave residues in food when used according to good agricultural practice" should be evaluated. The evaluations would include the estimate of an ADI and an explanation of its derivation.

To implement this recommendation, the first Joint Meeting of the FAO Committee on Pesticide Residues in Agriculture and the WHO Expert Committee on Pesticide Residues was convened in 1963 (FAO/WHO, 1964). This meeting adopted the concept of the ADI, which was based on:

- the chemical nature of the residue;
- the toxicity of the chemical based on data from acute, short-term and long-term toxicity studies and knowledge of metabolism, mechanism of action and possible carcinogenicity of residue chemicals (usually determined in animals);
- knowledge of the effects of these chemicals on humans; and
- the use of "safety factors" for extrapolating animal data to humans and to allow for variability within the human population.

The 1963 and 1965 meetings (FAO/WHO, 1964, 1965) were concerned solely with ADIs and did not consider tolerances (a term later replaced by MRLs). Separate meetings of an FAO Working Party on Pesticide Residues examined the issue of tolerances approximately 2 months after the 1963 and 1965 meetings and issued separate reports. The first report considered principles (FAO, 1964), and the second proposed tolerances for pesticides on raw cereals (FAO, 1966).

The 1966 JMPR (FAO/WHO, 1967) was the first to consider both ADIs and tolerances. Since then, JMPR has met yearly, with reports and evaluations published subsequently. The products of the meetings, which include ADIs, temporary ADIs, MRLs, temporary MRLs and extraneous residue limits, have remained essentially unchanged.

Principles and methods of toxicological and residue assessments have evolved continuously as new data have been evaluated by JMPR. In view of this, the 1985 JMPR (FAO/WHO, 1986b) recognized the need to consider the quality of data and provide general guidance on the methods used for toxicological evaluations. The Meeting recommended that an international meeting consider the toxicological basis and data requirements for the estimation of an ADI or temporary ADI and to provide general guidance on relevant toxicological methodology. The 1987 JMPR (FAO/WHO, 1987b) and 1988 JMPR (FAO/WHO, 1988b) noted the progress that had been made in preparation of

a monograph covering these issues, and the 1989 JMPR (FAO/WHO, 1989b) reviewed the draft monograph, which was published in 1990 as EHC 104 (IPCS, 1990).

Maximum residue levels for pesticide residues can be estimated and recommended by JMPR for use as MRLs by the Codex Committee on Pesticide Residues (CCPR), but their final recommendation and adoption as Codex MRLs are risk management decisions taken by CCPR and CAC.[1]

1.4.3 Relevant activities since the publication of EHC 70 and EHC 104

New activities not considered in the preparation of the earlier monographs include:

- the evaluation of residues of veterinary drugs in food;
- the development and refinement of methods for estimating the dietary exposure to chemicals in food;
- safety evaluation related to acute exposure; and
- the development of the Procedure for the Safety Evaluation of Flavouring Agents.

These activities are described in more detail below (see sections 1.4.3.1–1.4.3.4). Another new activity not considered previously is the formalization of the risk analysis framework by FAO, WHO and CAC.

An FAO/WHO Conference on Food Standards, Chemicals in Food and Food Trade (in cooperation with the General Agreement on Tariffs and Trade) was held in Rome in March 1991 (FAO/WHO, 1991). This Conference recognized the importance of JECFA and JMPR in providing evaluations based on sound science and risk assessment principles. The Conference recommended that FAO and WHO review the terms of reference of JECFA to ensure that it has the authority and responsibility to review food products derived from contemporary biotechnology. It also recommended that WHO should seek to develop

[1] JMPR distinguishes between a "maximum residue level", which is a scientific estimate with its attendant uncertainty, and a "maximum residue limit" (MRL), which is equivalent to a legal limit.

internationally agreed principles for risk assessment of substances that had been shown to be carcinogenic in animal studies.

1.4.3.1 *Evaluation of veterinary drug residues*

Several antibiotics used as veterinary drugs were evaluated at the twelfth meeting of JECFA (FAO/WHO, 1969), and the two agents proposed for use as growth promoters were considered at the twenty-sixth (FAO/WHO, 1982) and twenty-seventh (FAO/WHO, 1983) meetings. However, the extensive efforts that FAO and WHO have put into the evaluation of residues of veterinary drugs in food did not really begin until 1987 with the thirty-second meeting of JECFA (FAO/WHO, 1988a), which was the first meeting dedicated exclusively to veterinary drugs.

A Joint FAO/WHO Expert Consultation was held in Rome in 1984 (FAO/WHO, 1985) to consider various issues relating to the presence in food of chemicals used in animal husbandry and veterinary medicine. The Consultation recommended inter alia that immediate consideration should be given by CAC to the establishment of CCRVDF. It also recommended that the Directors-General of FAO and WHO convene an appropriate scientific body to advise Member governments and CCRVDF on questions pertaining to residues of veterinary drugs in foods of animal origin, in terms of both potential public health hazards and barriers to international trade. FAO and WHO gave this task to JECFA and set up separate meetings for this purpose.

The development of principles governing the safety evaluation of residues of veterinary drugs in food was begun at the thirty-second meeting (FAO/WHO, 1988a) and has continued since. At its thirty-second meeting, the Committee considered it appropriate and helpful to outline these general principles, but believed that it was desirable to encourage innovation and further developments in such areas as toxicology and residue analysis and did not wish to be unduly rigid in its requirements for data and their interpretation.

Although similar procedures for toxicological assessments are used by JECFA and JMPR, differences in assessment methods exist between JECFA in its assessment of residues of veterinary drugs and JMPR in its assessment of pesticide residues. This became apparent when JECFA and JMPR began evaluating residues of the same chemicals but from different sources. A meeting to harmonize

the work of JECFA and JMPR was therefore held in 1999 (FAO/WHO, 1999a), at which issues relating to the evaluation of chemicals used as both pesticides and veterinary drugs were discussed. It was noted that differences in the evaluation procedures used by the two scientific committees had led to different approaches to the definition of residues, estimation of dietary exposure, description of commodities for analysis and recommendations for MRLs. Other topics discussed at the meeting included risk assessment and tissue matrices used for the analysis of residues in meat/muscle, fat, milk and eggs.

The recommendations of this meeting were reviewed by the 1999 JMPR (FAO/WHO, 1999c) and the fifty-fourth meeting of JECFA (FAO/WHO, 2001a), the responses of which are included in the respective reports. Both scientific committees agreed to implement the recommendations to the extent feasible. Two issues were the different ways in which dietary exposure was estimated (see section 1.4.3.2) and differences in the way in which MRLs are derived by JECFA and JMPR. The MRLs for veterinary drug residues recommended by JECFA are based on the approved conditions of use in accordance with Good Practice in the Use of Veterinary Drugs (GPVD) and in compliance with the ADI, whereas the MRLs for pesticide residues established by JMPR are based on Good Agricultural Practice (GAP). This aspect is explained further in chapter 8. In order to bring its definitions more closely in line with those of JMPR, the fifty-fourth meeting of JECFA (FAO/WHO, 2001a) proposed revised definitions for egg and meat and a new definition for fat, foods included in the "food basket" used to estimate dietary exposure to veterinary drug residues (see chapter 8, section 8.2.2).

The Committees agreed that when JECFA and JMPR have recommended MRLs for the same chemical with the same residue/marker definition for the same commodity, the higher MRL will prevail.

1.4.3.2 Dietary exposure assessments

The procedures used for estimating dietary exposure to various types of chemicals in food have to some extent been developed separately by JECFA and JMPR. An FAO/WHO Consultation on Food Consumption and Exposure Assessment of Chemicals was held in 1997 (FAO/WHO,

1997b) to collate information on different approaches used for different food chemicals, followed by a joint workshop of risk assessors and risk managers (JECFA/CCFAC) on approaches to dietary exposure for contaminants and natural toxicants (FAO/WHO, 2000a). A more recent expert consultation on dietary exposure assessment was held in 2005 to harmonize approaches for the different types of chemicals considered by JECFA and JMPR, where possible. The outcome of that workshop (FAO/WHO, 2008) forms the basis of chapter 6 on dietary exposure assessment, with the history of consideration of dietary exposure estimates for different food chemicals outlined below.

(a) Pesticide residues

JMPR has been publishing chronic dietary exposure assessments as an integral component of its dietary risk assessments since 1998. The CCPR, at its eighteenth and nineteenth sessions in 1986 and 1987 (FAO/WHO, 1986c, 1987c), recommended that guidelines be developed for estimating the intake of pesticide residues, which would provide a procedure to ensure that MRLs adopted by Codex would be such that total dietary exposure to the residue did not exceed the ADI. Guidelines for predicting dietary intake of pesticide residues were published in 1989 (WHO, 1989) and revised in 1995 (WHO, 1997).

The original approach outlined in the 1989 guidelines (WHO, 1989) was a stepwise one, which first calculated a theoretical maximum daily intake (TMDI) as a screening step, assuming residue concentrations at the MRL for the pesticide and a hypothetical global diet. If the estimated dietary exposure exceeded the ADI on the basis of this worst-case calculation, a refined estimate was undertaken, the estimated maximum daily intake (EMDI), which included corrections for edible portion and losses on storage, processing and cooking. If dietary exposure exceeded the ADI on the basis of this calculation, an estimated daily intake (EDI) could be undertaken at a national level based on national diets and including information on the known residue level, corrections for edible portion and losses on storage, processing and cooking, national diets and known uses of the pesticide.

The revised guidelines of 1995 (WHO, 1997) moved away from a screening approach and recommended use of the best available data to calculate an international estimated daily intake (IEDI), based on the

WHO Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) diets for different regions in the world (discussed in chapter 6, section 6.2.2.5) and the supervised trials median residue (STMR) level with plausible correction factors for edible portion and processing (see chapter 8). These guidelines also considered the calculation of acute or short-term dietary exposure for comparison with reference values for acute toxicity (see section 1.4.3.3 below; also chapter 6, section 6.3.6.2, appendix 6.1).

(b) Veterinary drug residues

From the beginning of its work on veterinary drug residues, JECFA used a "food basket" or model diet combined with residue levels at the MRL to estimate the maximum dietary exposure to veterinary drug residues (TMDI), ensuring that MRLs consistent with good veterinary practice would not result in chronic dietary exposures higher than the ADI (FAO/WHO, 1989a, 2001a). Since 2006, the median veterinary drug residue level for foods in the model diet has been used to estimate potential dietary exposure as an EDI to better align with the JMPR approach (FAO/WHO, 2006c) (see also chapter 6, section 6.3.4.1; chapter 8).

(c) Food additives and contaminants

CCFAC developed guidelines for the simple evaluation of contaminant intake (WHO, 2000) and food additive intake (FAO/WHO, 1989c, Annex IV). With the development of the General Standard for Food Additives and the General Standard for Contaminants and Toxins in Foods, CCFAC recognized the need to ensure that the acceptance of a standard would not result in dietary exposures exceeding the ADI for food additives or the tolerable intake for contaminants. In recognition of this need, JECFA further developed principles for dietary exposure assessments, which have been used on a routine basis since the fifty-first meeting of JECFA in 1998 (FAO/WHO, 2000b). In general, the GEMS/Food diets are used by JECFA in the estimation of dietary exposure to contaminants and natural toxicants, but these diets are not suitable for an assessment of food chemicals added to processed foods, such as food additives. JECFA evaluates dietary exposure estimates for food additives, novel foods and nutrients used as additives submitted by individual countries, which are usually based on national

food consumption data. Since 2008, JECFA has also had access to summary food consumption data for processed food categories for various European countries (EFSA, 2008) for use in its evaluations.

1.4.3.3 *Assessment of acute toxicity*

Most work in this area was instigated by JMPR when it was recognized that some pesticide residue–crop combinations could give rise to wide unit-to-unit (e.g. carrot-to-carrot) variation in residue levels, which could result in sporadic high dietary exposures to the pesticide residue. In response to observations by CCPR that the traditional ADI was probably not an appropriate toxicological benchmark to be used in assessing risks due to short-term exposure to acutely toxic pesticides, the assessment of acute toxicity has been a regular item on the agenda of JMPR since 1994. The 1995 JMPR (FAO/WHO, 1996) developed and defined the acute reference dose (ARfD) and established ARfDs for several pesticides. The 1998 JMPR (FAO/WHO, 1999b) published procedures for estimating an ARfD and concluded that, in future, the possibility of establishing an ARfD would be considered for all pesticides, unless, on the basis of its toxicological profile, a pesticide was considered unlikely to present an acute hazard.

The 2000 JMPR (FAO/WHO, 2001b) provided further guidance on the establishment of the ARfD, and additional guidance on the derivation of the ARfD was published in the 2002 and 2004 JMPR reports (FAO/WHO, 2002, 2004b). All the guidance to date on ARfDs has been collated into one publication (Solecki et al., 2005). JECFA has also adopted the principles of establishing ARfDs when needed. Further details on ARfD setting are given in chapter 5 (section 5.2.9).

It has been clear from the beginning of JMPR's consideration of acute toxicity that it was not appropriate to use chronic dietary exposure estimates to compare with the ARfD as part of the risk characterization of acutely toxic pesticide residues. The FAO/WHO Consultation on Food Consumption and Exposure Assessment of Chemicals that was held in 1997 (FAO/WHO, 1997b) developed procedures for estimating short-term or acute dietary exposure, termed the international estimated short-term intake (IESTI), which have been used by JMPR since 1999. A number of different cases were developed for different

commodities that were blended (e.g. grains, milk) or consumed as a single entity (e.g. fruit, vegetables), which have been refined by JMPR at subsequent meetings (FAO/WHO, 2002, 2004a,b); these are discussed in more detail in chapter 6, appendix 6.1.

1.4.3.4 *Evaluation of flavouring agents*

EHC 70 (IPCS, 1987) recognized that there were special issues associated with the safety evaluation of flavouring agents related to the very large number of substances used as food flavouring agents, many of which occur in natural products, and to the generally low and self-limiting levels of use. Most flavouring agents have not been subjected to detailed and comprehensive toxicity tests.

A paper outlining a procedure for the safety evaluation of flavouring agents in a consistent and timely manner was considered at the forty-fourth meeting of JECFA (FAO/WHO, 1995). It incorporated a series of criteria that took account of available information on annual production data for flavouring agents, structure–activity relationships, metabolism and toxicity data and is a form of risk characterization that relates dietary exposure estimates to the potential for toxicity. The production data for the flavouring agents were used to derive a population-based estimate of chronic dietary exposure to each flavouring agent for use in the procedure.

The procedure was developed further at the forty-sixth meeting of the Committee (FAO/WHO, 1997a), at which time 46 flavouring agents in three chemical groups were evaluated. The procedure was refined at the forty-ninth meeting (FAO/WHO, 1999d) and formally adopted as the Procedure for the Safety Evaluation of Flavouring Agents; 224 flavouring agents in seven chemical groups were evaluated. Between 100 and more than 200 flavouring agents have been evaluated at each of several subsequent meetings of JECFA. At the sixty-ninth meeting of JECFA (FAO/WHO, 2009), the Procedure was again revised to include an additional dietary exposure estimate based on added use levels for flavouring agents in foods and typical food portions, to account for consumers who regularly consume a certain food containing a flavouring agent and the potential for an uneven distribution of dietary exposures to that agent. The procedure for flavouring agents is discussed in detail in chapter 9.

1.5 Framework for identification, evaluation, development and incorporation of new principles and methods

The development of new principles and methods and the re-evaluation of existing principles and methods are conducted at regular meetings of JECFA. Special meetings or working groups are convened as appropriate.

Historically, new general principles have been developed for issues relative to the deliberations of the meeting at hand. The conclusions of the meeting with regard to general principles and methods will continue to be published as part of the report of the meeting.

1.6 References[1]

Barlow S, Dybing E, Edler L, Eisenbrand G, Kroes R & Van den Brandt P eds (2002) Food safety in Europe (FOSIE): risk assessment of chemicals in food and diet. Food Chem Toxicol, **40**(2/3): 237–427.

EFSA (2008) Concise European Food Consumption Database. Parma, European Food Safety Authority (http://www.efsa.europa.eu/en/datex/datexfooddb.htm).

FAO (1959) Report of the FAO Panel of Experts on the Use of Pesticides in Agriculture, Rome, 6–13 April 1959. Rome, Food and Agriculture Organization of the United Nations (FAO Meeting Report, No. 1959/3).

FAO (1964) Report of the first meeting of the FAO Working Party on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Meeting Report, No. PL/1963/16).

FAO (1966) Report of the second meeting of the FAO Working Party on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Meeting Report, No. PL/1965/12).

FAO (2000) Report of the conference on international food trade beyond 2000: science-based decisions, harmonization, equivalence and mutual recognition. Melbourne, 11–15 October 1999. Rome, Food and Agriculture Organization of the United Nations (unpublished FAO document ALICOM 99/25; http://www.fao.org/docrep/meeting/X4015E.htm).

[1] Internet links provided in these references were active as of the date of final editing.

FAO/WHO (1955) Fourth report of the Joint FAO/WHO Expert Committee on Nutrition. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Nutrition Meetings Report Series, No. 9; WHO Technical Report Series, No. 97).

FAO/WHO (1956) Joint FAO/WHO Conference on Food Additives. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Nutrition Meetings Report Series, No. 11; WHO Technical Report Series, No. 107).

FAO/WHO (1957) General principles governing the use of food additives. First report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Nutrition Meetings Report Series, No. 15; WHO Technical Report Series, No. 129; http://whqlibdoc.who.int/trs/WHO_TRS_129.pdf).

FAO/WHO (1958) Procedures for the testing of intentional food additives to establish their safety for use. Second report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Nutrition Meetings Report Series, No. 17; WHO Technical Report Series, No. 144; http://whqlibdoc.who.int/trs/WHO_TRS_144.pdf).

FAO/WHO (1961) Evaluation of the carcinogenic hazards of food additives. Fifth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Nutrition Meetings Report Series, No. 29; WHO Technical Report Series, No. 220; http://whqlibdoc.who.int/trs/WHO_TRS_220.pdf).

FAO/WHO (1962a) Evaluation of the toxicity of a number of antimicrobials and antioxidants. Sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Nutrition Meetings Report Series, No. 31; WHO Technical Report Series, No. 228; http://whqlibdoc.who.int/trs/WHO_TRS_228.pdf).

FAO/WHO (1962b) Principles governing consumer safety in relation to pesticide residues. Report of a meeting of a WHO Expert Committee on Pesticide Residues held jointly with the FAO Panel of Experts on the Use of Pesticides in Agriculture. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Plant Production and Protection Division Report, No. PL/1961/11; WHO Technical Report Series, No. 240).

FAO/WHO (1964) Evaluation of the toxicity of pesticide residues in food. Report of a Joint Meeting of the FAO Committee on Pesticides in Agriculture and the WHO Expert Committee on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Meeting Report, No. PL/1963/13; WHO/Food Add./23).

FAO/WHO (1965) Evaluation of the toxicity of pesticide residues in food. Report of the second Joint Meeting of the FAO Committee on Pesticides in Agriculture

and the WHO Expert Committee on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Meeting Report, No. PL/1965/10; WHO/Food Add./26.65).

FAO/WHO (1967) Pesticide residues in food. Joint report of the FAO Working Party on Pesticide Residues and the WHO Expert Committee on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Agricultural Studies, No. 73; WHO Technical Report Series, No. 370).

FAO/WHO (1969) Specifications for the identity and purity of food additives and their toxicological evaluation: some antibiotics. Twelfth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Nutrition Meetings Report Series, No. 45; WHO Technical Report Series, No. 430; http://whqlibdoc.who.int/trs/WHO_TRS_430.pdf).

FAO/WHO (1974) Report of the third Joint FAO/WHO Conference on Food Additives and Contaminants. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Miscellaneous Meeting Reports Series, No. ESN:MMS 74/6; WHO/Food Add./74.43).

FAO/WHO (1981) Evaluation of certain food additives. Twenty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 669; http://whqlibdoc.who.int/trs/WHO_TRS_669.pdf).

FAO/WHO (1982) Evaluation of certain food additives and contaminants. Twenty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 683; http://whqlibdoc.who.int/trs/WHO_TRS_683.pdf).

FAO/WHO (1983) Evaluation of certain food additives and contaminants. Twenty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 696, and corrigenda; http://whqlibdoc.who.int/trs/WHO_TRS_696.pdf).

FAO/WHO (1984) Evaluation of certain food additives and contaminants. Twenty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 710; http://whqlibdoc.who.int/trs/WHO_TRS_710.pdf).

FAO/WHO (1985) Residues of veterinary drugs in foods. Report of a Joint FAO/WHO Expert Consultation, Rome, 1984. Rome, Food and Agriculture Organization of the United Nations (FAO Food and Nutrition Paper, No. 32).

FAO/WHO (1986a) Evaluation of certain food additives and contaminants. Twenty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 733; http://whqlibdoc.who.int/trs/WHO_TRS_733.pdf).

FAO/WHO (1986b) Pesticide residues in food—1985. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 68).

FAO/WHO (1986c) Report of the eighteenth session of the Codex Committee on Pesticide Residues, The Hague, 21–28 April 1986. Rome, Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission (unpublished FAO document ALINORM 87/24).

FAO/WHO (1987a) Evaluation of certain food additives and contaminants. Thirtieth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 751; http://whqlibdoc.who.int/trs/WHO_TRS_751.pdf).

FAO/WHO (1987b) Pesticide residues in food—1987. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 84).

FAO/WHO (1987c) Report of the nineteenth session of the Codex Committee on Pesticide Residues, The Hague, 6–13 April 1987. Rome, Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission (unpublished FAO document ALINORM 87/24A).

FAO/WHO (1988a) Evaluation of certain veterinary drug residues in food. Thirty-second report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 763; http://whqlibdoc.who.int/trs/WHO_TRS_763.pdf).

FAO/WHO (1988b) Pesticide residues in food—1988. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 92).

FAO/WHO (1989a) Evaluation of certain veterinary drug residues in food. Thirty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 788; http://whqlibdoc.who.int/trs/WHO_TRS_788.pdf).

FAO/WHO (1989b) Pesticide residues in food—1989. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 99).

FAO/WHO (1989c) Report of the twenty-first session of the Codex Committee on Food Additives and Contaminants, The Hague, March 1989. Rome, Food

and Agriculture Organization of the United Nations, Codex Alimentarius Commission (unpublished FAO document ALINORM 89/12A).

FAO/WHO (1991) Report of the FAO/WHO conference on food standards, chemicals in food and food trade, Rome, 18–27 March 1991. Rome, Food and Agriculture Organization of the United Nations (unpublished FAO document ALICOM 91/22).

FAO/WHO (1995) Evaluation of certain food additives and contaminants. Forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 859; http://whqlibdoc.who.int/trs/WHO_TRS_859.pdf).

FAO/WHO (1996) Pesticide residues in food—1995. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 133; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/95_rep/Report1995.pdf).

FAO/WHO (1997a) Evaluation of certain food additives and contaminants. Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 868; http://whqlibdoc.who.int/trs/WHO_TRS_868.pdf).

FAO/WHO (1997b) Food consumption and exposure assessment of chemicals. Report of an FAO/WHO Consultation, Geneva, 10–14 February 1997. Geneva, World Health Organization (WHO/FSF/FOS/97.5).

FAO/WHO (1999a) JECFA/JMPR informal harmonization meeting, Rome, 1–2 February 1999. Rome, Food and Agriculture Organization of the United Nations and World Health Organization (unpublished FAO document; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/99_JECFA/JEC-JMPR.pdf).

FAO/WHO (1999b) Pesticide residues in food—1998. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 148; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/98_rep/REPORT1998.pdf).

FAO/WHO (1999c) Pesticide residues in food—1999. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 153; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/99_rep/REPORT1999.pdf).

FAO/WHO (1999d) Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives.

Geneva, World Health Organization (WHO Technical Report Series, No. 884; http://whqlibdoc.who.int/trs/WHO_TRS_884.pdf).

FAO/WHO (2000a) Methodology for exposure assessment of contaminants and toxins in food. Report of a Joint FAO/WHO workshop, Geneva, 7–8 June 2000. Geneva, World Health Organization (WHO/SDE/PHE/FOS/00.5; http://www.who.int/foodsafety/publications/chem/en/).

FAO/WHO (2000b) Evaluation of certain food additives. Fifty-first report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 891; http://whqlibdoc.who.int/trs/WHO_TRS_891.pdf).

FAO/WHO (2001a) Evaluation of certain veterinary drug residues in food. Fifty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 900; http://whqlibdoc.who.int/trs/WHO_TRS_900.pdf).

FAO/WHO (2001b) Pesticide residues in food—2000. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 163; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/2000_rep/cont.pdf).

FAO/WHO (2002) Pesticide residues in food—2002. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 172; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/2002_rep/2002JMPRReport2.pdf).

FAO/WHO (2004a) Pesticide residues in food—2003. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 176; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/2003.pdf).

FAO/WHO (2004b) Pesticide residues in food—2004. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 178; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/DOWNLOAD/2004_rep/report2004jmpr.pdf).

FAO/WHO (2006a) A model for establishing upper levels of intake for nutrients and related substances: report of a Joint FAO/WHO technical workshop on nutrient risk assessment, 2–6 May 2005. Geneva, World Health Organization (http://www.who.int/ipcs/highlights/full_report.pdf).

FAO/WHO (2006b) Food safety risk analysis: a guide for national food safety authorities. Rome, Food and Agriculture Organization of the United Nations and World Health Organization (FAO Food and Nutrition Paper, No. 87; http://whqlibdoc.who.int/publications/2006/9789251056042_eng.pdf).

FAO/WHO (2006c) Evaluation of certain veterinary drug residues in foods. Sixty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 939; http://whqlibdoc.who.int/publications/2006/9241209399_eng.pdf).

FAO/WHO (2008) Dietary exposure assessment of chemicals in food. Report of a Joint FAO/WHO Consultation, Annapolis, MD, 2–6 May 2005. Geneva, World Health Organization (http://whqlibdoc.who.int/publications/2008/9789241597470_eng.pdf).

FAO/WHO (2009) Evaluation of certain food additives. Sixty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 952; http://whqlibdoc.who.int/trs/WHO_TRS_952_eng.pdf).

IPCS (1987) Principles for the safety assessment of food additives and contaminants in food. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 70; http://www.inchem.org/documents/ehc/ehc/ehc70.htm).

IPCS (1990) Principles for the toxicological assessment of pesticide residues in food. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 104; http://www.inchem.org/documents/ehc/ehc/ehc104.htm).

IPCS (2000) Human exposure assessment. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 214; http://www.inchem.org/documents/ehc/ehc/ehc214.htm).

Renwick AG, Barlow SM, Hertz-Picciotto I, Boobis AR, Dybing E, Edler L, Eisenbrand G, Grieg JB, Kleiner J, Lambe J, Müller DJG, Smith MR, Tritscher A, Tuijtelaars S, Van den Brandt PA, Walker R & Kroes R (2003) Risk chacterization of chemicals in food and diet. Food Chem Toxicol, **41**: 1211–1271.

Solecki R, Davies L, Dellarco V, Dewhurst I, Van Raaij M & Tritscher A (2005) Guidance on setting of acute reference dose (ARfD) for pesticides. Food Chem Toxicol, **43**: 1569–1593.

WHO (1989) Guidelines for predicting dietary intake of pesticide residues. Report prepared by the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme in collaboration with the Codex Committee on Pesticide Residues. Geneva, World Health Organization.

WHO (1997) Guidelines for predicting dietary intake of pesticide residues (revised). Prepared by the Global Environment Monitoring System – Food

Contamination Monitoring and Assessment Programme (GEMS/Food) in collaboration with the Codex Committee on Pesticide Residues. Geneva, World Health Organization (WHO/FSF/FOS/97.7; http://www.who.int/foodsafety/publications/chem/en/pesticide_en.pdf).

WHO (2000) Methodology for exposure assessment of contaminants and toxins in food. Geneva, World Health Organization (WHO/SDE/PHE/FOS/00.5; http://www.who.int/foodsafety/publications/chem/exposure_june2000/en/index.html).

2. RISK ASSESSMENT AND ITS ROLE IN RISK ANALYSIS

2.1 Introduction

The Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) have provided scientific advice to Member States of FAO and WHO since 1956 and 1961, respectively, and to several general subject committees of the Codex Alimentarius Commission (CAC) since its formation in 1963. However, the structural framework for the interaction between both scientific bodies and the Codex committees was not formalized until the development and the adoption of the risk analysis paradigm.

Risk analysis has been defined by CAC as "a process consisting of three components: risk assessment, risk management and risk communication", which are themselves defined as follows (FAO/WHO, 2008):

For acronyms and abbreviations used in the text, the reader may refer to the list of acronyms and abbreviations at the front of this monograph. Definitions of select terms may be found in the glossary at the end of the monograph.

- *Risk assessment*: A scientifically based process consisting of the following steps: 1) hazard identification, 2) hazard characterization, 3) exposure assessment and 4) risk characterization.

- *Risk management*: The process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices and, if needed, selecting appropriate prevention and control options.

- *Risk communication*: The interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions.

The risk analysis paradigm (see Figure 2.1) is a formal description of the risk analysis process that emphasizes the functional separation of its three components while at the same time demanding the need for communication and interaction between those with responsibility for each of the three components. Within risk analysis, the functional separation between risk assessors and risk managers is essential to ensure scientific objectivity of the risk assessment process. Further background information can be found in an FAO/WHO publication on food safety risk analysis (FAO/WHO, 2006).

The use of a structured risk analysis process facilitates consistent, science-based and orderly decision-making in the area of food safety. The scientific part of this process, the risk assessment for food safety matters, is undertaken at an international level by joint FAO/WHO expert bodies. JECFA and JMPR, the expert committees that deal mainly with chemical risks in food, base their evaluations on scientific principles and ensure necessary consistency in their risk assessment determinations. CAC and its respective committees that deal with chemicals in food are responsible, as risk managers, for the final decisions on establishing maximum limits for pesticide residues, veterinary drug residues, contaminants and additives in food and adopting other related measures.

Fig. 2.1. Risk analysis (adapted from FAO/WHO, 1997)

As part of the discussion that led to the adoption of the risk analysis paradigm, CAC recognized the need to revisit existing risk analysis approaches as applied by Codex committees and JECFA/JMPR. At its request, three consecutive expert consultations were held by FAO and WHO, which focused on risk assessment (1995), risk management (1997) and risk communication (1998) as related to food safety (FAO/WHO, 1995, 1997, 1999).

2.2 Definitions of hazard and risk

The first consultation (FAO/WHO, 1995) explored the risk analysis domain and focused on risk assessment. The consultation was also aware of the need for uniform terminology on risk analysis in the work of Codex and considered risk analysis definitions from different sources. The consultation drafted definitions of risk analysis terms related to food safety and recommended them to CAC. CAC subsequently amended these definitions and published them in the Procedural Manual (FAO/WHO, 2004). The definitions of two terms,

hazard and risk, should be mentioned in particular, as they are fundamental in the risk analysis process, but differentiating words for these two terms do not exist in many languages. Codex has adopted the following definitions for hazard and risk in relation to food that cover not only chemical agents, but also biological and physical agents:

- *Hazard*: A biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect.

- *Risk*: A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food.

The Codex definition of hazard differs from that of other bodies, notably those dealing with risk assessment of chemicals, for which a hazard is a property associated with a chemical or an agent rather than the chemical or the agent itself. Thus, a single chemical could represent multiple hazards (e.g. it could be a reproductive toxicant and a carcinogen). As part of the project for the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals, the International Programme on Chemical Safety (IPCS) has defined hazard and risk slightly differently from Codex (IPCS, 2004):

- *Hazard*: Inherent property of an agent or situation having the potential to cause adverse effects when an organism, system or (sub)population is exposed to that agent.

- *Risk*: The probability of an adverse effect in an organism, system or (sub)population caused under specified circumstances by exposure to an agent.

These IPCS definitions apply to all areas of chemical risk assessment that most clearly describe the approaches of JECFA and JMPR, and therefore they are used in this monograph.

2.3 Role of risk assessment in risk analysis for food chemicals

Risk assessment is the central scientific component of risk analysis and was developed primarily because of the need to make decisions to protect health in the face of scientific uncertainty. Risk assessment

of food chemicals can be generally described as characterizing the potential hazards and the associated risks to life and health resulting from exposure of humans to chemicals present in food over a specified period.

Risk managers decide eventually whether a risk assessment is possible and necessary and commission the risk assessment, carrying out tasks such as describing the purpose of the risk assessment and the food safety questions to be answered, establishing a risk assessment policy, setting time schedules and providing the resources necessary to carry out the work.

Risk assessment of chemical substances used on or present in food is one of the key components of the work of JECFA and JMPR. Risk assessment provides the scientific basis for the risk management executed by CAC and its member governments. Accordingly, aspects of this component are examined in more detail in this monograph, whereas the other two components of risk analysis, risk management and risk communication, are not further discussed.[1]

2.4 The four steps of risk assessment for food chemicals

Risk assessment (in particular in the food context, also often called "safety assessment"), comprising the four steps of hazard identification, hazard characterization (including dose–response assessment), exposure assessment and risk characterization, is a conceptual framework that, in the context of food chemical safety, provides a mechanism for the structured review of information relevant to estimating health outcomes in relation to exposure to chemicals present in food. In this monograph, the terms "risk assessment" and "safety assessment" are used interchangeably.

Risk assessment can include a key component in which the probability of harm is estimated. As a probability calculation, a risk assessment will include both a statement of the nature of the harm and the basis for the assertion that the harm may occur (i.e. the probability).

[1] The interested reader is referred to other publications for further background reading, such as those recommended in FAO/WHO (2006).

The risk assessment is followed by either a risk management decision or a request for further analysis, which may influence any further research that is conducted. The record produced by a risk assessment stands as a scientific basis for any risk management decision at that time. However, the risk assessment or risk analysis may be reopened—for example, if additional information becomes available.

As discussed previously, the work of JECFA and JMPR is best described making reference to the definitions that have been developed and confirmed by IPCS in the ongoing project on Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals (IPCS, 2004). These definitions are the ones discussed in the following sections and used, where applicable, in this monograph. The differences between these definitions as applied by JECFA/JMPR and those used by Codex are important but do not affect communication and the joint work of risk assessors and risk managers, if taken into account consciously.

2.4.1 Hazard identification

Hazard identification is defined as follows (IPCS, 2004):

> The identification of the type and nature of adverse effects that an agent has an inherent capacity to cause in an organism, system, or (sub)population. Hazard identification is the first stage in hazard assessment and the first of four steps in risk assessment.

The purpose of food chemical hazard identification is to evaluate the weight of evidence for adverse health effects, based on assessment of all available data on toxicity and mode of action. It is designed to primarily address two questions: 1) the nature of any health hazard to humans that an agent may pose and 2) the circumstances under which an identified hazard may be expressed. Hazard identification is based on analyses of a variety of data, ranging from observations in humans or domestic animals and studies in laboratory animals and in vitro laboratory studies through to analysis of structure–activity relationships. From the range of studies and observations available, the nature of any toxicity or adverse health effects occurring and the affected target organs or target tissues are identified.

2.4.2 Hazard characterization

Hazard characterization is defined as follows (IPCS, 2004):

> The qualitative and, wherever possible, quantitative description of the inherent properties of an agent or situation having the potential to cause adverse effects. This should, where possible, include a dose–response assessment and its attendant uncertainties. Hazard characterization is the second stage in the process of hazard assessment and the second of four steps in risk assessment.

Hazard characterization describes the relationship between the administered dose of, or exposure to, a chemical and the incidence of an adverse health effect. The critical effect—that is, the first adverse effect observed as the dose or exposure is increased—is determined.

In cases where the toxic effect is assumed to have a threshold, hazard characterization usually results in the establishment of health-based guidance values—for example, an acceptable daily intake (ADI) for additives or residues or a tolerable intake (TI) for contaminants.

For some substances used as food additives, the ADI may not need to be specified; in other words, no numerical ADI is considered necessary. This may be the case when a substance is assessed to be of very low toxicity, based on the biological and toxicological data, and the total dietary intake of the substance, arising from the levels used in foods to achieve the desired function, does not represent a hazard.

2.4.3 Exposure assessment

Exposure assessment is defined by IPCS (2004) as follows: "Evaluation of the exposure of an organism, system, or (sub)population to an agent (and its derivatives). Exposure assessment is the third step in the process of risk assessment."

According to CAC, the exposure assessment of food chemicals may be described more narrowly as "The qualitative and/or quantitative evaluation of the likely intake of chemical agents via food as well as exposure from other sources if relevant" (FAO/WHO, 2008).

In the case of food chemicals, dietary exposure assessment takes into consideration the occurrence and concentrations of the chemical

in the diet, the consumption patterns of the foods containing the chemical and the likelihood of consumers eating large amounts of the foods in question (high consumers) and of the chemical being present in these foods at high levels. Usually a range of intake or exposure estimates will be provided (e.g. for average consumers and for high consumers), and estimates may be broken down by subgroup of the population (e.g. infants, children, adults).

2.4.4 Risk characterization

Risk characterization is defined by IPCS (2004) as follows:

> The qualitative and, wherever possible, quantitative determination, including attendant uncertainties, of the probability of occurrence of known and potential adverse effects of an agent in a given organism, system, or (sub)-population, under defined exposure conditions. Risk characterization is the fourth step in the risk assessment process.

This definition of the final step of risk assessment is, if restricted to the population of consumers only, practically identical to the one agreed to and used by Codex (FAO/WHO, 2008).

In risk characterization, the information from the intake or exposure assessment and the hazard characterization is integrated into advice suitable for decision-making in risk management. Risk characterization provides estimates of the potential risk to human health under different exposure scenarios. It should include all key assumptions and describe the nature, relevance and magnitude of any risks to human health.

The information and advice provided to risk managers may be qualitative or quantitative. Qualitative information may include:

- statements or evidence that the chemical is of no toxicological concern owing to the absence of toxicity even at high exposure levels;
- statements or evidence that the chemical is safe in the context of specified uses; and
- recommendations to avoid, minimize or reduce exposure.

Quantitative information may include:

- a comparison of dietary exposures with health-based guidance values;

- estimates of risks at different levels of dietary exposure;
- risks at minimum and maximum dietary intakes (e.g. nutrients); and
- margins of exposure.

The risk characterization statement should include a clear explanation of any uncertainties in the risk assessment resulting from gaps in the science base. It should also include, where relevant, information on susceptible subpopulations, including those with greater potential exposure or specific predisposing physiological conditions or genetic factors. The advice to risk managers can be in the form of a comparison of the relative risks among risk management options.

2.5 Interactions between risk assessment and risk management

More recent examinations of risk assessment and risk analysis methodology have paid much closer attention to the influence of risk management on the risk assessment process (USNRC, 1994; Stern & Fineberg, 1996; Presidential Commission, 1997; WHO, 2000; Renwick et al., 2003). Although it is desirable to separate the functional activities of risk assessment from those of risk management in order to ensure scientific independence, it is acknowledged that risk managers should communicate and interact with risk assessors during the process to establish the scope of the analysis, particularly during problem formulation (also known as risk profiling). Thus, the relationship between risk assessment and risk management is an interactive, often iterative, process (see Figure 2.2).

Within the framework of CAC, the responsibilities of the Codex committees as risk managers and the expert committees as risk assessors are defined in more detail in Section III of the Codex Procedural Manual (FAO/WHO, 2008). This section of the Procedural Manual also addresses specific risk analysis principles and risk assessment policies employed by JMPR and the Codex Committee on Pesticide Residues (CCPR) and by JECFA and the Codex Committee on Food Additives (CCFA), the Codex Committee on Contaminants in Food (CCCF) and the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) (FAO/WHO, 2008).

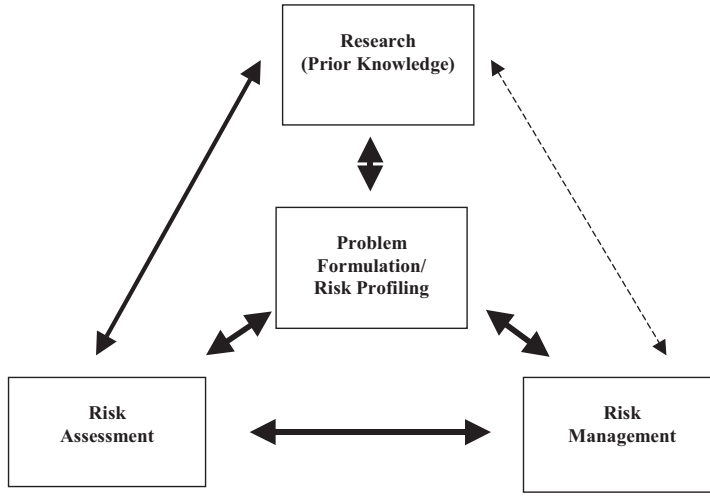

Fig. 2.2. Interactions of risk assessment with risk management

2.5.1 Problem formulation

As a general rule, formal risk assessments are preceded by a preliminary consideration of the necessity for a risk assessment and its objective. These may be subjective and informal and may be initiated either from inside or outside the risk management, risk assessment and scientific communities. The transition process from preliminary considerations to formal risk assessments has been described as problem formulation or risk profiling (Renwick et al., 2003). It is an iterative process involving risk assessors and risk managers that determines the need for—and, if needed, the extent of—a risk assessment. Communication with other interested parties (stakeholders) is particularly important during problem formulation.

Within the risk analysis process that addresses chemicals in foods, problem formulation describes the food safety problem and its context, in order to identify those elements of hazard or risk associated with a chemical that are relevant to potential risk management decisions. Problem formulation would include identifying those aspects relevant to prioritization in relation to other food safety problems, the establishment of risk assessment policy, including the choice of acceptable levels of risk, and identification of management options. A

typical problem formulation in case of chemical risk analysis might include the following:

- a brief description of the intended application of the product (e.g. food additive) and the commodities involved;
- the issues expected to be affected (e.g. human health, economic concerns) and the potential consequences;
- consumer perception of the hazards or risks;
- the distribution of possible risks among different segments of the population; and
- possible benefits associated with the use of the chemical in food.

The output is a plan for the risk assessment process for an identified chemical substance and potential hazard, which can be changed as the risk assessment progresses. The desired outcomes of problem formulation are 1) the questions that need to be answered under risk characterization to meet the needs of the risk manager, 2) determination of the resources that are needed and available and 3) the time frame for completing the assessment. For defined categories such as food additives or residues of pesticides, formal plans or procedures are in place that define the questions to be posed and the data necessary for initiating a risk assessment.

2.5.2 Priority setting for JECFA and JMPR

The selection of new or existing chemicals for consideration by JECFA or JMPR and recommending priorities for review are the responsibility of FAO and WHO, their Member countries and CAC, through its committees. For JECFA, these committees include CCFA, CCCF and CCRVDF. For JMPR, the primary source of input is CCPR. The protection of human health should be the main criterion for prioritization for risk assessment. The exposure levels and toxicity of the substance and the existence of particularly susceptible populations are key determinants that impact human health. However, the lack of available data may also be a factor in prioritization for risk assessment.

Re-evaluation may be particularly of high priority for substances for which new data raise suspicion of significant hazard, where there is

evidence to question the validity of the data submitted for the previous evaluation or with a previously allocated temporary ADI.

The FAO and WHO Joint Secretaries for JECFA and JMPR, as representatives of their respective organizations, have the final responsibility and authority for the determination of the priorities of substances to be evaluated in their respective areas. This can be dependent in part on available resources.

2.5.3 Periodic reviews and specific re-evaluations

JECFA and JMPR have indicated already during their initial deliberations on the principles they would apply in their work that it will be necessary to review assessed substances as new data become available. It was also recognized that safety assessments and resulting guidance such as an ADI for a specific substance would be subject to future modifications as a result of the accumulation of experience and improvements in toxicological methodology in general.

Reviews of past decisions on safety regarding food additives, contaminants and residues of pesticides and veterinary drugs may be necessary as a result of one or more of the following developments (adapted from FAO/WHO, 1970):

- a new manufacturing process;
- a new specification;
- new data on the biological properties of the compound;
- new data concerning the nature and/or the biological properties of the impurities present;
- advances in scientific knowledge relevant to the nature or mode of action;
- changes in consumption patterns, levels of use or dietary exposure estimates; and
- improved requirements for safety evaluation. These are made possible by new scientific knowledge and the quality and quantity of safety data considered necessary in the case of food additives and residues of pesticides and veterinary drugs.

For pesticide residues, at the request of CCPR or national governments, JMPR has always re-examined data supporting ADI estimates

and data on residue trials and registered use information supporting maximum residue limits (MRLs). Because MRLs are related to registered uses, when a registered use changes or is withdrawn, the remaining MRL may have to be revised. However, it is very difficult to know the registration status throughout the world, whether adequate data are available to support the current or revised MRL or if the MRL should be withdrawn. CCPR has a Periodic Review Programme in place that provides an opportunity for data submission for required compounds and MRLs, while introducing a timetable for ADIs and MRLs to be deleted if no data or inadequate data were provided. The first periodic reviews were carried out by JMPR in 1992 following wide discussion of the principles at CCPR sessions in 1991 and 1992 (FAO/WHO, 1991, 1992). CCPR applies criteria for periodic re-evaluation, such as the level of public health concern, available data, the elapsed time since the last toxicological review (>15 years) or issues in trade. JMPR will evaluate available studies according to modern scientific standards and will not rely on data submissions to FAO and WHO from previous years.

JECFA meetings on food additives, contaminants and residues of veterinary drugs and the relevant Codex committees have not established formal re-evaluation approaches as implemented for JMPR. On a case-by-case basis, either the risk assessor or the risk manager (or both together) will discuss and decide whether an existing risk assessment remains valid or requires an update in view of available data.

That a considerable amount of re-evaluation of substances is already carried out within the system is evident when the year-to-year agendas of JECFA and JMPR are examined. Temporary ADIs have been allocated by JECFA and JMPR to permit the acceptance of substances where there are sufficient data to conclude that the use of the substance is safe over the relatively short period of time required to produce further safety data, but are insufficient to conclude that the use of the substance is safe over a lifetime. An expiry date is generally established by which time appropriate data to resolve the safety issue should be submitted. JECFA, as part of its recommendations in the evaluation of specific contaminants, often makes requests for additional data and recommendations for subsequent re-evaluation.

Establishing a priority order for the re-evaluation of compounds requires input from a number of sources. Within the risk analysis

paradigm, the system for periodic review, including the determination of priorities for re-evaluation, is part of risk management and, for JECFA and JMPR, the responsibility of FAO, WHO and CAC, through its committees.

The following situations are triggers for prioritizing substances for re-evaluation:

- substances for which new data raise suspicion of significant hazard;
- substances for which there is evidence to question the validity of the data submitted for the previous evaluation;
- substances previously allocated a temporary ADI, where the requested additional data are available;
- substances whose re-evaluation has been requested by FAO or WHO; and
- substances whose re-evaluation has been requested by CAC.

The use of an international forum to devise and implement a system for the periodic review of chemicals used in or on food and contaminants of food could also be of great economic and practical value to Member States. It would ensure a uniform approach, duplication of effort would be minimized, and emphasis on such a programme would give added reassurance to consumers throughout the world that the food supply continues to be safe. Such a programme could be developed in cooperation with CAC.

2.6 References[1]

FAO/WHO (1970) Specifications for the identity and purity of food additives and their toxicological evaluation: some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances. Thirteenth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Nutrition Meetings Report Series, No. 46; WHO Technical Report Series, No. 445; http://whqlibdoc.who.int/trs/WHO_TRS_445.pdf).

FAO/WHO (1991) Report of the twenty-third session of the Codex Committee on Pesticide Residues, The Hague, 15–22 April 1991. Rome, Food and

[1] Internet links provided in these references were active as of the date of final editing.

Agriculture Organization of the United Nations, Codex Alimentarius Commission (unpublished FAO document ALINORM 91/24A).

FAO/WHO (1992) Report of the twenty-fourth session of the Codex Committee on Pesticide Residues, The Hague, 6–13 April 1992. Rome, Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission (unpublished FAO document ALINORM 93/24).

FAO/WHO (1995) Application of risk analysis to food standards issues. Report of the Joint FAO/WHO Expert Consultation, Geneva, 13–17 March 1995. Geneva, World Health Organization (WHO/FNU/FOS/95.3; http://www.who.int/foodsafety/publications/micro/en/march1995.pdf).

FAO/WHO (1997) Risk management and food safety. Report of a Joint FAO/WHO Consultation, Rome, 27–31 January 1997. Rome, Food and Agriculture Organization of the United Nations (FAO Food and Nutrition Paper, No. 65; http://www.fao.org/docrep/W4982E/w4982e00.HTM).

FAO/WHO (1999) The application of risk communication to food standards and safety matters. Report of a Joint FAO/WHO Expert Consultation, Rome, 2–6 February 1998. Rome, Food and Agriculture Organization of the United Nations (FAO Food and Nutrition Paper, No. 70; http://www.fao.org/docrep/005/x1271e/X1271E00.HTM).

FAO/WHO (2004) Codex Alimentarius Commission procedural manual, 14th ed. Rome, Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission (ftp://ftp.fao.org/codex/Publications/ProcManuals/Manual_14e.pdf).

FAO/WHO (2006) Food safety risk analysis—A guide for national food safety authorities. Rome, Food and Agriculture Organization of the United Nations and World Health Organization (FAO Food and Nutrition Paper, No. 87; http://www.who.int/foodsafety/publications/micro/riskanalysis06/en/).

FAO/WHO (2008) Codex Alimentarius Commission procedural manual, 18th ed. Rome, Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission (ftp://ftp.fao.org/codex/Publications/ProcManuals/Manual_18e.pdf).

IPCS (2004) IPCS risk assessment terminology. Geneva, World Health Organization, International Programme on Chemical Safety (Harmonization Project Document, No. 1; http://www.who.int/ipcs/methods/harmonization/areas/ipcsterminologyparts1and2.pdf).

Presidential Commission (1997) Risk assessment and risk management in regulatory decision-making. Final report. Vol. 2. Washington, DC, The Presidential/Congressional Commission on Risk Assessment and Risk Management (http://www.riskworld.com/Nreports/1997/risk-rpt/volume2/pdf/V2EPA.pdf).

Renwick AG, Barlow SM, Hertz-Picciotto I, Boobis AR, Dybing E, Edler L, Eisenbrand G, Grieg JB, Kleiner J, Lambe J, Müller DJG, Smith MR, Tritscher

A, Tuijtelaars S, Van den Brandt PA, Walker R & Kroes R (2003) Risk characterization of chemicals in food and diet. Food Chem Toxicol, **41**: 1211–1271.

Stern PC & Fineberg HV eds (1996) Understanding risk: informing decisions in a democratic society. Prepared by the Committee on Risk Characterization, Commission on Behavioral and Social Sciences and Education, National Research Council. Washington, DC, National Academy Press (http://www.nap.edu/openbook.php?isbn=030905396X).

USNRC (1994) Science and judgement in risk assessment. Prepared by the Committee on Risk Assessment of Hazardous Air Pollutants, Board on Environmental Studies and Toxicology, Commission on Life Sciences, National Research Council. Washington, DC, National Academy Press (http://www.nap.edu/openbook.php?isbn=030904894X).

WHO (2000) The interaction between assessors and managers of microbiological hazards in food. Report of a WHO Expert Consultation, Kiel, 21–23 March 2000. Geneva, World Health Organization (http://www.who.int/foodsafety/publications/micro/march2000/en/).

3. CHEMICAL CHARACTERIZATION, ANALYTICAL METHODS AND THE DEVELOPMENT OF SPECIFICATIONS

3.1 Introduction

Chemical characterization plays a critical role in risk assessment, in surveys and in regulatory monitoring activities. Suitable analytical methods are necessary for:

- the definition of the nature, including isomeric composition and chemical purity, of the materials investigated during in vitro and in vivo hazard identification and characterization studies;

For acronyms and abbreviations used in the text, the reader may refer to the list of acronyms and abbreviations at the front of this monograph. Definitions of select terms may be found in the glossary at the end of the monograph.

- the speciation of contaminants (e.g. determination of the various chemically bonded forms of elements);
- determination of the concentrations of the chemical under review and its relevant metabolites and breakdown products in body fluids, tissues and excreta of laboratory animals and of food-producing animals in pharmacokinetic/toxicokinetic and residue depletion studies;
- determination of the concentrations of contaminants and of incurred residues of veterinary drugs and pesticides of concern; and
- the identification and quantification of the substances for which maximum residue limits (MRLs) and maximum levels (MLs) are recommended by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR).

Analytical requirements of JECFA and JMPR for food additives, pesticides, veterinary drug residues, contaminants and substances consumed in large amounts are given in sections 3.4, 3.5, 3.6, 3.7 and 3.8, respectively.

Chemical characterization is also necessary for the preparation of specifications for the identity and purity of food additives.

3.2 Criteria for the review of analytical methods and required technical competence of testing laboratories

At the time of the review of the analytical methods by JECFA and JMPR, they must at least have been validated in accordance with accepted criteria of single-laboratory validation carried out by a laboratory accredited according to the applicable international standard for testing laboratories or operating an equivalent system of quality management and exhibiting equivalent technical competence.

JECFA and JMPR review the suitability of the methods on the basis of the available validation data. Therefore, the methods should be described in an internationally recognized format, and the information on method validation should include the data generated in the process of determining the following performance characteristics: specificity,

limit of detection (LOD), limit of quantification (LOQ), accuracy and precision (repeatability within the laboratory). A mathematical/statistical description of calibration curves should also be given if such curves form the basis for the quantification of the analytes. Definitions and interpretations of the above performance characteristics, requirements with regard to single-laboratory validation and further references to relevant Codex Alimentarius Commission (CAC) documents are provided and regularly updated in the Procedural Manual of CAC, which is published on its web site (FAO/WHO, 2008). However, JECFA and JMPR always review the above performance characteristics in the light of contemporary scientific and technical development.

For methods developed solely for the purpose of generating the database required for the risk assessment, every suitable analytical approach is acceptable. However, methods recommended for monitoring of compliance of commodities with recommended regulatory limits should meet additional criteria, such as applicability, practicability and ruggedness. For such methods, the validation study must also include the analysis of incurred residues in a suitable number of independent tissues or commodities. The definitions of these criteria are subject to change in view of the rapid progress observed in the development of analytical technology, including instrumentation. JECFA and JMPR carry out a full scientific review with regard to these additional criteria. A further evaluation with regard to collateral criteria is carried out by the competent CAC committees—the Codex Committee on Methods of Analysis and Sampling (CCMAS), the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) and the Codex Committee on Pesticide Residues (CCPR).

It is known that methods based on certain principles, such as microbiological inhibition or ligand–protein interactions in the determinative step of a method, cannot meet all of the above criteria. If such methods are proposed, JECFA and JMPR will review them on a case-by-case basis and discuss them in sufficient detail in the monographs prepared to enable national authorities to judge whether these methods could serve as screening methods in monitoring programmes.

The currently applicable international standard laying down the general requirements for the competence of testing and calibration laboratories is the norm ISO/IEC 17025 (ISO, 2005). If laboratories

comply with the requirements of this international standard, which incorporates relevant elements of Good Laboratory Practice (GLP), they will operate a quality management system for their testing and calibration activities that also meets the quality management principles of ISO 9001 (ISO, 2008). An important additional requirement for obtaining and maintaining accreditation is the regular successful participation in proficiency tests. JECFA and JMPR will judge on a case-by-case basis whether the infomation on method validation provides sufficient evidence that it has been carried out under conditions equivalent to those required by the above-mentioned international standard and whether partial absence of such evidence has an impact on the credibility of the results of the validation.

3.3 The significance of multilaboratory method trials and collaborative studies

Relatively few of the analytical methods reviewed by JECFA and JMPR have been subjected to properly designed multilaboratory studies, which provide information on method performance in the hands of different analysts in different laboratories. In view of the currently established framework for single-laboratory validation, it is generally not necessary to conduct multilaboratory studies in order to enable JECFA and JMPR to review and assess analytical methods with regard to fitness for purpose. If such studies are performed, the international harmonized protocol agreed upon by the competent international organizations (Thompson & Wood, 1993) should be followed. However, JECFA and JMPR will perform an independent review of available studies based on an accurate record of the design and conduct of the study and the raw concentration data obtained in the analysis of the samples used in the study.

Multilaboratory trials that do not meet all criteria for the conduct of collaborative studies and subsequent statistical evaluation of the results may still provide useful information on the expected performance of the method tested.

Multilaboratory and collaborative studies of methods usually do not encompass all possible combinations of the analyte and commodities for which regulatory limits have been recommended and to which the method may subsequently be applied. These methods may

be extended to related analytes and sample materials not included in the original multilaboratory study by completing additional properly designed within-laboratory studies, provided such activities are covered by the scope of the accreditation of the laboratory involved. JECFA and JMPR will review all available information with a view to scientifically assess the fitness for purpose of a method.

3.4 Food additive specifications

3.4.1 General considerations

Specifications of identity and purity are necessary products of JECFA safety evaluations for food additives. Evaluations of food additives by JECFA depend on studies performed with a chemical substance or product of defined identity, purity and physical form. The acceptable daily intake (ADI) is valid only for products that do not differ significantly in identity and quality profile from the material used to generate the data used in the evaluation.[1]

The specifications of identity and purity established by JECFA are intended to ensure that the Committee's safety evaluations apply, with a high degree of confidence, to all products manufactured to comply with those specifications. The first Joint FAO/WHO Conference on Food Additives (FAO/WHO, 1956) was asked to formulate general principles governing the use of food additives and to recommend suitable methods for the chemical, physical, pharmacological, toxicological and other properties of individual food additives.

The first two meetings of JECFA prepared reports on general principles governing the use of food additives (FAO/WHO, 1957) and procedures for the testing of intentional food additives to establish their safety for use (FAO/WHO, 1958) and recommended the need for specifications. Since then, specifications have been an important part of JECFA evaluations of food additives. JECFA specifications have three purposes:

[1] For an overview of the purpose, function and format of JECFA food additive specifications and the interaction of JECFA and CAC, see the introduction to the Combined Compendium of Food Additive Specifications (FAO, 2005/2006).

1) to identify the substance that has been tested biologically;
2) to ensure that the substance is of the quality required for safe use in food; and
3) to reflect and encourage Good Manufacturing Practice (GMP) and maintain the quality of additives on the market.

Since 1956, the meetings of JECFA have designated specifications as either full or tentative. Until the twenty-third meeting of JECFA, specifications were designated as tentative either because the chemistry data were inadequate or because a temporary ADI was assigned to the additive. At and since the twenty-third meeting of JECFA, a tentative specification has been assigned only when the data were inadequate for preparing full specifications.

A food additive may be a single chemical substance, a manufactured chemical mixture or a natural product. Complete information on chemical composition—including description, methods of manufacture, raw materials and impurities—is equally important for each type of additive. However, implementation of the requirement for chemical composition data may vary, depending on the type of substance.

For additives that are single chemical substances, it is virtually impossible to remove all impurities arising from their commercial production; therefore, analyses are generally performed on the major component and predicted impurities, especially those with potential toxicity.

For commercially manufactured complex mixtures, such as monoglycerides and diglycerides, information is needed on the range of substances produced, with emphasis on descriptions of manufacturing processes, supported by analytical data on the components of the different commercial products.

Natural products present particularly difficult problems because of their biological variability and because the chemical constituents are too numerous for regular analytical determinations. For additives derived from natural products, it is vital that the sources and methods of manufacture be defined precisely. Chemical composition data should include analyses for general chemical characteristics. These might include proximate analyses of protein, fat, moisture, carbohydrate

and mineral content. Analyses should be undertaken for specific toxic impurities carried over from raw materials or chemicals used in the manufacture of the product. Further information necessary for the evaluation of substances used in large amounts, which are often derived from natural products, is provided in section 3.8.

JECFA policy has been to prepare specifications whenever constituents of the substance added to food had the potential to be present in the finished food. Initially, specifications were prepared only for intentional food additives—that is, those that are added directly to a food to accomplish a technical effect (e.g. a preservative or colour). The fourteenth meeting of JECFA (FAO/WHO, 1971) prepared specifications for extraction solvents; although these "processing aids" are largely removed from food, evaluation of their safety in use depends on their identity and purity. Since then, specifications have been prepared for all processing aids (e.g. antifoaming or clarifying agents, enzyme preparations, filtering aids, packing gases, release agents and others) used in conjunction with food manufacture.

The twenty-seventh meeting of JECFA (FAO/WHO, 1983) decided that chemical reagents used in the preparation of food additives or processing aids (e.g. glutaraldehyde in the preparation of immobilized enzyme preparations or acetic anhydride in the manufacture of modified starches) do not usually need specifications. Carryover of these reagents or their contaminants into food may be controlled by the specifications for purity of the specific additive or processing aid.

Many food additive specifications have identical analytical methods or test procedures. To avoid repetition in each individual specification, these methods and test procedures were assembled in a volume entitled "Guide to Specifications" (FAO, 1978), and subsequent specifications referred to that volume when appropriate. The volume was revised and updated in 1983 (FAO, 1983) and 1991 (FAO, 1991). In 2006, the information contained in the volume was completely revised and rewritten and was published as Volume 4 of the Combined Compendium of Food Additive Specifications (FAO, 2005/2006).

Food additives may be marketed as formulated preparations, such as a mixture of a main ingredient with a solvent vehicle and emulsifier. Specifications refer to each ingredient in the formulated preparation

as individual commercially manufactured food additive substances. Mixtures should not be formulated in such a way that the absorption or metabolism of any ingredient is altered; otherwise, the biological data, derived using the individual component, will be invalidated (FAO/WHO, 1966, 1972). Added substances, such as anticaking agents, antioxidants and stabilizers, may influence the results of analytical tests given in specifications. Therefore, in its nineteenth report, JECFA recommended that manufacturers of food additives should indicate the presence of such added substances (FAO/WHO, 1975).

3.4.2 Formulation of specifications and information requirements

The formulation of satisfactory specifications requires that detailed information be made available to JECFA on the method of manufacture of the additive, including information on raw materials and on its chemical characterization. The Committee requires such information to be provided as part of the total data package whenever an additive is submitted for risk assessment; all such information is regarded as suitable for being made publicly available unless requested otherwise and agreed by the JECFA Secretariat. Those submitting data for a JECFA evaluation are advised to consult existing specifications for further guidance, which is available in the Combined Compendium of Food Additive Specifications (FAO, 2005/2006), where the individual criteria used in the elaboration of JECFA specifications are described. The same criteria are used for most additives; however, because of their particular characteristics, separate criteria have been developed for enzyme preparations and for flavouring substances.

Specifications may be revised when there is new information available on methods of manufacture or on the characteristics of the product or when changes or revisions in analytical methods are needed. Such specification changes may trigger a review of the safety evaluation; conversely, a review of the specifications may be needed if the safety is re-evaluated.

Although all the individual criteria in specifications monographs must be met, additives are mainly defined by a combination of 1) a description of their manufacture, 2) a minimum requirement for the content of the principal functional components of the additive and 3)

maximum limits for undesirable impurities. The relative importance of these criteria depends on the nature of the additive; for example, additives composed largely of single components are mainly defined in terms of their chemical purity, whereas the definition of more complex materials relies more on a description of the raw materials and the method of manufacture.

3.4.3 Stability and fate of additives in food

Specifications are intended to apply to the additive as marketed and supplied for food use. In considering whether specifications apply to food additive quality as manufactured or as added to food, JECFA has decided to prepare specifications to cover the normal shelf-life of the additive. Limits are set for decomposition products that may form during normal storage. Manufacturers and users of food additives should ensure good packaging and storage conditions and use good handling practices to minimize deleterious changes in quality and purity (FAO/WHO, 1975). Information on changes in the composition of food additives during storage should be submitted for evaluation by the Committee.

Certain food additives perform their functional effect by reaction with undesirable food constituents (e.g. antioxidants react with oxygen in food, and ethylenediaminetetraacetic acid [EDTA] reacts with trace metals) or by reactions that modify food constituents (e.g. flour improvers). Food additives may also degrade under certain conditions of food processing, even though such degradation is detrimental to their functional effect. For example, the sweetener aspartame is transformed to a diketopiperazine derivative at rates that vary with the acidity and the temperature of the food. For such additives, the Committee has evaluated analyses for additive reaction products in food as consumed and biological testing data on either specific reaction products or samples of food containing the reaction products as consumers would ingest them.

In order to ensure that test data are relevant to the way in which the additive is used in food, the Committee requires information on potential reactivity to be provided as part of submissions for the safety evaluation of all intentional food additives (FAO/WHO, 1981). Four types of data related to reactivity are required:

1) the general chemical reactivity of the additive;
2) stability of the additive during storage and reactions in model systems;
3) reactions of the additive in actual food systems; and
4) the metabolism of the additive in living organisms.

These data are important for relating toxicological data to the actual use of the additive in food.

Processing aids are substances that come into contact with food during processing and may unintentionally become part of food because of their incomplete removal. JECFA has evaluated a number of processing aids, such as extraction solvents and enzyme preparations, for their safety in use. When evaluating a processing aid, information should be provided on its use and either analytical data on or a computed estimate of the amount of the processing aid carried over into food. Particular attention should focus on any component of the processing aid that may have the potential for biological effects, such as ethylenimine leaching from polyethylenimine, an immobilizing agent used in the preparation of immobilized enzyme preparations.

3.4.4 Analytical methods

Information submitted to JECFA on the identity and purity of food additives should always include details of the analytical methods that can be used to verify the information. Information on the potential compositional variability of the substance should also be given, together with details of any sampling protocols used to assess this. Insufficient information on analytical methodology is one reason why JECFA may be unable to elaborate suitable specifications or why it may decide that it is able to assign only a "tentative specification" pending receipt of the further information required.

JECFA specifications incorporate guidance on the analytical techniques that should be used to verify the information. Wherever possible, this should be done by reference to Volume 4 of the Combined Compendium of Food Additive Specifications (FAO, 2005/2006). If this is not possible, details of the test procedures are set out in the individual specifications monographs.

Because JECFA specifications are elaborated for worldwide use, the Committee prefers to quote methods that require the use of apparatus and equipment that are available in most laboratories, provided that such methods give results appropriate to the specified criteria. Methods involving more recently developed techniques or equipment will therefore not normally be quoted until such techniques are accepted internationally and are generally available at reasonable cost. However, reference to specific methods of analysis should not be taken as precluding the use of other methods, provided that these are validated as giving results of at least equivalent accuracy and specificity to those quoted.

3.5 Pesticide characterization

3.5.1 General considerations

When an active ingredient is evaluated by JMPR for the first time or during a periodic review, it is identified by its International Organization for Standardization (ISO) common name, International Union of Pure and Applied Chemistry (IUPAC) and Chemical Abstracts Service (CAS) systematic chemical names, CAS and Collaborative International Pesticides Analytical Council (CIPAC) numbers, structural formula (with stereochemistry when needed), molecular formula and relative molecular mass.

For relatively pure synthetic compounds, the identity is straightforward, but for isomer mixtures, clear identification needs special attention. A CAS number is not necessarily a unique identifier for a compound, even for a specific isomer. Information is required on the proportions of different components when the compound is a mixture (e.g. of stereoisomers), because the isomers may have different toxicological properties (Green, 1978; FAO/WHO, 1980). For example, an ADI for permethrin (40% *cis* : 60% *trans*) was allocated in 1982 (FAO/WHO, 1982), whereas an ADI for permethrin (25% *cis* : 75% *trans*) was not allocated until 1987 (FAO/WHO, 1987).

The considerations of identity, purity and stability of pesticides were explained in chapter 4 of Environmental Health Criteria (EHC) 104 (IPCS, 1990). Toxicological evaluations are strictly valid only for the technical-grade material being examined, and special care and

knowledge of the detailed specifications are required to extrapolate the findings to other products.

The 1987 JMPR (FAO/WHO, 1987) noted that ADIs based on studies using compounds of specific purity can be relevant to products of different origin or purity (i.e. equivalent products), but that there are examples where changes in the amount or type of impurity in the technical material can markedly influence the toxicity of a compound.

The International Code of Conduct on the Distribution and Use of Pesticides (FAO, 2005) defines equivalence broadly as:

> the determination of the similarity of the impurity and toxicological profiles, as well as of the physical and chemical properties, presented by supposedly similar technical material originating from different manufacturers, in order to assess whether they present similar levels of risk.

JMPR (FAO/WHO, 1985), after noting the influence on toxicity of impurities such as dimethylhydrazine, dioxins and hexachlorobenzene, stressed "the importance of determining whether the toxicity of a technical pesticide is due to the inherent toxicity of that compound or also due to the presence of toxic impurities".

In 1999, FAO, in cooperation with WHO, introduced a revised procedure for evaluating data to establish specifications for pesticides (FAO/WHO, 1999c). The Joint FAO/WHO Meeting on Pesticide Specifications (JMPS) now establishes specifications for technical-grade material and formulations. The specifications include minimum permitted content of active ingredient and maximum permitted concentrations for relevant impurities. A relevant impurity is a by-product of the manufacture or storage of a pesticide that, compared with the active ingredient, is toxicologically significant to health or the environment, is phytotoxic to treated crops, causes taint in food crops, affects the stability of the pesticide or causes any other adverse effect. The long-term aim was for FAO/WHO specifications for technical material to be developed before the establishment of an ADI or an acute reference dose (ARfD).

Data required to support the development of pesticide specifications by JMPS include the identity of the active ingredient, physical and chemical properties, route of manufacture, minimum active

ingredient content, maximum limits for impurities present above 1 g/kg, maximum limits for impurities proposed as relevant at <1 g/kg, the identity and nominal content of compounds intentionally added to the technical material, toxicological and ecotoxicological summaries, properties of formulations, and methods for the analysis and testing of technical material and formulations (includes methods for relevant impurities).

A IUPAC project examined the significance of impurities in the safety evaluation of pesticides and made recommendations on assessment, analysis and monitoring of pesticide quality (Ambrus et al., 2003).

JMPR takes account of the JMPS specifications for a pesticide where available. In other cases, the technical-grade pesticide is characterized by its minimum purity, isomer composition and the limits for content of impurities that might impact on the hazard assessment. Because data on impurities and the composition of technical-grade materials could provide valuable information to competitors, they are normally confidential information and are not published in the JMPR reports or monographs. In 2005, JMPR reiterated the previous conclusions that specifications for the technical material should be developed for a pesticide before it is evaluated within the periodic review programme of the CCPR and for new pesticides, but that this should not delay evaluation of pesticides by JMPR (FAO/WHO, 2005a).

Data on the shelf-life stability of the technical-grade material are also important, because the percentage of the active material will decrease and that of potentially relevant breakdown products may increase with time if a test compound is unstable under the conditions of storage.

As well as the importance of possible changes in products offered for sale, shelf-life stability may be critical in studies where a single batch of technical material is utilized for a long-term study or a multigeneration study. Also, variable percentages of degradation occurring in different batches (i.e. batches of different post-manufacturing age) may complicate the interpretation of a study. Further, components of the test diet might promote degradation of the active compound, which may result in the production of toxic reaction products in the diet. In

cases where the percentage of active parent compound decreases or the breakdown products are more toxic than the parent compound, no-observed-adverse-effect levels (NOAELs) derived from the toxicity tests may not be representative of the product as used.

To date, JMPR has evaluated only the active ingredients (pure and technical grade) of pesticide formulations. The toxicity of other ingredients of the formulations—such as solvents, emulsifiers and preservatives—that may occur as residues in food has not been considered.

3.5.2 Identity and purity

Guidance on the development and use of specifications for pesticides evaluated by JMPR was elaborated in 2002 by the first meeting of JMPS (FAO/WHO, 2002) and updated in 2006 (FAO/WHO, 2006a).

For the purposes of the characterization:

- A detailed specification of the test material used in each individual study must be provided.
- Where isomeric mixtures exist, the ratio of isomers in the test material must be clearly specified.

For purity considerations:

- The percentage of the active ingredient in any technical material used in a toxicity test or proposed for marketing must be specified.
- Percentages of all identifiable impurities should be specified.
- Data on manufacturing processes may be required to permit determination of potential impurities; however, because of confidentiality, such data will not be published in JMPR monographs.

3.5.3 Stability

The stability of the test material during storage and in the diet must be adequately investigated and reported.

Where instability in diets is observed, the possible reaction products and the nutritional quality of the diet should be investigated.

3.5.4 Physical and chemical properties

Data submitted on the physical and chemical properties of the pure active ingredient are evaluated in order to recognize the influence of these properties on the behaviour of the pesticide during and after its application on crops or animals. JMPR receives data on the pesticide's physical appearance, solubility in water (including pH effects) and in organic solvents, vapour pressure, dissociation constant, *n*-octanol–water partition coefficient (K_{ow}), hydrolysis and photolysis.

The volatility of the compound, its stability in water and its sensitivity to irradiation with ultraviolet light may considerably affect its disappearance after application.

Epimerization may sometimes be observed during hydrolysis studies. For example, esfenvalerate (2S,αS) was epimerized to the 2S,αR isomer more quickly than it was hydrolysed under experimental conditions (FAO, 2003). The proportion of epimers may influence the toxicity.

The solubility of the pesticide is of great importance, because the ability of the compound to penetrate plant and animal tissues is dependent on its solubility in water and organic materials.

JMPR (FAO/WHO, 1991) chose the K_{ow} of a pesticide as the physical property to represent solubility in fat. In general, the compound would be designated fat soluble when log K_{ow} exceeded 4, but not when log K_{ow} was less than 3. Subsequently, JMPR (FAO/WHO, 2005b) examined the available data and concluded that partitioning in meat between fat and muscle is essentially independent of log K_{ow} for compounds with values greater than 3. In consequence, and when no evidence is available to the contrary, the compound is designated fat soluble when log K_{ow} exceeds 3, but not when log K_{ow} is less than 3. Although log K_{ow} of an individual component of a residue is an initial indicator, it is not the only or prime factor used to assess fat solubility. The distribution of the residue (as described in the residue definition) between muscle and fat obtained from livestock metabolism and feeding studies should be the prime indicator of fat solubility.

3.5.5 Analytical methods

Pesticides are very diverse chemical compounds with a wide range of physical and chemical properties. Analytical chemists have devised methods for the analysis of pesticide residues, including their transformation products, in a wide range of situations.

Methods should be validated to provide the supporting information on accuracy, selectivity and reliability of the data generated by the method. Hill & Reynolds (1999) explained the practicalities and compromises in validating analytical methods for pesticide residues in food and animal feeds.

Analytical methods should be suitable for the required purpose, which usually falls into one of three areas of residue analysis:

1) data generation for registration;
2) MRL enforcement and surveillance; and
3) total diet studies.

JMPR evaluates the analytical methods used for generation of residue data to check that the methods are suitable for the relevant analytes and sample types. The methods should be supported by adequate validation data, especially on analytical recoveries, LOQ and selectivity.

JMPR also reports information on methods that are suitable for MRL enforcement and whether particular compounds are suitable for analysis by multiresidue methods.

Most analytical methods for residues of simple organic compounds in a food commodity matrix consist of three main steps: 1) extraction, 2) cleanup and 3) determination or measurement, usually involving gas chromatography or liquid chromatography. However, some analytes require other approaches. For example, a chemical reaction may be needed to release an analyte from the residue, or a derivative of the analyte may have to be prepared for the chromatography step (e.g. the analytical method for residues of dithiocarbamates is nonspecific and measures carbon disulfide released by treatment with acid).

JMPR evaluates methods used for generating preregistration residue data that are needed for analysis of samples from:

- supervised residue trials;
- food processing studies;
- livestock feeding studies and direct animal treatment; and
- sample storage stability studies.

Analytes include compounds to be specified in the residue definitions (i.e. the MRL enforcement residue definition and the dietary intake risk residue definition). This substance would, in the majority of cases, be the parent compound, with inclusion of one or more metabolites or other transformation products when appropriate, based on the metabolism of the pesticide in plants and animals.

The LOQ of the analytical method for residue trials would be typically 0.01–0.05 mg/kg. Lower LOQs may be needed in some circumstances. For example, dietary intake calculations for a pesticide with a low ADI or ARfD might suggest that residues need to be measured at levels less than 0.01 mg/kg, necessitating a method with a lower LOQ. Total diet studies may need especially low LOQs for some analytes.

The FAO Panel of JMPR defines the LOQ of an analytical method for residues in specified commodities as being the lowest level where satisfactory recoveries were achieved. The LOQ is the smallest concentration of the analyte that can be quantified. It is commonly defined as the minimum concentration of analyte in the test sample that can be determined with acceptable precision (repeatability) and accuracy under the stated conditions of the test (FAO, 2002b).

Analytical recovery data support JMPR decisions on the acceptability or non-acceptability of the associated residue data. Recoveries in the 70–120% range are considered satisfactory. JMPR does not normally adjust or correct residue data using analytical recovery data.

Residue methods should normally be tested and validated on representative commodities (chosen because of expected residue occurrence), such as:

- plant material with a high moisture content (e.g. lettuce, tomatoes);
- plant material with high oil and protein contents (e.g. soybeans, peanuts, avocados);

- plant material with high starch or sugar content (e.g. cereal grains, potatoes);
- acidic commodities (e.g. citrus fruits);
- low-moisture feed materials (e.g. maize fodder);
- animal tissues (e.g. beef muscle, fat, liver, kidney); and
- milk and eggs.

Some matrices may cause particular problems (e.g. poor recoveries or interferences). For example, onions, broccoli and cabbage release carbon disulfide from endogenous precursors when treated with acid, which interferes with the measurement of dithiocarbamate residues (FAO, 1993a). In another example, recoveries of approximately 50% were obtained when racemic glufosinate was spiked into transgenic glufosinate-tolerant soybean plants, because the transgenic plant material very rapidly metabolized the L-enantiomer, leaving only the D-enantiomer for measurement (FAO/WHO, 1999b).

Interference from the matrix could add to the measured residue or cause losses during the procedure, and such problems are often encountered. For example, the chromatographic response to indoxacarb residues was enhanced by the crop extract, necessitating the preparation of standard solutions in crop extract (FAO, 2006).

The analysis of ethylenethiourea residues in the presence of parent ethylenebisdithiocarbamate (mancozeb) presents special problems that may not be covered by normal validation testing. Mancozeb residues may be converted to ethylenethiourea under some conditions during the analytical procedure (estimated conversion rates 0.22–8.5%). In samples where mancozeb is present at concentrations up to 1 mg/kg, it is possible that ethylenethiourea residues close to but above the LOQ (0.02 mg/kg) may have been produced during the analytical procedure (FAO, 1993b).

The extraction efficiency for residues bound within the matrix cannot be tested by spiking samples shortly before analysis, but bound ^{14}C-labelled residues from metabolism studies may be used to check extractability. Samples of plant and animal tissue from the radiolabelled metabolism studies containing bound ^{14}C residue levels may subsequently be analysed by the routine residue method (or, at least, the extraction procedure of the routine method) in order to define the extractability of the bound ^{14}C residues.

The 1998 JMPR (FAO/WHO, 1999a) recommended that

> Comparative extraction efficiency studies including the frequently used extraction solvents, such as acetone/water, ethyl acetate and acetonitrile/water should be carried out on samples from metabolism studies for the compounds which are expected to be included in the residue definition(s).

A IUPAC report (Skidmore et al., 1998) stated that

> The extraction procedures used in residue analytical methods should be validated using samples from radiolabelled studies where the chemical has been applied in a manner consistent with the label and Good Agricultural Practices.

In analytical chemistry, the term "common moiety" means that structural portion of different compounds that is the same and that tends to remain intact during chemical reactions. A common moiety analytical method relies on this feature to measure the concentration of a group of related compounds all together. Such a method may be useful when a number of metabolites with the common moiety need to be included in the estimates of dietary intake or when the composition of the residue is quite variable and the common moiety is easier to measure than a specific component. An example of this is the analysis of dithiocarbamate pesticides using acid-release carbon disulfide as the final analyte.

An analytical method used for testing the stability of residues during frozen storage needs to be reproducible for the duration of the test (perhaps 2 years), and it should distinguish the starting compound from degradation products. If analytical recoveries are too variable, the variability will obscure conclusions about stability, and only large losses during storage will be observable.

3.6 Veterinary drug residues

3.6.1 General considerations

The basic data requirements were established by the thirty-second meeting of JECFA (FAO/WHO, 1988). The Committee must be assured that any veterinary drug it evaluates is well characterized, with details of the chemical and physical properties of the drug and the identity

and concentrations of any major impurities. In addition, the manufacturing process should be described and the consistency and quality of the final products demonstrated. This information should be included in the dossier submitted for review by the Committee and is used to define the substance used in the studies that lead to the establishment of the MRLs for a veterinary drug (MRLVDs)[1] and the ADI.

Veterinary drugs cover a broad range of chemical structures and usually undergo metabolism after administration to an animal. Modes of administration include injection, implantation, dermal application by spray or pour-on, and inclusion in feed or water, all of which may result in different rates of absorption, with possible differences in the tissue distribution and nature of the residues. The form and the distribution of the residues that result from each authorized mode of application in each species should be determined, and the depletion of the residues from edible tissues or animal-derived foods should be studied. A marker residue should be identified, which is usually the form of the drug (parent compound or metabolite) that is found at the highest concentration for the longest period in the target food. The relationship of this marker residue to the total residue of the drug should be determined, usually through treatment of experimental animals with an isotope-labelled form of the drug. The tissue in which the highest residues are found is usually designated as a "target tissue" for routine monitoring purposes.

Analytical methods, whether intended for use in pharmacokinetic and metabolism studies, in residue depletion studies or in regulatory control programmes for residues of veterinary drugs, share a common subset of validation criteria. However, additional criteria are to be met for methods used in routine monitoring of compliance of commodities with MRLVDs. Performance characteristics to be determined for all methods include specificity, accuracy, precision, LOD, LOQ, susceptibility to interference and information on method calibration. Practicability, applicability under normal laboratory conditions and ruggedness are the additional criteria for the evaluation of regulatory methods. Validation thus addresses all aspects of performance

[1] Both JECFA and CCRVDF use the acronym MRL for this limit throughout its stepwise elaboration; however, MRLVD is the acronym of the final standard adopted by CAC on the recommendation of CCRVDF.

characteristics of the analytical methods. Target values for method precision and recovery have been established by CCRVDF for the concentrations typically required to support MRLVDs (FAO/WHO, 1993).

3.6.2 Analytical methods

The first meeting of the Committee devoted exclusively to the evaluation of veterinary drugs (FAO/WHO, 1988) recognized that analytical methods are required to

> detect, quantify and positively identify residues of veterinary drugs; support toxicological, drug metabolism, and pharmacokinetic studies; support residue studies of compounds to be evaluated by the Committee; and satisfy the needs of public health agencies.

The initial focus of JECFA was to ensure that methods used in the pharmacokinetic and residue depletion studies evaluated by the Committee had been suitably described and appropriately validated. The ninth session of CCRVDF decided that no MRLVD could be accepted without a suitable method being identified to support the MRLVD. This decision added emphasis to the role of JECFA in identifying analytical methods suitable for regulatory use as part of their review (FAO/WHO, 1997). The eleventh session of CCRVDF (FAO/WHO, 1999d) determined that JECFA would have primary responsibility for review of methods for compounds. This was taken into account at the fiftieth (FAO/WHO, 1999e) and all subsequent meetings of JECFA. A guidance document entitled "JECFA Requirements for Validation of Analytical Methods" was published with the residue monographs of the fifty-eighth meeting of JECFA (FAO, 2002a).

During JECFA review, the primary requirement for methods used in pharmacokinetic and residue depletion studies is that the method has been shown to have performed reliably in the hands of the analyst or analysts involved in that specific study. The dossier reviewed by JECFA usually includes a complete validation report for the method, particularly if the method has not been published in the peer-reviewed scientific literature.

For some compounds evaluated by JECFA, no residues were detected in one or more of the four edible target tissues (muscle,

liver, kidney, fat) from any of the animals to which the drug had been administered at any time of sampling. In such cases, CCRVDF has requested that JECFA establish MRLVDs for these tissues in which no residues have been detected, based on the LOQ of the available residue control method, provided that such MRLVDs are consistent with adequate health protection.

In the past, JECFA and CCRVDF have not usually recommended analytical methods for residues of substances for which no ADI or MRLVD has been established. This practice has since been changed, and the Committee now recommends validated methods for substances without a recommended ADI or MRLs, provided such methods are made available to the Committee.

3.7 Contaminants

3.7.1 General considerations

Contaminants in the diet may include environmental pollutants, such as heavy metals and industrial chemicals, mycotoxins, migrants from packaging materials and other substances not authorized for use in food.

The data required for the characterization of a contaminant should include its concentrations in foods and the total diet from as many countries as possible. The sixty-fourth meeting of JECFA (FAO/WHO, 2006b) recommended that the data should be formatted using the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) to facilitate the collation and quality control of the data. The data should be accompanied by additional details on sampling plans and analytical methods used to generate the data.

Contaminants in food commodities may result from environmental contamination by persistent compounds formerly used as pesticides (e.g. persistent organochlorine pesticides). JMPR proposes limits (extraneous maximum residue limits [EMRLs]) for such contaminants when they originate from environmental sources and not from direct or indirect uses on the crop or farm animals. In 1990 (FAO/WHO, 1990), JMPR explained that EMRL assessments rely on monitoring data and supporting information, including:

- country;
- year;
- commodity and portion analysed;
- pesticide and residue definition;
- sample classification as import, export or domestic production and consumption; and
- sampling plan described as random monitoring or target sampling.

Ideally, for reasonable EMRL estimates to cover international trade, JMPR should have current and geographically representative data (FAO/WHO, 1996), but typically data are available from only three or four (usually developed) countries. JMPR requests the submission of all relevant data, including nil results. Because residues gradually decrease, new data should be assessed every few years with a view to EMRL revision.

3.7.2 Analytical methods

The LOQs of the analytical methods to measure the concentrations of contaminants in foods (on a raw basis or an as consumed basis) should be as low as reasonably possible (usually much lower than the regulation limit). This consideration is of critical importance in exposure estimations, because low levels of contaminants are frequently present in foods, and the censored data (data points with non-quantified results) represent a bias source in calculations of exposure. If the LOQ is not sufficiently low, then there is a risk of underestimation if all non-detects are taken as zero or overestimation if all non-detects are taken as the LOQ. To minimize this bias, it is recommended that the censored data should be treated following the statistical approach discussed in chapter 6.

3.8 Substances consumed in large amounts

Thorough chemical analysis should be performed on high-consumption substances, such as bulk additives, to measure potential impurities and to provide information on nutritional adequacy, especially when such substances replace traditional food.

It is not possible to provide a checklist of necessary chemical studies to cover all high-consumption compounds. The substance should

be subjected to a full analysis, and particular attention should be paid to the points discussed in the following paragraphs.

Because the exposure to undesirable impurities (e.g. heavy metals) concomitant with the intake of high-consumption materials is potentially high, special effort should be made to identify the impurities. Information on the production process, including the materials and procedures involved, will point to the types of contaminants for which limits may need to be specified. The specifications should be accompanied by details of product variability and of the analytical methods used to check the specifications and details of the sampling protocols. If the substance is so complex that comprehensive product specifications on chemical composition are impracticable (as they might be for a microbial protein), the description of the substance in the specifications may include relevant aspects of its manufacturing process. If manufacturing data are based on production on a pilot scale, the manufacturer should demonstrate that, when produced in a large-scale plant, the substance will meet the specifications established on the basis of pilot data.

The permissible limits for impurities may in some cases correspond to the levels accepted for natural foods that have similar structure or function or that are intended to be replaced by the new material. If the substance is prepared by a biological process, special attention should be paid to the possible occurrence of natural toxins (e.g. mycotoxins).

If the nature of the substance or manufacturing process indicates the possible presence of naturally occurring or adventitious anti-nutritional factors (phytate, trypsin inhibitors, etc.) or toxins (haemagglutinins, mycotoxins, nicotine, etc.), the product should be analysed for them specifically. Biological tests, either as part of the nutritional evaluation in the case of enzyme inhibitors or more specifically as part of a mycotoxin screening programme, will provide useful backup evidence concerning the presence or absence of these contaminants.

Finally, if under the intended conditions of use the substance may be unstable or is likely to interact chemically with other food components (e.g. degradation or rearrangement of the substance during heat processing), data should be provided on its stability and reactivity. The various tests should be conducted under conditions relevant to the use

of the substance (e.g. at the acidity and temperature of the environment and in the presence of other compounds that may react).

3.9 References[1]

Ambrus A, Hamilton DJ, Kuiper HA & Racke KD (2003) Significance of impurities in the safety evaluation of crop protection products (IUPAC Technical Report). Pure Appl Chem, **75**(7): 937–973 (http://old.iupac.org/publications/pac/2003/pdf/7507x0937.pdf).

FAO (1978) Guide to specifications: general notices, general methods, identification tests, test solutions, and other reference materials. Rome, Food and Agriculture Organization of the United Nations, Joint FAO/WHO Expert Committee on Food Additives (FAO Food and Nutrition Paper, No. 5).

FAO (1983) Guide to specifications: general notices, general methods, identification tests, test solutions, and other reference materials. Rome, Food and Agriculture Organization of the United Nations, Joint FAO/WHO Expert Committee on Food Additives (FAO Food and Nutrition Paper, No. 5, Rev. 1).

FAO (1991) Guide to specifications: general notices, general methods, identification tests, test solutions, and other reference materials. Rome, Food and Agriculture Organization of the United Nations, Joint FAO/WHO Expert Committee on Food Additives (FAO Food and Nutrition Paper, No. 5, Rev. 2).

FAO (1993a) Maneb. In: Pesticide residues in food—1993 evaluations. Part I. Residues. Rome, Food and Agriculture Organization of the United Nations, p 705 (FAO Plant Production and Protection Paper, No. 124; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/93_eva/jm93eval.pdf).

FAO (1993b) Mancozeb. In: Pesticide residues in food—1993 evaluations. Part I. Residues. Rome, Food and Agriculture Organization of the United Nations, p 673 (FAO Plant Production and Protection Paper, No. 124; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/93_eva/jm93eval.pdf).

FAO (2002a) Residues of some veterinary drugs in animals and foods. Rome, Food and Agriculture Organization of the United Nations (FAO Food and Nutrition Paper, No. 41/14).

FAO (2002b) Submission and evaluation of pesticide residues data for the estimation of maximum residue levels in food and feed, 1st ed. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 170; http://www.fao.org/ag/agp/agpp/pesticid/JMPR/Download/FAOM2002.pdf).

[1] Internet links provided in these references were active as of the date of final editing.

FAO (2003) Esfenvalerate. In: Pesticide residues in food—2002 evaluations. Part I. Residues. Rome, Food and Agriculture Organization of the United Nations, pp 600–601 (FAO Plant Production and Protection Paper, No. 175/1; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/2002_eva/Esfenvaljj.pdf).

FAO (2005) International code of conduct on the distribution and use of pesticides (revised version). Rome, Food and Agriculture Organization of the United Nations (ftp://ftp.fao.org/docrep/fao/009/a0220e/a0220e00.pdf).

FAO (2005/2006) Combined compendium of food additive specifications. Vols. 1–4. Rome, Food and Agriculture Organization of the United Nations (FAO JECFA Monographs, No. 1).

FAO (2006) Indoxacarb. In: Pesticide residues in food—2005 evaluations. Part I. Residues. Rome, Food and Agriculture Organization of the United Nations, p 518 (FAO Plant Production and Protection Paper, No. 184; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/2005_eva/Residue%20Evaluations%20JMPR%202005%20.pdf).

FAO/WHO (1956) Joint FAO/WHO Conference on Food Additives. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Nutrition Meetings Report Series, No. 11; WHO Technical Report Series, No. 107).

FAO/WHO (1957) General principles governing the use of food additives. First report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Nutrition Meetings Report Series, No. 15; WHO Technical Report Series, No. 129; http://whqlibdoc.who.int/trs/WHO_TRS_129.pdf).

FAO/WHO (1958) Procedures for the testing of intentional food additives to establish their safety for use. Second report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Nutrition Meetings Report Series, No. 17; WHO Technical Report Series, No. 144; http://whqlibdoc.who.int/trs/WHO_TRS_144.pdf).

FAO/WHO (1966) Specifications for the identity and purity of food additives and their toxicological evaluation: some antimicrobials, antioxidants, emulsifiers, stabilizers, flour-treatment agents, acids, and bases. Ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Nutrition Meetings Report Series, No. 40; WHO Technical Report Series, No. 339; http://whqlibdoc.who.int/trs/WHO_TRS_339.pdf).

FAO/WHO (1971) Evaluation of food additives: specifications for the identity and purity of food additives and their toxicological evaluation: some extraction solvents and certain other substances; and a review of the technological

efficacy of some antimicrobial agents. Fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Nutrition Meetings Report Series, No. 48; WHO Technical Report Series, No. 462; http://whqlibdoc.who.int/trs/WHO_TRS_462.pdf).

FAO/WHO (1972) Evaluation of food additives: some enzymes, modified starches, and certain other substances: toxicological evaluations and specifications and a review of the technological efficacy of some antioxidants. Fifteenth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Nutrition Meetings Report Series, No. 50; WHO Technical Report Series, No. 488; http://whqlibdoc.who.int/trs/WHO_TRS_488.pdf).

FAO/WHO (1975) Evaluation of certain food additives: some food colours, thickening agents, smoke condensates, and certain other substances. Nineteenth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Nutrition Meetings Report Series, No. 55; WHO Technical Report Series, No. 576; http://whqlibdoc.who.int/trs/WHO_TRS_576.pdf).

FAO/WHO (1980) Pesticide residues in food—1979. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 20).

FAO/WHO (1981) Evaluation of certain food additives. Twenty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 669; http://whqlibdoc.who.int/trs/WHO_TRS_669.pdf).

FAO/WHO (1982) Pesticide residues in food—1982. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 46).

FAO/WHO (1983) Evaluation of certain food additives and contaminants. Twenty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 696 and corrigenda; http://whqlibdoc.who.int/trs/WHO_TRS_696.pdf).

FAO/WHO (1985) Purity of pesticides for toxicological evaluation. In: Pesticide residues in food—1984. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations, p 9 (FAO Plant Production and Protection Paper, No. 62).

FAO/WHO (1987) Pesticide residues in food—1987. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 84).

FAO/WHO (1988) Evaluation of certain veterinary drug residues in food. Thirty-second report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 763; http://whqlibdoc.who.int/trs/WHO_TRS_763.pdf).

FAO/WHO (1990) Data requirements for extraneous residue limit estimations. In: Pesticide residues in food—1990. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations, pp 4–5 (FAO Plant Production and Protection Paper, No. 102).

FAO/WHO (1991) Fat-soluble pesticides. In: Pesticide residues in food—1991. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations, pp 15–16 (FAO Plant Production and Protection Paper, No. 111).

FAO/WHO (1993) Residues of veterinary drugs in foods, 2nd ed. Vol. 3. Rome, Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission.

FAO/WHO (1996) JMPR approach to estimating extraneous residue limits (ERLs). In: Pesticide residues in food—1995. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations, pp 21–23 (FAO Plant Production and Protection Paper, No. 133; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/95_rep/Report1995.pdf).

FAO/WHO (1997) Report of the ninth session of the Codex Committee on Residues of Veterinary Drugs in Foods. Rome, Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission (ALINORM 97/31; http://www.fao.org/docrep/meeting/005/w0062e/w0062e00.htm).

FAO/WHO (1999a) Data requirements for the validation of analytical procedures. In: Pesticide residues in food—1998. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations, p 11 (FAO Plant Production and Protection Paper, No. 148; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/98_rep/REPORT1998.pdf).

FAO/WHO (1999b) Glufosinate-ammonium. In: Pesticide residues in food—1998. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues

in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations, p 135 (FAO Plant Production and Protection Paper, No. 148; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/98_rep/REPORT1998.pdf).

FAO/WHO (1999c) Relevance of pesticide specifications for JMPR evaluations. In: Pesticide residues in food—1999. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations, pp 16–17 (FAO Plant Production and Protection Paper, No. 153; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/99_rep/REPORT1999.pdf).

FAO/WHO (1999d) Report of the eleventh session of the Codex Committee on Residues of Veterinary Drugs in Foods. Rome, Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission (ALINORM 99/31; http://www.fao.org/docrep/meeting/005/x0203e/x0203e00.htm).

FAO/WHO (1999e) Evaluation of certain veterinary drugs in food. Fiftieth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 888; http://whqlibdoc.who.int/trs/WHO_TRS_888.pdf).

FAO/WHO (2002) Manual on development and use of FAO and WHO specifications for pesticides, 1st ed. Rome, World Health Organization and Food and Agriculture Organization of the United Nations, Joint FAO/WHO Meeting on Pesticide Specifications (FAO Plant Production and Protection Paper, No. 173; http://whqlibdoc.who.int/publications/2002/9251048576.pdf).

FAO/WHO (2005a) Joint FAO/WHO Meeting on Pesticide Specifications. In: Pesticide residues in food—2005. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations, pp 33–34 (FAO Plant Production and Protection Paper, No. 183; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/DOWNLOAD/2005_rep/report2005jmpr.pdf).

FAO/WHO (2005b) Definition of fat-soluble pesticides in meat and fat. In: Pesticide residues in food—2005. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations, pp 27–31 (FAO Plant Production and Protection Paper, No. 183; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/DOWNLOAD/2005_rep/report2005jmpr.pdf).

FAO/WHO (2006a) Manual on development and use of FAO and WHO specifications for pesticides. March 2006 revision of the first edition. Rome, World Health Organization and Food and Agriculture Organization of the United Nations, Joint FAO/WHO Meeting on Pesticide Specifications (http://whqlibdoc.who.int/publications/2006/9251048576_eng_update_2006.pdf).

FAO/WHO (2006b) Evaluation of certain food contaminants. Sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 930; http://whqlibdoc.who.int/trs/WHO_TRS_930_eng.pdf).

FAO/WHO (2008) Codex Alimentarius Commission procedural manual, 18th ed. Rome, Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission (ftp://ftp.fao.org/codex/Publications/ProcManuals/Manual_18e.pdf).

Green WI (1978) Mechanisms of action of anti-thyroid compounds. In: Werner SC & Ingbar SH eds. The thyroid. New York, NY, Harper and Row, pp 77–87.

Hill ARC & Reynolds SL (1999) Guidelines for in-house validation of analytical methods for pesticide residues in food and animal feeds. Analyst, **124**: 953–958.

IPCS (1990) Principles for the toxicological assessment of pesticide residues in food. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 104; http://www.inchem.org/documents/ehc/ehc/ehc104.htm).

ISO (2005) General requirements for the competence of testing and calibration laboratories, 2nd ed. Geneva, International Organization for Standardization (ISO/IEC 17025:2005).

ISO (2008) Quality management systems—Requirements. Geneva, International Organization for Standardization (ISO 9001:2008).

Skidmore MW, Paulson G, Kuiper HA, Ohlin B & Reynolds S (1998) Bound xenobiotic residues in food commodities of plant and animal origin. Pure Appl Chem, **70**: 1423–1447.

Thompson M & Wood R (1993) International harmonized protocol for proficiency testing of (chemical) analytical laboratories. Pure Appl Chem, **65**: 2132–2144.

4. HAZARD IDENTIFICATION AND CHARACTERIZATION: TOXICOLOGICAL AND HUMAN STUDIES

For acronyms and abbreviations used in the text, the reader may refer to the list of acronyms and abbreviations at the front of this monograph. Definitions of select terms may be found in the glossary at the end of the monograph.

4.1 Introduction

Toxicological studies may be broadly divided into in vitro studies, using cultured organisms or cells or tissue preparations from laboratory animals or humans, and in vivo studies in laboratory animals or humans. Such studies serve a number of purposes, including:

- identification of potential adverse effects;
- definition of the exposure conditions necessary to produce the effects;
- assessment of dose–response relationships for the adverse effects, including definition of dose levels that do not produce the effects; and
- interpretation of experimental data for risk assessment purposes, such as information on the mode of action and its relevance to humans and metabolism and toxicokinetic data that allow extrapolation of the data from laboratory animals to humans and to population subgroups.

A number of factors can influence the selection of appropriate methods for the toxicological testing of substances in food. Not all substances in food can or need to be tested toxicologically to the same degree or subjected to the same range of toxicity tests. The following text lists important factors to consider in the selection of test methods.

4.1.1 Nature of substances to be evaluated

The nature of the substance and its uses and levels of use can all influence the extent of toxicity testing necessary for risk assessment:

- The selection of test methods is governed to an extent by the nature of the substances to be tested.

- Substances evaluated by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) range from single chemicals ingested in small amounts, such as contaminants, flavours, pesticides and certain food additives, to complex substances that may comprise a substantial portion of the diet, such as major food ingredients and whole foods.

- Substances consumed in small amounts can readily be subjected to appropriate and relevant toxicity tests, in which high dose levels can be used to increase the sensitivity of hazard identification. The majority of the tests discussed in this chapter are most readily applicable to low molecular weight, single-chemical entities.

- For substances consumed in large amounts, standard toxicity studies, while applicable, need to be designed and interpreted with caution because of possible physiological or nutritional perturbations that may be induced in test animals.

- For substances consumed in large amounts, human studies can play a significant role in assessing the tolerability of such substances.

4.1.2 Knowledge requirements for substances to be tested and evaluated

Prior to embarking on any toxicological testing of substances found in or intended for use in food, data should be available in several key areas:

- For a substance added either directly or indirectly to foods, information should be available on its source, including data on its manufacture (including aspects of Good Manufacturing Practice [GMP]) and appropriate information on its purity and specifications as a food-grade material. It is important that the substance being tested and evaluated is representative of that added to or present in food (see chapter 3).

- Knowledge of potential interactions of the substance with components of the foodstuff during processing and storage is essential in some cases to ensure that the appropriate chemical species are being tested and evaluated.

- Chemical speciation is important to consider for contaminants, residues of pesticides, packaging materials and residues of veterinary drugs, in order to ensure that toxicological and other studies are related to the chemical form or species that occurs in food.

4.1.3 Role of structure–activity relationships and metabolic fate

Careful examination of the composition, structure and known or presumed metabolic fate of the test substance should be undertaken prior to toxicity testing of substances added to or found in food. Examination of substances for structural alerts for toxicity can provide valuable guidance in the design of appropriate safety tests.

The general approach to safety evaluation should begin with an evaluation of the molecular structure of the substance in question. Some substances used as food additives and a large number of flavours are known to be endogenous substances or known or predicted to be readily converted in vivo into endogenous substances. Other substances may be known or presumed to be readily converted to metabolic products that could be considered harmless under the intended conditions of use of the parent substance. This may limit the extent to which such substances need to be subjected to toxicological testing.

Substances with structural alerts for specific forms of toxicity, such as neurotoxicity in the case of organophosphorus compounds or genotoxicity in the case of certain epoxides, nitrosamines, etc., should be subjected to detailed toxicological investigation, paying particular attention to that specific toxicity alert. Literature sources of knowledge regarding structure–activity relationships should be fully consulted before designing and conducting toxicity tests, especially to determine the need for any special studies related to identified safety concerns.

For substances intended to be consumed in large amounts, knowledge of the structure and metabolic fate may provide guidance on the interpretation of certain toxicological or physiological end-points. Substances that undergo colonic fermentation or produce caecal or colonic enlargement when given in large amounts or substances that raise the osmotic pressure of the colon often produce a cascading series of physiological events culminating in toxicological responses that may not be relevant to exposures encountered under conditions of practical use. Examples are polyols, which can produce hyperplasia of the adrenal medulla and phaeochromocytomas indirectly associated with abnormal calcium homeostasis, and the fat replacer olestra, which can produce adverse effects in high-dose animal studies by interfering with the absorption of fat-soluble vitamins.

For substances consumed in large amounts, secondary effects may limit the usefulness of conventional toxicological tests in assessing their safety, leading to an increased need to conduct appropriate and relevant studies in humans.

For substances for which there is no prior available knowledge of metabolic fate and pharmacokinetics (see section 4.2), such studies should be conducted prior to initiating large-scale toxicological studies.

4.1.4 Integrating data on dietary exposure

The extent and nature of testing that are considered adequate for a toxicological evaluation of a substance that is present in food should be based not only on any data on structure–activity relationships and metabolic fate, but also on presumed or known exposure:

- Exposure assessment should consider the likely duration and pattern of exposure (acute, short-term, long-term, intermittent, etc.) and the nature of the population that is likely to be exposed (e.g. the whole population or specific subgroups), as well as the potential for changes in exposure over time.

- Toxicological valuation of substances present in the diet at very low levels, such as flavouring agents (see chapter 9, section 9.1.2), may be based on data for structural analogues or more general thresholds of toxicological concern (TTCs) (chapter 9, section 9.1.1).

- TTCs (FAO/WHO, 1995, 1997, 2000b; Munro et al., 1996; Kroes et al., 2004), which define human exposure thresholds for different structure-based chemical classes, may be used to provide guidance on the degree of testing required (see also chapter 9, section 9.1.1).

4.1.5 General approach to toxicity testing

Several internationally recognized organizations, such as the Organisation for Economic Co-operation and Development (OECD), provide guidance for minimum standards for the design and conduct of toxicological studies. Hence, the following is a guide to general

principles. All studies used in the risk assessment of a substance in food should be assessed for adequacy of design and conduct; for recent studies, this should include compliance with Good Laboratory Practice (GLP) (see chapter 3).

In making an assessment of the need for and extent of toxicity testing required for substances added to food, the following information needs to be considered in an integrated fashion: 1) structure–activity relationship, 2) metabolic fate and 3) exposure. The stepwise approach to assessing toxicity testing needs is illustrated in Figure 4.1.

4.1.5.1 *Role of in silico and in vitro studies*

It is generally accepted that animal testing should be reduced, refined or replaced as far as is practicable, and this has led to an increased use of alternative approaches. While recognizing the desirability of this, it is important that scientifically sound methods and approaches are used for the safety testing of food chemicals. Hence, although advances are being made in the development of in silico and in vitro approaches, at the present time these do not permit the replacement of animal testing for most end-points of concern.

In silico approaches encompass a wide range of methods, ranging from simple quantitative structure–activity relationships (QSAR) to sophisticated multiparametric simulation and even prediction based on quantum chemistry and other fundamental approaches.

At the present time, only a limited number of in silico and in vitro methods have been adopted by the OECD and other organizations involved in method approval. In a few instances, in vitro methods have been recognized as generally valid for risk assessment purposes, particularly in genotoxicity testing, but also for assessing some non-genotoxic end-points, such as corrosivity and phototoxicity. The use of in vitro methods for these purposes can provide robust data for risk assessment. Where non-standard methods are used as part of a data submission, evidence of their performance characteristics and validation should be provided.

In silico methods are a practical means of comparing the sequence of proteins and peptides with those of known allergens to determine whether there are epitopes in common, although the reliability of this approach is not high. In vitro methods are useful in determining

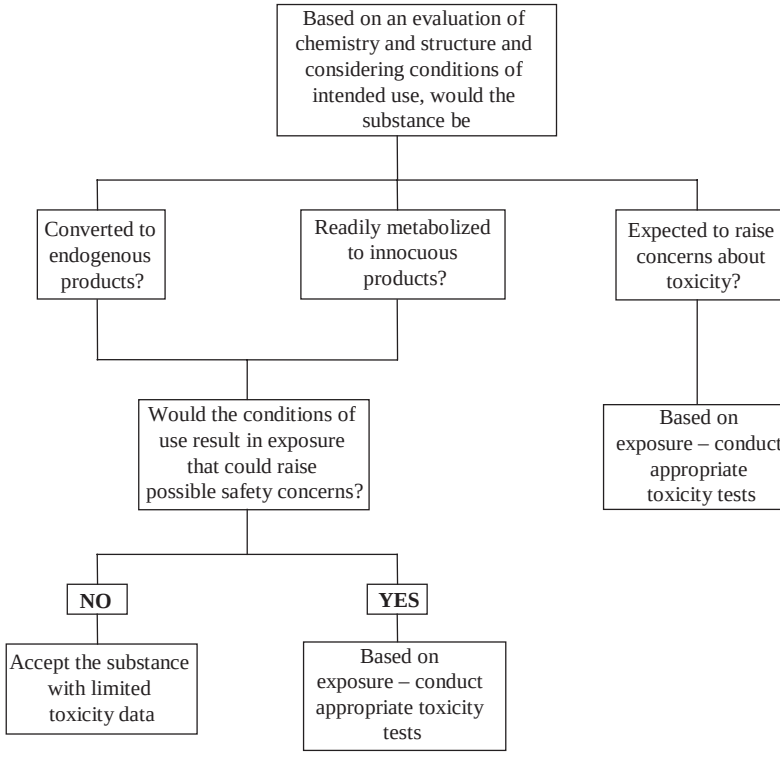

Fig. 4.1. A stepwise approach to assessing toxicity testing needs

the stability of proteins and peptides in digestive juices, such as gastric acid.

Mechanisms of toxicity are often investigated using in silico and in vitro methods. The results of such studies should be incorporated into a weight of evidence consideration of toxicity. In addition, such studies can provide insight into the relevance to humans of findings in experimental animals.

Also, in silico and in vitro methods are being used increasingly to characterize the metabolism of chemicals. Often, these data provide an invaluable bridge between laboratory animals and humans. Data derived from in silico and, even more so, from in vitro methods provide the basis for many physiologically based toxicokinetic (PBTK)

models. Information that may be obtained in this way includes kinetic parameters for metabolism of the chemical, blood–tissue partition coefficients and plasma protein binding. Data can be obtained for both laboratory species and humans.

4.1.5.2 *Digestion and impact on gut flora*

Many substances in food have the potential to affect the gut flora, but some effects occur in experimental animals only when fed very high doses—for example, with poorly absorbed substances, such as polyols and modified starches. For such substances, effects in humans are extremely unlikely if the maximum human exposure is only a small fraction of the doses used in laboratory animal studies.

During the testing for systemic toxicity, experimental animals should be monitored routinely for possible direct and indirect effects on the gastrointestinal tract, by assessment of behaviour and clinical signs, biochemistry (serum and urine), gross morphology and histopathology. Where there are indications from toxicity tests of an effect on the gastrointestinal tract (e.g. caecal enlargement, diarrhoea), the reasons for this should be investigated.

Specific tests on the gut microflora should be carried out when there is an obvious potential for an effect on the gut flora, such as from an antibiotic. In testing for effects on the gut flora, several aspects should be considered, such as alteration of barrier effect and emergence of antimicrobial resistance. The choice of test system should be informed by the end-point of concern. Due consideration needs to be given to the nature of the microflora to be tested and the conditions under which the test will be conducted.

Where there is concern for an effect of the microflora on the substance—for example, in digestion or the production of microflora-specific metabolites—ex vivo studies could be undertaken using an appropriate selection of microflora of laboratory animal or human origin (see section 4.12).

4.1.5.3 *Absorption, distribution, metabolism and excretion (ADME)*

Studies on the fate and behaviour of substances in food are important in the design and interpretation of toxicity studies and in extrapolation

to humans (IPCS, 1986a; Lipscomb & Ohanian, 2007). Interspecies and intraspecies differences in the kinetics of a substance are often a major contributory factor to interspecies and interindividual variation in response. Hence, a detailed understanding of the kinetics of the substance may enable some of the default uncertainty factors to be replaced with a chemical-specific adjustment factor (CSAF) (see IPCS [2005] and also chapter 5 for further discussion of uncertainty factors and CSAFs). ADME is described in section 4.2.

4.1.5.4 *Considerations in the selection of appropriate in vivo studies and relevant species (models)*

Although no experimental species is an ideal substitute for humans, there is extensive evidence that studies in test animals generally provide an effective means for evaluating the potential toxicity of substances in food, provided that the data are interpreted critically. Studies in experimental animals allow evaluation of toxicity to all mammalian organs and tissues and to physiological and metabolic processes and integrative functions. An important pragmatic factor influencing the choice of species and strain is the availability of historical control data; the absence of such data can severely limit the interpretation of equivocal findings.

The species selected should reflect the underlying biology of the end-point of concern and be of relevance to human biology. Hence, for studies of effects on fertility or development, animals of the appropriate life stage and reproductive capacity need to be selected, whereas animals of the appropriate sex (and often both sexes) would be used for potential effects on endocrine systems. However, not all such issues are resolved. For example, it is debatable as to which is the appropriate life stage in experimental animals for certain life stages in humans (e.g. children aged 1–3 years).

In selecting an animal model, its potential relevance to humans needs to be considered. There may be strain-specific or species-specific differences in metabolism or response such that findings for certain types of substance will not be relevant. For example, the CF-1 mouse is not a good animal model for investigating substances that show P-glycoprotein-dependent limits to their absorption.

The species and strain selected should be susceptible to the type of toxic effect being investigated. For example, some species or

strains are known to be less susceptible to developmental toxicity than others.

Although test species and humans have many common pathways of foreign compound metabolism, it is unlikely that a species will be found that exhibits exactly the same metabolic profile for a substance as humans. Ideally, the species used in toxicity studies should produce all of the metabolites formed in humans. If human-specific metabolites are identified, it might be necessary to conduct toxicity studies with the metabolites themselves.

4.1.5.5 Types of animal studies and their role in safety assessment

Studies should be such that the toxicity of the substance can be assessed for all known or predicted exposure scenarios, for all relevant subgroups of the population and for all potential effects. As discussed above (section 4.1.4), the extent of testing necessary for regulatory purposes is related to the extent of human exposure.

Most end-points are adequately addressed by current study designs, such as the OECD testing guidelines (http://masetto.sourceoecd.org/vl=2781582/cl=14/nw=1/rpsv/cw/vhosts/oecdjournals/1607310x/v1n4/contp1-1.htm), but there are some specific types of toxicity or circumstances of exposure where there may be a need for modification of or even novel study designs. An example is the assessment of acute toxicity other than lethality, for which there is currently no approved study protocol. The exact choice of studies will depend on considerations of likely human exposure duration, the population to be exposed and any prior information on the substance.

It is not always necessary to test the substance specifically to cover all situations. It may be possible to adopt conservative assumptions, using a non-optimal study. For example, in the case of acute risk, if the predicted human exposures are well below the health-based guidance value, such as an acute reference dose (ARfD; see chapter 5, section 5.2.9) derived using data from a 90-day study, further refinement of the risk assessment would not be necessary. Conversely, should exposure assessment indicate a possible risk, a specific study of acute toxicity could be undertaken to help refine the risk assessment.

The lethality of the substance should be determined, but only up to a limit dose. This has been set at 2 or 5 g/kg body weight. Any non-lethal effects should be reported, as these may provide evidence for mechanism of lethality or of non-lethal acute toxicity.

In both short- and long-term studies, a wide range of end-points is investigated, including clinical signs, body and organ weights, clinical chemistry and haematology, urinalysis, and gross and histopathological examination of organs. These may be supplemented by validated biomarkers for specific effects.

The effects of the substance when administered short term should be assessed; this usually involves studies for about 10% of lifespan (e.g. 90 days in rat, 1 year in dog), although valuable data may be derived from extensive studies of shorter duration in rats or dogs. The need for two species, one non-rodent, should be considered.

Long-term studies for chronic toxicity and carcinogenicity should be conducted; these are usually of 2 years' duration in rodents, which is more or less equivalent to "lifetime" exposure. Such an extended duration may increase the sensitivity to detect cancer at the expense of a reduced sensitivity for other effects because of masking by age-related changes, although data obtained from interim results at 1 year could avoid this complication in evaluating toxicity.

The genotoxicity of the substance should be evaluated using a range of appropriate in vitro tests for mutation (bacteria), chromosomal damage and changes in chromosome number. Positive results should be confirmed in an in vivo genotoxicity study. In the absence of evidence to the contrary, a substance that is an in vivo genotoxin would be presumed to be a genotoxic carcinogen.

The relevance to humans of any tumorigenic response observed on administration of the substance to experimental animals should be assessed using a structured framework (Boobis et al., 2006).

The need for two species for the cancer bioassay, or indeed the need for a bioassay at all, should be considered. Alternative strategies might include a tiered approach involving genotoxicity testing, investigation of precursor effects for non-genotoxic carcinogenicity

in short-term studies and the use of genetically modified animals (Gulezian et al., 2000).

The effects of the substance on reproductive performance of both males and females should be determined, if appropriate. The duration of exposure of the animals, relative to life stage, needs to be considered. For most substances, it will be necessary to consider the effects on embryonic and fetal development by treating pregnant dams. The need for two species for developmental testing should be considered.

The potential accumulation of the chemical also needs to be taken into account in the design and interpretation of such toxicity studies (e.g. the body burden of dioxins accumulates over a period of weeks of treatment).

Although studies such as those mentioned above should detect functional and structural effects on most tissues and organs, there are some systems for which additional testing may be required as appropriate. These include nutritional effects, neurobehavioural effects and neurotoxicity, both in adults and during development, and immunotoxicity. Appropriate further testing should be undertaken where there is reason to suspect such an effect, based on structure, prior knowledge or alerts from the results of more conventional tests.

Specific studies on mechanism of toxicity or mode of action, particularly for end-points that may be used in establishing reference values, such as health-based guidance values, may provide useful data.

For all study designs, careful consideration needs to be given to:

- dose spacing and number of study groups;
- maximum dose utilized;
- number of animals in each group;
- choice of controls and whether there is a need for a positive control group;
- dosing regimen;
- confirmation of dose administered compared with nominal dose;
- dose ingested (e.g. palatability, wastage of food); and
- incidental disease, such as infection.

Increasingly, the utility of studies of precursor effects, long used to help in the risk assessment of non-genotoxic carcinogens, needs to be considered. Often, measurements reflecting such precursor effects are being developed as biomarkers. High-volume profiling techniques (e.g. metabonomics) are now being utilized in the search for novel biomarkers (USNRC, 2004).

When biomarkers have been used in toxicity studies, consideration should be given to their interpretation. The relevance of a biomarker to toxicological effects needs to be assessed critically. Biomarkers are of particular value in studies of mechanism and mode of action—for example, on the interspecies relevance of a mode of action. Biomarkers need to be adequately characterized and assessed for fitness for purpose (IPCS, 2001c; Gundert-Remy et al., 2005). This is especially true for data derived from studies using "omic" techniques (e.g. transcriptomics, proteomics, metabonomics). In addition to their application in biomarker discovery and development, these technologies are particularly useful in mechanistic toxicology (Heinje et al., 2005; Gatzidou et al., 2007). However, use of such data in risk assessment provides appreciable challenges, both in bioinformatics and in biological interpretation. The changes observed do not necessarily reflect an adverse effect, but may simply be a result of homeostatic regulation or adaptation. A number of these issues were discussed at an International Programme on Chemical Safety (IPCS) workshop in 2003 (IPCS, 2003).

The methods for statistical analysis should be addressed with care. The numbers of animals used per dose group will affect the power of the study, so both type I (false positive) and type II (false negative) errors need to be considered. Paired or two-sample comparisons are often undertaken, and the statistical test should apply a correction when multiple comparisons of non-independent data are analysed. A trend analysis may be helpful for dose-dependent effects. The power of the study to identify a measurable effect needs to be considered when large numbers of end-points are compared in a small number of animal groups. If isolated significant findings are identified, such as in a single clinical chemistry parameter, particular attention should be given to biological consistency with other observations in the database.

The study design should be adequate to determine the reference point selected for hazard characterization, such as the no-observed-

adverse-effect level (NOAEL), benchmark dose (BMD) or other points of departure (see chapter 5). This includes adequacy of dose range and spacing, numbers of animals, variation within groups and nature of end-point measured.

4.1.5.6 *Role of human studies*

In general, data from humans are preferable to data from experimental animals, as they will have been obtained in the species of interest (see section 4.11). However, there are ethical and practical difficulties in obtaining such information. Administration to humans would be considered unethical if the safety of the substance is unknown and there has been no prior exposure of humans. In observational studies, there can be difficulties in obtaining adequate information on the extent of exposure.

Information from humans can arise in a number of different ways. These include:

- controlled studies in volunteers from whom informed consent has been obtained;
- studies of incidentally exposed subjects through epidemiological assessment;
- surveillance of occupationally exposed individuals;
- case-studies of subjects who have accidentally or deliberately consumed the substance (usually acutely);
- supervised trials of those substances where the level of human intake precludes the normal application of large uncertainty (safety) factors to data from animal studies (e.g. novel foods); and
- clinical trials on substances that also have potential use in human medicine.

Where the effect observed in animals is mild, acute and readily reversible, it may be possible to investigate this in healthy volunteers. Data obtained from such studies should be considered in risk assessment when the study is of a suitable design.

Surveillance-type studies, even when the data are inadequate for risk assessment, can provide a very useful reality check on the results obtained in experimental animals, often enabling a lower bound for any

effect in humans to be established (using conservative assumptions for exposure assessment). Post-marketing surveillance data can be useful in supporting tolerability in humans, but should not be used as a justification for reduced premarketing safety assessment.

When the reference point used for hazard characterization, such as the NOAEL, cannot be derived from human data, it may be possible to compare kinetic data from animals with in vivo human data obtained at low doses or to incorporate in vitro human data into a PBTK model. Such information can be invaluable for interspecies comparison and for interpreting the results of studies in experimental animals.

Human tissues or preparations may also be studied in vitro; such information can provide useful insights into the relevance of effects for humans and interspecies extrapolation.

The design of studies in humans needs to consider:

- choice of doses;
- duration of administration (usually acute);
- number of subjects;
- sex of volunteers; and
- how representative the subjects are of the potentially exposed population; important variables include age, genetics, concurrent disease/drug treatment, diet and lifestyle factors, such as alcohol use and smoking.

In using human data, the adequacy of study design in addressing all possible subgroups in the population needs to be considered. For example, toxicokinetic studies in adult male volunteers may not be representative of females or the very young. Uncertainties in the interpretation and use of data from studies in humans can be allowed for by the application of appropriate uncertainty or adjustment factors (see chapter 5).

4.2 Absorption, distribution, metabolism and excretion (including residues of toxicological concern)

4.2.1 Introduction

The relationship between the external, or administered, dose of a substance and biological responses can be divided into two aspects:

- *toxicokinetics*, which relates to the delivery of the chemical to and its removal from the site of action as the parent substance and/or any active metabolites; and
- *toxicodynamics*, which relates to the interaction between the chemical and/or any active metabolites at the site of action and the final outcome or toxicological response.

Knowledge of the biological disposition of a chemical (i.e. its ADME) is a key part of any hazard characterization and risk assessment (Lipscomb & Ohanian, 2007; Renwick, 2008). Such information can be important for two main aspects of risk characterization:

- the design of appropriate animal studies for identifying and characterizing the hazards associated with exposure to the chemical; and
- the interpretation of the resulting data in relation to the mechanism or mode of toxicity, consideration of interspecies scaling and consideration of potential human variability.

Historically, the ADME of substances were studied by following the biological fate of the radiolabelled substance (usually ^{3}H-labelled or ^{14}C-labelled) using nonspecific techniques to measure total radioactivity, combined with separation methods, such as chromatography, to identify the radiolabelled constituents in the biological sample. In recent years, basic ADME studies have been supplemented by the generation of toxicokinetic data in which the concentrations of the chemical or its circulating active metabolites are measured in plasma and body tissues and used to provide a mathematical description of the concentration–time course of internal exposure (Renwick, 2008).

The term *toxicokinetics* describes the movement of a substance around the body and therefore relates to its absorption from the site of administration, its distribution from the general circulation into, and out of, body tissues and its elimination, usually by metabolism and excretion. It is clear from this that toxicokinetics should cover both radiolabelled ADME studies and plasma concentration–time curves. Some texts maintain a largely artificial distinction between metabolism and toxicokinetics, probably related to the nature of the studies used to develop the data.

The principles of toxicokinetic studies were outlined in Environmental Health Criteria (EHC) 57 (IPCS, 1986a); such studies basically provide a biochemical, physiological and mathematical description of the fate of the chemical in the body. In EHC 70 (IPCS, 1987), such information is under the heading "The use of metabolic and pharmacokinetic studies in safety assessment", whereas in EHC 104 (IPCS, 1990), it is under "Absorption, distribution, metabolism, and excretion". The term "pharmacokinetics" is sometimes used, because many of the mathematical approaches and models were developed for studies on therapeutic drugs in humans. In consequence, toxicokinetic studies are most readily applicable to single-chemical entities, whether an additive, pesticide, veterinary drug or contaminant. Limited data may be produced for mixtures, by the use of nonspecific techniques that detect all constituents in a mixture or chemical-specific analysis of principal components. Simple studies on digestibility and caloric value may be all that is practicable for novel foods or macroingredients (see chapter 9, section 9.2).

Guidance on the design of toxicokinetic studies has been developed for pharmaceutical agents by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) for both single-dose studies (ICH, 1994a) and repeated-dose investigations (ICH, 1994b). The guidance is broadly applicable to studies on single-chemical entities in food, such as additives and residues of pesticides and veterinary drugs, except that the possible impact of the food matrix on the rate and extent of absorption is of major potential importance.

The different components of ADME are outlined below, followed by discussion on the value of such data in the design and interpretation of toxicological studies.

4.2.2 Absorption

Absorption is the process by which the substance is transferred from the site of administration into the circulation. For chemicals in food, absorption usually refers to passage across the gut wall into the circulation, although for some chemicals, uptake may be only as far as the epithelium of the gastrointestinal tract. Absorption may be as the parent compound or as metabolites formed within the lumen or the

wall of the gastrointestinal tract. Because the term *absorption* does not define the nature of the absorbed material, it can give rise to confusion; for example, a substance might be completely absorbed from the gut, but with none of the parent compound detectable in the blood or tissues. To allow for this possibility, the pharmacokinetic term *bioavailability* is used to describe the fraction or percentage of the administered dose that enters the general circulation as the parent compound (Duffus & Worth, 2006). The term bioavailability is one of the most misused toxicokinetic terms (see Duffus & Worth [2006] for alternative and less specific definitions).

The main routes by which humans are exposed to chemicals are via ingestion in food or drinking-water, inhalation and across skin, with the last two being of relevance to occupational exposure to pesticides. These data may be useful for route-to-route extrapolation (see section 4.2.9).

The most important process involved in the transfer of foreign chemicals from the site of administration into the general circulation is passive diffusion down a concentration gradient. For each of the main routes of administration, the substance has to cross cell membranes before it enters the general circulation. In consequence, low molecular weight, lipid-soluble molecules are absorbed more rapidly and to a greater extent than highly water-soluble or larger molecules. Highly lipid-soluble substances, such as paraffin waxes, β-carotene and polyhalogenated dibenzodioxins, show incomplete absorption from the gut because they do not form a molecular solution in the gut lumen. Diffusion across the gastrointestinal wall is usually rapid for lipid-soluble molecules, because of the large surface area of the small intestine, but there may be a delay because of physiological processes such as gastric emptying. Diffusion of volatile substances across the airways may be extremely rapid, especially if the substance is delivered to the finer airways and alveoli. Absorption across the dermis is usually extremely slow and limited to lipid-soluble molecules only.

Although active transport processes are important in the absorption of nutrients from the gastrointestinal tract, they are highly specific to the normal nutrient substrate of the carrier protein; very few foreign chemicals are substrates for any of the physiological transporters in the gastrointestinal tract. An exception to this generalization is the

efflux transporter known as P-glycoprotein, which transports a wide range of low molecular weight organic foreign molecules from the cytosol of enterocytes into the gut lumen. This efflux transporter may limit the absorption of some foreign compounds and can be a source of non-linear kinetics at high dietary concentrations (see below).

Information on absorption may relate to the rate at which the chemical is transferred into the general circulation or to the extent to which the administered dose enters the circulation or is excreted in urine, either as the administered substance or as its metabolites:

- The *rate of absorption* can be determined by serial measurements of the concentrations of the substance, or its metabolites, in plasma or their excretion in urine, as part of a toxicokinetic study. The absorption rate constant can be determined from the increase in plasma concentrations following the administration of a single dose by the appropriate route. The rate of absorption from the gastrointestinal tract and lungs is usually rapid and first order (i.e. the rate of absorption is proportional to the concentration available for absorption). The absorption rate is most likely to be important in relation to acute toxic effects and the establishment of short-term guidance values such as the ARfD. The rate of absorption across the skin tends to be slow and may result in low, but relatively constant, plasma concentrations.

- The *extent of absorption* is important for both acute and chronic toxicity. The extent of absorption may be estimated in two ways. The extent of total absorption following the administration of a radioactive dose can be estimated from the urinary excretion of the radiolabel after oral and intravenous administration. (The use of an intravenous dose allows correction for any compound in the general circulation that may be eliminated by other routes, such as biliary excretion or exhalation. Such information can also be obtained by bile duct cannulation and trapping of expired air.) Such data usually relate to the combined excretion of the administered substance and its metabolites in urine and would not indicate the extent of any metabolism that may occur prior to the substance reaching the general circulation (i.e. first-pass or presystemic metabolism). The extent of absorption as the parent compound (i.e. bioavailability) may also be determined from chemical-specific measurements of

the compound in either the general circulation or urine following both oral and intravenous administration. (The use of an intravenous dose is essential, as it provides reference data corresponding to 100% "absorption" into the general circulation.)

The term bioavailability has a strict meaning and definition in pharmacokinetic terms, and its nonspecific use in other contexts can lead to confusion and misunderstanding. For food additives, contaminants and pesticide residues, the term is used in the toxicokinetic sense given above. For veterinary drug residues in food, it is used to reflect the fraction that can be released from the food matrix and is available for absorption, but this is only one of the factors that can determine the true bioavailability of the residue to the general circulation. Confusion can also arise when the calculated bioavailability is compared with the results from studies measuring the urinary excretion of radioactivity following an oral dose; for example, 100% of a radioactive dose may be eliminated in the urine, but the bioavailability would be only 10% if the substance undergoes 90% first-pass metabolism in the gut or liver prior to entering the general circulation.

The extent of absorption is of particular importance when the substance undergoes extensive first-pass metabolism or is only poorly absorbed from the gastrointestinal tract or site of administration, such that the bioavailability and the extent of absorption, as the parent compound plus metabolites, are low. Under such circumstances, the absorption process may be the source of wide differences between species or between different human individuals, adding greater uncertainty to the hazard characterization process. The bioavailability of a chemical can be affected considerably by the experimental conditions (e.g. diet versus gavage) and the vehicle used for gavage doses. Saturation of presystemic metabolism in the gut or liver at high oral doses results in a non-linear relationship between internal concentrations of the parent compound and the external dose.

4.2.3 Distribution

Distribution is the process by which the substance or its metabolites present in the general circulation move around the body and partition into and out of different body tissues.

Transfer from the general circulation into tissues is primarily by passive diffusion of the chemical down a concentration gradient. In consequence, tissue levels increase as the plasma concentrations rise during the absorption of the substance, and tissue concentrations fall when the plasma concentration decreases during the elimination of the substance from the body. Transfer from the general circulation into tissue cells requires that the substance cross the cell membrane, and again this occurs more rapidly for lipid-soluble molecules than for highly polar or larger molecules.

The entry of molecules into some organs, especially the brain, is largely limited to lipid-soluble molecules, because there are tight junctions between adjacent endothelial cells that prevent water-soluble molecules from leaving the lumen of the blood vessels. The small size of membrane pores in the endothelial cell membrane and the presence of active transporters, including P-glycoprotein, also contribute to the so-called "blood–brain barrier". Active transporters in endothelial cells supplying the brain are important in the delivery of essential nutrients, such as glucose and amino acids, but, again, they are not available to the vast majority of non-nutrient chemicals.

The vasculature of certain organs, such as the liver, kidneys and brain, contains transporters that can either actively take up the chemical from the circulation or transport chemicals from the tissues back into blood. Tissue efflux transporters, such as P-glycoprotein and multidrug resistance associated protein (MRP), have low specificity and can be induced by chronic exposure to some substrates, which can affect tissue distribution on repeated administration. Membrane transporters can show species differences, sex differences and genetic polymorphisms. The toxicity of the pesticide abamectin shows wide differences between strains of mice, which can be related to the lower activity of P-glycoprotein in the gut wall and blood–brain barrier in the more sensitive strains (FAO/WHO, 1998).

As for absorption, distribution may be thought of in terms of the rate of the process and its extent—i.e. what proportion of the body burden of the substance moves out of the general circulation into body tissues:

- The *rate of distribution* is largely dependent on the rate of perfusion of those organs that show the highest affinity for the substance.

For example, if the substance is very lipid soluble, there will be a much higher concentration in adipose tissue than in the plasma, and therefore the rate at which the substance can enter adipose tissue is limited by the low perfusion rate of this tissue. The rate of distribution is usually determined by toxicokinetic measurements following an intravenous bolus dose.

- The *extent of distribution* is determined by the relative affinity of the circulation and of the organs of the body. Substances may dissolve in lipoproteins or cell membranes present in the general circulation, as well as intracellular and extracellular membranes within the tissues. In addition, many substances show reversible binding to plasma and tissue proteins. In consequence, the ratio of the concentration of the substance in the tissue to that in the plasma depends on the overall affinity of the tissue compared with plasma and may be extremely high in some organ systems; for example, lipid-soluble substances may show very high adipose tissue to plasma ratios.

The extent of distribution may be measured both using nonspecific radiochemical methods and from chemical-specific analyses. The former will provide information on the pattern of distribution of the parent compound plus its metabolites, but may also represent material that is covalently bound to tissue proteins, ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) (which is really an elimination process in relation to the parent compound). Consideration needs to be given to the position and chemical stability of the radioisotope within the molecule, as misleading data on tissue distribution could be obtained if the label were labile and entered general intermediary metabolism—for example, as tritiated water or a ^{14}C-labelled methyl residue. Chemical-specific analysis of the concentrations of parent compound in plasma and tissues can be used to indicate the pattern of distribution. Data from the plasma concentration–time curve following a single intravenous bolus dose can be analysed to determine the apparent volume of distribution, which reflects the ratio between the total body burden and the plasma concentration; this parameter can also be calculated from studies in humans. For highly lipid-soluble substances, such as polyhalogenated dibenzodioxins, the relationship between the total body burden and the concentrations present in adipose and other tissues depends on body composition and the

percentage of body fat, which can vary between species and also between individuals (USNRC, 2006).

4.2.4 Metabolism

Metabolism (biotransformation) is the process by which the administered substance is changed structurally into molecules that are eliminated from the body.

Although metabolism is often thought of as representing a detoxification process, in many cases target organ toxicity can arise from the actions of a metabolite rather than those of the parent compound. In some cases, the metabolite may be so unstable that it interacts covalently with tissue proteins, RNA or DNA to produce cellular changes that are part of the mode of action of the toxic effect. In such cases, metabolism of the substance becomes an important part of the mode of action and may be a major source of species differences and human variability in sensitivity to the chemical.

It is important that toxicokinetic measurements used for hazard characterization relate to the active chemical entity in the circulation or tissue. Depending on the biological activity of the parent compound and its metabolites, toxicokinetic measurements based on the parent compound may not provide an adequate basis for consideration of species differences or human variability.

PBTK models (see below) can incorporate data on enzyme kinetics as part of the overall elimination process (Krishnan & Andersen, 2007). Some PBTK models also include local target organ metabolism, thereby providing a particularly powerful method for predicting the target organ dose of the active chemical entity in the experimental animals and predicting equivalent target organ doses in humans.

Although some food additives are metabolized by the enzymes of normal intermediary metabolism, the majority of additives, pesticides and veterinary drugs are low molecular weight, “foreign” organic molecules, and these are metabolized by a variety of phase I and phase II “drug-metabolizing” enzymes that are present largely in the liver. Phase I metabolism involves the oxidation, reduction or hydrolysis of the molecule with the introduction of groups suitable for subsequent

phase II or conjugation reactions. Phase II reactions involve the conjugation of the foreign compound, or its phase I metabolite, with a molecule such as glucuronic acid or sulfate; this serves to mask potential active functional groups and generally leads to an increase in water solubility (Kemper et al., 2007).

Both phase I and phase II metabolic reactions usually lead to a decrease in toxicity and the generation of excretable products; however, they may also lead to the generation of reactive chemical species that are important in the toxicity of the molecule. In consequence, studies of metabolism should aim to define the processes involved in the elimination of the parent compound and any toxicity associated with that molecule, as well as the generation of any active chemical products of the substance and their subsequent detoxification and elimination from the body.

Consideration should be given to factors that might affect metabolism during the conduct of toxicity tests. These include strain and species differences, sex differences, route dependency, dose dependency (e.g. saturation, competing pathways with different kinetic parameters), time dependency (e.g. induction, inhibition) and concurrent pathology. The extent to which such differences can be extrapolated to humans should be evaluated; for example, many sex differences in metabolism observed in rats do not occur in humans. The enzymes involved in the metabolism of foreign compounds represent the most important source of interspecies differences and human variability in the biodisposition of the compound and, for many cases, in the generation of toxic effects.

At low substrate concentrations, the rate of metabolism is proportional to the substrate concentration, which means that toxicokinetic parameters, such as clearance and half-life (see below), are constant and independent of dose level. However, the amounts of metabolizing enzymes in the body are limited, and saturation of metabolism can occur at high dose levels; saturation of metabolism results in slower elimination at higher doses and a disproportionately increased body burden with increase in dose level during repeated dosing. Saturation of metabolism is not always a feature of toxicity studies, because adverse effects are often found at doses that do not saturate metabolism; however, saturation that occurs over the dose range used

for toxicity studies complicates analysis of the dose–response data and their extrapolation to humans.

Metabolism is only one possible route of elimination from the body, and the measured rate of elimination from the body—for example, the plasma half-life—is the sum of all elimination processes.

4.2.5 Excretion

Excretion describes the processes involved in the elimination of the substance or its metabolites from the general circulation into a biological waste product, such as urine, faeces or exhaled air.

The urine is the major route of elimination of low molecular weight foreign compounds from the body. However, it is efficient only for low molecular weight, highly water-soluble molecules, because lipid-soluble molecules will be reabsorbed from the renal tubule and re-enter the general circulation. It is for this reason that low molecular weight, lipid-soluble molecules tend to be retained in the body and undergo metabolism prior to their excretion. The rate of renal excretion of a compound may be very high if it is a substrate for the various anionic or cationic carriers that transport molecules from the general circulation into the lumen of the renal tubule, but may be very slow for compounds that are highly bound to plasma proteins. There are a number of different transporters for organic anions (organic anion transporters, or OAT, transporters for acids), organic cations (organic cation transporters, or OCT, transporters for bases), peptide transporters and nonspecific transporters (members of the MRP family). These may occur on either the basolateral or apical membranes of the renal tubule or both, are important in extracting chemicals from blood and transferring them into the tubule lumen, and show species and sex differences (Lee & Kim, 2004). In addition, compounds filtered at the glomerulus may undergo pH-dependent passive reabsorption from the renal tubule back into the general circulation.

Another important route of elimination is via the bile, where the molecule is incorporated into the micellar constituents of bile and passes into the lumen of the gastrointestinal tract. Biliary excretion can also involve a number of efflux transporters, such as P-glycoprotein and MRP. Although the excretion effectively removes the compound from

the general circulation, it is possible that the metabolites eliminated in bile may be further metabolized within the lumen of the gastrointestinal tract and reabsorbed. For example, the glucuronic acid conjugate of a compound may be formed in the liver, eliminated in bile and hydrolysed back to the original compound in the gut lumen; the compound is then absorbed from the lower bowel to re-enter the general circulation. Such a process is known as enterohepatic circulation.

Compounds eliminated in the exhaled air are usually of low molecular weight and volatile or are fragments of larger administered substances that possess these characteristics.

4.2.6 Overall elimination from the body

The overall rate of elimination of a chemical from the body, which can be measured from the decrease in plasma concentration with time, reflects the sum of all the processes contributing to the elimination of that chemical—i.e. metabolism plus renal excretion plus biliary excretion plus exhalation plus any other minor routes of elimination.

Because physiological and metabolic processes are first order with respect to substrate at low concentrations, decreases in plasma concentrations with time are usually exponential in nature and can be defined by measurement of the appropriate elimination rate constant or its associated half-life. The rate of elimination and half-life are important parameters, as they indicate the duration of exposure of the body and its tissues to the substance, and they also indicate the potential for accumulation on repeated dosing.

Again, it is important to recognize the difference that may be obtained from measurements based on total radioactivity (parent compound plus metabolites) and chemical-specific assays that will measure separately the parent compound and characterized metabolites. A major advantage of nonspecific methods such as the use of radioisotopically labelled substrates is the ability to measure all metabolic products, including those that have not been characterized. However, such information could be misleading if the measured half-life reflected that of an inactive and non-toxic metabolite and therefore was not related to the body burden or the accumulation of the toxic moiety. The same criticism would apply if a chemical-specific method

were applied to an inactive moiety. In cases where the active chemical entity is produced within the target organ and does not enter the circulation, the plasma toxicokinetics should relate to the circulating precursor molecule (usually the parent compound).

4.2.7 The role of toxicokinetic studies in the design of animal toxicity tests

ADME and toxicokinetic studies are important in selection of the appropriate test species and the dosing regimen. There are major species differences in the routes and rates of elimination of test substances in different animal species compared with humans. Quantitative differences between the species used in toxicity studies and humans are an almost inevitable part of hazard characterization.

Although it is frequently suggested that the animal species used in toxicity studies should be as metabolically similar to humans as possible, in reality only a few species are used in toxicity tests. This is because of the need for background knowledge of the animal's histopathology and physiology combined with practical aspects, such as size, housing conditions and longevity. In consequence, despite known differences compared with humans in the rates and extents of metabolism and excretion, most studies are performed in a relatively small number of test species. Under these circumstances, knowledge of the qualitative and quantitative nature of any differences between the test species and humans can be a very important part of hazard characterization.

Although the primary aims of dose selection are to identify hazards and to define their dose–response characteristics, toxicokinetic information can help to inform this process. The biological processes outlined above are essentially first order at low concentrations, but the exaggerated dosages used in animal toxicity studies for the identification and characterization of hazards may lead to saturation of transporters or metabolic enzyme systems, such that the relationship between dose and target organ exposure to the parent compound or its metabolites is not a simple linear relationship. Saturation of metabolism may lead to lower than predicted concentrations of the metabolites formed by the metabolic pathway that is saturated, but higher than predicted concentrations of the parent compound and other

metabolites. The toxicological consequences of this may be a non-linear dose–response relationship with exaggerated toxicity at high, saturating doses, if the parent compound is the active toxicant, but reduced toxicity at high doses, if the product of the saturated enzyme is the primary toxicant. Specifically designed toxicokinetic studies can provide the key to interpreting dose–response relationships derived from toxicity studies.

4.2.8 The role of toxicokinetic studies in the interpretation of data from animal toxicity studies

Toxicokinetic studies are designed to produce information on the profile of exposure to the active chemical entity at the site of toxicity under the conditions that produce the toxicity and that are the basis for determining the NOAEL and hazard characterization. Important toxicokinetic data relate to:

- the internal dose in animals based on plasma, serum or blood concentrations of the parent compound or its active metabolites; the most commonly made measurements are the area under the concentration–time curve (AUC), the observed peak concentration (C_{max}) and the time of the peak concentration (T_{max});
- the relationship between the external dose given to animals and the internal dose (as indicated by the AUC for plasma or tissue);
- the relationship between the plasma or blood concentrations (AUC or C_{max}) and those at the site of toxicity; and
- information on appropriate plasma or blood concentrations after the administration of tracer doses to human volunteers in order to allow extrapolation of animal data to humans.

Data on the AUC and C_{max} of the parent compound in blood or plasma derived from specifically designed, single-dose toxicokinetic studies (ICH, 1994a) can be used to calculate related toxicokinetic parameters that describe the basic handling of the substance in the body. These parameters can then be used to predict the fate of the substance on repeated dosage and assist in interspecies extrapolation (Renwick, 2008). Important toxicokinetic parameters are:

- *Clearance (CL)*: the volume of blood or plasma cleared of the substance per unit time; units are volume per unit time (e.g. ml/min or

ml/min per kilogram body weight); value is dependent on the in vivo functional capacity of the organs of elimination, which may be limited by organ blood flow or tissue activity; calculated as [AUC/ intravenous dose].

- *Apparent volume of distribution (*V*)*: the volume of blood or plasma in which the body burden appears to be dissolved; units are volume (e.g. ml or ml/kg or l/kg); value is dependent on the extent of distribution from the general circulation into tissues, which is affected by protein binding, the lipid solubility of the compound and body composition; calculated as [intravenous bolus dose/C_{max}], but other more robust methods are normally used in practice (Renwick, 2008).

- *Elimination half-life (*$t_{½}$*)*: the time taken for the post-peak blood or plasma concentration to halve; units are time (e.g. min or h); value is dependent on CL and *V*, which are independent physiologically related variables; calculated from regression analysis of the concentration–time course data or as [0.693*V*/CL].

- *Bioavailability (*F*)*: the fraction (or percentage) of the administered dose that reaches the general circulation as the parent compound; a unitless fraction; for oral doses, the value is dependent on the extent of transfer from the gut lumen and any presystemic metabolism in the gut lumen, gut wall and liver; calculated as [$AUC_{oral} \times dose_{iv} / AUC_{iv} \times dose_{oral}$] or [$AUC_{oral}/AUC_{iv}$] when the same dose levels are given by each route (oral and intravenous, or iv).

Each of the above parameters is independent of concentration at doses that do not saturate the enzyme systems or transporters involved in the biological fate of the compound. Non-linear kinetics may also arise from physicochemical non-linearity, such as the saturation of solubilization at the site of administration. Dependent on the nature of the plasma or blood concentration–time curve, a compartmental model containing one, two or more exponential terms may be fitted to the data.

Quantification of systemic exposure or body burden in the test species during the performance of toxicity studies provides important information that can assist in the interpretation of similarities and differences in toxicity across species, dose groups and sexes (ICH, 1994a). Suitable data may sometimes be obtained from all animals on

a toxicity study or from representative subgroups, but because of the invasive nature of toxicokinetic methods, data are usually obtained from specially established satellite groups or from separate studies.

ADME studies based on the elimination of radioactive compound and metabolite after a single oral dose may be useful in defining the extent of species differences and of saturation of metabolic pathways in the biodisposition of the compound in the test species. When comparable data are available from studies in humans, these can be used to define the adequacy of the test species as a model for humans, providing that the biological consequences of metabolism (i.e. detoxification or bioactivation) have been characterized. In some cases, data are available for small numbers of human subjects given a single oral dose of the radiolabelled substance, and such information can be very informative.

Of greater potential value are data relating to the circulating concentrations of the parent compound and any active metabolites in the test species under the experimental conditions giving rise to the hazard that will be the basis for hazard and risk characterization. Suitable toxicokinetic data from studies in experimental animals and humans can reduce the uncertainties associated with interspecies extrapolation and also give insights into the potential human interindividual variability.

When the toxicity database on a substance is to be used to estimate a health-based guidance value, such as an acceptable daily intake (ADI), the most relevant toxicokinetic data are for the test species under the experimental conditions giving the NOAEL for the critical effect and matching information for humans at the projected ADI or health-based guidance value. Although there are ethical considerations with respect to the intentional administration of non-therapeutic agents to humans, it is difficult to envisage objections to intentional exposures to doses of food additives or pesticides that would represent the ADI for unintentional exposure in the absence of any such study in humans.

In vitro data can provide extremely important information relating to the enzymes involved in the metabolic detoxification or activation of the substance. Definition of the enzyme kinetics of the major pathways in organs taken from the test animal species and from humans can be particularly valuable in defining species differences and in the

development of PBTK models that characterize species differences. Unlike the basic toxicokinetic parameters given above, PBTK models can provide data on the concentrations in potential target organs and describe how they change with time and with repeated dosage. In some cases, such models can be extended to include local tissue bioactivation and detoxification processes within the target organ for toxicity and therefore provide insights that are not possible from in vivo pharmacokinetic measurements. In principle, PBTK models could be used to predict human variability in target organ doses, providing there were sufficient data on human variability in the key parts of the PBTK model, such as organ blood flows and enzyme kinetics.

In addition to the development of PBTK models, in vitro studies using livers with characterized expression patterns for different isoenzymes can be useful in identifying the isoenzymes responsible for different metabolic processes; similar information can also be obtained from in vitro enzyme expression systems. Such information may be particularly valuable in predicting the likely human variability in metabolism of the substance.

A major uncertainty associated with most forms of hazard characterization arises from the relatively limited number of data available from studies in humans and the inadequacy of such data to define the extent of human variability in biodisposition. Information on human variability is rarely available from studies using radioactive substrates; more extensive information may be available in some cases where chemical-specific assays have been used to describe the toxicokinetics following administration of low doses of the unlabelled substance.

Knowledge and understanding of the major pathways involved in the detoxification and any bioactivation of the substance can be used to predict likely human variability in the biodisposition of the substance based on known human variability for substrates that are metabolized by the relevant pathways. For example, a substrate metabolized extensively by an enzyme exhibiting genetic polymorphism would show considerably more interindividual variability within the human population than would a substrate eliminated primarily unchanged via renal excretion. Such potential human variability in toxicokinetics needs to be considered as part of hazard characterization and assessment of the adequacy of the default uncertainty factors.

Parameters, such as bioavailability, clearance and half-life, derived from a single-dose toxicokinetic study can be used to predict the concentrations in plasma or blood following chronic administration, providing that repeated dosage does not alter the biovailability, clearance or distribution. The body burden during chronic administration is called the "steady-state body burden". The term "steady state" relates to the condition during repeated dosing in which the daily dose of a substance is eliminated from the body within 24 h (i.e. there is no overall change in the average body burden of the substance). However, this term should not be confused with a constant unvarying plasma concentration and body burden. For substances that are rapidly absorbed and eliminated from the body, there will be significant peak and trough concentrations between each dose. Peaks and troughs are most apparent when a substance with a short half-life is given as a single daily bolus gavage dose; in contrast, when such a substance is incorporated into the diet, the plasma and tissue concentrations of the substance will reflect the diurnal pattern of food intake. For substances with long half-lives, such as the dioxins and other chlorinated hydrocarbons, there will be significant accumulation during repeated dosage. The daily pattern of dose input will represent a small fraction of the total body burden or plasma concentrations at steady state, and there will be little diurnal variation, so that the "steady-state" condition will actually be represented by relatively constant plasma and tissue levels.

Problems of accumulation on repeated dosing and saturation of elimination are particularly pertinent to high-dose animal toxicity studies, and information on these areas can be obtained readily from suitably designed in vivo toxicokinetic studies.

During repeated dosing, the average or steady-state plasma concentration is determined by the rate of dose administration and the systemic clearance and bioavailability of the substance, parameters that are readily determined from a single oral dose. Therefore, single-dose toxicokinetic studies can be used to predict the average steady-state plasma concentration and body burden. Similarly, single-dose tissue distribution data can be used to predict steady-state tissue concentrations based on the plasma concentration at steady state and the single-dose tissue to plasma ratios.

Inherent in the use of single-dose data for predictions about steady-state conditions is the assumption that repeated dosing does not alter

either the bioavailability or the clearance of a substance. Although this is a reasonable assumption in the majority of cases, the bioavailability and clearance can be altered by prior treatment for substances that are either inducers or inhibitors of their own metabolism. Under these circumstances, the single-dose data would either overpredict or underpredict, respectively, the steady-state plasma and tissue concentrations of the parent compound. In addition, substances that produce adverse effects on the liver or kidneys may affect the elimination of the substance itself during repeated administration at doses that give rise to such toxic effects. Comparison of the plasma toxicokinetics of a substance following a single oral dose given as gavage with the concentration–time profile for a dose interval at steady state (e.g. over a 24 h period) can give useful insights in relation to the possible influence of repeated dosage on both the absorption and elimination of the substance.

Single-dose toxicokinetic studies in experimental animals can be important for route-to-route extrapolation (see section 4.2.9). Data following treatment with gavage doses, incorporation of the compound into the diet and other routes of administration that are relevant to the hazard characterization can be used in the interpretation of hazard characterization data that were generated using routes or vehicles that are not of direct relevance to human exposure.

It is important that the life stage investigated in toxicokinetic studies is the same as that which becomes the focus for hazard and risk characterization. Absorption and elimination processes vary during the life of both experimental animals and humans; they are immature in the neonatal period, but then increase rapidly to adult levels, followed by a slow decline as the organism ages. In consequence, an apparently constant dosage regimen expressed in milligrams per kilogram body weight may be associated with elevated plasma and tissue concentrations during the later phases of a chronic bioassay. At the period when toxicokinetic processes are most immature (i.e. the neonate), the principal route of exposure is via maternal milk, and this may be of particular significance for neonatal exposure to lipid-soluble substances. Transfer of chemicals into milk may be an important measurement component of the exposure profile of animals during reproductive toxicity and two-generation carcinogenicity studies.

Both health-based guidance values and the starting points for their determination, such as the NOAEL (see chapter 5), are expressed on

a body weight basis (e.g. mg/kg body weight per day), with an uncertainty factor used to allow for possible species differences and human variability in both toxicokinetics and toxicodynamics. The clearance of foreign compounds is usually greater in rodent species than in humans on a body weight basis, and this difference in toxicokinetics is an important reason for the application of an interspecies uncertainty factor. Many physiological and metabolic characteristics relate more closely to body surface area or body weight$^{0.7}$ (Rodricks et al., 2007). The use of surface area for interspecies scaling to convert the NOAEL into an ADI would reduce the need for an interspecies uncertainty factor. Such an approach would be most valid for compounds that are metabolized by normal intermediary metabolism, but would be less valid for compounds eliminated by phase I and phase II foreign compound metabolizing enzymes, because these show wide species differences that do not scale closely with body surface area. In contrast, the use of body weight$^{1.0}$ is more conservative than the use of body weight$^{0.7}$ when considering the kinetics in children compared with adults, because children show greater elimination capacity on a simple body weight basis, and therefore their internal dose would be lower than in an adult given the same external dose expressed as milligrams per kilogram body weight.

4.2.9 Route-to-route extrapolation

The target site dose is the ultimate determinant of risk. Substances that do not establish an internal dose by a given route would not be presumed to produce internal toxicity by that route. Conversely, substances that cause internal toxicity by one route of exposure would be assumed to do so by any other route that also produces a comparable internal dose of the active chemical entity at the target tissue. The differences in biological processes between different routes of exposure (oral, inhalation, dermal, intravenous) can be great. In oral studies, even the mode of administration (gavage versus diet versus drinking-water) may be an issue for extrapolation within the same route.

If the route for the kinetic studies in either animals or humans varies from that on which the critical effect level is based, then route-to-route extrapolation may be necessary, and the data will need to be assessed critically on a case-by-case basis (Pepelko, 1987), including for use for the development of a CSAF. Toxicokinetics in general, and PBTK modelling in particular, are useful for quantifying route-to-route

extrapolations, including using a combination of existing data and modelling approaches.

4.3 General systemic toxicity

4.3.1 Introduction

Tests of general systemic toxicity are conducted to identify target organs for toxicity and to confirm or mitigate the need for additional or more specific testing. Principles that are common to tests for general systemic toxicity, utilizing repeated-dose protocols, are described in this section. To a large extent, the designs of toxicity studies have been standardized, and common parameters are evaluated at different time points in studies of different durations. Standardized toxicity testing guidelines have been produced by the OECD (see http://masetto.sourceoecd.org/vl=2781582/cl=14/nw=1/rpsv/cw/vhosts/oecdjournals/1607310x/v1n4/contp1-1.htm) for:

- Repeated Dose 28-Day Oral Toxicity Study in Rodents (Test Guideline No. 407; OECD, 1995a) (updated for endocrine effects, adopted in 2008; OECD, 2008);
- Repeated Dose 90-Day Oral Toxicity Study in Rodents (Test Guideline No. 408; OECD, 1998a);
- Repeated Dose 90-Day Oral Toxicity Study in Non-Rodents (Test Guideline No. 409; OECD, 1998b);
- Chronic Toxicity Studies (Test Guideline No. 452; OECD, 1981b); and
- Combined Chronic Toxicity/Carcinogenicity Studies (Test Guideline No. 453; OECD, 1981c).

Additional information is available in United States Environmental Protection Agency (USEPA) test guidelines (USEPA, 1998d,e,f, 2000; see also http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/index.html), in the United States Food and Drug Administration (USFDA) Redbook 2000 (USFDA, 2000) and in Jacobson-Kram & Keller (2006).

Tests of general systemic toxicity assess the effects of a test substance on a wide range of end-points indicative of toxicity, including observational, functional, biochemical and pathological end-points.

The goal of such tests is to determine which organs are affected by the test substance and how they are affected. Testing is done in a manner that best relates to human exposure scenarios; for substances present in foods, administration of the substance in repeated-dose animal studies is usually via the diet, by gavage or via drinking-water.

Reproductive or developmental toxicity, neurotoxicity and immunotoxicity are not assessed adequately in tests of general systemic toxicity. There is more information on tests for these forms of toxicity in sections 4.7, 4.8 and 4.9 (reproductive and developmental toxicity, neurotoxicity and immunotoxicity, respectively).

4.3.2 Tests for general systemic toxicity

Tests for general systemic toxicity are multidose studies of various durations. Ideally, the dose levels are selected such that toxic effects, but not death or severe suffering, are produced at the highest dose level, with lower dose levels producing graded responses and no adverse effects observed at the lowest dose level (NOAEL). Dose selection may be based on prior knowledge, but often a range-finding study may be necessary to define the doses to be used in the toxicity studies. Data from studies of shorter duration are normally used in the selection of dose levels for long-term or chronic studies. All studies should include a control group of animals; the handling of controls should be identical to that of the treated animals, including the administration of the dosing vehicle if relevant.

Whereas conventional acute toxicity studies (section 4.4) are conducted to determine a single maximally tolerated or lethal dose, tests for general systemic toxicity are conducted using repeated dosing over various periods of time, from days to years. In general, studies are conducted for 14–28 days, 13 weeks, 52 weeks or longer. Two-year carcinogenicity studies in rats are often combined with a 1-year study of toxicity by including satellite groups for toxicological evaluations. The terms subacute (14–28 days), subchronic (13 weeks) and chronic (52 weeks) are used to describe tests of general systemic toxicity, but these designations are not precisely defined; tests of shorter or longer duration (e.g. 7 days, 26 weeks or 2 years) are also common. The terms used are less important than understanding that the objective is to test for a defined proportion of an animal's lifespan.

4.3.3 Testing strategies

Studies of variable duration are typically conducted in sequence, with shorter-duration studies conducted before studies of longer duration. In this way, information gained early on in testing can be used to determine appropriate methods and doses or to otherwise optimize study designs for subsequent tests of longer duration or to evaluate specific end-points (e.g. immunotoxicity or neurotoxicity studies).

The type and amount of data needed to evaluate various substances should be determined on a case-by-case basis, so testing strategies will vary from substance to substance. Knowledge of the anticipated human exposure to and chemical structure of the substance will help in the design of an appropriate testing strategy.

4.3.4 Study design and data interpretation

4.3.4.1 Good Laboratory Practice

Non-clinical laboratory studies should be conducted according to the principles of GLP (see http://www.oecd.org/document/63/0,3343,en_2649_34381_2346175_1_1_1_1,00.html) and related national regulations, or similar guidelines. These cover the care, maintenance and housing of experimental animals as well as other general study considerations, such as resources, protocols and written procedures, characterization of test items and test systems, documentation and quality assurance. The use of GLP helps to ensure that studies are conducted appropriately and that the results can be used with confidence for risk assessment purposes. Studies not conducted to GLP or similar standards can provide valuable data (e.g. related to mode of action) and should not be ruled out for consideration when setting health-based guidance values.

4.3.4.2 Test substance

The test substance should be thoroughly characterized with respect to chemical identity, purity, stability and other properties, such as pH or solubility. For commercial substances, such as additives, pesticides and veterinary drugs, the substance tested should be the (intended) article of commerce. If the article of commerce is not the test substance, its relationship to the test substance must be accurately described.

The effect of a vehicle or other formulation aids on the test substance should also be considered; for example, they may affect the rate or extent of absorption from the gastrointestinal tract. Use of a single lot of test substance throughout a study will help to minimize inconsistent results due to differences in composition or levels of contaminants between batches, but relevant stability data on the test substance are then necessary to ensure consistency of the material dosed throughout the study.

4.3.4.3 *Species, number and sex*

General systemic toxicity studies are typically conducted in two species, a rodent and a non-rodent species or two rodent species, to maximize the opportunity to find an effect (hazard identification). The animals most often tested are rats and dogs, but other species may be used. Pigs, for example, may be the animal of choice for testing a fatty substance, because the metabolism of fat in pigs most closely approximates fat metabolism in humans. When other species are used, existing protocols may need to be modified to account for the unique characteristics of the selected test species. It is essential that all protocol modifications are reported so that the results can be properly interpreted.

Both sexes should be tested. Equal numbers of males and females of each species and strain should be tested to allow for an evaluation of potential hormonal influences, differences in metabolism or other sex differences. The animal's sensitivity in relation to the nature of the toxicity of the test substance needs to be considered in both designing and interpreting a study.

Longevity has become an issue for some strains of rats, with rates of survival so low that data collection from and interpretation of long-term studies are compromised. The anticipated survival of the animals should help influence the number of animals entered into a study so that there are enough animals available at termination to provide meaningful study results. In general, more animals are tested as the duration of the study increases. For a 13-week study, a minimum of 20 rodents per sex per group or at least 4 dogs per sex per group are common recommendations. Fewer animals may be included in a range-finding study, whereas more animals may be included if interim necropsies are planned.

Animals should be randomly assigned to control and treated groups to help minimize bias and assure comparability of pertinent variables across groups. As an example, mean body weights and body weight ranges should not differ substantially across groups at the start of an experiment if group data are to be evaluated. In some situations, additional control groups are useful—for example, when dietary imbalances are suspected (e.g. the highest dose causes significant caloric dilution).

4.3.4.4 *Dose selection*

The dose selection should take into account the anticipated human exposure, the frequency of exposure and the duration of exposure. Dose selection for toxicity studies should also be based on information known about the test substance and any prior results of toxicity tests. In general, responses require higher doses in studies of shorter duration than in long-term studies; in shorter studies, higher doses may be tolerated.

Three to five dose levels of the test substance and a concurrent control group are ordinarily sufficient to be able to relate toxicity to level of exposure. As a primary aim of any study is to define the quantitative relationship between exposure and effect (i.e. the dose–response; see chapter 5), more doses instead of fewer are generally desired. At a minimum, three dose levels of the test substance and a concurrent control group should be used in tests of general systemic toxicity. The dose range selected should allow for the expression of toxicity at the highest dose (e.g. 10% reduction in body weight) and no toxicity at the lowest dose tested; intermediate toxicity would be expected at intermediate doses (e.g. 5% reduction in body weight). For essentially non-toxic substances, the top dose studied may be set by an accepted limit dose, such as 5% addition to the diet. Other factors that need to be considered include the potential human exposure and the possibility of non-linear kinetics at high doses, which can complicate data interpretation and extrapolation to humans.

4.3.4.5 *Administration of the test substance*

Differences in toxicity related to route of administration are common, and therefore the route of administration of the test substance should approximate that of normal human exposure. For risk

assessment of chemicals in food, studies in which the test substance is administered orally are the most useful. However, in some instances (e.g. contaminants), most of the available data may be from routes other than the oral route; for resource and animal welfare reasons, it is important to utilize such data where possible. Toxicokinetic data can be used to correct for route-dependent differences in systemic exposure in cases where the available data were derived using a route different from that by which humans are exposed.

For food chemicals (e.g. food additives, residues of pesticides and veterinary drugs), the test substance is often added to the diet. The diet selected must meet the nutritional requirements of the test species. Control and test diets should ordinarily be isocaloric and nutritionally equivalent; the percentage of test substance in the diet and use of a vehicle are relevant issues to address in this regard. Subtle differences in the diet have the potential to result in nutritional imbalances or underfeeding or overfeeding, thereby confounding study results and their interpretation. Pair-feeding can be useful if effects on feed and nutrient intake are suspected—for example, if palatability is an issue. Caloric restriction, intentional or otherwise, can have profound effects on toxicity; for example, it reduces the background tumour burden in animals and thus has the potential to increase the ability of a study to detect a test substance–related increase in incidence. Administration by encapsulation (common in dog studies) or oral intubation (gavage) may be used if the diet does not provide satisfactory delivery; however, such bolus administration is often associated with higher peak blood levels than would occur by dietary administration of the same daily dose. Delivery in drinking-water may be appropriate for a substance used in a beverage; however, measurement of water intake may be inaccurate if, for example, the animals play with water spouts. Addition of microencapsulated test substance into the diet has proved useful for administration of volatile substances, which would otherwise be lost from the diet.

4.3.5 Observations and measurements

Standardized protocols for tests of general systemic toxicity define a range of end-points and indicators of toxicity. These include, but are not limited to, mortality, cage-side observations, haematology, blood chemistry, gross pathology, histopathology and functional assessments.

4.3.5.1 Mortality

Except for lifetime studies, mortality greater than 10% in any treatment or control group is a cause for concern. High mortality in high-dose groups may be an indication of poor dose selection. High rates of mortality increase the chances for autolysis of tissues and organs, possibly resulting in incomplete data collection. High mortality may also be indicative of infection or other problems not associated with the test substance that could compromise study results and interpretation.

4.3.5.2 Observations of test animals

Routine cage-side observations are made on all animals at least once or twice a day throughout the study to assess general signs of pharmacological or toxicological effects and to detect morbidity and mortality. Expanded sets of observations, including functional evaluations performed inside or outside of the cage, are commonly incorporated in tests of general systemic toxicity. Such observations provide a general indication of the overall state of health of the animal, and they may identify the need to conduct additional testing with either standard or modified experimental designs (e.g. ataxia or seizures indicate central nervous system toxicity and call for a comprehensive neurotoxicity assessment).

4.3.5.3 Body weight and feed intake data

Test animals and controls are weighed on a regular basis (usually weekly for 13 weeks, then monthly thereafter), and food intake is assessed during the conduct of a study. Reductions in body weight or decrements in body weight gain are sensitive indicators of toxicity; in some cases, however, diet palatability rather than toxicity may be the reason for changes in feed intake and body weight. Failure to monitor feed intake or to regularly measure body weight seriously compromises the interpretation of toxicity studies on food chemicals.

4.3.5.4 Ophthalmology

Eye examinations in all animals are typically conducted at the start and end of a study. Anatomical differences in eye structure among various species have to be factored in to the interpretation of any findings. Although ophthalmology rarely reveals changes, it was a key

investigation in the evaluation of the toxicity of the food and feed colour canthaxanthin (FAO/WHO, 1995).

4.3.5.5 Haematology

Blood is sampled in either fasting or non-fasting animals at variable time periods throughout the study, usually at the start and at the end of the study or, in a chronic study, at other time intervals in between. Measurements include haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte counts, mean corpuscular haemoglobin, mean corpuscular volume and mean corpuscular haemoglobin concentration. Clotting time, prothrombin time, thromboplastin time and platelet count are measured to assess clotting potential. Reticulocyte counts and changes in bone marrow cytology are also appropriate measures to include in assessing injury to the haematopoietic system.

The interpretation of results may be difficult as a result of turnover of cell types in the bone marrow or lymphoid tissue. Other sources of variability in the data may come from stress or nutritional factors and age of the animals, to name but a few. In addition, adaptation or tolerance may alter the responses observed over time. Because of their variability, interpretation of the toxicological significance of haematological changes requires careful consideration of consistency of effect, dose–response and comparison with historical control ranges.

4.3.5.6 Clinical chemistry

Clinical chemistry tests in general include measurements of electrolyte balance, carbohydrate metabolism, and liver and kidney function. Serum enzyme levels indicative of hepatocellular function that are typically evaluated include alanine aminotransferase (ALT, previously known as serum glutamate–pyruvate transaminase, or SGPT), aspartate aminotransferase (AST, previously known as serum glutamate–oxaloacetate transaminase, or SGOT), sorbitol dehydrogenase and glutamate dehydrogenase. Assessment of hepatobiliary function may include measurements of serum alkaline phosphatase, bilirubin (total), gamma-glutamyl transpeptidase (GGT), 5′-nucleotidase and total bile acids. Markers of cellular function or change include albumin, calcium, chloride, cholesterol (total), cholinesterase, creatinine, globulin (calculated), glucose (in fasted animals), phosphorus, potassium,

protein (total), sodium, triglycerides (fasting) and urea nitrogen. Other tests for acid/base balance, hormones, lipids, methaemoglobin or proteins may be indicated, depending on the nature of the test substance.

Changes in serum enzyme levels are commonly associated with target organ toxicity, because enzymes are released from injured cells. Thus, changes in clinical chemistry parameters may signal renal, cardiac or hepatic toxicity. They may be particularly useful for interpretation of study results where there are changes in organ weight, such as liver or kidney, but no overt histopathological changes, as alterations in clinical chemistry parameters associated with organ function can be the first indication of toxicity. A number of enzyme changes are associated with cardiotoxicity, for example, including increases in AST, lactate dehydrogenase and creatinine kinase. Changes in plasma lipids may indicate liver toxicity, whereas changes in blood glucose suggest the possibility of renal toxicity. Concentrations of electrolytes vary with food intake and hydration status, so they are not very sensitive indicators of toxicity.

Clinical chemistry data are subject to a number of sources of variability. Temperature and humidity are two environmental factors that could influence results. Attributes of the test animals, such as sex and age, and study conditions, such as time of sampling and extent of handling, may cause variability in the data recorded. Thus, as with haematological changes, interpretation of changes in clinical chemistry parameters requires careful consideration of consistency of effect, dose–response and comparison with historical control ranges.

Measurement of the test substance in blood samples can provide important information on systemic exposure. Absorption and presystemic metabolism are important factors in determining how much of the test substance reaches the systemic circulation. Toxicokinetics, which defines the movement of a substance around the body and delivery to its site of action, is addressed in section 4.2. Toxicokinetic data from short-term studies can provide useful information for the design of long-term studies, especially in relation to dose selection.

4.3.5.7 Urinalyses

Urinalyses consist of determining the volume of urine produced, specific gravity, pH, glucose and protein. In addition, microscopic

evaluation for sediment and presence of blood or blood cells is typically done. These analyses are usually conducted during the last week of the study. Analysis of urine, and faeces if indicated, may provide important information relating to changes in normal excretory functions caused by the test substance.

4.3.5.8 Necropsy

Gross necropsy, including examination of external surfaces, orifices, cranial, thoracic and abdominal cavities, carcass and all organs, is typically conducted on all animals. Necropsy should be performed soon after an animal is killed or found dead, or steps need to be taken so that interpretation of the data is not compromised by loss of tissues due to autolysis. Tissue specimens should be taken from the animals and placed in appropriate fixatives during necropsy for subsequent histopathological examination.

4.3.5.9 Organ weight

Organs that are typically weighed include the adrenals, brain, epididymides, heart, kidneys, liver, lung, spleen, testes, thyroid/parathyroid, thymus, ovaries and uterus. Data are often expressed as absolute weights and relative to the animal's body weight. Ratios of organ weight to brain weight may be more reliable indicators of organ-directed toxicity than are ratios of organ weight to body weight; this is because brain weight is rarely affected nonspecifically by toxicity, whereas body weight is more variable and may change as a result of toxicity. Organ weight changes may be indicators of possible morphological or functional changes.

4.3.5.10 Histological examination

In rodents, gross lesions and all scheduled tissues from the animals in the control group and high-dose group should be microscopically examined. When effects are observed, histological examination is extended to other dose groups until a dose level is examined at which no effects are observed. Any animals found dead or terminated early in the study must also be examined histologically. If a small number of animals are tested (e.g. in studies using dogs), histological examinations are normally performed on the controls and all treated groups.

The appropriateness of the fixation and staining techniques for various types of tissues may influence the ability to interpret study results. For example, artefacts such as vacuoles may be produced inadvertently and confused with manifestations of toxicity if fixation is done incorrectly. Ineffective visualization of tissue components and inclusions could result if routine stains (e.g. haematoxylin and eosin) are used when special stains (e.g. silver staining) are required. Properly conducted histological examination is usually the most powerful means of assessing toxicity. As with other toxicological end-points, adaptation or tolerance may alter the responses observed over time. Thus, minor changes observed in short-term studies may no longer be evident in the terminal kills in chronic studies. More commonly, changes observed in short-term studies may become more severe in chronic studies. In addition, normal age-related pathological changes may mask the toxic effects of a chemical in chronic studies.

4.3.5.11 Neurotoxicity and immunotoxicity

Tests of general systemic toxicity commonly incorporate some end-points that are useful for an initial evaluation of the neurotoxic and immunotoxic potential of the test substance. These assessments can be used to define additional testing requirements. The incorporation of additional end-points, however, should not compromise the original purpose of the study. More information on neurotoxicity and immunotoxicity can be found in sections 4.8 and 4.9, respectively.

4.3.5.12 Reversibility

Additional animals are sometimes included in short-term general systemic toxicity studies to determine if effects that might have been observed in earlier studies are reversible. Studying reversibility can assist in deciding whether a change is a physiological or adaptational effect, rather than a toxic effect. The relevance of the reversibility of a toxic effect will depend on the pattern of human exposure. For example, if exposure to a particular chemical in the diet could be more or less daily, then reversibility does not lessen the potential risk.

4.3.5.13 Other considerations

The comparison of data from treated groups with data from concurrent controls is the most important part of the analysis. However,

comparison with data from historical controls may be necessary to understand the significance of a finding. Historical control data should be from the same strain of animals, preferably from the same test facility and relatively concurrent (e.g. over 5 years centred on the study of interest).

Statistical analyses are essential for evaluating data from rodent studies. For dogs, the data collected for each animal may be evaluated individually, with each dog serving as its own control (to the extent possible). There are limitations in interpreting results of studies conducted in dogs when too few animals are entered into the studies.

Dose–response relationships should be analysed to determine if the effect is significantly related to treatment and also to provide the information necessary for risk characterization (see chapters 5 and 7). Risk characterization frequently focuses on data from long-term, general systemic toxicity studies, as these often show the greatest effects at the lowest doses.

Studies of general systemic toxicity with durations of a year or less are not adequate to determine the carcinogenic potential of a test substance. However, in rodents, it is possible to conduct combined chronic toxicity/carcinogenicity studies, which are usually 18 months (mice) or 2 years (rats) in duration. As with indicators of immunotoxic or neurotoxic potential, indications of carcinogenic potential obtained from a shorter-duration toxicity study may be a signal that appropriately designed and conducted carcinogenicity tests may be needed (see section 4.6).

Conclusions from tests of general systemic toxicity should be made taking into account everything that is known about the test substance and test conditions. Data on intermediate or precursor effects identified in short-term studies can be useful both for dose selection in long-term studies and also in assessing the possible mode of action.

4.4 Acute toxicity

4.4.1 Introduction

Acute toxicity describes the responses of an organism that are observed within a short time of exposure to, or administration of, a

chemical, either as a single exposure or dose or (less commonly) as multiple exposures or doses received over a period of 24 h or less. The nature of the toxicity ascertained normally involves severe adverse reactions or death. Formal acute toxicity tests in animals usually record such reactions for a period of 14 days after the administration of the chemical. In relation to most chemicals in food, acute toxicity tests are not generally useful for hazard identification or risk assessment, because human exposures usually are considerably lower and continue for much longer than the exposures that give rise to acute toxicity. Moreover, other types of toxicity usually occur at doses well below those that are acutely toxic, and it is these other toxicities that are normally pivotal to the risk assessment. However, in certain circumstances, such as the sporadic presence of high residues of an acutely toxic pesticide or a microbial contaminant, there is the potential for acute effects, and acute toxicity needs to be assessed.

JECFA and JMPR routinely consider the toxicity of chemicals in food and establish ADIs or tolerable daily intakes (TDIs), usually on the basis of data from repeated-dose studies, such as chronic toxicity or multigeneration studies. Some substances (e.g. certain metals, mycotoxins, marine biotoxins, veterinary drug residues, pesticide residues or low-digestible carbohydrates, such as polyol sweeteners) could give rise to acute health effects in relation to short periods of intake. JECFA has included in its evaluations an assessment of acute effects (e.g. for inorganic tin) and, where appropriate, the possibility of acute effects in sensitive individuals. JMPR has also set ARfDs for some pesticides and now routinely considers the need to set an ARfD for all pesticides it evaluates.

The appropriateness, or otherwise, of using doses and end-points from subchronic and chronic studies to establish ARfDs needs to be carefully considered. Particular weight should be given to observations and investigations at the beginning of repeated-dose studies. In the absence of information to the contrary, all toxic effects seen in repeated-dose studies should be evaluated for their relevance in establishing an ARfD.

The guidance prepared by JMPR on the setting of ARfDs is outlined in chapter 5 (section 5.2.9). It offers a stepwise approach for setting ARfDs for agricultural pesticides, but the principles are also

applicable to other chemical residues in food and drinking-water. In particular, the detailed guidance (Solecki et al., 2005) discusses some toxicological end-points that may be particularly relevant as key acute toxicity alerts. JMPR has also proposed a protocol for a single-dose study, described below.

4.4.2 Guidance for a single-dose study

Currently available data sets usually do not allow accurate evaluation of the acute toxicity of compounds. JMPR has therefore developed a protocol for a single-dose study, with the aim of enabling more accurate derivation of ARfDs. The protocol describes a targeted study suitable for substances with a well-defined toxicity profile but an inadequate database for derivation of an ARfD. Such a single-dose study should not be regarded as routinely required, but rather as a higher-tier study that is necessary only when refinement of the acute risk assessment is required. For example, if a compound has negligible residues, such that dietary intake calculations indicate an adequate margin of safety even when measured against a conservative ARfD derived from a repeated-dose study, then it should be considered unnecessary to perform a single-dose study.

A specific study designed to enable an accurate ARfD to be set should be undertaken only once the toxicological profile of an active substance is reasonably well documented and understood (i.e. at least the short-term toxicity has been evaluated in rats and dogs). The most sensitive species and relevant toxicological end-points for an active substance should be known, enabling a focused study to be designed to investigate the end-points. A flexible approach is necessary, depending on the species and the observed or expected effects with a given substance. Only the minimum number of animals necessary for a thorough safety assessment should be used, while ensuring the minimum amount of distress in the animals in the test.

The principle of the study is to administer the test substance orally as a single dose at several dose levels to groups of experimental animals. A control group is also included. The animals are followed closely for signs of toxicity, with termination of subgroups at one of two time periods (within 24 h and up to 14 days post-treatment). Dose levels and study design will be influenced by the quantitative and qualitative outcome of the repeated-dose studies and findings in

existing high-dose acute studies and will be supported by relevant data on toxicokinetics.

The aim of the single-dose study is to identify the most appropriate NOAEL or lowest-observed-adverse-effect level (LOAEL) to derive an ARfD, to provide further information on the dose–response curve, time to peak effects and reversibility for the acute toxic effects, and to provide a flexible approach for an adequate characterization of relevant acute effects. The single-dose study does not aim to identify any lethal doses or provide data on mortality or morbidity after acute exposure to a chemical. The information should be considered with a view to possible refinement of safety factors used in the derivation of the ARfD.

4.5 Genotoxicity[1]

4.5.1 Introduction

Genetic toxicology—the study of toxic effects on the inherited genetic material in cells—originated with the experiments of Müller (1927), who observed "artificial transmutation of the gene" by ionizing radiation in the fruit fly, *Drosophila melanogaster*. Chemically induced mutation also has a long history, the first scientific publication dating from 1947, when Auerbach and co-workers, using Müller's fruit fly model, described mutations arising from exposure to sulfur mustards (Auerbach et al., 1947). Deep concern over mutagenesis was first expressed in the mid-1960s with the discovery of "supermutagens", as exemplified by chemicals such as the heterocyclic nitrogen mustard ICR-170, AF-2, hycanthone and β-propiolactone, which induce high levels of mutation at high levels of survival. Several leading geneticists were concerned that supermutagens might be widely distributed (Crow, 1968) because either they had passed through traditional toxicity screens without showing adverse effects or they had never been tested at all. In spite of these concerns, the major impetus given to mutagenesis as a toxicological topic came from the belief that carcinogenic activity was predictable by examining the potential of a chemical to interact with DNA. Thus, in 1973, Ames and co-workers pronounced that "carcinogens are mutagens" (Ames et al., 1973).

[1] The text in section 4.5 has been published in similar form in McGregor (2006), because the author developed the two documents in parallel.

Although this may have been the spark that became a blaze of activity in developing and validating new tests for genetic toxicity, the concern that the human germline should be well protected in its own right also benefited. The result is that tests for genetic toxicity serve to identify not only potential somatic cell mutagens that may lead to cancer via a genotoxic mode of action, but also potential human germ cell mutagens as well.

The importance of including germ cell effects specifically in genotoxicity testing is, however, questionable. Identification of substances that are germ cell mutagens, even under experimental conditions in mammals, is difficult, and quantitative studies can require large numbers of animals. In contrast, identification of potential somatic cell mutagens can be done in vitro, or with fewer animals in vivo, and to date there is no evidence of any substances that are germ cell mutagens but not somatic cell mutagens (see section 4.5.4.2). Thus, in risk assessment, a default assumption can be made that a somatic cell mutagen may also be a potential germ cell mutagen. Knowledge that a substance is a germ cell mutagen does not mean that it should be treated differently from a substance that is a somatic cell mutagen but has not been tested for germ cell mutagenicity. Regulatory decisions declaring that such hazards exist should not have different consequences in these cases. If the individual is protected from the genotoxic and carcinogenic effects of a substance, then so is the population from the heritable genetic effects. Although national regulatory authorities might take a different view, this is the practical standpoint of JMPR and JECFA at this time, and there is no clear scientific reason why this should be changed.

4.5.2 Tests for genetic toxicity

4.5.2.1 Test categories

To address the need for identifying all aspects of genetic toxicity, more than 100 different in vitro and in vivo test methods have been developed. However, only a few are commonly used. It is the diversity of potentially damaging events that has encouraged this development, and for this reason it should not be expected that there will always be consistency among the results of different classes of assays. The tests can be grouped as:

- genetic toxicity tests that measure types of DNA damage known to be precursors of genetic alterations (e.g. formation of DNA adducts or DNA strand breaks) or cellular responses to DNA damage (e.g. unscheduled DNA synthesis); and
- mutagenicity tests that measure expressed genetic damage (e.g. gene mutations, chromosomal rearrangements or deletions, and loss or gain of chromosomal segments or of whole chromosomes, the last also known as aneuploidy).

4.5.2.2 *Commonly used tests*

Commonly used tests (Table 4.1) include those for:

- gene mutation in bacteria;
- gene mutation in mammalian cell lines;
- chromosomal aberrations (including micronuclei) and aneuploidy in cultured mammalian cells;
- DNA damage in primary cultures of mammalian cells (commonly rat hepatocytes);
- in vivo tests for DNA damage (such as DNA binding, unscheduled DNA synthesis in the liver or the comet assay in a number of tissues);
- in vivo tests for chromosomal damage (including micronuclei) using mammalian haematopoietic cells; and
- in vivo tests for gene mutations.

Less commonly used testing methods, with more limited validation, make use of yeast, moulds and insects (*Drosophila*) as test organisms.

Some useful information can also be provided by tests for cell transformation in vitro. Positive results obtained with such tests, however, are not necessarily indicative of genetic toxicity in the form of reactivity with DNA; they may also represent a consequence of an epigenetic event (any heritable influence in the progeny of cells or of individuals on chromosome or gene function that is not accompanied by a change in DNA nucleotide sequence).

4.5.3 *Testing strategy*

For comprehensive coverage of the potential mutagenicity of a substance, information on the ability to induce gene mutations, structural

Table 4.1. Some assays for genetic toxicity

DNA damage/repair	Gene mutation	Chromosomal damage
In vitro assays DNA adduct measurement using cell cultures Unscheduled DNA synthesis using primary cultures (often hepatocytes) DNA strand breakage and alkali-labile sites monitored by single-cell gel electrophoresis (comet assay) or by sucrose gradient or filter elution, using cell cultures **In vivo assays** DNA adduct measurement Unscheduled DNA synthesis (usually in liver) Strand breakage and alkali-labile sites monitored by single-cell gel electrophoresis (comet assay) or by sucrose gradient or filter elution in tissue DNA	**In vitro assays** *Microbial tests* Reversion to a specific nutrient independence using: • *Salmonella typhimurium* • *Escherichia coli* Forward mutation to resistance to a specific anti-metabolite using: • *Salmonella typhimurium* • *Escherichia coli* *Mammalian tests* Forward mutation at the hypoxanthine-guanine phosphoribosyl transferase (*hprt*) gene using cell lines such as: • Chinese hamster ovary (CHO) • Chinese hamster lung (V79) • Human lymphocytes Forward mutation at the thymidine kinase (*tk*) gene using cell lines such as: • Mouse lymphoma L5178Y • *cII* and *lacI* transgenes in cultured Big Blue® mouse and rat embryonic fibroblasts **In vivo assays** Somatic cell assays: • *LacZ* (Muta™Mouse) or *lacI or cII* (Big Blue® mouse or rat) Germline cell assays: • Specific locus test in mice	**In vitro assays** Sister chromatid exchange (SCE) Chromosomal aberrations, micronuclei and aneuploidy using: • CHO and V79 cell lines and human lymphocytes **In vivo assays** Somatic cell assays: • SCE Chromosomal aberrations, micronuclei and aneuploidy using: • Bone marrow cells and lymphocytes (rodent) Germline cell assays: • Chromosomal aberrations, heritable translocations and dominant lethals using mice and rats

chromosomal aberrations and aneuploidy is required. Usually a small number of well-validated in vitro assays are selected to cover different genetic end-points. Commonly used test batteries include a gene mutation test in bacteria (i.e. the *Salmonella*/microsome assay) and one or two tests in mammalian cells detecting point mutations or chromosome damage (clastogenicity/aneugenicity).

Completely negative results in the in vitro test battery are normally considered sufficient to conclude that a substance is devoid of genotoxic potential, unless there are reasons for special concern (e.g. high or sustained human exposure, structural considerations). Conversely, one or more positive in vitro tests normally require follow-up by in vivo testing. The choice of the appropriate in vivo test is made case by case taking into account the results of in vitro assays and information on the toxicokinetics and toxicodynamics of the substance. For substances with adequate systemic availability, tests on rodent erythropoietic or liver cells are generally performed. For directly reactive, short-lived substances, tests on tissues at the initial site of contact are selected. If the first in vivo test is negative, the need for further in vivo tests is decided case by case taking into account the quality of available data, the evidence of target tissue exposure and any other relevant information.

4.5.4 Data assessment

Given the variety of test methods applied, which are designed to cover different genetic end-points, a weight of evidence approach should be used to decide whether a substance is genotoxic. A clear positive result at a single mutagenicity end-point is generally sufficient to classify a substance as positive, even when multiple negative results at other end-points are reported. On the other hand, contrasting results at the same end-point, or in the same test method, should be evaluated case by case with consideration of study design, reproducibility and biological plausibility of the results. With so many different types of assay, so many standard protocols and so many protocol variations possible for special studies, it is not practicable, other than in very general terms, to describe the process of data assessment. This process should, of course, include a judgement of the standards under which the experiments were conducted. Guideline protocols for many of these assays have been published

by the OECD (see http://masetto.sourceoecd.org/vl=2781582/cl=14/nw=1/rpsv/cw/vhosts/oecdjournals/1607310x/v1n4/contp1-1.htm).

Following an initial quality assessment of individual studies, the second scientific requirement is that any observation that is made should be reproducible. Although this principle applies to any kind of study, the resource and animal welfare constraints that hinder replication of many mammalian toxicity studies are less of a hindrance in genetic toxicology, particularly for in vitro studies. The strength of a finding is increased if it can be demonstrated in a number of laboratories. Indeed, where an observation is made in a single laboratory—even if made on a number of occasions—it is generally viewed with suspicion if other laboratories fail to achieve the same result. This suspicion may not be justified in some cases, but it is nevertheless an understandable view in data evaluation.

The third step is to look for a plausible pattern in the hierarchy of results. Such hierarchical patterns can be used only as general guides, because there can always be exceptions. It is expected that a substance that is clastogenic in vivo will also be clastogenic in vitro; and that, in vivo, a germline cell clastogen will also be clastogenic to somatic cells. Deviations from this pattern may occasionally occur, but these should be scrutinized with special care. The basis for suggesting this procedure for cytogenetic assays is given below. Unfortunately, apart from the data in section 4.5.4.3, a similar basis cannot be presented for induction of gene mutation because of the current paucity of in vivo tests for gene mutation. This situation may be expected to change as more data accumulate from the increasing use of transgenic mouse models.

4.5.4.1 Cytogenetic assays in vivo and in vitro

Thompson (1986) reviewed the literature and found 216 chemicals that had been tested both in vitro and in rodent bone marrow tests for clastogenicity. Definitive results were obtained with 181 of them, among which there was concordance between in vivo and in vitro results for 126 chemicals. Of the 55 chemicals for which there were discordant results, 53 were positive in vitro and negative in vivo. Only D-ascorbic acid and ethinylestradiol were negative in vitro while inducing significant clastogenicity in vivo in bone marrow. This leads to the conclusion that a chemical that fails to induce a significant response in

an in vitro clastogenicity assay is unlikely to be clastogenic in in vivo bone marrow assays.

4.5.4.2 Germline and somatic cell in vivo cytogenetic assays

Holden (1982) reviewed the literature and found 76 compounds that had been tested for chromosomal effects in vivo in both somatic and germline cells. Of these, concordant results were obtained for 58 chemicals. The remaining 18 chemicals for which there were discordant results were all positive (i.e. induced damage) in somatic cells only. At that time, therefore, the available evidence suggested that a negative somatic cell response is highly predictive of a negative germline cell response. Subsequently, it was suggested by a USEPA Gene-Tox workshop that six chemicals could be uniquely germline cell mutagens (Auletta & Ashby, 1988), but a re-evaluation of the Gene-Tox Program literature on these chemicals indicated that they had been misclassified (Adler & Ashby, 1989). Thus, as of 1989, there was no reason to change the presumption that all germ cell clastogens are also somatic cell clastogens. This kind of thinking led the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) to propose a testing strategy in which agents without somatic cell genotoxicity in vivo could be assumed to have no potential for germline cell genotoxicity (Arni et al., 1988).

4.5.4.3 In vivo gene mutation assays in germline cells

Data on the mouse specific locus test from the National Toxicology Program (NTP) of the United States of America (USA), reviewed by Shelby et al. (1993), identified only 6 chemicals out of 12 tested as being clearly positive. The chemicals identified as germ cell mutagens are all highly reactive and induce a wide variety of toxic effects in humans and other animals; they are also clastogenic in germ cells.

4.5.5 Genetic toxicity in relation to carcinogenicity

4.5.5.1 Validation of genetic toxicity tests for the prediction of carcinogenicity

The outcomes of different in vitro genetic toxicity tests have been compared with experimental results from rodent carcinogenicity bioassays, such as those from the NTP and its predecessor at the United States National Cancer Institute. The comparisons are expressed in

terms of sensitivity, specificity, concordance, positive predictivity and negative predictivity, as defined by Cooper et al. (1979). The first two aspects are considered to be more important than the last three, and together they provide an adequate description of assay performance. (The proportion of carcinogens that give a positive result in the test is termed the sensitivity, and the proportion of non-carcinogens that give a negative result in the test is termed the specificity.) However, determination of whether or not a compound is genotoxic should be based on an overview assessment of all the available data (see section 4.5.4); therefore, comparisons of the results from carcinogenicity bioassays with the results from a single genotoxicity testing system reflect the relationship only to that test and not to the outcome of a comprehensive testing strategy.

There were hopes in the early 1970s that it would be possible to predict whether or not a chemical was carcinogenic on the basis of a relatively simple bacterial gene mutation test. Using a database consisting of 283 chemicals (prevalence of carcinogens 62%), it was found initially that the *Salmonella*/microsome test had a sensitivity of 90% and a specificity of 87% (Ames et al., 1975). However, a subsequent analysis based on data for 301 chemicals tested by the NTP for carcinogenic activity (prevalence of carcinogens 62%) found that the sensitivity was 56% and the specificity was 75% (Ashby & Tennant, 1991).

Similar results were found in an NTP validation study of four in vitro tests using 73 chemicals (Tennant et al., 1987). The tests investigated were the *Salmonella*/microsome test, the Chinese hamster ovary (CHO) cell test for sister chromatid exchange (SCE), the CHO test for chromosomal aberrations and the mouse lymphoma L5178Y cell $tk^{+/-}$ locus test for gene mutations. Similar test performance evaluations have been made on the basis of published data (McGregor, 1996; McGregor & Anderson, 1999; Quillardet & McGregor, 1999) and on the basis of experience within single laboratories for four additional assays (Matthews et al., 1993; Kitchin & Brown, 1994; Storer et al., 1996; LeBoeuf et al., 1999). For all of these tests, the concordance was about 60%, with sensitivities ranging from 45% to 73% and specificities ranging from 43% to 86%. Because of the heterogeneity of the carcinogenicity test reporting and the variations in genetic test protocols used, the rigor of the Tennant et al. (1987) study is lacking in

some of these analyses. Overall, these data suggest that none of the results of the short-term tests considered can alone provide a reliable prediction of whether or not a chemical is a carcinogen in rodents.

More recently, the ability of a battery of three of the most commonly used in vitro genotoxicity assays (*Salmonella*/microsome assay, mouse lymphoma $tk^{+/-}$ test and chromosomal aberrations/micronucleus tests) to discriminate between rodent carcinogens and rodent non-carcinogens from a large database of over 700 chemicals has been evaluated (Kirkland et al., 2005). All test batteries displayed high sensitivity but insufficient specificity, mainly due to the high incidence of false-positive results produced by mammalian cell systems. Thus, an understanding of the mechanism of action and consideration of the weight of evidence are recommended to assess the carcinogenic risk from genotoxicity test results.

4.5.5.2 *Evidence of mode of action*

The dogma that cancer is primarily a genetic disease has led to a default assumption in the evaluation of chemicals for hazard and risk—namely, if the substance is mutagenic or clastogenic, then this is its mode of action as a carcinogen. This assumption has driven the manner in which carcinogenic chemicals are dealt with in regulatory arenas at the national and supranational levels. Its origins are simple to understand: all studied neoplasms contain mutations of one type or another; there is a single copy of DNA in every cell; therefore, it is reasoned, there can be no threshold of damage below which DNA damage has no consequence; hence, there can be no safe level of exposure to a carcinogen that is genotoxic. These considerations do not apply to substances that do not react with DNA, such as those that affect spindle function and organization, inducing aneuploidy, or that affect chromosome integrity through topoisomerase inhibition. For these compounds, which interact with redundant cellular targets, threshold-based mechanisms are assumed.

The assumption that genotoxic carcinogens act through direct DNA modification is probably useful only as a guide, as there are indications that epigenetic events are more important than hitherto believed, in both experimental animal and human cancer. Many of the schemes of epigenetic modes of action that have been developed from observations on rodents and humans involve disturbance of hormonal regulating

networks, whereas others involve enzyme induction, enzyme inhibition or other expressions of toxicity. It is also clear that a property common to the proposed modes of action is the persistent stimulation of cell populations to divide as a hyperplastic response either to toxicity or to mitogenesis; inhibition of apoptosis may also play a role. In many examples, the mode of action has been reasonably well established, up to the point where there is a cell population increase. Why hyperplasia should ever result in neoplasia is not well established, but the assumed mechanism until now has been that a shortened cell cycle time reduces the time for repair of "background" damage to DNA and increases the probability of mutation.

Whereas intermitotic DNA damage and inefficient repair may be parts of the process, it is known that relatively short cell cycle times are also characteristic of several normal cell populations (e.g. dermis, intestinal epithelium, haematopoietic tissues and, not least, throughout embryos and early fetuses). Another possibility is emerging from recent studies in molecular biology and protein chemistry. If a chemical reacts with DNA, then it is highly probable that it is also reacting with various amino acids in proteins and peptides that, with DNA and many other types of molecules, constitute chromatin. These proteins, including histones, are often involved in normal gene regulatory function. It has been proposed that there is a "histone code", based upon patterns of acetylation, methylation, phosphorylation and ubiquitylation of basic amino acid histone tails protruding from the nucleosomes, which enables other proteins to recognize specific regions of the genome (Strahl & Allis, 2000). These patterns determine whether particular genes are expressed or not. Interference with the activity or function of methyl transferases, acetylases and deacetylases, etc., may very well cause inappropriate phenotypic changes in histones, which could include the silencing of repair genes or of tumour suppressor genes.

Genetic and epigenetic mechanisms can cooperate in chemical carcinogenesis. Consequently, the International Agency for Research on Cancer (IARC) has recommended that there should be no genetic toxicity associated with a substance if an epigenetic mechanism of carcinogenesis is to be accepted. Thus, because of its implications in risk characterization and for the definition of a health-based guidance value, the elucidation of the genotoxic potential of a chemical carcinogen plays an important role in risk assessment.

4.5.6 Conclusions

The use of genetic toxicology in hazard identification, both for effects on somatic cells that may lead to cancer and for heritable effects on the germline, has been accepted in academic, industrial and regulatory circles. The tests that are used provide evidence that the chemical under study can react with biologically important molecules, either directly or after metabolism. Validation studies, using rodent carcinogenicity data as the yardstick, have shown that any prediction of carcinogenic hazard will be imperfect, but certain tests perform better than others in this respect. Similarly, evidence of a particular mode of carcinogenic action that might be derived from positive results of genetic toxicity tests will always have an element of uncertainty about it. As long as these weaknesses are not forgotten and the strengths are not overemphasized, the results can provide useful guidance in chemical risk assessment.

4.6 Carcinogenicity

4.6.1 Introduction

The purpose of testing chemicals for carcinogenicity in experimental animals is to identify potential cancer hazards for humans. Tests are usually conducted for the majority of the lifetime of experimental animals at high multiples of potential human exposures. Under these conditions, the absence of cancer indicates a likely absence of human risk. Positive findings require careful interpretation in relation to mode of action, possible interspecies differences in background incidence and in response and high dose to low dose extrapolation. Virtually all chemicals associated with cancer in humans have been found to increase the incidence of neoplasms in experimental animals (McGregor et al., 1999), although not necessarily the same type of tumour is seen in exposed humans. Accordingly, chronic cancer bioassays are established as relevant for human hazard identification and characterization.

4.6.2 Mechanisms of carcinogenicity and mode of action

In the early days of chemical carcinogenesis, it was initially suspected that carcinogens operated through a common mechanism (Miller & Miller, 1979). With advances in the understanding of the

molecular effects of carcinogens, concepts of differing modes of tumour induction were developed (Williams, 1992). It is now widely accepted that two general types of mode of action can be distinguished—genotoxic mechanisms involving chemical interaction of the carcinogen with DNA, and non-genotoxic mechanisms involving other cellular and extracellular effects (Vaino et al., 1992). These different modes of action have major implications for hazard characterization, because a biological threshold is believed to occur for non-genotoxic mechanisms, and a level of human exposure without significant risk can be established. As a precautionary approach, it is considered that a threshold may not exist for direct-acting (alkylating) genotoxic chemicals or that if a threshold does exist, it may be below the level of human exposure; in consequence, any level of human exposure could be associated with some degree of risk. In contrast, a threshold might exist for some forms of genetic damage (genotoxicity) that do not result in potentially irreversible change to DNA leading to a mutation.

The concept of initiation and promotion as distinct steps in carcinogenesis was developed in mouse skin, and a two-step or multistep process is now known to occur in most tissues (McClain, 1993). In general, initiation is produced by DNA-reactive carcinogens, whereas promotion is produced by non-genotoxic carcinogens.

4.6.2.1 *Genotoxic or DNA-reactive mechanisms*

Genetic changes induced by carcinogens are a fundamental part of carcinogenesis (Vaino et al., 1992) and for alkylating compounds arise from the reactivity of the carcinogen with DNA. DNA-reactive carcinogens usually operate as electrophilic reactants to bind to DNA (Williams, 1992). Carcinogens that act through such genotoxic mechanisms are usually multiorgan and trans-species carcinogens, can be active with a single dose and are effective at low exposures.

4.6.2.2 *Non-genotoxic mechanisms*

Non-genotoxic mechanisms of carcinogenesis do not involve a direct chemical attack on DNA, but rather are produced by other effects of the carcinogen on target cells or on the extracellular matrix (Williams, 1992). There are several non-genotoxic effects that can lead to enhancement of tumour development. Adaptive effects may

lead to carcinogenicity with chronic, high-level exposure (Dybing et al., 2002; Williams & Iatropoulos, 2002). Thus, carcinogens that act through non-genotoxic mechanisms usually require high, sustained exposure. A common feature of the effects of non-genotoxic carcinogens is enhanced cell proliferation.

4.6.3 Chronic bioassays for the identification and characterization of cancer risk

Methods for the conduct of chronic cancer bioassays are well described (OECD, 1981a; Kitchin, 1999; Williams & Iatropoulos, 2001; VICH, 2002). For regulatory purposes, carcinogenicity bioassays usually consist of a 2-year rat study plus an 18-month mouse study, with 50 animals of each sex per group. Normally, there are at least three dose levels in addition to a concurrent control group; the highest dose should be associated with minimal toxicity as indicated by changes such as a slight decrease in weight gain, without affecting survival, to ensure that the bioassay provides suitable sensitivity for hazard identification purposes. For substances of low toxicity, the substance would normally be added to the diet at up to 5% by weight. Demonstration of a toxic effect in a cancer bioassay that does not compromise survivability or physiological homeostasis ensures that the animals were sufficiently challenged and provides confidence in the reliability of a negative outcome (VICH, 2002).

A positive response in either test species should be considered indicative of carcinogenic potential. With the development of alternative test systems (see section 4.6.4), carcinogenicity studies (e.g. for therapeutic drugs) are sometimes performed in one rodent species, preferably the rat, plus one or more alternative methods. Such an approach may become acceptable for WHO advisory committees in the future.

Extensive results using rats and mice are available (Gold & Zeiger, 1996), and such tests remain the standard. However, issues have arisen over the relevance to humans of an increase in certain types of neoplasms (section 4.6.6) and of mouse bioassays per se (Van Oosterhout et al., 1997).

4.6.3.1 Statistical methods

The statistical analysis of multidose cancer bioassays with potential treatment-related differences in survival is a complex and specialist

issue. The methods provided by Peto et al. (1980) are widely accepted for statistical analysis, although other methods may be used.

4.6.3.2 Evaluation

Important criteria in the evaluation of positive findings are consistency and reproducibility. Results are more compelling if carcinogenic effects are seen in both rats and mice. In a single experiment, dose-related trends in specific tumour types, the nature and type of tumour, the occurrence of cancer in non-sex-related tissues in both sexes and the presence of related non-neoplastic findings (e.g. hyperplasia or toxicity) are important indicators of treatment-related neoplastic and preneoplastic effects.

4.6.3.3 Interpretation

The interpretation of bioassay results for human risk involves consideration of the relevance of the tumour type to humans and the dose–response in relation to the magnitude of human exposure. Further information is given in sections 4.6.6 and 4.6.7.

4.6.4 Alternative methods for carcinogenicity testing

A variety of alternative tests for carcinogenicity have been introduced in which tumorigenic responses are enhanced and the duration of bioassays is thereby reduced (McGregor et al., 1999; Cohen et al., 2001; Goodman, 2001). None of these have yet been applied to the same extent as the chronic bioassay.

4.6.4.1 Initiation/promotion models

Based upon distinct steps of initiation and promotion in carcinogenesis, models have been developed in which the substance is tested either as an initiator by administration before a promoter for the target organ of interest or as a promoter by administration after an initiator for the target organ (reviewed in Enzmann et al., 1998a,b). As these studies are generally less than 1 year in duration, the background of spontaneous neoplasms is negligible.

One of the major contributions of these models is that they provide information on the mode of action for observed effects. For example, McGregor et al. (1999) concluded that in such models, the

> appearance of tumours after administration of a test chemical as an initiator provides evidence of carcinogenic activity.... Additional evidence of promoting activity makes the evidence compelling. When data are available only on promoting activity, the evidence is suggestive of carcinogenicity..., but the information should be evaluated in conjunction with other data….

On the other hand, caution is needed in data interpretation, as these models assume that the added promoter or initiator is biologically relevant to the corresponding initiator and promoter under test.

4.6.4.2 *Neonatal mouse model*

In this model, newborn mice, usually of the CD-1 strain, are given the test substance by intragastric instillation on days 8 and 15 postpartum and observed for up to 1 year (Flammang et al., 1997; McClain et al., 2001). At the end of the study, the incidence of spontaneous neoplasms is negligible.

Data suggest that this model responds only to genotoxic carcinogens; as such, its utility for testing unknown substances is limited. In the International Life Sciences Institute (ILSI)–Health and Environmental Sciences Institute (HESI) Collaborative Program on Alternative Models for Carcinogenicity Assessment (ILSI, 2001), only 1 non-genotoxic chemical (17β-estradiol) of the 18 compounds that were evaluated was reported positive (McClain et al., 2001). Thus, a positive response in this model indicates that the test substance probably produced cancer via a genotoxic effect.

4.6.4.3 *Transgenic mouse models*

Through selective gene activation or deletion, mice of unique genotypes can be produced that may be more susceptible to carcinogenesis (Gulezian et al., 2000). These models have been widely applied in the testing of pharmaceuticals (ICH, 1997) and were evaluated in the ILSI-HESI Collaborative Program on Alternative Models for Carcinogenicity Assessment (ILSI, 2001). Usually the duration of bioassays is 26 weeks (rather than 2 years or 18 months for the rat and mouse, respectively) because of the increase in spontaneous tumours in transgenic animals beyond this time.

(a) $p53^{+/-}$ mice

This model employs mice in which one allele of the *TP53* tumour suppressor gene is disrupted (Donehower et al., 1992); hence, the model is believed to be responsive to genotoxic carcinogens (French et al., 2001). Initially, the inactivated null *Trp53* allele was implanted into C57BL/6 female mice, which produced, after numerous crossings, the C57BL/6-based model (Donehower et al., 1992; French et al., 2001). In a widely used version of this model based on the C57BL/6 mouse, the most common spontaneous neoplasm is subcutaneous sarcoma (Mahler et al., 1998), and increases have been provoked by implantation of devices (Mahler et al., 1998) or injection of irritant materials (Youssef et al., 2001). In addition, malignant lymphoma (both sexes) and osteosarcoma (males) are also known to occur spontaneously (French et al., 2001).

In the ILSI-HESI evaluation (ILSI, 2001), 6 of the 21 compounds tested were human carcinogens. In this model, four of these were positive (cyclophosphamide, melphalan, cyclosporin A and diethylstilbestrol), one was negative (phenacetin) and one was equivocal (17β-estradiol). Moreover, 12 of the 16 genotoxic human or rodent carcinogens were positive, and 2 (chloroform and diethylhexylphthalate) of the 22 non-genotoxic rodent carcinogens were judged equivocal (Storer et al., 2001).

(b) TG.AC model

Homozygous TG.AC mice were developed in the FBV/N strain by the introduction of a construct containing an activated v-Ha-*ras* oncogene (Leder et al., 1990). Either the homozygous TG.AC line or a heterozygous line derived by mating homozygous TG.AC males with FBV/N females can be used for chemical evaluation. Thus far, this model has been used largely for topical application in which the test substance is applied to the shaved dorsal skin (ILSI, 2001). Test substances have been administered in a variety of vehicles.

One issue with this model is the potential for chronic dermal irritation resulting from repeated shaving together with application of irritant vehicles (e.g. acetone) to enhance responses to test substances. This model is not an adequate replacement for a chronic mouse

bioassay, as five of seven non-genotoxic mouse carcinogens were negative (Tennant et al., 2001).

(c) K6/ODC

Recently, K6/ODC mice have been evaluated as an alternative for short-term dermal carcinogenicity testing (Miller et al., 2008), as this strain develops epidermal tumours when exposed to genotoxic carcinogens. In a recent study, mice that received 7,12-dimethylbenz[*a*]-anthracene dermally developed papillomas as early as 6 weeks, but progressive adverse health and decreased survival suggested that K6/ODC mice may be an inappropriate alternative model.

(d) Xpa

$Xpa^{-/-}$ homozygous knockout mice have a defect in genes controlling the DNA repair pathway known as nucleotide excision repair. Xpa mice develop skin tumours at high frequency when exposed to ultraviolet light and are susceptible to genotoxic carcinogens given orally (Van Steeg et al., 2001). In an attempt to further increase both the sensitivity and specificity of the Xpa model in carcinogenicity testing, Xpa mice were crossed with $p53^{+/-}$ mice; the resulting Xpa/$p53^{+/-}$ double-knockout mice developed tumours earlier and with higher incidences upon exposure to carcinogens compared with their single-knockout counterparts. There appears to be a good correlation between compounds identified as positive in the Xpa/$p53^{+/-}$ model and human carcinogenicity (Van Steeg et al., 2001).

(e) Tg-rasH2

Unlike the $p53^{+/-}$ mouse, the Tg-rasH2 mouse is sensitive to both genotoxic and non-genotoxic carcinogens, but develops more spontaneous neoplasms compared with wild-type mice (Morton et al., 2002). In carcinogenicity testing, 4 of 6 known/suspected human carcinogens were positive; for 19 non-mutagenic agents testing positive in conventional rodent bioassays, 7 chemicals were positive, 10 chemicals were negative and 2 were equivocal. Results for 15 of 18 mutagenic chemicals agreed with the results of conventional rodent bioassays, and 3 results were equivocal. Thus, the Tg-rasH2 mouse model appears to predict known or suspected human carcinogens as well as

the traditional mouse bioassay, but with fewer positive results for non-genotoxic compounds that are not considered human carcinogens (Morton et al., 2002).

(f) Other models

Several other transgenic models are available (Robinson & MacDonald, 2001) but are less widely used and lack adequate validation for regulatory purposes.

4.6.4.4 Interpretation of the data from alternative methods

McGregor et al. (1999) considered these alternative models appropriate for identifying carcinogens in rodents. However, the basis for a tumour increase can be obscure. For example, certain agents enhance the development of spontaneous neoplasms only; these could simply arise from a shortening of the latent period for these tumours, which appear in high incidence later.

In medium-term assays with preneoplasia as the end-point, McGregor et al. (1999) concluded that "the occurrence of preneoplasia ... within a period of 20–40 weeks provides evidence of potential carcinogenic activity".

More recently, IARC suggested that under certain circumstances, data from alternative assays could be used in safety evaluation in place of a second bioassay and that some of these models might be useful in hazard identification if used in conjunction with information from other sources in a weight of evidence, integrated analysis approach to risk assessment (Cohen et al., 2001).

4.6.5 End-points in carcinogenicity studies

4.6.5.1 Spontaneous neoplasms

The rodent strains used in chronic cancer bioassays have high incidences of certain tumour types (Williams & Iatropoulos, 2001) that may be irrelevant for human health, especially if increases are found only in such common neoplasms. Any increase may have arisen by enhancement of an endogenous spontaneous rodent mechanism, providing evidence of a cancer-promoting potential rather than a

cancer-initiating potential. As such, the dose–response would be expected to exhibit a threshold.

4.6.5.2 Pathological classification of neoplasms

Standard criteria for the diagnosis of rodent neoplasms have been developed (Faccini et al., 1992). These are generally used in studies conducted for regulatory purposes, but not always in investigator-originated studies. The precision with which diagnostic criteria are applied is, of course, a function of the skill of the study pathologist. Guidance for the performance of the pathological evaluation is available (Williams & Iatropoulos, 2001).

For veterinary drugs, it has been recommended that in-life observations and pathological examination, consistent with OECD Test Guideline No. 451 (OECD, 1981a), are undertaken in carcinogenicity studies and that clinical pathology (haematology, urinalysis and clinical chemistry) is not considered necessary and does not contribute to the assessment of neoplastic end-points.

A valuable component of the pathological evaluation is peer review, in which a second pathologist examines a representative sampling of the material. Such peer review is particularly valuable when the pathologist is not informed as to which slides are from treated animals and which are from control animals (blind analysis).

4.6.5.3 Benign and malignant neoplasms

The distinction between benign and malignant neoplasms in experimental animals is usually made on the basis of histopathology; neoplasms classified as benign are usually not invasive or metastatic. There is controversy over whether an agent that induces only benign neoplasms should be classified as carcinogenic, and these data should therefore be used in an overall weight of evidence approach. Often a combination of histogenetically related benign and malignant neoplasms is used to arrive at a conclusion that the test substance is carcinogenic (Faccini et al., 1992; Williams & Iatropoulos, 2001).

4.6.5.4 Preneoplastic lesions

Preneoplastic lesions are part of the continuum of neoplastic development (Williams, 1999). Accordingly, their presence in a tissue at

the end of a bioassay, together with related neoplasms, supports the conclusion of a chemical-induced carcinogenic effect. By themselves, however, they do not justify the conclusion that the substance is carcinogenic.

4.6.6 Characterization of carcinogenic effects

IARC has developed guidelines on the use of information on mechanisms in evaluating carcinogenicity findings of this type (Capen et al., 1999), which have been applied to assessment of human hazard of specific chemicals (McGregor et al., 1999).

IPCS developed a conceptual framework on the evaluation of an animal mode of action for chemical carcinogenesis. This framework provides a generic approach to the principles commonly used for evaluating mode of action. It outlines a list of elements to be considered in analysing whether available data support a particular mode of action (Sonich-Mullin et al., 2001).

Subsequently, this framework was extended to address the issue of human relevance of animal cancer data. The IPCS framework for analysing the relevance of a cancer mode of action for humans, along with three case-studies, was published in 2006 (Boobis et al., 2006). The application of this framework is intended to increase transparency in analysing and interpreting cancer data and will result in improved communication of the bases for scientific conclusions and decision-making.

4.6.6.1 *Mechanisms relevant to humans*

(a) DNA reactivity or genotoxicity

Carcinogens that are DNA reactive are usually trans-species carcinogens and therefore are presumed to be potential human carcinogens (McGregor et al., 1999); indeed, most human carcinogens are clearly DNA reactive (Thorgeirsson et al., 1994; Williams & Iatropoulos, 2001). Thus, assessment of genotoxicity is an important component of chemical evaluation and critical in the hazard characterization approach adopted (see chapter 7). Barlow et al. (2002) concluded that “specific markers of DNA damage or adducts will not only assist mechanistic understanding, but can assist in risk

assessment". It should be noted that some forms of genotoxicity may exhibit a threshold—for example, aneugenicity as a consequence of spindle inhibition (Parry et al., 1994). In rare circumstances, toxicokinetic factors may be such that there is a de facto threshold for genotoxicity in vivo—for example, for phenol when exposure is via the oral route (EC, 2006).

Substances that produce cancer via modes of action that do not involve direct DNA reactivity and alkylation tend to show species differences in susceptibility and are often associated with cancer incidence at a single site. In addition, these non-genotoxic carcinogens usually show a biological threshold in their dose–response relationship. Normally, other effects that may be precursors are seen at doses below those that increase the incidence of cancer, and these effects are usually the focus of hazard characterization and derivation of a health-based guidance value.

4.6.6.2 Mechanisms not relevant to humans

(a) Surface and luminal tissue chronic irritation

It has long been known that wounding of surface and luminal tissues can elicit tumour development at the wound site. As blocking of cellular communication channels, an increase in the intensity of tissue metabolic reactions and even induction of sustained tissue ischaemia differ between laboratory animals and humans, their relevance to humans is limited.

(b) Mouse liver neoplasms

The relevance of the production of increases only in mouse liver neoplasms has long been questioned (Stevenson, 1990). No agent that produces increases only in mouse liver tumours is associated with comparable effects in humans (Williams, 1997).

(c) Hormonal disruption

Several hormone systems in rodents are more susceptible to disruption with consequent increase in neoplasia than the corresponding systems in humans. For example, thyroid tumours in rats can arise from thyroid–pituitary disruption, whereby reduced thyroid hormone

levels lead to a negative feedback increase in thyroid-stimulating hormone levels and subsequent hyperplasia and neoplasia (Thomas & Williams, 1991; Hill et al., 1998; Rice et al., 1999) that are of negligible relevance to humans.

(d) Inhibition of tissue trophic activity

Interference with neuroendocrine immune feedback pathways can result in neoplasia that is species or sex specific and not relevant to humans (Iatropoulos & Williams, 1996; Williams & Iatropoulos, 2001).

(e) α2u-Microglobulin-induced rat nephropathy

Kidney tumours in male rats arising indirectly through binding to and increases in renal excretion of α2u-microglobulin are considered not relevant to humans, because humans do not synthesize α2u-microglobulin (USEPA, 1991d).

(f) Rat stomach neuroendocrine neoplasm

Neoplasia of gastric neuroendocrine cells is stimulated by gastrin in rats and to a lesser degree in mice, because rodents have a high density of neuroendocrine cells, giving high levels of gastrin (>1000 pg/ml). Because these high gastrin levels are not achieved in humans and other primates, this type of neoplasm is not relevant to humans (Tuch et al., 1992; Thake et al., 1995).

(g) Peroxisome proliferation

Rodent hepatic peroxisome proliferators cause tumours in rodent liver but do not produce these effects in primate or human liver (Williams & Perrone, 1996) as a result of species differences in levels of the peroxisome proliferator activated receptor of the class α (PPARα) (Tugwood & Elcombe, 1999) and other mechanistic differences between rodents and humans (Klaunig et al., 2003). Because of this, IARC (1995) has recommended that a tumour response in mice or rats secondary to peroxisome proliferation should modify the evaluation of carcinogenicity.

(h) Cytotoxicity and regenerative hyperplasia

Sustained, chemically induced cytotoxicity of various types can lead to regenerative hyperplasia and subsequent preneoplastic foci

and tumours. However, the relevance of this to human exposure is questionable, as this mechanism is often a "high-dose" phenomenon that may be species specific.

4.6.7 Assessment of carcinogenic response

Carcinogenicity is a major concern in the risk assessment of chemicals in food, particularly if a genotoxic mechanism in known or suspected. In part, this is because risk management options for such substances can vary with jurisdiction. Hence, it is important that any possible carcinogenic effect be fully and consistently assessed. There are a number of issues that should be considered.

4.6.7.1 Nature of the test substance

The chemical purity of the substance and the possibility that impurities or co-formulants such as the vehicle (e.g. corn oil) might have influenced the response should be considered. The physicochemical form of the substance tested should be appropriate to the substance to which the population may be exposed. For example, the carcinogenicity of some metals (e.g. chromium) depends markedly on speciation. In the case of airborne particulates, the geometry and solubility of the particle will profoundly influence the response.

4.6.7.2 Relevance of study design

The route of exposure needs to be considered. Where irritant substances are administered at high local concentrations—for example, by oral gavage—they may produce tumours at the site of contact that are of limited or no relevance to humans under the exposure scenarios of concern. Some routes of exposure—for example, intraperitoneal—are not relevant to human exposure. These need to be considered on a case-by-case basis. In some instances, the avoidance of presystemic metabolism may lead to quantitatively, or even qualitatively, erroneous conclusions.

Duration of exposure should also be considered. Where study duration is less than that recommended by the relevant test guidelines, the likelihood that carcinogenic effects would have been missed needs to be assessed. This also applies to situations where survival at the end of a study is less than the minimum recommended. In some instances,

it may still be possible to obtain meaningful conclusions from the study—for example, where survival is still high until a couple of months before the normal end of the study.

4.6.7.3 Are the tumours substance related?

As discussed above, the possibility that tumours are a consequence of the vehicle used or the method of administration—for example, physical irritation by the gavage needle—should be considered, particularly where the response is specific to a particular set of experimental conditions and is negative in other studies with different experimental conditions (e.g. when using another vehicle). The statistical significance of the tumour response should be considered, together with historical control data. For example, was the tumour incidence in the control group lower or higher than the extremes in the historical control data?

The nature of the dose–response relationship can be of value in interpreting the data. For example, where a statistically significant response is observed only at the lowest dose and no response is seen in any of the higher dose groups, the plausibility of a substance-related response needs to be considered carefully. The lesion in question should be a malignant tumour, although, on occasion, benign tumours may be informative in assessing carcinogenicity, as discussed above. However, the relationship between preneoplastic and neoplastic effects needs to be considered; where there is no substance-related malignancy, the relevance of preneoplastic findings alone needs to be addressed.

Food intake can influence longevity and tumour incidence as a consequence of nutritional status or altered lifespan. Hence, substance-related effects and other factors influencing food consumption may indirectly affect tumour incidence, and due consideration should be given to this possibility when there are appreciable changes in either food consumption or lifespan (increased) in a study.

4.6.7.4 Can a mode of action for the tumour response be established?

A mode of action has been defined as a series of key events leading to an observed effect supported by robust experimental observations and mechanistic data (Boobis et al., 2006). Examples of key events

include specific metabolic transformation, receptor–ligand changes, increased cell growth and organ weight, and hormonal or other physiological perturbations. Identification of the mode of action for a carcinogenic response in experimental animals can be of considerable value in addressing issues such as human relevance, dose–response and CSAFs. Identification of a mode of action is based on a weight of evidence approach that has been described in detail in publications from IPCS (Sonich-Mullin et al., 2001; Boobis et al., 2006). Whereas formal mode of action analysis may not be necessary for every carcinogenic response, some consideration of mode of action will be necessary in all cases, if only to determine whether the response is likely to exhibit a threshold or not (see section 4.6.2).

4.6.7.5 *Is the mode of action relevant to humans?*

IPCS has published an analytical framework for assessing whether the mode of action for a tumour response observed in an experimental study is relevant to humans (Boobis et al., 2006). A number of modes of action are not relevant on the basis of qualitative or quantitative considerations (see section 4.6.6.2). Application of the framework will not be necessary in all cases—for example, where a compound is clearly a direct-acting DNA-reactive genotoxic carcinogen. However, in other cases, the framework can be invaluable in determining the strength of evidence of a conclusion regarding human relevance, in a transparent and consistent manner. Hence, in cases where there is possible ambiguity as to the conclusion regarding human relevance, it is recommended that the framework be applied and the results presented in the report of the assessment. Even where human relevance cannot be excluded, application of the framework can provide insight into species differences, dose–response relationships and potential susceptible subpopulations—for example, on the basis of life stage.

4.6.7.6 *Historical control data*

The incidence of spontaneous tumours can vary, sometimes appreciably, among control groups of the same species and strain in different studies, even when conducted within the same laboratory under carefully controlled conditions. Hence, for a response to be considered substance related, not only should it differ significantly from that in the control group, but in general it should also differ from the background

incidence in that species and strain of experimental animal. Hence, suitable data on historical controls should be available to help in interpretation of the findings. Although historical control data can be of considerable value in data interpretation, they should not be viewed as a substitute for concurrent control data. An overall weight of evidence approach is necessary.

Ideally, historical control data will have been obtained in the same species and strain, from the same supplier, and maintained under the same conditions in the same laboratory as that generating the study data being evaluated. The data should be from control animals over a 5-year period, centred as closely as possible on the date of the study being evaluated. The historical control data should be presented for each discrete group, indicating sex and age of the animals. In addition, information on the following should be provided:

- species, strain, name of the supplier and specific colony identification if the supplier is based in more than one location;
- name of the laboratory and date on which the study was performed;
- description of general conditions under which the animals were maintained, including details of diet and, where possible, the amount consumed;
- the approximate age, in days, of the animals at the beginning of the study and at the time of death;
- details of the mortality pattern observed during or at the end of the study and of any other relevant observations (e.g. infections);
- identity of the pathology laboratory and the pathologist responsible for analysing the pathology data from the study; and
- which tumours were combined, if any, in generating the incidence data.

In evaluating historical control data, the following points should be considered:

- If the tumour incidence in the concurrent control group is lower than that in the historical control groups but is within the historical control range in the treated groups, it would be concluded that there is no biologically relevant substance-related response.

- If the tumour incidence in the treated groups is above the historical control range but not statistically significantly different from that of the concurrent controls, it would be concluded that there is no substance-related response (although it is always possible that this was a false negative).

- Where the tumour incidence in the treated groups is significantly greater than that in the concurrent controls and is above the historical control range, it would be concluded that the carcinogenic effect is likely to be substance related, with a low probability of a false positive.

4.7 Reproductive and developmental toxicity

4.7.1 Introduction

Adverse effects on reproduction may be expressed through reduced fertility or fecundity in either the parents or offspring as a result of morphological, biochemical, genetic or physiological disturbances. Adverse effects on development may be expressed through altered viability, growth or structural or functional abnormalities due to either mutations or biochemical/physiological disturbances. Adverse effects on development induced by chemicals may be expressed immediately or they may be delayed, sometimes for many years, as exemplified by transplacental carcinogens.

Typical developmental toxicity studies investigate the effects of exposure to test substances starting at implantation and continuing through the period of organogenesis. More recent study protocols extend the period of exposure to include the fetal period. Effects due to chemical exposure during the fetal period, the developmental period after the major organ systems have formed, generally involve growth retardation and functional disorders, although the external genitalia and the central nervous system are also susceptible to injury during this period. These studies were previously called "teratogenicity studies" but are now called "prenatal toxicity" or "developmental toxicity" studies in recognition that they cover more than just structural malformations. Subtle structural or functional abnormalities often do not become obvious until some time after birth and in some cases not until adulthood.

Because of the differential rates of development between species and the relative states of maturity of neonates at birth, it is important

to understand equivalencies of developmental stages when comparing exposure scenarios across species (i.e. what is the equivalent human stage for a particular window of exposure in a rodent?). Comparative rates of development, as well as spontaneous rates of malformations for a number of species and strains, are provided by Schardein (2000). The developmental processes at risk and their critical stages of vulnerabilities during prenatal and postnatal life have been reviewed by IPCS (2006b).

Neonatal development may be influenced by chemicals (or their metabolites) that are present in the maternal diet and subsequently transferred into maternal milk. Chemical exposure of the mother may also affect neonatal development by influencing maternal behaviour, hormonal balance or nutrition. Direct neonatal exposure to xenobiotic compounds can also occur via consumption of infant formula. Examples include the limited number of additives that are used in infant formula, phytoestrogens in soy-based formula and migrants from infant feeding bottles.

Guidelines for reproductive and developmental toxicity tests have been developed by various legislative and international organizations, including the OECD (see http://www.oecd.org/department/0,2688,en_2649_34377_1_1_1_1_1,00.html), the ICH (1994c), the USEPA (1991b, 1996; see also http://www.epa.gov/opptsfrs/home/guidelin.htm) and IPCS (2001b). A guideline for developmental neurotoxicity has also been developed by OECD, in which postnatal function and behaviour can be investigated in offspring exposed to chemicals during the prenatal and in the early postnatal period (OECD, 2007). Such studies are discussed in section 4.8.3.3 and will not be further addressed here.

4.7.2 End-points of concern

The range of reproductive functions that are observed in reproductive toxicity studies includes gametogenesis, mating, fertility, maintenance and duration of pregnancy, parturition, litter numbers, lactation, puberty, viability and growth of offspring and reproductive senescence. These aspects can be investigated in the parental and filial generations through end-points such as the following:

- Parents and offspring:
  - Sperm measures (number, motility, morphology, sperm production rate)

  - Vaginal cytology (estrous cycles)
  - Hormone measurements
  - Evidence of mating
  - Pregnancy rate
  - Organ weights (gonads, uterus, epididymis and accessory sex glands)
  - Histopathology of the reproductive tissues
  - Reproductive behaviour
- Offspring:
  - Litter size and viability
  - Body weight
  - Sex ratio
  - Anogenital distance
  - Nipple/areola retention in males
  - Vaginal opening
  - Testes descent
  - Preputial separation

For all the outcomes and end-points, it is necessary to determine the normal range and the extent of deviation that should be considered adverse.

The range of adverse effects on offspring arising from maternal exposure to chemicals during pregnancy includes death and resorption of the embryo or fetus, teratogenic defects (structural malformations), growth retardation or specific developmental delays, and decreased postnatal functional capabilities.

For a developmental toxicant, the effects that will be expressed depend on the level and gestational timing of the dose of the chemical and the duration of the treatment period. Thus, a substance given at one dose level may result in growth retardation, whereas at a higher level it may result in death and resorption of the embryo. Sometimes the slope of the dose–response curve for these effects is very steep. The concept of critical period is important to recognize, as an exposure at one developmental stage could be without effect, whereas the effect could be severe at another developmental stage because the target tissue is at an exceptionally vulnerable point as a result of the progression of developmental events that are occurring. Similarly, an

exposure at one point in development may induce growth retardation, whereas malformations could be observed during a different exposure window. In addition, because of differences in the rates of development and toxicokinetics, it is not expected that a particular experimental outcome will translate with fidelity across species. Thus, an agent that induces, for example, limb malformations in a mouse would not necessarily yield that same result in humans (but for human risk assessment purposes, it would generally be assumed to have the potential to produce some manifestation of developmental toxicity). Because all of these outcomes are adverse, the most important consideration when evaluating these studies should not be what effect is observed, but rather at what dose level the adverse effect became evident (USEPA, 1991b) and whether there was also any evidence of maternal toxicity.

4.7.3 Study design

4.7.3.1 Overview

A number of reviews of procedures and methodologies for assessing the effects of chemicals on reproductive function are available (USEPA, 1996, 1998b,c, 2002; IPCS, 2001b). The procedures described in these publications are designed to assess the potential for reproductive and developmental toxicity of test substances using lower mammals as model systems. It is important to take into account the existing toxicological database on the chemical to make sure that appropriate end-points are being adequately covered. The knowledge can be used for more individualized study designs that go beyond the minimum core guideline requirements in order to better understand the full potential of the chemical to affect reproductive function and development.

Regardless of the actual experimental design, the goal of reproductive and developmental toxicity protocols is to assess the sensitivity of various processes and life stages to alterations brought about by exposure to the substance under study and to characterize the most vulnerable target tissue. Therefore, the highest dose of a food chemical that is administered is generally the amount that would be expected to cause slight systemic toxicity, with lower doses being geometrically spaced to a level not expected to induce significant

adverse effects. If there is a significant reduction in maternal body weight or other indication of excessive maternal toxicity, caution should be applied in interpreting any adverse outcomes in the offspring, as the effects could be secondary to maternal toxicity. It is important that appropriate sensitive end-points be evaluated, that exposures cover all of the known critical periods and that sufficient sample sizes be used in order to ensure adequate statistical power to detect effects when present. Thus, in the case of developmental toxicity studies, where either half or all (depending on the particular protocol) of the fetuses are examined for soft tissue and skeletal morphology, it has been estimated (USEPA, 1991b) that the minimum change detectable is an increased incidence of malformations of 5- to 12-fold over control levels and a 3- to 6-fold increase in embryonic or fetal death. This contrasts with the ability to detect a 0.15- to 0.25-fold reduction in fetal weight, which is a continuous variable. As a number of chemicals have now been identified as endocrine disruptors that can cause malformations of the reproductive tract that would not be readily observable in the fetal examinations conducted in developmental toxicity tests (e.g. hypospadias), it is likely that in reproductive toxicity tests, the numbers of offspring evaluated in filial (F_1, F_2, etc.) generations (where subsequent postnatal development allows the malformations to be expressed and readily observed) will need to be increased.

4.7.3.2 *Reproductive toxicity*

Generally, effects on reproduction are evaluated in multigeneration studies such as OECD Test Guideline No. 416: Two-Generation Reproduction Toxicity Study (OECD, 2001b), the USEPA's Reproduction and Fertility Effects test guideline (USEPA, 1998b) and the Reproductive Assessment by Continuous Breeding protocol of the United States NTP (Chapin & Sloane, 1997). Rats are the usual species of choice for multigeneration-type studies, and generally only one species is tested because of the length, cost and complexity of such studies.

For hazard identification, several other protocols exist that evaluate various aspects of reproduction and development, such as OECD Test Guideline No. 415: One-Generation Reproduction Toxicity Study (OECD, 1983), OECD Test Guideline No. 421: Reproduction/

Developmental Toxicity Screening Test (OECD, 1995d), OECD Test Guideline No. 422: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (OECD, 1996) or the NTP 35-day screening protocol (Harris et al., 1992). One-generation studies usually evaluate the effects of subchronic exposure of adult animals in the parental generation and the F_1 generation through to weaning, whereas in multigeneration studies, exposure of the F_1 generation continues through weaning to adulthood, at which point they are mated to produce the F_2 generation. Because the parental and subsequent filial generations have different exposure histories, different outcomes may be observed. In particular, effects may be observed in the F_1 and F_2 generations that are not apparent in the parental generation because of their exposure during the full period of development. More recently, with the concerns raised for chemicals that could interact with the endocrine system and thus disrupt a number of processes critical for successful development and reproduction, a series of screening assays have been proposed that evaluate specific aspects of physiology related to estrogen, androgen and thyroid hormone action (see section 4.7.3.5).

It should be borne in mind that some end-points in reproductive toxicity studies are also inherently insensitive to chemical exposure (USEPA, 1996). For example, because of a large reserve capacity in sperm numbers, daily sperm production can be drastically reduced in the adult male rat without any apparent effect on fertility. This is in contrast to the situation in humans, where relatively small decrements in sperm production would be expected to elevate the probability of infertility or subfertility. To address this discrepancy and to add more sensitive end-points, recent revisions to test guidelines (e.g. USEPA, 1998b; OECD, 2001b) include guidance for the assessment of testicular function (e.g. daily sperm production and epididymal sperm counts, sperm motility and sperm morphology). Similarly, to be more sensitive to endocrine-active agents, some designs include determination of the age at vaginal opening in the female and preputial separation in the male as indices of puberty and options for measurement of anogenital distance, an androgen-dependent, sexually dimorphic trait, in the neonate and nipple retention in male offspring.

Single-generation and multigeneration reproduction studies are particularly useful for assessing potentially deleterious effects on

reproduction and development through birth to weaning. Although the basic protocols have been in existence for at least 30 years, new end-points have been added to them over time in order to increase the breadth of the end-points covered, as well as the sensitivity of the end-points to perturbations (Kimmel & Makris, 2001). There is also discussion about the sample sizes used to evaluate the offspring in multigeneration studies for malformations. Existing guidelines generally require one male and one female from each of the litters to be evaluated for malformations. Such small sample sizes require that a very high incidence of an effect be present before it would be confirmed statistically (see discussion of statistical power in section 4.7.3.1).

Conversely, other components of earlier multigeneration test protocols have been dropped over time, most notably the need to rear two litters per generation (nowadays, only one is recommended) and the need to use three generations (nowadays, only one or two is recommended). The general consensus now is that these additional components did not provide qualitatively new information.

4.7.3.3 *Developmental toxicity*

Effects on prenatal development are examined using protocols such as OECD Test Guideline No. 414: Prenatal Developmental Toxicity Study (OECD, 2001a) and the USEPA's Prenatal Toxicity Study (USEPA, 1998c), which expose pregnant animals during the period of major organ formation and examine fetuses for growth and structural development. Generally, developmental toxicity tests are conducted in two species, usually a rodent and a non-rodent, as greater confidence is gained when results are available from more than one species. This is especially true in instances where the lack of developmental toxicity is noted in the first species tested. However, in situations where the first study shows evidence of developmental toxicity, it may be possible to complete the assessment with adequate confidence (see section 4.7.3.4). The species of choice for routine studies are usually rat and rabbit, but in cases where the rabbit is unsuitable (see section 4.7.4), the mouse is often used.

The basic protocol for the evaluation of developmental toxicity has been largely unchanged for more than 25 years, although later modifications have increased their scope and sensitivity (Kimmel &

Makris, 2001). One change has involved the extension of the dosing period from just covering the period from implantation through to closure of the palate (known as "organogenesis" and corresponding to days 6–15 of pregnancy in the rat) to include the late gestation period to the day before sacrifice. This allows better coverage of late-developing organ systems, such as the reproductive tract and the central nervous system. There are still recognized limitations in detecting alterations in some systems using the standard fetal examination process that focuses on morphology and examines tissues that are not fully mature (and hence may not yet express the developmental effect), such as the central nervous system (Rodier et al., 1994; Harry, 1998), the immune system (Holladay & Luster, 1994) and the heart, lungs and kidneys (Lau & Kavlock, 1994). These limitations can be addressed, at least partially, in the newer multigeneration and developmental neurotoxicity study protocols (e.g. OECD, 2007), which include assessments of animals after birth. Another significant change to developmental toxicity protocols has been to increase the numbers of non-rodents per dose group from 12 to 20 animals. This change was made in recognition of the fact that studies in non-rodents were statistically underpowered relative to those in rodents, which themselves still have limitations in terms of detecting rare events. A final modification relates to the examination of cartilage in addition to bone, as this can provide information for judging whether a skeletal alteration represents a variation or a true structural malformation.

As in reproductive toxicity studies, rats are commonly used in developmental toxicity studies, but experience has indicated that the use of a second species (generally a non-rodent like the rabbit) affords greater confidence in identifying agents that are likely to be hazardous to humans because of the recognized variability among species in response to developmental toxicants. Additional information on the use of rabbits in reproductive and developmental toxicity studies has been summarized by Foote & Carney (2000).

Regardless of the approach taken, evaluation of developmental toxicity data is facilitated by the use of common terminology. Glossaries of common developmental abnormalities (Wise et al., 1997) and skeletal anomalies (Solecki et al., 2001), as well as accompanying images, are available on the Internet at http://www.devtox.org/.

4.7.3.4 *Tiered and combined approaches to reproductive and developmental toxicity testing*

A proposal has been developed recently, in the context of pesticide safety assessment, for a tiered approach to toxicity testing at different life stages (Cooper et al., 2006). The aim of the approach is to assess the potential of a chemical to cause adverse effects on reproduction and assess the nature and severity of any effects on development and adolescence. It proposes, for Tier 1, an F_1-extended one-generation reproduction study in the rat and a prenatal developmental toxicity study in the rabbit. Pharmacokinetic studies are rarely performed routinely in pregnant or young animals, but such information is helpful in better understanding dose–response relationships and in placing the results in context with potential human exposure situations. This proposed approach emphasizes the value of using kinetic data in the design and interpretation of life stage studies. A draft protocol for an extended one-generation reproduction study is currently under development by OECD.

The International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) also recommends a tiered approach to testing for the safety assessment of veterinary drug residues in human foods. In the first instance, a two-generation reproduction study in the rat and a developmental toxicity study in the rat should be conducted. If clear evidence of teratogenicity is observed, regardless of maternal toxicity, testing for developmental toxicity in a second species would not be required, unless teratogenicity in the rat was the critical effect for the setting of the ADI. If a negative or an equivocal result for teratogenicity is observed in the rat, a developmental test in a second species, preferably the rabbit, should be conducted. In the absence of teratogenicity in the rat, a developmental toxicity test in a second species would be required even if there were other signs of developmental toxicity in the rat (i.e. fetotoxicity or embryolethality). The VICH guidelines are available at http://www.vichsec.org/en/guidelines2.htm.

4.7.3.5 *Endocrine toxicity*

The state of the science in the area of endocrine toxicity was extensively reviewed by IPCS (Damstra et al., 2002). It is now recognized that the well-established tests for reproductive and developmental

toxicity described above do not necessarily cover the full range of effects that might be induced by chemicals that interfere with the endocrine system. Moreover, these tests are resource intensive and not suited to the initial screening of large numbers of chemicals for endocrine toxicity. Spurred on by the concerns raised during the last decade about chemicals acting as endocrine disruptors and by legislative mandates such as the Food Quality Protection Act of 1996 in the USA, considerable effort has been directed at developing a battery of assays that can evaluate chemicals that interact with the estrogen, androgen and thyroid signalling pathways.

A tiered screening battery was proposed by the United States Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC, 1998) and is in the process of being validated through international cooperation between the USEPA and OECD. Tier 1 of the battery includes in vitro tests of receptor binding and gene activation for estrogens and androgens, a uterotrophic assay to identify estrogens, a Hershberger assay to identify androgens/anti-androgens, a female pubertal assay to evaluate neuroendocrine (estrogenic and thyroid) control of puberty, a frog metamorphosis test to evaluate thyroid effects and a short-term fish reproduction test to evaluate alterations in steroid hormone homeostasis in a lower vertebrate (Gray et al., 2002). As the Tier 1 screening tests are directed at detecting modes of action and not necessarily adverse effects, they serve primarily to trigger other tests (e.g. multigeneration tests) that could confirm a hazard and establish dose–response relationships. Because they can provide insight into potential modes of action, these screening assays should be highly informative at directing attention to specific outcomes in any follow-up dose–response studies, which could be customized to detect the more sensitive end-points. However, it should be noted that for many of the food chemicals that are evaluated by JECFA and JMPR, a reproductive toxicity test is conducted routinely, irrespective of whether the chemical is suspected to be an endocrine disrupter.

It is clear that the methodology for investigating endocrine toxicity is still evolving, and there are currently no generally accepted core requirements beyond the standard developmental and reproductive testing guidelines. The current status of the validation and use of the EDSTAC screening battery (EDSTAC, 1998) by the USEPA can be found at http://www.epa.gov/scipoly/oscpendo/index.htm. The current

status of method validation by the OECD through its programme on Endocrine Disrupter Testing and Assessment can be found at http://www.oecd.org/document/62/0,3343,en_2649_34377_2348606_1_1_1_1,00.html.

4.7.4 Issues specific to category of chemical

There are relatively few examples in reproductive or developmental toxicity where a species is inappropriate for evaluation of a particular class of chemicals. One such example is chemicals that interfere with prolactin, which is essential for the maintenance of early pregnancy in the rat but not in humans. Another example, relevant to the work of JECFA on veterinary drug residues, is oral administration of certain Gram-negative antibiotics in rabbits. The intestinal flora of rabbits is particularly sensitive to this type of antibiotic, and treated dams can develop diarrhoea with reductions in food consumption and body weight, resulting in abortions, resorptions, malformations and fetal growth retardation (reviewed in Chernoff et al., 1989).

Schardein (2000) discussed the appropriateness of various animal models for assessing human risk. As with any toxicity test, it would be most appropriate to utilize a species that metabolizes a chemical in a manner similar to that of humans. However, in practice, such information is usually not available. Another consideration is whether the type of placentation in a particular species influences the degree or nature of the outcome in the fetus. For example, trypan blue is a developmental toxicant in rodents because of its effects on the yolk sac placenta, which is critical for the nutrition of the embryo in rodents. Such effects do not occur in other species in which, like humans, the embryo does not rely on the yolk sac for nutrition.

4.7.5 Interpretation of data

There are a number of publications, mostly developed by regulatory agencies or other bodies, that provide excellent information on the evaluation of reproductive and developmental toxicity data (e.g. USEPA, 1991b, 1996; IPCS, 2001b; Hood, 2006). In addition, the Center for the Evaluation of Risks to Human Reproduction (CERHR), established by the United States National Institute of Environmental Health Sciences, convenes expert panel meetings dealing with chemicals, chemical classes or generic issues related to the evaluation of

data. The basis for the CERHR evaluative process can be found at http://cerhr.niehs.nih.gov/aboutCERHR/index.html#evalprocess.

In interpreting data from both reproductive and developmental toxicity studies, it is important to look for biologically related patterns of response and the relationship of outcomes across end-points and to relate any findings to the larger body of toxicological data available from other bioassays. Outcomes from other toxicity studies can be useful in targeting those end-points in developmental or reproductive toxicity tests that might be expected to be responsive to the agent, as well as assisting in determining potential modes of action. The incidence and severity of the findings should be noted, with comments on the extent to which the effects might be expected to be reversible upon cessation of exposure. Attention should be paid to which life stage is the most sensitive to exposure, although initial studies may not pinpoint the origin of the adverse effect because of the possibility of delay in its appearance.

In developmental toxicity studies, a *malformation* is usually defined as a permanent anatomical structural change that may adversely affect survival, development or function. The term *variation* is used to indicate an alteration in anatomical structure that generally does not adversely affect survival or health. When interpreting the significance of some structural variants, it is important to consider the stage of the fetus at the time of observation. Under most regulatory guidelines, fetuses are removed from the mother 12–14 h prior to the anticipated time of birth, a period of very rapid growth. Even slight perturbations in the growth trajectory can lead to changes in the rate of ossification and increases in the number of variants recorded. Double-staining the skeleton for bone with alizarin R and for cartilage with alcian blue can help distinguish whether bone development is merely delayed or whether there is an underlying morphological alteration. However, distinguishing between variations and malformations is difficult, as there is a continuum of responses from the normal to the extremely abnormal. There is no generally accepted classification of malformations and variations. Other terms that are often used, but no better defined, include anomalies, abnormalities, birth defects, deformations and aberrations.

Appropriate historical control data can sometimes be very useful in the interpretation of data on the incidence of malformations and

variations. Comparison of data from treated animals with data from concurrent study controls should always take precedence over comparison with historical control data. The most appropriate historical control data are those from the same laboratory in which studies were conducted. Even data from the same laboratory, however, should be used cautiously and examined for subtle changes over time that may result from genetic alterations in the strain or stock of the species used, changes in environmental conditions, both in the breeding colony of the supplier and in the laboratory, and changes in personnel conducting studies and collecting data. Study data should be compared with recent as well as cumulative historical data. Although a dose-related increase in malformations is readily interpreted as an adverse developmental effect of exposure to a chemical, the biological significance of an altered incidence of anatomical variations is more difficult to assess and must take into account what is known about developmental stage (e.g. with skeletal ossification), background incidence of certain variations (e.g. 12 or 13 pairs of ribs in rabbits) or other strain-specific or species-specific factors. However, if variations are significantly increased in a dose-related manner, these should also be evaluated as a possible indication of developmental toxicity (USEPA, 1991b).

Because standard study designs require that the top dose exert some minimal indication of maternal toxicity (e.g. a 10% reduction in maternal body weight gain during pregnancy), there is sometimes difficulty in distinguishing whether a developmental effect seen at such a dose is a direct result of the action of the chemical on the embryo or fetus or an indirect result of altered maternal homeostasis. Although there have been several examples of the latter, it is important not to infer causation from an association of developmental toxicity with maternal toxicity without additional analysis and experimentation. Some aspects that should be considered include the following: Is the nature of the developmental manifestation a rare or common event in control offspring? What is the statistical power to detect a maternal versus a developmental event? Does the incidence or intensity of the effect tend to correlate with the intensity of the corresponding maternal response? Does the response occur in common across a number of members of a chemical class? Chernoff et al. (1989), Daston (1994) and Schardein (2000) have discussed various aspects of this issue. For example, significant impairment of maternal renal function by mercury(II) chloride in the rat has relatively minimal effect on rat embryonic development

(Kavlock et al., 1993), whereas the induction of maternal nutritional deficiencies (e.g. zinc deficiency following metallothionein induction) has been causally related to altered pregnancy outcomes (Keen et al., 2003). In any event, maternal and developmental toxicity should not be causally linked merely because of their concurrent appearance on the dose–response curve. However, the larger the spacing between the dose causing a maternal effect and a lower dose causing a developmental effect, the more likely a chemical will pose a developmental hazard to humans, as there would be no warning from maternal toxicity of the impending developmental effect. It is also important to note that some human developmental toxicants, such as lead, methylmercury and alcohol, exert effects on the embryo and fetus at doses that induce maternal toxicity, but the adverse effects are not secondary to the maternal toxicity, and thus the expected exposure conditions for humans are also an important consideration in interpreting such data.

4.7.6 Other considerations

4.7.6.1 *In vitro tests*

A number of assays have been proposed for use in screening chemicals for developmental toxicity. These include the use of lower organisms (e.g. *Drosophila* or *Xenopus* embryos), cell lines (e.g. human epithelial mesenchymal cells, mouse ovarian tumour cells, chick embryo neural retinal cells and various embryonic stem cell lines), primary cell cultures (e.g. neuronal and limb bud cells), avian embryos in ovo and mammalian embryos in culture. None of these tests has yet achieved international acceptance for use in hazard assessment, but they have proven valuable in some situations for understanding structure–activity relationships within chemical classes, as well as potential modes of action for toxicity.

4.7.6.2 *Paternally mediated effects*

Paternally mediated effects are those that are expressed in the offspring via exposure of the male prior to mating. A workshop (Robaire & Hales, 2003) reviewed evidence showing that such effects can occur with certain types of chemical. Most of the emphasis on paternally mediated effects has traditionally been in relation to infertility (e.g. dominant lethal effects), as opposed to evaluations of abnormal pregnancy outcomes (e.g. structural malformations or transplacental carcinogenesis).

In general, chemicals that have been associated with the induction of paternally mediated effects are DNA reactive and exert effects through DNA damage to the sperm. As a consequence, a number of new tests have been developed to serve as biomarkers of genetic and chromosomal integrity of sperm (e.g. chromosome-specific fluorescence in situ hybridization probes, the sperm chromatin structure assay and the comet assay). Because these biomarker tests tend to be technically difficult to perform, they have not received widespread use. For risk assessment purposes, it is important to understand the exposure paradigm in relation to the spermatogenic cycle, the nature of the end-points evaluated and the characterization of any dose–response relationships.

4.7.7 Information gaps

There are also several gaps in current approaches for the assessment of reproductive toxicity, including 1) the lack of longitudinal studies that assess exposed individuals through to senescence, 2) little evaluation of reproductive senescence in particular, 3) very limited evaluations of endocrine function, 4) little or no information regarding pharmacokinetics (this includes age-related studies, sex studies and target organ dosimetry) and 5) no use of acute or chronic exposures for the evaluation of reproductive effects or consideration of latent effects.

Likewise, there are gaps in the testing protocols for assessment of developmental toxicity. These include 1) the limited exposure of the neonatal animal, 2) the general limitation that the studies focus primarily on morphological changes and do not evaluate functional alterations in important systems such as the immune, cardiovascular, respiratory and renal systems, 3) the lack of pharmacokinetic information and 4) the paucity of information related to identification of latent manifestations of toxicity.

4.8 Neurotoxicity

4.8.1 Introduction

Neurotoxicity has been defined as an adverse change in the structure or function of the central nervous system and/or peripheral nervous system following exposure to a chemical (natural or synthetic) or physical agent (Tilson, 1990b; ECETOC, 1992; Ladefoged et al., 1995). The Nordic Council of Ministers defined neurotoxicity as the

capability of a chemical to induce adverse effects in the central nervous system, peripheral nervous system or sense organs and cause a consistent pattern of neural dysfunction or lesion (Johnsen et al., 1992). The crucial term within these definitions is "adverse". Exactly what defines an effect as adverse remains a major point of debate. In a toxicological sense, "adverse" can indicate a detrimental change in structure or function of the nervous system. A commonly accepted definition of adversity is an exposure-related alteration from baseline functioning that diminishes an organism's ability to survive, reproduce or adapt to its environment (ECETOC, 1992; Ladefoged et al., 1995; USEPA, 1998a; IPCS, 2001a). IPCS has also defined an adverse effect as a change in morphology, physiology, growth, development or lifespan of an organism that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other environmental influences (IPCS, 2004).

Neurotoxic effects include a spectrum of biochemical, morphological, behavioural and physiological abnormalities whose onset can vary from immediate to delayed following exposure to a toxic substance and whose duration may be transient or persistent. These effects may be due to a direct action of the substance or metabolites on the nervous system or an indirect action on other biological systems that in turn adversely affect the nervous system (ECETOC, 1992, 1998; O'Donoghue, 1994; Ladefoged et al., 1995; USEPA, 1998a; USFDA, 2000).

4.8.2 Nervous system features

The basic structure and function of the nervous system, as they relate to neurotoxicity, have been comprehensively presented in EHC 60 (IPCS, 1986b) and EHC 223 (IPCS, 2001a). Additional descriptions are available in USEPA testing and risk assessment guidelines (USEPA, 1991a,c), in the IPCS-sponsored workshop efforts on in vitro techniques for neurotoxicity (Harry, 1998) and in other reports (United States Congress, Office of Technology Assessment, 1990; USNRC, 1992; SGOMSEC, 1996).

4.8.3 Evaluation of neurotoxicity

Conventional toxicity studies do allow some evaluation of neurotoxicity; however, these studies provide little information concerning

less severe, but important, types of neurotoxic effects, including behavioural and physiological dysfunction and developmental neurotoxicity. Historically, neurotoxicity was equated with structural changes involving frank neuropathological lesions or overt neurological dysfunctions, such as seizure, paralysis or tremor. However, a significant body of scientific literature has demonstrated a variety of functional and structural abnormalities associated with chemically induced changes at the cellular and molecular level that may occur in the absence of evident structural changes identified using routine neuropathological techniques. Thus, reliance on routine neuropathology does not adequately reflect contemporary concerns about the broader spectrum of potential neurotoxic effects on the organism.

Methods to assess morphological, physiological, biochemical, behavioural and interactive components of nervous system functioning have been included in specific testing guidelines. Current guidelines for neurotoxicity studies have been developed by various national and international bodies, including assessments of general toxicity, gross histopathology and evaluations of behavioural functions (USEPA, 1991a,c, 1998a; ICME, 1994; OECD, 1995b,c, 1997; USFDA, 2000). Guidelines for developmental neurotoxicity studies recommend dosing during defined periods of gestation and lactation and the assessment of postnatal physical and behavioural development, including learning and memory, and neuroanatomical alterations, as appropriate (USEPA, 1991b; USFDA, 2000; OECD, 2007).

4.8.3.1 *Morphological evaluations*

The complexity and integrative nature of the nervous system make reliance on a single end-point problematic. The presence of a gross histopathological lesion in the brain would clearly identify a compound as being neurotoxic; however, discrete lesions are not always detected, even with known neurotoxicants. Any requirement that histopathological or morphological changes must be present as evidence of neurotoxicity is inappropriate and limits the discovery of neurotoxic potential (Ladefoged et al., 1995). Dissociation of neuropathology from functional changes may involve a number of factors, including the intrinsic toxicity of a chemical, the dose and regimen of exposure, the age of the animals exposed and the sensitivity of the tests. In addition, the nervous system maintains a level of compensatory capacity

as a mechanism of repair and has been shown to possibly retain a level of regenerative capacity in certain brain regions. However, although such repair processes exist, they are not fully understood and do not appear to result in the nervous system returning to a completely normal state. Rather, the nervous system returns to a relatively normal state in which it remains somewhat altered and possibly compromised in its response to future insults. Greater understanding of the structural complexity, connectivity and various cell–cell interactions has clearly demonstrated that the level of examination required to identify such discrete changes is significantly greater than that conducted in a general morphological or histopathological examination. However, the level of sensitivity in detection of neuropathological changes can be enhanced by a more careful histopathological examination of the nervous system.

Various types of neuropathological lesions may be classified according to the site where they occur (Spencer et al., 1980; Spencer & Schaumburg, 1985; IPCS, 1986b; Krinke, 1989; Griffin, 1990). Within each general class of nervous system structural alteration, there are various histological changes that can occur. The degenerative process of the nerve cell can be either relatively rapid or prolonged, depending on the underlying mechanism responsible. For example, neurons can degenerate following a direct action on the cell body, following loss of synaptic target site influences, loss of trophic factors or loss of stimulus innervation from other neurons. Each process may require examination along the neuronal projection field to detect the level of injury induced. Guidelines exist for tissue preparation and examination of the nervous system (IPCS, 1986b). However, guidance remains sparse regarding the neuroanatomy of the brain, such as specific brain regions for examination, associated neural pathways, types of cellular alterations and other unique features of "screening" nervous system tissue for damage as compared with other organ systems.

Histological evaluation often relies solely on routine stains such as haematoxylin and eosin; however, the addition of immunohistochemical staining for specific cell types and cell processes can serve to complement traditional histological evaluations. One special stain recommended in various guidance documents is an immunological stain for the major structural protein of astrocytes, glial fibrillary acidic protein. In response to injury and excessive neural activity, the astrocytes

will increase in size, resulting in an increase in this structural protein. This can occur at both the primary site of injury as well as the projection sites of injured neurons. The detection of astrocyte hypertrophy in distinct brain regions can serve as an indicator for additional detailed examination. More recently, microglia, associated with inflammatory processes, have been examined in brain tissue following chemically induced injury, with the initial data suggesting that this response may serve as an early indicator of injury. Unlike the neuron, the astrocyte/microglia response does not appear to be influenced by ischaemia/hypoxia and cell shrinkage that can occur with immersion fixation. At low exposure levels, gross neuronal necrosis and astrocyte hypertrophy may not be evident and indeed may not even play a significant role in the neurotoxicity.

Issues with regard to histological examination of the developing brain have been extensively discussed by Garman et al. (2001). Structural evaluation of adverse effects on the developing nervous system poses a set of questions additional to those associated with histopathology. While acute degenerative lesions can occur in the developing brain, quite often the neuropathology assessment is primarily one of identifying chemically induced alterations in determination of cell fate (numbers and locations) and the normal developmental process. With low levels of exposure, one may assume that a gross necrotic lesion would not be the likely manifestation of damage, but rather a disarrangement of the normal cytoarchitecture of the brain. Some of the proposed methods to evaluate such effects have included both qualitative and quantitative morphological assessment. In addition to histological assessment, quantitative evaluations can be conducted, including end-points such as brain weight and, although not yet validated, morphometric dimensions. Differential sensitivity in the degrees of retardation of brain development may be expected from one area of the brain to another. For example, areas that mature after birth (e.g. cerebral cortex, cerebellum and hippocampus) might be more affected by chemical exposure than are subcortical structures that develop in utero. When examining a delay in development of the brain or an effect on a specific cellular structure, biochemical and molecular methods can be used to more closely examine such effects. For example, ontological profiles of developmentally regulated structural proteins and associated messenger RNAs (mRNAs) can provide evidence of delayed or altered synapse formation, astrocyte maturation or

myelin formation (Toews & Morell, 1999) that can be used to complement morphological findings.

Unlike other organs, the actual size and weight of the brain are relatively unaffected by mild to moderate changes in total body weight. Such "brain sparing" is typically seen in undernourished adult animals but may also occur in the developing animal and does not necessarily preclude delayed or otherwise abnormal brain development. Delayed brain development and smaller brains can be seen in undernourished juvenile animals, yet the ratios of brain weight to body weight for undernourished pups are generally equal to or slightly greater than the ratios for adequately nourished rat pups. Undernutrition can be the result of increased litter size, decreased lactation, decreased maternal nutrition or maternal neglect. Thus, it is critical to control these factors in order to adequately interpret study findings as evidence of chemical-specific neurotoxicity.

Quantitative neuropathological approaches include morphometric evaluation of specific regional structures using linear (linear measurements of a brain or brain region, such as width or length between two specific sites), areal (measurements of the two-dimensional area of a brain region) or stereological measurements (measurements that are assumed to provide a more three-dimensional compilation of two-dimensional measurements of a brain region). Although such quantitative evaluations may offer discrete measurements, there is considerable debate as to the validity of such methods to uniformly represent the brain region of interest, both within a subject as well as between subjects. This debate involves, for example, the variability of these measurements, the many factors that can contribute to these measurements, such as plane of cut through the brain that must be standardized in each study, ill-defined topographical markers, insufficient database, lack of validation of methods for toxicological assessment and varied assumptions underlying each method. More recent imaging methods allow for three-dimensional reconstruction of a brain and the determination of total volume of any specific brain region. Magnetic resonance imaging may allow for an accurate evaluation of altered brain development and identification of specific target sites. However, this is based on the assumption that structural components of the region would be disrupted in a manner that would cause a change in volume. Alterations in the connectivity of a region would

not necessarily be detected using any of these types of structural evaluations.

4.8.3.2 Neurobehavioural evaluation

Evaluation of neurotoxicity is not performed routinely for all chemicals, but only when indicated (e.g. from structure–activity considerations or the results of other toxicity tests). Among the various approaches for assessing neurotoxicity, behavioural testing in conjunction with neuropathological evaluation has been considered a practical approach to assess functional integrity of the nervous system. Behaviour is an adaptive response of an organism, orchestrated by the nervous system, to internal and external stimuli. A behavioural response represents the integrated end-product of multiple neuronal subsystems, including sensory, motor, cognitive, attention and integrative components, as well as an array of physiological functions. Thus, behaviour can serve as a measurable index of the status of multiple functional components of the nervous system.

Behavioural testing has been established as a reliable toxicological index, and considerable progress has been made in the standardization and validation of neurobehavioural testing procedures (IPCS, 1986b, 2001a; Tilson, 1990a; Eisenbrandt et al., 1994; OECD, 1995a,b, 1997; EC, 1996, 1997; Catalano et al., 1997; Moser, 1997; Moser et al., 1997a,b,c,d; Tilson et al., 1997). Neurobehavioural assessment methods are used routinely to evaluate the effects of developmental neurotoxicants on sensory, motor and cognitive functions (Tilson, 1998; Cory-Slechta et al., 2001). It is important to recognize that as neural function interacts dynamically with the status of other organ systems (e.g. cardiovascular, endocrine and immunological systems), certain patterns of behavioural change may indirectly reflect significant primary toxicity in those other organ systems.

4.8.3.3 Developmental neurotoxicity

Developmental neurotoxicity has been defined as any effect on the developing nervous system before or after birth that interferes with normal nervous system structure or function. IPCS (1986b, 2001a) addressed some of these concerns and highlighted specific differences between the adult and immature nervous systems. The developing

nervous system as a unique target system for adverse effects has been addressed in an ILSI-sponsored workshop with a review of testing methods and assessments of nervous system injury. This review considered available testing guidelines and identified approaches that can be used to assess adverse effects following exposure during development (Cory-Slechta et al., 2001; Dorman et al., 2001; Garman et al., 2001; Mileson & Ferenc, 2001). Since then, the OECD has adopted a guideline for developmental neurotoxicity (OECD, 2007). Additional concern for adverse effects on the developing nervous system has been presented in many reviews regarding endocrine disrupting agents (USNRC, 1993, 1999; USEPA, 1998a,b; EC, 1999; Damstra et al., 2002).

It has long been known that critical windows of vulnerability exist during the formation and maturation of the nervous system (e.g. the period of the brain growth spurt) (Rodier, 1990; Isaacson & Jensen, 1992a,b). The mammalian central and peripheral nervous systems are complex structures resulting from critically timed developmental processes, including cell proliferation, differentiation, apoptosis, migration, synaptogenesis and myelination. Each brain region develops according to specific and unique temporal profiles, with a critical interdependence between each structure for stimulus input and projection target sites. The final neural network pattern is dependent upon the integration of selective neural connections between all cell types of the brain. This process begins during prenatal life and continues through adolescence, with plasticity throughout adult life.

In evaluating the potential of a chemical to disrupt the formation and maturation of the neural network, a number of factors must be considered. These include 1) the developmental stage of the target tissue or the specific nervous system component, 2) the mode or mechanism of action of the toxic agent, 3) the dose of the agent delivered to the target tissue, 4) the toxic end-point of interest, 5) the age of the offspring during testing and 6) the method used to evaluate the outcome. Toxicological effects on the nervous system depend on the delivered dose, exposure duration and the developmental stage at which exposure occurred. Pharmacokinetic processes governing chemical disposition within the adult and in the offspring will also have an influence (see review by Dorman et al., 2001). In addition, unique physical features such as the placental barrier and the maturation of the blood–brain

and blood–nerve barriers significantly influence chemical disposition. Neonatal exposure may depend on maternal pharmacokinetic processes and transfer of the substance through the milk, although direct exposure can occur from other routes.

4.8.4 Tiered testing strategy

A number of expert groups have recommended tiered testing strategies for the evaluation of chemically induced neurotoxicity (e.g. IPCS, 1986b; United States Congress, Office of Technology Assessment, 1990; USNRC, 1992; EC, 1996; USFDA, 2000). The initial phase of a tiered testing strategy is the identification of neurotoxicity at some dose level (hazard identification). Tests designed to measure the presence or absence of an effect are usually different from those used to assess the degree of toxicity or type of toxicity or to determine the lowest exposure level required to produce an effect (Tilson, 1990a).

Screening procedures are first-tier tests typified by their capability to assess a large number of animals. Such procedures do not require extensive resources, are usually simple to perform and can yield semiquantitative data (Moser, 1989, 1995; O'Donoghue, 1989; Schulze & Boysen, 1991; Moser et al., 1997a,b). Systematic clinical observation, such as the USEPA's functional observational battery, is considered an essential part of first-tier testing. Clinical signs have been criticized as being highly variable and poorly documented. Thus, numerous efforts have been made to place observation of clinical signs under a systematic protocol. For any first-tier test, a screening technique should include the following: 1) clearly defined methods and end-points, 2) quantified end-point using an explicitly stated rating scheme, 3) trained and experienced observers and 4) an adequate number of end-points assessed to evaluate multiple modalities of nervous system function. Observations should detect signs of significant neurological disorders, behavioural abnormalities, physiological dysfunctions and any other signs of nervous system toxicity. In addition to the animal's physical appearance, body posture and weight, the clinical screen should provide sufficient information to assess the incidence and severity of such end-points as seizure, tremor, paralysis or other signs of neurological disorder, the level of motor activity and alertness, the animal's reactivity to handling or other stimuli, motor coordination and strength, gait, sensorimotor response to primary

sensory stimuli, excessive lacrimation or salivation, piloerection, diarrhoea, polyuria, ptosis, abnormal consummatory behaviour and any other signs of abnormal behaviour or nervous system toxicity. Assessment of cognitive functioning is not usually a component in first-tier screens. The specific composition of the screen and the end-points to be recorded should be consistent with the particular focus of the study and be appropriate for the age and species of the animals to be tested.

Although observational methods are conceptually the most straightforward, they are also the easiest to confound and can sometimes be difficult to interpret without some internal or external corroboration of results. A quantitative measure of locomotor activity, limb grip strength and hindlimb foot splay can be considered as first-tier tests. Often, such functional tests are used in conjunction with other methods, including neuropathology. Given the various biological modalities encompassed in nervous system function and the numerous end-points examined, questions can arise concerning the significance of a change in any one specific screening end-point. As a result of the IPCS-sponsored international collaborative study on neurobehavioural methods for the functional observational battery, motor activity and grip strength, a clustering approach was proposed as one method to deal with such data (Moser et al., 1997a,b,c,d). This approach clusters the various observations into functional domains that represent common neurobiological processes (i.e. autonomic, motor and sensory function), generating a composite response to reflect the functional integrity of a given subset of neurological processes. This approach would allow data to be evaluated within a small number of neurobiologically meaningful clusters rather than numerous isolated end-points. In all cases, it is important that the neurotoxicity screening information be supplemented with any other relevant toxicological findings.

There are a number of publications to guide the design and conduct of testing appropriate for neurotoxicity screening of the adult (Deuel, 1977; Tupper & Wallace, 1980; Gad, 1982, 1989; Vorhees, 1987; O'Donoghue, 1989; Broxup, 1991; Schulze & Boysen, 1991; USEPA, 1991c; Tilson & Moser, 1992; Chang & Slikker, 1995; Moser et al., 1997a,b) and the developing organism (Buelke-Sam et al., 1985; Wier et al., 1989; Rees et al., 1990; Rodier, 1990; Nelson, 1991; USEPA, 1991b; Slikker, 1997).

The second tier of neurotoxicity testing utilizes more specific tests than the first tier and is designed to characterize the nature and dose–response for the neurotoxic effect. A decision to test at the next tier is based on data suggesting that an agent produces neurotoxicity, including neurotoxicological data already in the literature, structure–activity relationships, data from first-tier testing or reports of specific neurotoxic effects in humans. The choice of the most appropriate approach is dependent on the scientific questions generated by the results of the first-tier testing or other available data. These specialized tests are often more sensitive, may contribute information concerning mode of action and are aimed at objectively quantifying effects and determining NOAELs or BMDs. Second-tier tests often yield graded or continuous data amenable to routine parametric statistical analysis.

Third-tier testing may involve mechanistic studies that attempt to establish a detailed profile of a chemical's effect at several levels of nervous system organization (i.e. behavioural, physiological, cellular, molecular). Such tests could provide detailed information on enzyme function, ionic balance, signal transduction, transmitter systems, receptor modulation and underlying molecular mechanisms as they relate to the pathogenesis of effects. It is from such studies that understanding of the processes underlying neurotoxicity and specificity of effect is gained. Mechanism or mode of action studies, when linked to the pathogenesis, provide a basis for the development of biologically based models of neurotoxicity.

4.8.5 Cholinesterase-inhibiting compounds

Inhibition of a specific enzyme, acetylcholinesterase (AChE), has been shown to occur with some neurotoxicants, such as the organophosphate and carbamate pesticides. This enzyme hydrolyses the neurotransmitter acetylcholine, and inhibition results in prolonged action of acetylcholine at receptor sites. Objective clinical measures of cholinergic overstimulation (e.g. salivation, sweating, muscle weakness, tremor, blurred vision) can be used to identify such an effect and the dose–response relationship (Moser, 1995). Generally, the acute cholinergic effects of anticholinesterase compounds are viewed as reversible (ECETOC, 1998), although longer-lasting effects have been reported in animals (Tandon et al., 1994; ECETOC, 1998). Tolerance may be observed following repeated exposure to cholinesterase-inhibiting

chemicals; however, the cellular mechanisms associated with this process may lead to other effects not present at the time of initial exposure (Bushnell et al., 1991). There is currently no experimental evidence for lasting or persistent effects of repeated exposure to organophosphates at levels that do not produce significant inhibition of brain AChE (Ray, 1999). Depending on magnitude and time course, a given depression in red blood cell or brain AChE activity may or may not be accompanied by clinical manifestations. Reductions in brain AChE are usually considered as adverse, whereas reductions in plasma and red blood cell cholinesterase are considered as indicative of possible adverse effects. Reductions in plasma butyrylcholinesterase serve as biomarkers of exposure. Low levels of inhibition of AChE are tolerated, whereas inhibitions of 20% or more are considered to be significant for risk assessment purposes. All available data on brain, blood and other tissue cholinesterase activity, as well as the presence or absence of clinical signs and neuropathology, should be evaluated for cholinesterase-inhibiting chemicals on a case-by-case basis using a weight of evidence approach (ECETOC, 1992; Padilla et al., 1994; USEPA, 1998a).

A subset of organophosphate agents, such as tri-*o*-cresylphosphate and leptophos, can produce a delayed neuropathy (organophosphate-induced delayed neuropathy [OPIDN]) after acute or repeated exposure. This degenerative process involves primarily demyelination of long axons of both the peripheral nerves and the spinal cord. It is not clear whether this process occurs in all species; however, humans are known to be highly susceptible, and the adult hen is the experimental animal model of choice. Chemicals that can cause OPIDN in the hen are generally regarded as unacceptable for use as pesticides. The observed ataxia is clinically "irreversible", although the picture can change from a flaccid paralysis (peripheral nerve plus central nervous system lesions) to a spastic paralysis (central nervous system lesions only). Initiation of OPIDN has been associated with the inhibition and "ageing" of neuropathy target esterase (NTE) (Johnson, 1990; Richardson, 1995). Comparison of the semi-log relationship between dose and NTE inhibition and clinical manifestation suggests that more than 70% of NTE inhibition/ageing is required for OPIDN to develop.

4.8.6 Alternative test methods

Attention has been directed to the development of in vitro systems for assessing the neurotoxicological impact of chemical agents (United

States Congress, Office of Technology Assessment, 1990; Harry, 1998; USEPA, 1998a; USFDA, 2000; IPCS, 2001a). The nervous system is composed of highly specialized, heterogeneous, integrated populations of cells. Thus, it is unlikely that a single in vitro test or even a battery of in vitro tests would be able to mimic the responses of the nervous system to a broad range of chemically induced toxicity. Given the complicated nature of the interdependent interactions of the various cell types and network processes in the nervous system, it would be unwise to conclude that a chemical does or does not have neurotoxic potential based upon data from in vitro systems alone. However, batteries of in vitro tests do offer the possibility of developing additional or more appropriate first-tier screening methods for inclusion in a test battery.

This does not diminish the value of information gained from in vitro test systems; it just emphasizes the requirement that any such data be placed within the framework of a limited representation of nervous system function and the toxicokinetics of a given substance. In general, the consensus is that in vitro/alternative test systems offer the greatest strength in hypothesis-based mechanistic studies (Harry, 1998) that may allow one to refine subsequent second-tier study designs, resulting in an overall reduction in animal use.

4.8.7 Interpretation of data

Neurotoxicity is one of several non-cancer end-points that share common default assumptions and principles. The evaluation of the validity of the database is a primary step in the interpretation of data as indicative of a potential neurotoxic effect. This requires four principal questions to be addressed to provide a useful framework for evaluating either laboratory animal or human studies or the weight of evidence for any given chemical (McMillan, 1987; Sette & MacPhail, 1992; Health Canada, 1994; Hertel, 1996; IPCS, 2001a):

1) Do the effects result from exposure?

2) Are the effects neurotoxicologically significant?

3) Is there internal consistency among behavioural, physiological, neurochemical and morphological end-points?

4) Are the effects predictive of what will happen under various conditions?

Although there are known differences between experimental animals and humans in sensitivity to some neurotoxicants, available data support the general assumption that an agent that produces an effect in the laboratory animals will pose a potential hazard to humans (Kimmel et al., 1990; Kulig, 1996; Spencer et al., 2000). Criteria for the quality of data necessary for use in risk assessment to represent the pattern of effects seen in vivo or to define neurotoxicity have been addressed in detail by IPCS (2001a). In general, the value of test methods for quantitative neurotoxicity risk assessment is related to a number of criteria, including 1) sensitivity of the test method to detect differences between exposed and non-exposed groups, 2) specificity for neurotoxicity end-point in a chemical exposure, 3) reliability (consistency of measurement over time) of both the measurement and the effect and 4) validity (concordance with other behavioural, physiological, biochemical or anatomical measurements of neurotoxicity). A relationship between exposure level and severity of response or inclusion of additional functional effects adds support for the observed neurotoxicity. Impairment across a number of functional domains lends support to characterization of an effect within a specific component of the nervous system (e.g. motor, sensory). Comparability of test methods across experimental animals and humans as well as information on underlying mechanisms associated with the neurotoxic response are of particular value. These issues are discussed in detail in USEPA (1998a) and IPCS (1986b, 2001a).

4.9 Immunotoxicity

4.9.1 Introduction

Immunotoxicology focuses on unintended modulation of the immune system. Effects that may occur include immunosuppression, immunostimulation, hypersensitivity and autoimmunity. These may result in outcomes such as increased incidences of infectious or neoplastic diseases, allergy/asthma or autoimmune diseases. To date, immunotoxicity risk assessment efforts have focused primarily on the potential for chemicals to suppress the immune system, as there is a general acceptance of the relevance of immunosuppression end-points in humans and experimental animals for the determination of human risk (see reviews by Vos & Van Loveren, 1998; Descotes, 2003; Luebke et al., 2006), and on identifying allergic contact sensitizers (see section

4.10 and reviews by Basketter et al., 2002; Gerberick et al., 2007; Van Loveren et al., 2008).

Numerous studies have been published suggesting that while immunosuppression is not a common occurrence in the human population, it is not rare. A number of epidemiological studies suggest that alterations in immune responses have arisen as a result of exposure to chemical contaminants in foods (reviewed in Luster et al., 2005).

4.9.2 Assessment of immunotoxicity

4.9.2.1 Laboratory animal studies

Although the toxicokinetics of some chemicals may differ between experimental animals and humans, rodents have proven to be useful models for examining the immunotoxicity of compounds that do not have species-specific effects because of the similarities in rodent and human immune systems. However, some degree of caution must be exercised, as there are instances where concordance between the effects in humans and other species, or even between different rodent species, does not occur. Toxicokinetic data may provide useful information with regard to interspecies differences. Immune system changes observed at overtly toxic dose levels should be interpreted cautiously, as stress and malnutrition are known to impair immune responsiveness. Inclusion of a positive control group, exposed to a well-characterized immunosuppressant, is important in data interpretation and to validate the robustness of the assays conducted.

(a) Standard toxicology studies

Data from standard toxicology studies, such as those conducted in accordance with OECD Test Guideline No. 407 (OECD, 2008) and the ICH S8 guideline (ICH, 2005), provide insensitive, but sometimes useful, information on immunological end-points. Changes in immune system parameters may accompany generalized toxicity affecting all organ systems, reduced body weight secondary to reduced food consumption and significantly reduced protein or micronutrient intake, or stress responses that induce increased corticosteroid production. Under these conditions, altered immune system end-points should be interpreted with caution, as they are unlikely to occur at doses that

do not cause generalized toxicity. In the absence of overt toxicity, lymphoid organ weights (absolute and relative) are useful, as they are suggestive of dystrophic or dysplastic changes. However, alterations in mean organ weights are by themselves poor predictors of immunotoxicity, and changes in immune system organ weights should not be the sole criteria used to determine immunotoxicity. Instead, these data should be considered along with other changes (e.g. functional immune response, histopathological parameters) as part of a weight of evidence approach to evaluate whether immunosuppression has occurred.

Haematological data, including erythrocyte counts, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelet count, total number of leukocytes and leukocyte differentials, as well as clinical chemistry data, such as the ratio of albumin to globulin, total immunoglobulin levels (if available) and a liver enzyme panel, are often included in standard toxicology studies. These end-points provide baseline information on other organ systems that may affect the immune system, as well as basic information on the supply of immune cells. For example, changes in erythrocyte parameters or leukocyte counts may indicate altered bone marrow function and the potential for decreased production of immune cell precursors, and shifts in the ratio of albumin to globulin may signal decreased antibody synthesis. Changes in these end-points may suggest that specific immune function assays are necessary to determine the existence of immunosuppression; however, these data alone are not considered to be reliable predictors of immunotoxicity, as these end-points may be within normal limits, even in children with primary immunodeficiencies.

(b) Immunology studies

Immunotoxicologists have applied tiered panels of assays to identify suppressive immunomodulatory agents in laboratory animals. The configurations of testing panels vary, but they typically include assessment of more than one of the following: 1) lymphoid organ weights and histopathology, 2) quantitative assessment of lymphoid tissue cellularity and peripheral blood haematology, 3) immune cell function at the effector or regulatory level and 4) host resistance studies involving infectious or neoplastic challenge. The first tier is usually a screen for

immunotoxicity, whereas subsequent tiers consist of more specific or confirmatory studies, host resistance studies or in-depth mechanistic studies.

Histopathology. From a histological standpoint, assessment of the mammalian immune system is fairly complex. It is composed of multiple organs and tissues, some of which are responsible for haematopoiesis (bone marrow), others for lymphocyte maturation (thymus) and still others that generate responses to antigen (lymph nodes and spleen). In addition, there are specialized tissues located throughout the body that are responsible for responding to antigens or pathogens locally (e.g. lymphoid tissues associated with the skin, lung and gut). Alterations in function in these tissue-associated lymphoid tissues can result in unique adverse effects. The biological processes responsible for the immune response suggest that immunotoxic chemicals that operate by altering antigen recognition or antigen-dependent responses would most likely manifest histopathology in secondary lymphoid organs (spleen, lymph node), coinciding with an active immune response. In contrast, agents that operate through nonspecific cytotoxic or antiproliferative processes would be expected to present histopathology in both primary (thymus) and secondary lymphoid organs, being more apparent in lymphoid organs that undergo extensive proliferation and self-renewal.

Gross and microscopic examinations of lymphoid tissues are important steps in the assessment of the potential for compounds to induce immunotoxicity. A number of studies indicate that histopathological evaluations of lymphoid tissues can be good predictors of potential immunotoxicity, provided that an appropriate level of stringency (histological score) is applied when assessing lesions and that standardized scoring, quality assurance and controls are used to ensure that subtle histopathological lesions can be consistently identified (ICICIS Group Investigators, 1998; Harleman, 2000; Germolec et al., 2004a,b). Histological lesions, particularly in the thymus, have been shown to be sensitive indicators of immunotoxicity, and lesions in the thymic cortex correlate well with altered antibody production. The use of histopathology as a screening tool for immune system toxicity would be advantageous, as these evaluations could be conducted during routine toxicology studies, such as the 28-day rodent study, without the need for additional animals (Kuper et al., 2000).

A working group within the Society of Toxicologic Pathology has developed and published a Best Practice Guideline for the routine pathology evaluation of the immune system, which identifies specific methodology and standardized terminology most appropriate for the detection and reporting of histopathological alterations to immune tissues (Haley et al., 2005). This working group agreed that three primary points should be emphasized when following the recommended "semiquantitative" evaluation of changes in lymphoid tissues: 1) lymphoid tissue sections should contain separate compartments that support specific immune functions, 2) these separate compartments should be evaluated individually for changes and 3) descriptive, rather than interpretive, terminology should be used to document changes within each compartment.

Histopathological evidence may be available from a range of tissues, and the utility of the data for risk assessment would depend on the degree of pathology, the extent of involvement of multiple organs and the biological rationale and likelihood of the histopathology to represent an adverse response to chemical exposure. For example, a lesion within the thymus or bone marrow may suggest suppression. However, a bone marrow lesion that is characterized by reduced progenitor cells in the bone marrow with a resulting reduction in specific cell types in the thymus or peripheral blood is stronger evidence that functional defects are likely to occur. Histopathology, haematology and clinical chemistry changes can also provide information in a weight of evidence approach to support immunotoxicity.

Lymphocyte phenotyping. Lymphocyte phenotyping is one of the most commonly utilized clinical measures of the immune system. Lymphocyte counts do not usually correlate with changes in immune function or host resistance unless marked changes occur. However, reductions in specific lymphocyte populations can be good indicators of overall changes in immune function (Luster et al., 1992). In addition, because lymphocyte phenotyping can be conducted in human studies, use of this measure in laboratory studies allows for comparison of effects across species. A number of different flow cytometry protocols are available for lymphocyte phenotyping, and standard protocols have been established following interlaboratory comparisons (e.g. Burchiel et al., 1997). To perform the assay, single-cell suspensions are prepared from blood or spleen (although thymus, lymph

nodes or bone marrow preparations are also used), stained with cell surface marker–specific antibodies and analysed by flow cytometry. A wide variety of commercial cell–type specific antibodies are available that bind to cell surface antigens, such as OX19+, the pan T cell marker in rats, or OX8+, which, when combined with OX19+ antibodies, identifies CD8+ T cells. Changes in lymphocyte subpopulations can be expressed as either a change in the absolute number of a specific cell type or a change in relative cell populations (i.e. ratio of CD4 to CD8).

Functional measures of immune responses. A detailed description of tests and methods used to screen compounds, evaluate resistance to infection or neoplastic challenge or determine mode or mechanism of action is beyond the scope of this chapter. Reference works (e.g. Burleson et al., 1995; Vohr, 2005) are an excellent source of detailed protocols and discussions of assay merits and shortcomings. The information that follows is a brief description of the tests that are commonly used to evaluate immune function in laboratory animals.

Humoral immunity—The utility of the T cell–dependent antibody response (TDAR) as a marker for immunosuppression hazard identification is 2-fold: 1) antibody synthesis is crucial for successfully controlling a wide range of infectious agents and associated toxins, whether immunity is the result of a previous infection or the result of deliberate immunization; and 2) antibody synthesis requires that a complex series of events take place, involving multiple cell types and multiple cellular products. The TDAR requires functional macrophages (antigen processing), T_H cells (source of stimulatory cytokines) and B cells (differentiation into antibody-producing plasma cells) and is generally considered to be an excellent indicator of overall immune function, especially when combined with certain routine toxicology tests, such as thymus weights (Luster et al., 1992). A variety of methods have been used to evaluate TDARs, particularly measuring antibody responses following immunization with sheep red blood cells or keyhole limpet haemocyanin. The number of antigen-specific antibody-producing cells can be measured in the spleen (plaque-forming cell assay or enzyme-linked immunosorbent spot [ELISPOT]) or from serum samples (enzyme-linked immunosorbent assay [ELISA] or haemagglutination assays). By varying the detecting antibodies in the latter assay systems, specific antibody subclasses can be quantified.

Cell-mediated immunity—Cellular immunity is traditionally thought of as reactions mediated by T cells, exclusive of the T_H component of antibody responses. Cytokines released by antigen-specific T cells amplify inflammatory responses against intracellular pathogens, down-regulate normal immune responses to prevent tissue damage, affect contact-dependent killing of altered host cells and suppress the activity of self-reactive cells associated with autoimmunity. In cell-mediated responses to pathogens, sensitized CD4+ T cells (from an earlier encounter or from immunization with specific proteins) respond to a challenge by producing cytokines that provide the activation signals required by macrophages to become bactericidal or cytolytic and participate in eliminating the infection. The delayed-type hypersensitivity (DTH) response provides a comprehensive assessment of the ability of T cells to respond to intracellular infections. The DTH response is used not only clinically to determine whether individuals have been previously exposed to a certain organism (e.g. *Mycobacterium tuberculosis*), but also as a measure of T cell reactivity, by testing with antigens that the majority of the population will respond to. Following intradermal injection of an extract of the organism, significant swelling and redness will be apparent 24–48 h later in individuals who have been sensitized by prior exposure to the organism. The response is referred to as "delayed" because of the time lag between antigen challenge and the host response. Immunotoxicologists evaluate the DTH response by immunizing animals to antigens such as egg or bovine serum albumin or keyhole limpet haemocyanin, typically by subcutaneous injection in combination with an adjuvant. The animal is subsequently challenged by intradermal injection of the same antigen, and swelling at the injection site is carefully measured after an additional 24 h.

Cytotoxic T lymphocytes play a central role in destroying chemically or virally modified host cells and neoplastic cells bearing tumour antigens. Their function is typically assessed by culturing antigen-primed T cells, generated either in vivo or in vitro, with labelled tumour cells or foreign lymphocytes and measuring label release. Because clonal expansion of antigen-specific cells is critical to immune function, the proliferative capacity of T cells has been used as an ex vivo correlate of clonal expansion, although the predictive value of the assay is limited (Vos & Van Loveren, 1998). Thus, an in vitro proliferative response to foreign cells such as allogeneic lymphocytes (e.g. the mixed lymphocyte response) or direct stimulation of the T cell receptor using an

antibody to the receptor (anti-CD3) can be used as a functional correlate of T cell replication. The potential ability of lymphocytes to proliferate in response to nonspecific agents, known as mitogens, which stimulate lymphocytes to enter the S-phase of the cell cycle, has also been utilized as an indicator of overall immune system health, both clinically and in experimental animals. Mitogens are commercially available that stimulate proliferation of T cells, B cells or both subsets of lymphocytes. Because antigen receptors are not engaged and the normal process of responding to an antigen is bypassed, these relatively nonspecific measures of cell-mediated and humoral-mediated immunity have proven to be of limited predictive value (Luster et al., 1992).

Innate immunity—Innate immunity refers to responses that do not require antigen recognition or cell division/maturation. Some measure of innate immune function is generally included in tiered testing panels, although the specific end-points may vary depending on potential targets or regulatory requirements. The methods employed to evaluate the functional status of macrophages and neutrophils following exposure to suspected immunotoxicants vary considerably, ranging from measures of phagocytic activity to release of a growing list of soluble mediators to complex bactericidal or tumoricidal activities, including the release of reactive oxygen or nitrogen. Tumor cell lysis by natural killer (NK) cells is one of the primary tests of innate immune function and immunotoxicity associated with chemical exposure. Lytic function is measured by quantifying the proportion of tumour cells (target cells) that have been lysed following co-incubation with NK cells (effector cells) collected from the spleen or peripheral blood.

Disease resistance measures or host resistance assays. The major function of the immune system is to protect the individual from infectious or neoplastic disease. As practised in immunotoxicology, experimental animals are challenged with sufficient numbers of transplantable tumour cells or pathogenic organisms to produce disease at a low level or in a small number of control animals. These "host resistance assays" are often considered particularly relevant for validating the usefulness of other methods to evaluate immune function and for extrapolating the potential of environmental agents to affect clinical disease in the human population. Host resistance models that utilize human pathogens have been developed for use in experimental animals; these and

others that closely mimic human disease processes are most commonly employed. In general, host resistance assays represent the final level of the screening process and are conducted only when there are indications of alterations in immune function in the primary screen. Although host resistance assays are often considered to be the ultimate predictor of adverse effects, functional immune tests are predictive of host resistance (Luster et al., 1993). Although it is relatively rare for compounds that produce no alterations in functional immune tests to affect disease resistance in the commonly used models with the increasing sensitivity of the end-points used in host resistance tests, these types of studies may detect suppression of immunity at dose levels where no effects are observed in specific functional tests (Van Loveren, 1995).

Because the immune mechanisms that mediate resistance differ for different pathogens, a single host resistance model is usually not suitable to study all possible consequences of immunosuppression. Selection of particular challenge models (see Table 4.2) is based upon experimental considerations, such as the route of chemical exposure and results obtained from initial immune evaluations, which provide an indication of which immune cells or processes are targeted by the toxicant. Although some models have been adapted for use in both rats and mice, to date, the majority of host resistance studies conducted have been in the mouse. Reference materials are available that contain background information and specific protocols for the conduct of these studies (e.g. Burleson et al., 1995; Coligan et al., 2005).

(c) Evaluation of allergic contact dermatitis

Guinea-pigs were traditionally used to test the sensitizing potential of chemicals, but animal costs, sensitivity issues and subjectivity of the assay end-point led to the development of other assays (Burleson et al., 1995). The mouse ear swelling test (MEST) is similar to the guinea-pig assay in that both immune sensitization and elicitation of an immune response phase are required. In the MEST, a compound is applied to the ear pinna and evaluated by measuring changes in ear thickness following challenge. An alternative test is the local lymph node assay (LLNA), in which the test material or appropriate control is applied topically in three successive daily applications to both ears of the test species, usually the mouse. Cell proliferation is subsequently measured in the lymph nodes draining the ears. At least one

Table 4.2. Commonly employed disease resistance models

Challenge agent	End-point measured
Listeria monocytogenes	Colony-forming units in spleen and liver, morbidity
Streptococcus pneumoniae	Morbidity
Plasmodium yoelii	Parasitaemia
Influenza virus	Morbidity, tissue burden
Cytomegalovirus	Morbidity, tissue burden
Trichinella spiralis	Numbers of parasites in muscle or intestine
PYB6 sarcoma	Tumour incidence (subcutaneous)
B16F10 melanoma	Tumour burden (lung nodules)

concentration of the test chemical must produce a 3-fold increase or greater in lymphocyte proliferation in the draining lymph nodes of test animals compared with vehicle-treated control mice to be considered a positive. The LLNA is currently the method of choice for determining skin sensitizing potential, as it provides a marked refinement and reduction in animal use compared with guinea-pig assays without a loss of accuracy (Dean et al., 2001; Basketter et al., 2002; Gerberick et al., 2007).

4.9.2.2 *Human studies*

Retrospective epidemiological studies have typically been employed to detect potential immunotoxicity in humans following inadvertent exposure to chemicals. The method has been used to evaluate individuals with transient high-level occupational exposure, small cohorts following accidental exposures or large cohorts with chronic low-level exposures. The assessment of immunotoxicity in humans is complicated by the need to account for confounding factors, such as genetic diversity, age and lifestyle factors (e.g. tobacco, alcohol or drug use). Testing strategies for assessing immunological effects in individuals potentially exposed to immunotoxic chemicals have been detailed in EHC No. 180 (IPCS, 1996), EHC No. 212 (IPCS, 1999) and EHC No. 236 (IPCS, 2006a), and the reader should refer to these documents for a more comprehensive discussion of the clinical measures that may be employed. In general, immunological testing has been limited to one or two assays that are relatively insensitive measures (e.g. lymphocyte counts or immunoglobulin levels) and are best at

identifying severe immunological effects, rather than mild to moderate changes in immune responses. Some of the more comprehensive immunotoxicology studies in humans have demonstrated immunosuppression in different populations of children following prenatal or postnatal exposure to persistent organochlorine compounds (e.g. polychlorinated biphenyls [PCBs]) via maternal diet and breast milk (reviewed in Luster et al., 2008).

Although human immune function data are generally not incorporated in human retrospective epidemiological studies, these types of data represent the strongest evidence of immunosuppression. However, a few studies have measured antibody titres to common vaccine antigens following immunization in adults (Sleijffers et al., 2003). Similar studies, conducted in conjunction with established vaccination programmes for newborns and young children (e.g. measles, diphtheria, tetanus and poliomyelitis), present a significant opportunity to assess chemical-induced alterations in immune status in populations with identified chemical exposure. Reduced antibody responses following immunization with several childhood vaccines have been observed in infants and children with perinatal exposures to PCBs (Weisglas-Kuperus et al., 2000; Heilmann et al., 2006).

Surface marker analysis (immunophenotyping) and serum immunoglobulin levels are the most commonly employed tests to evaluate immunological changes in human studies. These tests are routinely conducted in large hospitals and have provided considerable information on the ontogeny and activation state of the human immune system. In many human studies, statistically significant differences have been found between the control and case populations with respect to serum immunoglobulin levels and cell surface marker analysis of lymphocytes. However, because of the large variability in historical control values, case values may be significantly different from control values, while being within historical normal ranges. This was observed in a study of children with halogenated aromatic hydrocarbon exposure (Weisglas-Kuperus et al., 1995). However, exposure was also associated with a significant increase in inner ear and respiratory infections (Weisglas-Kuperus et al., 2000). These data indicate that exposure may result in minimal to mild shifts in observational end-points, essentially clustering at one end of the normal range. As such, when evaluating observational immune system data collected during epidemiological

studies, data obtained from routine toxicity testing (e.g. immunoglobulin levels, white blood cell counts, immunophenotyping) or functional data (e.g. vaccine titres) to identify potential immune system hazards, emphasis should be placed on statistically significant differences in values for exposed and appropriately matched controls, rather than on whether values for the exposed population fall within a broad range of normal.

4.9.3 Interpretation of data on immunotoxicity

As of 2009, formal guidance for chemical immunotoxicity risk assessment has not been published, although efforts are under way in the USA and Europe to develop guidelines.

In order to accurately predict the immunotoxic risk of exposures in human populations, a scientifically sound framework should be used to support an accurate and quantitative interpretation of experimental and epidemiological studies. Thus, when reviewing immunotoxicology data, it is important to examine multiple end-points and to determine that the results are biologically plausible. Regardless of the end-point being measured, data generated to assess immunotoxicity must be considered in their entirety, including dose responsiveness, general indications of toxicity, the appropriateness of the test methods and the historical predictive value of the data. It is important that information on immunosuppression be considered together with other health effects in the overall characterization of risk.

4.9.4 Conclusions

Immunosuppression represents a series of complex cascading cellular and organ-related events that can lead to an increased incidence or severity of infectious and neoplastic diseases. Unintended immune stimulation is not well understood, but can lead to increased allergic and autoimmune responses. Therefore, it is not surprising that the data from experimental immunotoxicology or epidemiological studies that are used to address quantitative risk assessment issues require careful interpretation. To improve risk assessment for immune system toxicity, it will be necessary to increase our understanding of the underlying immunomodulatory mechanisms that cause adverse effects and the quantitative relationships between the immunological

tests conducted in the laboratory and actual disease in human populations. This is particularly true when the magnitude of immunological effects is slight to moderate, as may be expected from inadvertent exposures to immunosuppressive agents in the food supply that have been linked to adverse health effects. It is therefore critical to address the potential risks of immune effects following dietary exposures to chemicals, as they have the potential to increase both the burden of disease and the costs of caring for affected individuals.

4.10 Food allergy and other food hypersensitivities

4.10.1 Introduction

Food allergy and other food hypersensitivities are adverse reactions to specific foods and food ingredients occurring in sensitive individuals within the general population (Ebo & Stevens, 2001). These food hypersensitivities are considered individualistic responses, in that most individuals are able to consume these foods without adverse consequences (Taylor & Hefle, 2001). Hence, these types of sensitivities do not include general toxic reactions to foods and food ingredients that could affect any consumer without discrimination provided the ingested dose of the toxic agent is sufficient.

Previously, food allergy was identified as a "form of food intolerance", where there existed "evidence of abnormal immunological reaction to a food" that is "mediated by immunoglobulin E" (IgE). Food intolerance has been defined as "a reproducible, unpleasant reaction to a food or food ingredient, including reactions due to immunological effects, biochemical factors such as enzyme deficiencies, and anaphylactoid reactions, which often include histamine release" (IPCS, 1987).

Since then, there have been several attempts to classify adverse reactions to food (Figures 4.2 and 4.3) (Sampson, 1999; Johansson et al., 2001).

The World Allergy Organization concluded in 2004 (Johansson et al., 2004) that the appropriate term is *food allergy* when immunological mechanisms have been demonstrated. If IgE is involved in the reaction, the term *IgE-mediated food allergy* is appropriate. *Non-IgE-mediated immunological reactions* are called either

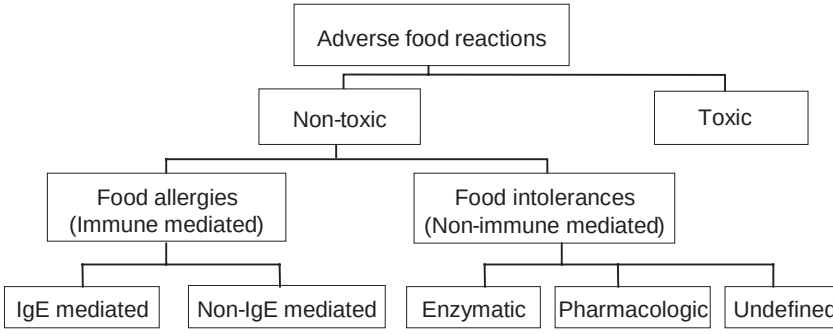

Fig. 4.2. Classification according to the European Academy of Allergology and Clinical Immunology nomenclature task force (adapted from Johansson et al., 2001)

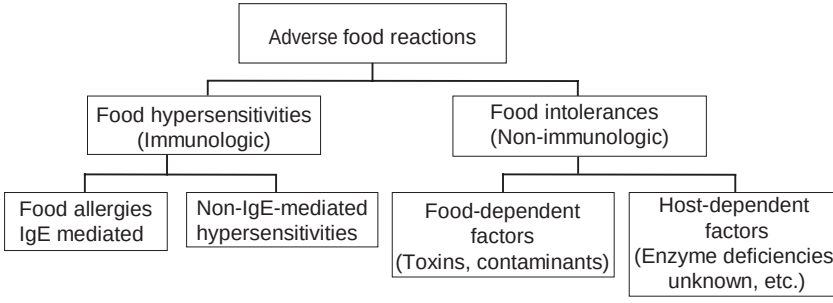

Fig. 4.3. Classification adapted from Sampson (1999)

non-IgE-mediated allergy or non-IgE-mediated hypersensitivity. All other reactions should be referred to as *non-allergic food hypersensitivity.*

A varied range of pathological mechanisms underlie food hypersensitivities. Some conditions involve immunological mechanisms, and others do not. The mechanism can be IgE mediated (Taylor & Hefle, 2001) or partially IgE mediated, as seen with conditions such as eosinophilic oesophagitis or asthma (Sampson, 1999). Immunological reactions can also be non-IgE mediated, being IgG mediated or cell mediated, as seen with disorders such as coeliac disease (Troncone et al., 2008). Finally, some adverse reactions do not involve the immune system (IPCS, 1987; Taylor & Hefle, 2001). These sensitivities may be attributed to the existence of metabolic disorders or the occurrence of reactions with unknown mechanism.

4.10.2 Prevalence

A meta-analysis of food hypersensitivity prevalence studies showed that it is not possible to make an overall worldwide estimate of the prevalence of food allergy or of the prevalence of specific foods, even based on well-conducted studies of prevalence, either self-reported or based on challenge studies (Rona et al., 2007; Zuidmeer et al., 2008).

The heterogeneity in the prevalence reported in different studies could be a result of differences in study design and methodology. Another possibility is that the findings reflect real differences between populations.

In studies of self-reported food allergies, 3–38% answer that they have food allergies, although only a few studies had figures above 20%. If those people who believe that they have a food allergy are challenged with the food that they think causes their allergy, only 1–11% have their food allergy confirmed. Most of the studies in which food allergy is clinically proven report percentages between 1% and 5% of the total population as having any food allergy. So there is a large gap between the percentage of people who think they have a food allergy and the percentage of people who are diagnosed as allergic. In general, the same effect is apparent when specific foods (with the exception of soy and wheat) are investigated: self-reported food allergy is overestimated compared with clinically proven food allergy (Rona et al., 2007; Zuidmeer et al., 2008).

4.10.3 IgE-mediated food allergy

4.10.3.1 Sensitization

The normal reaction to dietary proteins is development of tolerance, where the immune response is downregulated by an active immunological process (Brandtzaeg, 2002; Sampson, 2004).

Food allergies are a consequence of the undesired or uncontrolled immune response to a food antigen in susceptible individuals. They are based on the body's aberrant interpretation of certain dietary proteins as "foreign", which leads to a heightened response of the immune system.

Allergy develops through the process of sensitization. During the sensitization phase, exposure to the food allergen stimulates production of antigen-specific IgE (Taylor & Hefle, 2001).

Sensitization may occur via the intestinal tract. This is called traditional food allergy or class 1 allergy and is often caused by stable allergens. Class 2 food allergy develops after sensitization to airborne allergens via the lung and is typically caused by pollen cross-reacting with food allergens (Asero et al., 2007). Sensitization via the skin may also be possible (Lack et al., 2003). Class 1 food allergy is most prevalent in children, whereas class 2 food allergy is most prevalent in young adults and adults.

In general, milk and egg are the most common food allergens in children, and this is worldwide (Hill et al., 1997; Dalal et al., 2002; Osterballe et al., 2005). Eating habits may influence the development of food allergies. For instance, sesame allergy is frequent in Israel, probably because of early introduction of tahini (Dalal et al., 2002).

Most infants develop cows' milk allergy in the 1st year of life, but about 85% become clinically tolerant by the 3rd year of life (Host et al., 2002). Allergy to hen eggs often develops in the 2nd year of life. Approximately half of these patients become tolerant in 3 years, and up to 66% of children become tolerant in 5 years (Boyano Martínez et al., 2001). Peanut allergy tends to persist throughout adulthood, although up to 20% of peanut-allergic children lose their allergy (Skolnick et al., 2001; Hourihane, 2002).

The foods that most often cause allergy in adults are fruits and vegetables (Kanny et al., 2001; Zuberbier et al., 2004; Osterballe et al., 2005). Here, the primary sensitization comes mainly from pollen, and thus sensitization does not reflect eating habits, but rather exposure to flora.

Factors such as age, genetic predisposition and amount and frequency of food consumption may play a role in sensitization, but there is no current consensus regarding a threshold dose for sensitization for food allergens (see section 4.10.3.4).

It is important to remember that sensitization (e.g. the induction of specific IgE upon exposure to an allergen) is not the same as clinical

disease. This means that detection of specific IgE in serum or a positive skin prick test is not always accompanied by clinical disease (Asero et al., 2007).

4.10.3.2 *Symptoms and diagnosis*

The symptoms of food allergies range from mild discomfort to severe, life-threatening reactions (anaphylaxis), which require immediate medical treatment. Symptoms may be triggered in the skin (e.g. itching, redness, swelling), gastrointestinal tract (e.g. pain, nausea, vomiting, diarrhoea, itching and swelling of oral cavity), respiratory tract (e.g. itching and swelling of the nose and throat, asthma), eyes (e.g. itching and swelling) or cardiovascular system (e.g. chest pain, abnormal heart rhythm, very low blood pressure causing fainting, and even loss of consciousness). Fortunately, anaphylaxis is much less frequent than skin rashes or symptoms in the gastrointestinal tract.

Allergic reactions to foods may occur within a few minutes after eating the offending food, but symptoms may also (rarely) develop after hours, making the relationship with ingestion of food less clear. Symptoms can last for days. The specific symptoms and severity of an allergic reaction are affected by the type and amount of the allergen consumed, by the form in which the food containing the allergen was eaten, by the intake of alcohol, aspirin and other drugs such as beta-blockers and angiotensin-converting enzyme inhibitors, by exercise or stress, and by the sensitivity of the allergic person.

The most frequent symptoms of food allergies are itching and swelling of the mouth. Oral itching (known as oral allergy syndrome) can be an initial symptom in any kind of food allergy. Oral itching is, however, a well-known symptom in food allergy induced by cross-reaction with pollen, such as by apple, kiwi, hazelnut, walnut, celery, carrot, tomato, cherry and melon. Most of the allergens in cross-reacting foods will be destroyed in the gastrointestinal tract. This explains why the symptoms are frequently mild and limited to the mouth. Most of the allergens in the cross-reactive foods will be destroyed if the food is cooked. Many people allergic to birch pollen cannot eat raw apples without experiencing symptoms, but stewed apples and apple juice might not be a problem (Asero et al., 2007).

Anaphylaxis is an uncommon, acute, potentially life-threatening allergic reaction involving the whole body. A person who has this type of reaction will typically experience the following symptoms: itching of the skin or tingling in the mouth and throat followed quickly by feeling unwell and dizzy with an accelerated heart rate and nausea. At the same time, there may be a nettle rash or skin flushness, hay fever and asthma. Blood pressure may drop dangerously, and the person may collapse. Untreated anaphylaxis can rapidly result in death.

An unusual form of this condition can be triggered by eating problem foods within 2–3 h of vigorous exercising and is called "food-dependent, exercise-induced" anaphylaxis.

In Europe and the USA, peanuts and nuts are the foods most commonly reported to cause anaphylaxis (Pumphrey & Gowland, 2007; Shah & Pongracic, 2008). In Japan, milk, egg and wheat seem to be the most common foods associated with anaphylaxis (Immamura et al., 2008). Prompt administration of the medicine adrenaline after eating suspected problem foods has helped minimize life-threatening episodes. Applicators to administer adrenaline can be carried by people who are aware that they are at risk of anaphylaxis (Shah & Pongracic, 2008).

4.10.3.3 Common characteristics of food allergens

Virtually all known food allergens are proteins. The traditional food allergens (class 1) are water-soluble glycoproteins 10–70 kilodaltons in size and fairly stable to heat, acid and proteases (Sampson, 2004).

The food allergen component of a food represents only a few of a vast number of different proteins found in the complex mixture that comprises a food (Taylor & Lehrer, 1996; Becker & Reese, 2001). They can be less prominent proteins in the allergenic foods (Taylor & Lehrer, 1996). Most allergenic foods contain multiple allergenic proteins. When assessed with regard to the nature of their reactivity in sensitive individuals, the allergenic food proteins can be considered as a "major" food allergen or a "minor" food allergen, depending on whether, respectively, a majority or a minority of atopic or allergic individuals react to it (Taylor & Lehrer, 1996; Bredehorst & David, 2001).

A relatively small number of specific foods or food groups are responsible for the vast majority of food-related allergic reactions (Hefle et al., 1996; Sampson, 1999). The foods or food groups identified as key in this regard by an international expert panel (FAO, 1995) are cows' milk, eggs, peanuts, soybeans, wheat, tree nuts (e.g. almond, walnut, pecan), fish (e.g. finfish: cod, salmon) and crustaceans (e.g. shrimp, crab, lobster). Some food additives may also give IgE-mediated allergic reactions (Kägi et al., 1994; Wüthrich et al., 1997; Chung et al., 2001).

The relevant component of the primary protein structure of food allergen is an epitope. Epitopes are the part of the whole allergenic proteins or glycoproteins that are detected immunologically by antibodies (Lehrer et al., 1996; Becker & Reese, 2001). They serve as the interface between the chemical structure of the food allergen protein and the immune system. Different types of epitopes exist (Huby et al., 2000). Continuous epitopes are peptides of a length of 6–16 amino acid residues in a linear sequence (Lehrer et al., 1996; Becker & Reese, 2001). Discontinuous epitopes comprise different components or several different adjacent non-continuous amino acid sequences of the primary protein structure and depend on conformational or tertiary three-dimensional structure of the protein (Lehrer et al., 1996; Becker & Reese, 2001). The latter type of epitopes have the most potential to be altered or destroyed by denaturation and thus factor in the stability of food allergens, especially with respect to aspects of food processing (Becker & Reese, 2001). Epitopes can also be composed of glycoconjugate carbohydrate determinants, possibly causing glycosylated food allergens to be resistant to denaturation (Huby et al., 2000; Becker & Reese, 2001).

Systematic analysis of plant food allergens has shown that the majority belong to only a few protein structural families, the prolamin, Bet v 1 and cupin superfamilies (Breiteneder & Mills, 2005; Jenkins et al., 2005). Animal food allergens can be classified into three main families—tropomyosins, EF-hand proteins and caseins—along with 14 minor families, each composed of 1–3 allergens. The evolutionary relationships of each of the animal allergen superfamilies showed that, in general, proteins with more than approximately 62% sequence identity with a human homologous protein were rarely allergenic (Jenkins et al., 2007). These observations indicate that the structural features and properties of food proteins may play a role in determining their allergenicity.

For class 1 allergy, where sensitization occurs via the gastrointestinal tract, resistance to digestion may be important (Astwood et al., 1996). Thus, the ability of a protein to sensitize and to elicit allergic reactions via the gut may depend on the extent to which it survives digestion. This has been shown for a number of prolamin superfamily members, with IgE epitopes having been found to resist digestion for the 2S albumin allergens from Brazil nut (Moreno et al., 2005) and peanut (Sen et al., 2002) and for the lipid transfer protein allergens from grape and various Rosaceae fruits (Asero et al., 2000; Scheurer et al., 2004; Vassilopoulou et al., 2006). However, this hypothesis does not hold for the cupin allergens, such as the peanut allergen Ara h 1, which, despite being susceptible to proteolysis, retains its allergenic properties (Eiwegger et al., 2006). There is evidence that low molecular weight peptides form aggregates of a size sufficient both to sensitize and to elicit an allergic reaction (Bøgh et al., 2008).

In addition to digestive processes, allergenic food proteins are potentially altered by food preparation processes, including heat (e.g. roasting, cooking), proteolysis and hydrolysis (Bredehorst & David, 2001). The allergenicity of certain food proteins has been demonstrated to be less potent, more potent or, more commonly, unaltered to any significant degree after food processing or cooking procedures. These differences in reactivity that result from changes in food allergen proteins may vary across allergic individuals. Recently, a workshop concluded that it is not currently possible to identify specific variables that could be used to reliably determine how processing will influence protein allergenicity (Thomas et al., 2007).

Class 2 food allergy develops as a consequence of an allergic sensitization to inhalant allergens cross-reacting with allergens in fruits and vegetables. These class 2 allergens are in general more labile than allergens causing class 1 allergy and most often cause oral allergy syndrome (e.g. typical for the birch–apple syndrome), but they can also cause anaphylaxis, which is not rare in the mugwort–celery syndrome (Breiteneder & Ebner, 2000).

Not all allergies to fruits and vegetables are caused by labile pollen cross-reacting with allergens (Fernandez-Rivas et al., 2006). For example, lipid transfer proteins in peach and apple are very resistant to processing (Asero et al., 2000).

4.10.3.4 Thresholds

(a) Sensitization

There is no current consensus regarding a threshold dose for sensitization for food allergens. Nor is there information delineating the differences in sensitization threshold across age groups, routes of sensitization or the combination of both. In addition, the parameters that define the process of sensitization—for example, the amount of allergen ingested per exposure, the number of exposures, the duration of exposure, the pattern of exposures and even the total dose of exposure—are not well defined.

(b) Clinical food allergy (elicitation)

Exposure to low or minimal amounts of an allergenic food is potentially hazardous to individuals with an allergy to that food. Hence, determination of a "safe" or tolerable level of exposure is critical to those individuals with an allergy to a specific food. Risk assessment methodologies allow for the estimation of this level.

For food allergy, knowledge about hazard and adverse effect levels comes from case-reports and case-series or from challenge studies performed on sensitive individuals. Food challenge tests are typically conducted to diagnose the presence of a food allergy in individuals suspected of sensitivity to a particular food. The data from challenge tests available in the literature are from open challenge tests, single-blind placebo-controlled food challenge (SBPCFC) tests, meaning only the patient is unaware of the food or placebo being tested, and double-blind placebo-controlled food challenge (DBPCFC) tests, meaning neither the patient nor the test administrator is aware of the food or placebo being tested. Of these food challenge tests, the findings from DBPCFC test protocols are considered the more reliable and valid source of dose–effect information (e.g. Bock et al., 1988; Hourihane et al., 1997; Taylor et al., 2002; Bindslev-Jensen et al., 2004). It is sometimes referred to as the "gold standard" protocol.

Oral food challenge trials have shown large individual differences in human reactivity to allergenic food, from 0.01 mg to several grams of protein (Taylor et al., 2002; Wensing et al., 2002; Ballmer-Weber et al., 2007).

Over recent years, more focus has been directed towards the performance of low-dose DBPCFC tests to determine the NOAEL as well as the LOAEL for allergenic foods (e.g. Hourihane et al., 1997; Taylor et al., 2002, 2004; Wensing et al., 2002; Flinterman et al., 2006; Ballmer-Weber et al., 2007). A part of this process has been to publish consensus standardized clinical protocols for low-dose DBPCFC tests. The goal of these protocols is to be able to more confidently compare food challenge results across studies and to reduce the variability in these results (Taylor et al., 2004; Crevel et al., 2008).

Different allergenic foods may have different NOAELs or LOAELs. This may reflect real differences in potency or differences in the allergic population investigated in challenge trials. Reviews of challenge data can be found in Taylor et al. (2002), in EFSA (2004) and at http://www.foodallergens.info.

Because of potentially severe reactions (anaphylaxis), some patients are excluded from food challenge procedures. In addition, patients are included in challenge trials when their symptoms are stable and they have no infections. For these reasons, it is often debated whether results from challenge trials reflect the reactivity in the whole population allergic to the food investigated. On the other hand, low-dose DBPCFC trials are conducted at university allergy clinics where the patient group may be more sensitive than the ordinary food-allergic patient (Crevel et al., 2008).

4.10.3.5 *Risk assessment in food allergy*

It is assumed that food-allergic persons are able to avoid the food to which they are allergic if the allergenic food is an ingredient in the food they eat. This means that risk assessment is typically conducted in situations where the allergenic food occurs not as an ingredient, but as a "contaminant" (e.g. milk in dark chocolate). Another important area is the exemption from labelling requirements (e.g. to determine if the level of residual protein in highly refined soybean oil is so low that there is no risk for persons with soy allergy).

In food allergy, risk assessment is based on data from challenge trials in food-allergic patients, intake data, levels of contamination with the allergenic food and, if possible, prevalence data. Most risk assessments

have been done on a case-by-case basis, taking relevant information into account. The risk assessment concludes whether or not a level of allergen contamination will result in adverse reactions in food-allergic persons (EFSA, 2004). One of the big challenges for the risk assessor is that there is consensus that a threshold for food allergy reactions exists (Taylor et al., 2002), but it is not possible, based on current data, to set scientifically based thresholds for allergenic foods (EFSA, 2004).

Food allergy risk assessment is a relatively new discipline, and there is no general consensus on how it should be conducted. Three approaches have been suggested, using 1) NOAEL and uncertainty factors, 2) BMD and margin of exposure (MOE) and 3) probabilistic risk assessment (Madsen et al., 2009). The three approaches are described below (Madsen et al., 2009).

Risk assessment in food allergy using thresholds and uncertainty factors depends on the use of data from challenge trials that identify a NOAEL or a LOAEL. The relevant study that reports the lowest NOAEL (or LOAEL if a NOAEL cannot be identified) is used. The NOAEL can be based on either subjective or objective symptoms. The NOAEL is then divided by an uncertainty factor. There is no consensus on the use of uncertainty factors in food allergy, but it has been suggested that a factor of 10 be used to account for intraspecies differences and an additional factor of 10 to account for potential severity of reaction in the highly sensitive population (Buchanan et al., 2008). The advantage of this approach is that it is very simple and uses a methodology well known from toxicology. The disadvantage is that it is based on a single data point from a single study and may result in thresholds that are too low to be of practical use. For further discussion, see Madsen et al. (2009).

Instead of using a single data point from a single study, the use of mathematical modelling based on distribution of positive challenges from a single study or from a combination of challenge studies with the same allergenic food has been suggested. This allows the determination of a BMD (in food allergy, also called the eliciting dose) for this food based on all available relevant data (Crevel at al., 2007). A collection of data from peanut challenges of 185 patients from 12 studies was used to estimate the BMD using distribution models. The ED_{10} (i.e. the dose expected to give reaction in 10% of the peanut-allergic

population) was found to be 17.6, 17.0 or 14.6 mg whole peanut, depending on the model used (Taylor et al., 2009).

The MOE approach generally uses the lower 95% confidence limit of the BMD. This is called the benchmark dose lower limit (BMDL) (see chapter 5).

The BMDL is divided by the estimated intake of the allergenic food, resulting in an MOE. Different intake scenarios can be compared as well as MOEs for different allergenic foods, in order to identify susceptible subgroups (e.g. high consumers) or to judge relative potencies of allergenic foods.

The advantage of the approach is that it uses all relevant data to establish a BMD. The disadvantage is that it does not describe the risk quantitatively. For examples and discussion, see Madsen et al. (2009).

The probabilistic risk assessment model calculates the most likely number of allergic reactions that might result from the accidental presence of an allergenic constituent in a food product. This calculation uses the distribution of positive challenges, together with those associated with variables determining the intake of the allergenic constituent. These include presence and concentration in the affected food, likelihood that an allergic person consumes the food and amount of the food consumed per eating occasion (Spanjersberg et al., 2007). The advantage of this approach is that it results in a quantitative estimate of a risk. The disadvantage is the demand not only for challenge data, but also for distribution of intake data.

As in other areas, a good risk assessment relies on the quality and suitability of the data used. In food allergy, the data used originate from humans, but there may be limitations in using existing data, because they were generated for other purposes. More and more threshold data on allergenic foods are being generated using standardized protocols with an extended range of doses, often starting at low microgram levels, generating NOAELs and LOAELs that can be used in risk assessment (Taylor et al., 2004; Flinterman et al., 2006; Ballmer-Weber et al., 2007; Crevel et al., 2008).

A reaction to a food allergen is analogous to an episode of acute poisoning rather than chronic toxicity in terms of dosimetry. Therefore, the relevant exposure assessments should be based on "meal/eating occasions" rather than exposure throughout the entire day or from a single food.

There has been much focus on the development and use of challenge data in food allergy risk assessment and much less focus on how intake data should be used. Both the MOE and the probabilistic approach use intake data, which, depending on how they are used, may influence the outcome of the risk assessment. For further discussion, see Madsen et al. (2009).

4.10.3.6 *Evaluating potential allergenicity of genetically modified food*

A part of the evaluation of the safety of genetically modified (GM) foods is to assess whether newly introduced proteins have allergenic potential. The purpose of this is 2-fold: 1) to protect food-allergic persons from exposure to the allergen and 2) to protect the population from introduction of new food allergens.

To predict the potential allergenicity of novel food proteins, two decision tree strategy approaches have been described (Metcalfe et al., 1996; FAO/WHO, 2001b).

The Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology (FAO/WHO, 2001b) proposed a decision tree for assessing the allergenic risks posed by novel proteins, which is an update of the original decision tree described in Metcalfe et al. (1996).

FAO/WHO (2001b) suggested that cross-reactivity between the expressed protein and a known allergen (as can be found in the protein databases) should be considered when there is either:

1) more than 35% identity in the amino acid sequence of the expressed protein (i.e. without the leader sequence, if any), using a window of 80 amino acids; or
2) identity of six contiguous amino acids.

As an identity of six contiguous amino acids between an allergen and a given protein sequence has a high probability of occurring by

chance, verification of potential cross-reactivity would be warranted when criterion 1) is negative, but criterion 2) is positive. In this situation, suitable antibodies (from a human or animal source) would have to be tested to substantiate the potential for cross-reactivity.[1]

The decision tree suggested by FAO/WHO (2001b) shows that if a protein has an identity score that equals or exceeds 35%, the protein should be considered to be a likely allergen, and no further testing is suggested.

If there is no sequence homology between the novel protein and known allergens, the recommendation from the FAO/WHO (2001b) consultation is that the protein should be tested against patients' sera. In the case of a GM food, if the source of the gene is known to be allergenic, sera from patients allergic to the source should be tested in a so-called "specific serum screen". This indirectly identifies protein epitopes recognized by allergic patients' IgE, the presence of such epitopes conferring a risk of the novel protein triggering allergic reactions in individuals with a pre-existing sensitivity. If this specific serum screen is negative or if the source of the gene is not known to be allergenic, the protein should then undergo a "targeted serum screen". Thus, if the recombinant protein is derived from a monocotyledonous plant source, it is proposed that serum samples from patients with high levels of IgE antibodies to monocot allergens such as grass and rice be tested. Similarly, if the recombinant protein is derived from a dicotyledonous plant, serum samples from patients with high levels of IgE antibodies to dicot allergens such as tree pollen, weed pollen, celery, peanuts, tree nuts and latex should be used. A similar approach is suggested if the recombinant protein is derived from a mould, an invertebrate or a vertebrate. Such a screen should include 25 individual serum samples with high levels of IgE to the selected group of airborne allergens and (if applicable) 25 sera with IgE to the selected group of food allergens.

This targeted serum screen will determine whether the novel protein has IgE epitopes identical to those present in related inhalant or food allergens. This approach is pertinent, as a number of food allergies

[1] Using as few as six contiguous amino acids was later shown to be useless because of many false positives (Stadler & Stadler, 2003).

are caused by cross-reaction to inhalant allergens. However, with our current lack of knowledge regarding the mechanisms of food allergy, the positive predictability of the targeted serum screen is not known, making a risk assessment difficult.

The Codex Alimentarius Commission (CAC) later abandoned the decision tree strategy and described a risk assessment procedure based on a weight of evidence approach (FAO/WHO, 2003).

There are no validated animal models that can predict the allergenicity of an unknown protein. The risk assessment therefore relies on a combination of methods looking at protein structure, protein stability and binding properties to serum IgE from allergic patients.

The following elements are included in the Codex guideline (FAO/WHO, 2003):

- Identifying the source of the gene
  - Does it come from a known allergenic food?
    - If yes, screen with specific serum from allergic patients
- Sequence similarity with a known allergen
  - More than 35% identity in the amino acid sequence using a window of 80 amino acids
    - Screen with specific serum from allergic patients
- Resistance to pepsin digestion

It has been commonly accepted that for a protein to sensitize an individual and elicit an allergic reaction, it must survive the acidic and proteolytic environment of the gastrointestinal tract. Astwood et al. (1996) showed in a study comparing the in vitro stabilities of food allergens and non-allergenic proteins to simulated gastric fluid that there was an association between resistance to digestion and allergenic potential. This has led to pepsin resistance being used as a predictive parameter in the risk assessment of the allergenic potential of novel proteins, as suggested in all three approaches above. However, in recent years, the relationship between resistance to digestion and allergenic potential of a protein and the validity of taking this parameter into account in risk assessment have been questioned (Fu et al., 2002). It is still true that many allergens giving rise to class 1 food allergy are relatively resistant to digestion, but there are also important exceptions, such as the

cupin superfamily, represented by the major peanut allergen Ara h 1 (Eiwegger et al., 2006), and the milk allergen casein, which is degraded relatively quickly by proteases (Wal, 2001). There are also examples of stable proteins that rarely cause allergy, such as thaumatin-like proteins from grape and apple (Vassilopoulou et al., 2006).

For further discussion of the scientific basis for allergenicity testing of GM food, see Goodman et al. (2008) and the European Food Safety Authority's draft scientific opinion on the assessment of allergenicity of GM foods (EFSA, 2009).

4.10.4 Non-IgE-mediated food allergy

4.10.4.1 Coeliac disease

The most well-described and prevalent non-IgE-mediated disorder caused by an immunological reaction to a food component is coeliac disease, also called gluten intolerance. It is a disease of the small intestine triggered by ingestion of gluten, a protein found in wheat, barley and rye. When a person with coeliac disease ingests gluten, an immunological reaction in the small intestine leads to flattening of the mucosa.

In the present text, coeliac disease is classified as a non-IgE-mediated food allergy. This definition is easy to communicate. Most people know about food allergy, and the treatment for coeliac disease, avoidance diet, is the same as for food allergy. Coeliac disease may also be seen as a multiorgan autoimmune disease, primarily as a gastrointestinal disease, but also with effects on the skeletal system, the peripheral and central nervous systems, the reproductive system and the cardiovascular system.

It is estimated that about 1% of the population has antibodies connected to coeliac disease. Wheat can also trigger IgE-mediated food allergy, although this is not as common as coeliac disease.

Coeliac disease was for many years diagnosed mainly in small children. Within months of starting a gluten-containing diet, susceptible children would present with chronic diarrhoea or loose stools, vomiting, a distended abdomen and failure to thrive. Similarly, diarrhoea, weight loss and general weakness are the most common symptoms in adults.

Today, we know that coeliac disease is a complex disorder with symptoms occurring not just in the gastrointestinal tract. Many symptoms and diseases are associated with coeliac disease. For example, the flattened mucosa caused by coeliac disease leads to poor absorption of nutrients in the intestine. Poor absorption of iron can lead to anaemia, poor absorption of vitamin B_{12} can lead to dementia, and poor absorption of vitamin D and calcium can affect bones and teeth. Coeliac disease is also often found in connection with other immunological diseases, such as diabetes and rheumatoid arthritis.

Coeliac disease is diagnosed on the basis of histological findings on a biopsy from the small intestine. In addition, symptoms should disappear on a gluten-free diet.

Patients with coeliac disease have IgA antibodies in serum against gluten as well as autoantibodies directed towards the enzyme tissue transglutaminase. Measurement of antibodies cannot be used as positive proof for the disease. A blood test can, however, help decide whether to take a biopsy from the small intestine.

About 10% of first-degree relatives to patients with coeliac disease also develop coeliac disease. The principal known determinants of genetic susceptibility are the highly variable human leukocyte antigen (HLA) genes located in the major histocompatibility gene complex. It has been demonstrated that the HLA-DQ2 and HLA-DQ8 class II protein molecules present gliadin peptides to T cells in the gut in a particularly efficient way. The HLA-DQ2 and HLA-DQ8 antigens are present in more than 95% of persons with coeliac disease (Troncone et al., 2008).

However, it is clear that additional factors are critical for the development of coeliac disease. Up to 30% of persons of North European ancestry, most of whom eat wheat, express HLA-DQ2, but coeliac disease develops in only a small proportion of these carriers. In Sweden, an epidemic of coeliac disease was started because of the early introduction of gluten-containing cereals (Ivarsson et al., 2000). Altered processing of gluten by gut enzymes and changes in the permeability of the gut may also be important factors (for more information, see the review by Troncone et al., 2008).

The only treatment for coeliac disease is avoiding gluten in the diet. Products with wheat, rye and barley must be avoided. Most patients tolerate products with oats as long as they are free from contamination with other cereals containing gluten. Once a coeliac patient is on a gluten-free diet, the flattened mucosa in the small intestine heals and the symptoms disappear.

(a) Risk assessment

To establish tolerable levels of gluten intake for patients with coeliac disease, it is necessary to challenge the patients over a period of time (e.g. 90 days). Adverse reactions are monitored by following serum antibodies as well as histological changes in the small intestine. A tolerable level of gluten has to be determined for the intake over a period of time and not as with IgE-mediated food allergy, where the dose at a single challenge occasion is the relevant intake scenario. Most patients with coeliac disease should ingest less than 50 mg of gluten per day (Hischenhuber et al., 2006; Catassi et al., 2007).

As opposed to food allergy, a regulatory threshold for gluten has been established. According to the Codex standards for food, gluten-free foods must adhere to a special standard for special dietary use for persons intolerant to gluten (FAO/WHO, 2008). Two standards for "gluten-free" food have recently been established (FAO/WHO, 2008):

1) "gluten-free" products contain gluten at concentrations below 20 mg/kg; and
2) products with "very low gluten content" may contain gluten at concentrations from 20 mg/kg to a maximum of 100 mg/kg.

According to CAC (FAO/WHO, 2008), gluten should be detected by an R5 ELISA method for gluten/gliadin. It is based on a monoclonal antibody reacting with the specific gliadin pentapeptide, QQPFP. This method shows a sensitivity and limit of detection for gliadin of 1.5 mg/kg (Mendez et al., 2005).

4.10.5 Non-immune-mediated food hypersensitivity

4.10.5.1 Metabolic disorders

Metabolic disorders describe those conditions where adverse reactions result from a genetic deficiency in the ability to metabolize some

component of the consumed food. Common examples of metabolic food disorders include lactose intolerance, a deficiency of lactase. Lactose intolerance may be inborn (rare), but it mostly appears during adolescence or early adulthood. It is the normal condition in 75% of the human population, but it is relatively rare in northern Europeans, probably occurring in 3–6%. Lactose intolerance may be transient in connection with intestinal infections. Individuals with lactose intolerance are unable to digest lactose and experience adverse gastrointestinal effects associated with bacterial metabolism of lactose in the colon. Small portions of lactose rarely cause symptoms. This means that persons with lactose intolerance normally can eat cheese and smaller amounts of other dairy products.

Favism is a deficiency of erythrocyte glucose-6-phosphate dehydrogenase, with acute haemolytic anaemia resulting from oxidative damage to erythrocytes following the consumption of fava beans containing vicine and convicine.

4.10.5.2 Other

Hypersensitivity to food additives represents a condition for which a mechanism has not been determined; however, reactions are probably not based on an abnormal immune response.

There are few scientific investigations concerning food additives and hypersensitivity, probably because it is a difficult subject to investigate as a result of many different food additives and relatively few people who react to any individual substance. This means that most descriptions of food additive hypersensitivity are based on very few patients.

The one exception is sulfites. Hypersensitivity to sulfites is relatively well described, especially in people with asthma, and may also trigger skin reactions such as hives (urticaria) (Wüthrich, 1993; Taylor et al., 1997).

4.11 General principles of studies in humans

4.11.1 Introduction

The potential value of data from studies in humans has been recognized since the first meetings of JECFA and JMPR.

EHC 70 (IPCS, 1987) stated that JECFA "recognizes the value of human data, has sometimes requested such data, and has always used it in its evaluations when available", whereas EHC 104 (IPCS, 1990) stated that "All human data (accidental, occupational, and experimental exposures) are fundamental for the overall toxicological evaluation of pesticides and their residues in food". EHC 104 (IPCS, 1990) included the following three principles:

1) The submission of human data, with the aim of establishing dose–effect and dose–response relationships in humans, is strongly encouraged.

2) Studies on volunteers are of key relevance for extrapolating animal data to humans. However, attention to ethical issues is necessary.

3) The use of comparative metabolic data between humans and other animal species for the purpose of extrapolation is recommended.

The recent EHC on dose–response modelling (IPCS, 2009) also confirms the value of human data:

> In the evaluation of human health risks, sound human data, whenever available, are preferred to animal data. Animal and in vitro studies provide support and are used mainly to supply evidence missing from human studies. It is mandatory that research on human subjects is conducted in full accord with ethical principles, including the provisions of the Helsinki Declaration [see World Medical Association, 1997].

JMPR has repeatedly considered the use of human data in pesticide risk assessment, in particular when considering ARfDs (see chapter 5). Detailed considerations were given in the 2002 JMPR report (FAO/WHO, 2002a). JMPR noted that human data on a pesticide, whether from volunteer studies or from other investigations of human exposures in the workplace or environment, can be extremely valuable in placing the animal data in context and, when available, should always be evaluated, even when they are not used to derive an ARfD.

Evaluators should consider the following issues in determining whether to use a volunteer study in the derivation of an ARfD:

- The initial consideration should be the ethical acceptability of the study.

- The next consideration should be scientific merit. A poorly designed or conducted study in humans (as with experimental animals) should not be used for establishing an ARfD.
- The acceptable group size will depend on factors such as interindividual variation in response and the level of change considered not to be adverse. The studies should be assessed with particular consideration of their power to detect critical effects.
- The IPCS guidance for the use of CSAFs (IPCS, 2005) proposed a minimum group size of 5. Studies using small group sizes might be usable (e.g. by combining results from two or more dose levels or applying a higher safety factor).
- The critical end-points identified in animal studies should be investigated appropriately in human studies.
- If only one sex or a particular age group has been used, the general applicability of the results should be ascertained, if possible, using data from studies in animals.
- As recommended by the 1998 JMPR (FAO/WHO, 1999a), recent studies in humans should include clear statements that they were performed in accordance with internationally accepted ethical standards. For older studies, ethical considerations should take into account both current standards and the standards pertaining at the time the study was performed.
- Studies that have not been performed in accordance with ethical principles but are scientifically valid should be used only if the findings indicate that acceptable human exposure is lower than the level that would be determined without the use of such a study.

Information from humans is of potential importance in identifying and characterizing the hazards and evaluating the risks of macroingredients in foods and of substances such as food additives, contaminants and residues of veterinary drugs and pesticides. The information may come from:

- controlled experiments in human volunteers, usually related to specific end-points or toxicokinetics;
- surveillance studies, including post-marketing surveillance;
- epidemiological studies of populations with different levels of exposure, which may be particularly important for contaminants;

- experimental or epidemiological studies in specific subgroups of people; or
- clinical reports or case-series of individuals.

Investigations in humans may take the form of short-term experiments involving controlled exposure of a small number of intensively monitored subjects in a clinical laboratory, larger or longer-term and more loosely controlled studies of subjects living in the community but still receiving a controlled exposure, or epidemiological investigations of people in the community, leading a normal life and eating their ordinary diet.

End-points may include examination of safety or tolerance, nutritional and functional characteristics of foods or food components, the metabolism and toxicokinetics of the substance, mechanism or mode of action, possibly using biomarkers for effects identified in animal studies, and adverse health effects from unintentional exposures (e.g. to a contaminant).

The WHO Scientific Group on Procedures for Investigating Intentional and Unintentional Food Additives (WHO, 1967) highlighted

> the need, at a relatively early stage, to obtain information on the absorption, distribution, metabolism, and elimination of the chemical in human subjects, since this makes it possible to compare this information with that obtained in various animal species and to choose the species that are most likely to have a high predictive value for human responses.

Critical issues for any experimental study in humans are the ethical, professional and basic legal controls that govern whether a study in humans is necessary and the circumstances under which it may be properly performed (Royal College of Physicians, 1990a,b; USNRC, 2004). Consideration needs to be given to when the use of human tissues ex vivo or in vitro might be sufficient. Such data are likely to have increasing utility with the incorporation of human metabolic systems into in vivo and in vitro test systems. Prior to undertaking new in vivo experiments in humans, clinical information from other sources, such as investigation of any effects of exposure to the substance of interest in the workplace, reports of overdoses and accounts of human or veterinary medicinal usage of the same substance, should be analysed

to determine the necessity of additional research. Increasing ethical concerns about the necessity and safety of studies in humans mean that in the future it may become increasingly difficult to justify and obtain ethics approval for in vivo studies involving the administration of a non-therapeutic substance to humans (see also section 4.11.5).

Of particular value for JECFA and JMPR in evaluating submitted experimental studies in humans are the guidelines developed by VICH (2000) for Good Clinical Practice (GCP). These guidelines include sections on the principles of VICH GCP, the institutional review board/independent ethics committee, the investigator, the sponsor, the clinical trial protocol and protocol amendments, the investigator's brochure and essential documents for the conduct of a clinical trial.

A helpful account of human studies of non-pharmaceuticals, such as pesticides and household products, has been published by Wilks (2001). It discusses the ethical and some of the practical problems and guiding principles that are applicable to items in the diet. Lessons learnt from human studies of pharmaceuticals are described below.

4.11.2 Lessons learnt from pharmaceutical development

Studies in humans are not a formal requirement for international or national safety assessment or regulatory approval of food additives or residues of veterinary drugs and pesticides. However, the information that can be obtained from humans is extremely valuable, and every opportunity should be taken to obtain worthwhile data both before and after a product becomes available for human consumption. In this respect, the regulatory assessment of substances in food differs from that of pharmaceuticals, such as prescription and other medicines, for which studies of efficacy and safety in humans are a data requirement for premarketing evaluation by regulatory authorities.

There are many similarities between the study of substances in food and the study of pharmaceutical compounds, because the basic physiological, pharmacological, immunological and biochemical processes that might be affected by exposure are similar. In addition, many metabolic and toxicokinetic processes of therapeutic drugs are also relevant to other low molecular weight "foreign" compounds, such

as food additives, natural non-nutrients, contaminants and residues of veterinary drugs and pesticides.

Human experimental investigations of pharmaceuticals have been developed much further than the clinical evaluation of dietary components and have resulted in the principles and practices governing studies in humans. For that reason, the need for ethical review, professional obligations, laws and official guidelines developed for pharmaceuticals control the nature and circumstances of human studies.

The principles guiding studies in humans have been dominated by the objectives, needs and practices of pharmaceutical development. However, the investigation of drugs differs from some of the purposes, objectives and approaches appropriate to the study of non-pharmaceuticals, especially in the general area of substances in foods. Drug development generally focuses on treating identifiable diseases in population subgroups, often for short periods, and, where necessary, compares the potential benefit with the possible harm of the drug. In contrast, the diet (including food additives, natural non-nutrients, contaminants and residues of veterinary drugs and pesticides) is intended to be harmless and is consumed by all members of society throughout life. The conventional risk–benefit analysis applied to drugs and used to justify various investigations and trials in healthy humans and patients cannot be applied in the same way to studies of foods and dietary components. Ethics committee approval would require that any study on a food substance carries negligible risk to the participants. This leads to a much stricter evaluation of any potential for risk in clinical investigations, because there is no balancing "benefit" in the sense of relief from a disease.

Invaluable and up-to-date information about general and specific requirements for pharmaceuticals can be obtained by consulting the web site of the ICH (see http://www.ich.org/cache/compo/276-254-1.html). More local interpretations of the international guidelines can be obtained from the web sites of major agencies, such as the European Medicines Agency (see http://www.emea.europa.eu/) and those of France (Agence Française de Securité Sanitaire des Produits de Santé) (see http://www.afssaps.fr/), Germany (Bundesinstitut für Arzneimittel und Medizinprodukte) (see http://www.bfarm.de/EN/Home/homepage__node.html), the United Kingdom (Medicines and

Healthcare Products Regulatory Agency) (see http://www.mhra.gov.uk/home/idcplg?IdcService=SS_GET_PAGE&nodeId=5) and the USA (USFDA; see http://www.fda.gov/default.htm).

4.11.3 Types of studies in humans

The principal types of human studies are listed in Table 4.3.

The numbers of subjects entered into a study must be sufficient to realize the aims of the investigation. Ethics approval normally requires a calculation of the group size necessary to meet the study objectives, as it would be unethical to perform an underpowered study. One approach to deciding the size of the experimental groups is to consider normal variability in the end-point being examined and to employ standard statistical methods on the power of an experiment in order to calculate the number of subjects required to demonstrate a predefined magnitude of response. The numbers should include definition of the size of any control group and take into account the predicted drop-out rate. The drop-out rate will depend on various factors, including the nature of any effects produced (although for an ethical study on a food component, this should be minimal) and the overall convenience of the protocol for the subjects (of which duration will be an important consideration).

4.11.3.1 Short-term clinical laboratory studies

The key features of clinical laboratory studies are that 1) they are short term, 2) they are likely to involve relatively few subjects under close supervision, 3) the nature and extent of their exposure to the test material are strictly limited and 4) measures of general safety and tolerance are monitored intensively.

Examples include studies on the toxicokinetics of the substance and examination of any effects on physiological functions and processes, such as the absorption of dietary lipids, plasma cholesterol, uptake of calcium or iron, effects on or replacement of vitamins, actions on intestinal flora, etc.

For food additives, veterinary drugs and pesticides, the absorption, metabolism and excretion in humans can be defined by suitably designed, single-dose studies. The doses chosen would approximate

Table 4.3. Principal types of studies in humans relevant to JECFA and JMPR

Type of study	Principal features	Common reasons for considering it
Short-term	Control of exposure with the administration of low doses predicted to be non-toxic. Intensive monitoring of end-points, effect and safety. Usually in healthy volunteers. Special studies may be undertaken in population subgroups, such as diabetics taking intense sweeteners.	
Physiology	Functional effects on gastrointestinal tract or other body system.	Basic research. Effect of dietary component.
Pharmacology	Interference with normal functions.	Basic research. Potentially harmful effects of dietary components, such as inhibition of AChE. Identification and cause of intolerance.
Biochemistry	Mechanistic investigation of action on metabolic processes.	Basic research. Mechanism of potentially adverse effects, such as enzyme inhibition or enzyme induction. Identification and cause of intolerance.
Toxicokinetics	Absorption, disposition, metabolism and clearance of substance.	Identification of species differences to assist interspecies extrapolation. Identification of genotypic or phenotypic differences to assist identification of possibly vulnerable population subgroups. Validation of biomarkers of exposure.
Immunology	Effects on or via immune system.	Basic research. Potentially harmful effects of dietary components, such as allergic sensitization. Identification and cause of intolerance.

Table 4.3. ***(Continued)***

Type of study	Principal features	Common reasons for considering it
Nutrition	Effects on blood levels of essential nutrients or other biomarkers.	Interference with normal nutritional processes, such as the absorption of micronutrients.
Toxicology	Low exposure usually of limited duration. Sensitive indicator of minimal effect (biomarker).	Mechanistic investigations using reversible biomarkers of effect. Identification and cause of intolerance.
Long-term	In general populations or selected subgroups. Exposure via normal dietary matrix and conventional preparative methods.	
Epidemiology	Case-series, case–control or cohort studies, etc.	Identification and characterization of adverse effects, usually for inadvertent contaminants.
Toxicology	Tolerability	Assessment of general tolerability of an approved substance administered at or close to the health-based guidance value.

those likely to be established as a health-based guidance value based on the available toxicity data. Studies involving the uptake and disposition of labelled materials (e.g. radioactive or stable isotopes) are important in understanding the fate of the substance in the body.

Any immunological, pharmacological, physiological or pathophysiological actions of the substance might be studied using single doses or a small number of doses, but these should be selected so that only minimal and reversible effects would be predicted. Studies would normally involve readily reversible biomarkers of effect, rather than adverse health effects. Short-term studies could also be used to investigate any effect of the substance in food on normal physiological, nutritional, biochemical or other bodily processes, food palatability and taste.

Other short-term studies on the identified end-points of interest, whether biomarkers of kinetics or biomarkers of effect, might include experiments on volunteer patients suffering from a known disease, individuals taking prescription or proprietary medicines, individuals who are genotypically or phenotypically different when the data indicate that this could be a significant variable, and investigations on possible influences of dietary constituents.

It must be emphasized that any special study in a selected group of subjects would require the same justification and ethics approval as for a study in normal healthy volunteers.

The advent of food components prepared from GM organisms, such as enzymes that are evaluated by JECFA, has led to some interest, especially in Europe, in the place of clinical studies in evaluation of their acceptability. An assessment of how to undertake such studies and the criteria for their appropriateness and acceptability have been published by the United Kingdom Advisory Committee on Novel Foods and Processes (FSA, 2002). Most JECFA safety evaluations of food components and processing aids from GM organisms have been on the basis of 90-day studies in rodents.

4.11.3.2 *More prolonged clinical laboratory studies*

In principle, a dietary component might be administered to groups of healthy volunteers or patients for a period of days or even

a few weeks, still in a controlled clinical laboratory setting. In reality, interference with normal human activities would mean that if the study were longer than a few days, the design would probably involve the subjects continuing the treatment while pursuing their normal lifestyle and returning to the laboratory periodically for measurements and investigations. This method can provide useful data to support the safety and tolerability of an approved food ingredient; a good example of this approach is the study on aspartame in 53 subjects given 75 mg/kg body weight per day for 26 weeks (Leon et al., 1989).

4.11.3.3 Post-marketing surveillance and epidemiological studies

These investigations involve studying exposure to the substance of interest and effects in people living in their normal communities for periods extending from weeks to months and occasionally longer. They require comparison of the end-points of interest, such as general health status, in groups with different levels of exposure. The different exposures in the groups included in the study often arise from lifestyle or geographical differences.

(a) Post-marketing surveillance

Post-marketing surveillance following the release of the substance in the diet requires that groups with different levels of exposure are identified. This could be a comparison between premarketing and post-marketing or following restricted marketing; for example, the mycoprotein Quorn was initially released in only part of the United Kingdom, which allowed a comparison of any general change in health status for different geographical regions. Obviously, such an approach would be very insensitive and could give only limited reassurance after the event.

The intakes of approved food substances show wide interindividual variations within a group of consumers, and it would be difficult to associate any reported effects with specific levels of intake. Nevertheless, useful insights may be obtained from collation of consumer complaints by the marketing company or the regulatory agency. The USFDA has collated and evaluated claims of adverse effects arising from the consumption of aspartame and the fat replacer olestra (Allgood et al., 2001). It should be recognized that the nature and

frequency of anecdotal consumer complaints are likely to be highly influenced by the extent of media coverage of the subject matter.

The uncertainties in such data and the potential sources of unavoidable bias and error make definitive conclusions impossible. Anecdotal reports on individual patients have been historically important in identifying possible adverse effects of therapeutic drugs that were not detected by traditional toxicology testing. Therefore, anecdotal data from consumers should be evaluated to assess the possibility of a previously unrecognized effect from a substance in food.

(b) Epidemiological studies

Epidemiological studies comprise investigations on people in the community in relation to their exposure to the substance of interest. They have been of greatest value to JECFA and JMPR in relation to hazard identification and characterization of food contaminants.

An overview of epidemiological studies in relation to chemicals in the diet is given by Van den Brandt et al. (2002), and the place of and differences between epidemiological and other types of clinical investigation are considered by Duggan et al. (2002). Various guidelines for Good Epidemiological Practice (GEP) have been proposed. Information is available on the International Epidemiological Association web site (see http://www.dundee.ac.uk/iea/GEP07.htm).

In any survey, it is essential not to assume that an apparent association between two or more factors indicates a cause–effect relationship. There are many sources of confounding that may suggest an association that arises indirectly due to other, irrelevant processes and specious correlations; these sources of error are well discussed by Bradford Hill (1965) and in monographs on epidemiology (e.g. Bonita et al., 2006).

The central theme of any epidemiological investigation is the collection of information in such a way as to show whether there is a difference between groups of people exposed to the substance over a given period and an otherwise comparable group that had no exposure or was exposed to a lesser extent (Coggon et al., 1997). The studies are best performed prospectively but may be retrospective (including the use of biological samples collected and stored over many years).

Experience has led epidemiologists to classify ecological and case–control studies as "hypothesis generating"—i.e. the results may suggest that a substance has or lacks a particular action, but the evidence is inconclusive. They should be distinguished from prospective, cohort or intervention studies, which are capable of "hypothesis testing".

The different types of epidemiological studies are described briefly below:

- *Ecological studies or case-series*: These are simpler to undertake than other types of study, because they comprise the collection of a series of past cases of the target event combined with retrospective assessment of their exposure to the test substance for comparison with some local, national or even international data about occurrence of the target event. This type of study is very susceptible to unrecognized and uncontrollable biases and other confounding effects. The main value of such studies is in the recognition of possible associations, and they can act as a trigger for more definitive research.

- *Case–control studies*: These are a more powerful but still relatively simple type of formal epidemiological investigation; as with case-series, however, they have a limited ability to control or even assess many factors that may influence the result. The basis of the approach is a retrospective comparison of the exposure between two groups—patients with the adverse effect or disease of concern and unaffected controls; a higher exposure in the patient group would suggest a possible causative association. The basis of the approach is the collection of relevant information about exposure and perhaps other major factors in the "test" group—i.e. those who suffer from the effect of interest—and in a matched control group whose members do not suffer from the effect. In contrast to other types of epidemiological study, case–control studies can provide information only about the effect that was investigated. Dose–time and dose–response relationships may be suggested by the study results. Typical problems, especially as the data usually come from free-living individuals in the community, are the accuracy of information about exposure and the high possibility of recall bias if the subject matter of the exposure assessment is obvious from the exposure questionnaire.

- *Cohort studies*: These are inherently more precise and more powerful than case–control investigations, but they are more costly to perform, may last a long time and may be more intrusive for the subjects involved. The basis is comparison of the incidence of the target events between groups with different levels of exposure. In many cases, the development of health effects is monitored prospectively. The approach can also be applied retrospectively if the exposure data in the different groups relate to a period before the health assessments were undertaken. Cohort studies usually involve large group sizes and offer the opportunity for better analysis of confounding factors. Dose–response and time–response relationships can be examined, and cautious subset analyses can sometimes be done to indicate the role of other factors not originally considered. A common refinement of the method is to divide the total population studied into bands with different levels of exposure (e.g. tertiles, quintiles) in order to assess dose–response relationships. Cohort studies applied to occupational data may provide information at exposures that are much higher than would normally occur via the diet.

- *Analytical or interventional studies*: These are cohort studies in which the exposure of interest is controlled by the experimenter (i.e. subjects are asked to consume or to refrain from consuming sources of the substance of interest). They are really a large-scale variant of the controlled clinical trial, in this instance employing dietary intervention instead of administration of a medicine. Examples of formal dietary intervention trials include the Alpha-Tocopherol, Beta-Carotene (ATBC, 1994) and the Beta-Carotene and Retinol Efficacy Trial (CARET) (Omenn et al., 1996) studies on vitamins.

4.11.4 Other sources of information about effects in humans

4.11.4.1 Poisoning

Case-reports and case-series from surveillance of accidental or deliberate poisoning cases (e.g. from regional and national poison information centres) are further valuable indicators of the harm that very high doses of a substance can cause.

Like some occupational data, the reports must be interpreted with care in relation to more conventional, lower-dose exposure, but they

can still be invaluable in indicating target organs and effects and toxic dose levels. Information about effective therapies can also be a useful guide to the mechanism of the toxic action and to the toxicokinetics of the substance in humans.

4.11.4.2 *Human tissues and other preparations in vitro*

Experiments on human cells or tissues or using other preparations containing or expressing human enzymes, receptors and other subcellular factors in vitro are fundamentally different from studies in people, because they bypass absorption, distribution, aspects of integrated metabolism and excretion. However, an advantage is that they permit mechanistic studies under controlled conditions not feasible in the clinic. Concentration–effect relationships need to be related to the toxicokinetics and possible blood and tissue concentrations of the substance in order to identify those in vitro effects that are feasible in vivo.

These techniques are of considerable value in suggesting metabolic pathways and response mechanisms that may be important in humans and may be worth monitoring as biomarkers of exposure or effect. A further important role of such in vitro experiments is to investigate similarities and differences between humans and test species in the metabolism and effects of xenobiotics that may provide information critical to the extrapolations normally used in risk assessment. In vitro studies are likely to be important in defining CSAFs for toxicodynamics (see chapter 5). They are also of potential value in investigations on the influence of genotypic and phenotypic differences on the metabolism and activities of compounds.

4.11.5 *Ethical, legal and regulatory issues*

Ethical, legal and regulatory issues have to be considered for any study involving humans or human tissues. Some are applicable throughout the world, and others are specific to the locale where the study is done. Associated factors affecting any study in humans are national laws about liability should any harm result from the exposure or the trial, any requirement for insurance coverage against that risk and the legal protection afforded to confidentiality of personal information.

Many of the requirements are mandatory, and non-compliance or breach of them may prevent the study from being done, or there may

be legal sanctions and other penalties for all those involved, rejection by official bodies of the information obtained and refusal by editors to consider reports for publication in the biomedical press.

Experiments in humans are strictly controlled to ensure ethical, legal and medical protection of the subjects and the avoidance of foreseeable risks. It is mandatory, therefore, in planning clinical work to justify any proposal to do experimental investigations in humans, especially if it involves data to be used in risk assessment, which may imply uncertainty about risks to which the participants may be exposed. It is necessary to provide a clear, objective explanation as to why only results of experiments in people will provide information that is essential for risk assessment of the material or substance in question. It should be shown how findings from conventional, non-clinical experiments and in vitro and ex vivo studies using human tissues or preparations expressing human enzymes, receptors, etc. cannot give information of the same or similar value for risk assessment purposes.

The most important factor governing a study in healthy people is that a formal evaluation of any possibility of harm to participants and a documented judgement that there is no realistic likelihood of such a risk have been recorded. The fundamental assessment is the same in every type of human experiment, but the nature of the investigation has a considerable influence on the information required to support the evaluation of potential risk. Risk assessments on the proposed studies are an essential part of the evaluation by the institutional review board/independent ethics committee. Evaluation of studies on substances in food would be based on assessment of the likely overall value of the possible research findings and the lack of any predictable risk, based on appropriate non-clinical information.

4.12 Gastrointestinal tract considerations, including effects on the gut flora

4.12.1 General considerations

Interactions that may occur between chemicals in food, including food additives and residues of veterinary drugs, and the bacterial flora of the gastrointestinal tract should be considered in terms of the effects of the gut microflora on the chemical and the effects of the chemical on the gut microflora.

Because the gut microflora is important in the metabolic fate and toxicological activity of some chemicals, the safety assessment should consider the possibility that the chemical in food may affect the host microflora and thereby modify the host response to the chemical in food.

The gut microflora may influence the outcome of toxicity tests in a number of ways, reflecting their importance in relation to the nutritional status of the host animal, the metabolism of xenobiotics prior to absorption and the hydrolysis of biliary conjugation products. JECFA has recognized this and has drawn attention to the usefulness in toxicological evaluations of studies on metabolism involving the intestinal microflora (FAO/WHO, 1971).

4.12.1.1 Effects of the gut microflora on the chemical

The spectrum of metabolic activities performed by the gut flora contrasts markedly with that of the host tissues. Whereas hepatic metabolism of foreign compounds is predominantly by oxidation and conjugation reactions, the gut bacteria perform largely reductive and hydrolytic reactions, some of which appear to be unique to the gut flora. Typical reactions include 1) the hydrolysis of glycosides (including glucuronide conjugates), amides, sulfates and sulfamates, 2) the reduction of double bonds and functional groups and 3) the removal of functional groups, such as phenol and carboxylic acid moieties.

From a structural point of view, many chemicals present in food are potential substrates for microbial metabolism. Microbial metabolism of foreign compounds has the potential to convert the molecule into a more toxic form.

The gut bacterial flora is situated principally in the terminal parts of the intestinal tract in most host species and consists primarily of strict anaerobes. Thus, highly lipid-soluble compounds that are absorbed in the upper intestine will not undergo bacterial metabolism unless tissue metabolism produces conjugates that are excreted into the bile and delivered to the bacterial microflora. Clearly, the design of appropriate investigations with the gut microflora must be linked closely to in vivo studies on absorption and metabolism.

There are three primary in vivo methods for studying the role of the gut microflora in the metabolism of a compound:

1) parenteral administration of the compound, which should result in decreased microbial metabolism of poorly absorbed polar compounds, compared with oral dosing;
2) studies on animals in which the bacterial flora is reduced by the use of antibiotics; and
3) studies on germ-free animals and on (formerly) germ-free animals inoculated with known strains of bacteria (gnotobiotic animals).

In vitro incubation of the food additive or its metabolites with the bacteria of the caecum or faeces is a useful but difficult technique, with considerable potential for the generation of spurious data. Some of the pitfalls of prolonged incubations are that the use of a nutrient medium may allow the growth of a non-representative bacterial population and that the use of a non-nutrient medium may act as a powerful selective force for organisms able to use the additive as a source of carbon, nitrogen, sulfur or energy.

A number of factors may influence the metabolic activation of foreign chemicals by the host microflora:

- *Host species*: Species differences exist in the number and type of bacteria found in the gut and in their distribution along the gastrointestinal tract. In this respect, rats and mice are poor models for humans, because the higher pH of the stomach allows the presence of significant numbers of largely aerobic bacteria in the upper intestinal tract; this region is almost sterile in humans, dogs and rabbits, because ingested organisms do not survive the low gastric pH in these species. In addition, coprophagy occurs in rodents and rabbits, which may complicate the kinetics of poorly absorbed compounds and theoretically could enhance the potential for metabolic adaptation.

- *Individual variations*: There is wide variability between individuals within a species in the extent to which some compounds undergo metabolism by the gut flora. Interindividual variability in the hydrolysis of the sweetener cyclamate greatly exceeds the

variability in foreign compound metabolism in the liver. Many of these variations probably arise from differences in the enzymatic capacity of the gut flora rather than in the delivery of the chemical to the lower intestine. Thus, if animal studies show that a chemical in food is metabolized by the gut flora to an entity of toxicological significance, it is essential that its metabolic fate is characterized in a sufficient number of humans to define the extent of any variability.

- *Diet*: The composition of the gut flora depends on the diet, which may influence the extent of microbial metabolism of a chemical in food.

- *Medication*: The widespread oral administration of medications, such as antibiotics and antacids, in the human population is a potential source of variation in metabolism by the gut microflora.

- *Metabolic adaptation*: The metabolic capacity of the gut flora is far more flexible than that of the host. Thus, long-term administration of foreign chemicals can lead to changes in both the pattern and extent of microbial metabolism of the chemical. Because prior exposure to the compound under test may significantly alter the metabolic potential of the gut microflora, metabolic studies should be performed not only on previously unexposed animals, but also on animals that have been exposed to the test compound for sufficient time to allow metabolic adaptation (a period of weeks rather than days). For the same reason, any in vitro studies should be performed with caecal contents that have been collected both prior to and during long-term animal feeding studies.

4.12.1.2 Effects of the chemical on the gut microflora

During high-dose animal feeding studies, the gut microflora may be affected in two ways:

1) *Antibacterial activity*: A weak antibacterial activity, shown by, for example, a food additive, may manifest after long-term intake of near-toxic doses either as an alteration in the numbers of bacteria present, which can be measured directly, or as an abnormal

microbial metabolic pattern. The latter can be studied by measurement of certain endogenous metabolites produced only by the gut flora, such as phenol and *p*-cresol, which provide indirect evidence of alterations in the gut flora. Such information may also be of value in the interpretation of other variables, such as nitrogen balance.

2) *Increased substrate for gut microflora*: The chemical may act directly as a substrate for bacterial growth. This can be readily illustrated by appropriate high-dose pharmacokinetic studies, coupled with in vitro metabolic studies on the gut flora. Alternatively, the chemical may inhibit digestion or absorption of other dietary components so that these become available to the bacteria in the lower intestine in increased amounts.

Increased amounts of substrates in the lower intestine provide an increased osmotic effect in the caecum, which may result in caecal enlargement. The reason for caecal enlargement must be studied before the significance of the lesion can be assessed, because it may be indicative of 1) abnormal osmotic balance with consequent changes in permeability to minerals in the caecum, which could lead to nephrocalcinosis; 2) microbial metabolism of nutrients, which could result in the formation of potentially toxic metabolites and abnormalities in the nitrogen balance; or 3) microbial metabolism of the chemical, which might lead to the formation of toxic products.

4.12.2 Decision tree approach for determining the potential adverse effects of residues of veterinary antimicrobial drugs on the human intestinal microflora

The potential for antibiotics in food to alter the intestinal flora is an important safety consideration. The only class of veterinary drugs to date that JECFA has evaluated for which the ADI is based on the selection of resistant bacterial strains is the tetracyclines (FAO/WHO, 1999b). At its fifty-second meeting, JECFA developed a decision tree for evaluating the potential effects of veterinary drug residues on human intestinal microflora (FAO/WHO, 2000a). This approach has been used subsequently by JECFA in several evaluations of residues of veterinary antimicrobial drugs (FAO/WHO, 2001a, 2002b, 2004).

At its fifty-second meeting (FAO/WHO, 2000a), JECFA proposed a comprehensive decision tree that takes account of all relevant data from model in vitro and in vivo test systems and includes minimum inhibitory concentrations (MICs) when setting an ADI. Similar approaches have been subsequently developed and used by several regulatory authorities. In the interest of harmonization of methods, VICH published a guideline entitled Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: General Approach to Establish a Microbiological ADI (VICH, 2004). This VICH guideline is a refinement of the JECFA approach. In recognition of the importance of international harmonization, JECFA, at its sixty-sixth meeting (FAO/WHO, 2006), agreed to incorporate the VICH guideline in future assessments to ensure consistency and transparency in the determination of microbiological ADIs.

A summary of the recommendations is given below:

1. Additional microbiological data are not required if there is evidence that:
   - the veterinary drug and its residues do not have antimicrobial properties, and/or
   - ingested residues do not enter the lower bowel, and/or
   - the ingested residues are transformed to inactive metabolites before entering the lower bowel, and/or
   - the ingested residues are transformed quantitatively to microbiologically inactive metabolites, and/or
   - data on the effects of the veterinary drug on gastrointestinal microflora in vitro and in vivo provide a basis for concluding that the ADI derived from toxicological data is sufficiently low to protect the intestinal microflora, and/or
   - clinical data show that the incidence of toxicological effects after therapeutic use of the drug in humans is substantially higher than that of any gastrointestinal side-effects due to the disruption of the microflora.

2. If none of the above can be demonstrated, additional studies were proposed for establishing an ADI (for detailed guidance, see FAO/WHO, 2000a):
   - The class of drug should be considered in order to determine whether the main concern is the emergence of resistance or

the disruption of the intestinal microflora. If effects on the barrier to colonization are a concern, the MIC of the veterinary drug against bacterial strains representative of relevant genera of the microflora in the gastrointestinal tract of healthy individuals can be used as the basis for a conservative estimate of the ADI.

– If disruption of the barrier to colonization is the concern and data are not available, information should be provided to show either that addition of the veterinary drug at concentrations covering the range expected in the colon from an ADI based on other effects does not cause disruption of the barrier to colonization or that oral administration of the veterinary drug to a monogastric animal (e.g. rat, mouse or other rodent inoculated with human flora), at a dose that would result in the concentrations expected in the human colon at an ADI, shows no effect on the barrier to colonization.
– If emergence of antimicrobial resistance due to consumption of residues is the concern, data to show that the expected residue concentrations in the colon do not change the antibiotic resistance or resident populations of *Escherichia coli* or other bacteria appropriate for the drug class should be provided.
– If the concern is change in a specific enzymatic activity that is directly linked to adverse effects on human health, in vitro or in vivo tests should be conducted to determine the concentration of the drug that does not alter that specific enzymatic activity.

4.13 References[1]

Adler I-D & Ashby J (1989) The present lack of evidence for unique rodent germ-cell mutagens. Mutat Res, **212**: 55–66.

Allgood GS, Kuter DJ, Roll KT, Taylor SL & Zorich NL (2001) Postmarketing surveillance of new food ingredients: results from the program with the fat replacer olestra. Regul Toxicol Pharmacol, **33**: 224–233.

[1] Internet links provided in these references were active as of the date of final editing.

Ames BN, Durston WE, Yamasaki E & Lee FD (1973) Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection. Proc Natl Acad Sci USA, **70**: 2281–2285.

Ames BN, McCann J & Yamasaki E (1975) Methods for detecting carcinogens and mutagens with the *Salmonella*/microsome test: assay of 300 chemicals. Mutat Res, **31**: 347–364.

Arni P, Ashby J, Castellino S, Engelhardt G, Herbold BA, Priston RAJ & Bontinck WJ (1988) Assessment of the potential germ cell mutagenicity of industrial and plant protection chemicals as part of an integrated study of genotoxicity in vitro and in vivo. Mutat Res, **203**: 177–184.

Asero R, Mistrello G, Roncarolo D, de Vries SC, Gautier MF, Ciurana LF, Verbeek E, Mohammadi T, Knul-Brettlova V, Akkerdaas JH, Bulder I, Aalberse RC & Van Ree R (2000) Lipid transfer protein: a pan-allergen in plant-derived foods that is highly resistant to pepsin digestion. Int Arch Allergy Appl Immunol, **122**: 20–32.

Asero R, Ballmer-Weber BK, Beyer K, Conti A, Dubakiene R, Fernandez-Rivas M, Hoffmann-Sommergruber K, Lidholm J, Mustakov T, Oude Elberink JNG, Pumphrey RSH, Skov PS, Van Ree R, Vlieg-Boerstra BJ, Hiller R, Hourihane JO, Kowalski M, Papadopoulos NG, Wal J-M, Mills ENC & Vieths S (2007) IgE-mediated food allergy diagnosis: current status and new perspectives. Mol Nutr Food Res, **51**: 135–147.

Ashby J & Tennant RW (1991) Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the US NTP. Mutat Res, **257**: 229–306.

Astwood JD, Leach JN & Fuchs RL (1996) Stability of food allergens to digestion in vitro. Nat Biotechnol, **14**: 1269–1273.

ATBC (1994) The effect of vitamin E and beta-carotene on the incidence of lung cancer and other cancers in male smokers. The Alpha-Tocopherol Beta-Carotene (ATBC) Cancer Prevention Study Group. New Engl J Med, **330**: 1029–1035.

Auerbach C, Robson JM & Carr JG (1947) The chemical production of mutations. Science, **105**(2713): 243–247.

Auletta A & Ashby J (1988) Workshop on the relationship between short-term test information and carcinogenicity: Williamsburg, Virginia, January 20–23, 1987. Environ Mol Mutagen, **11**: 135–145.

Ballmer-Weber BK, Holzhauser T, Scibilia J, Mittag D, Zisa G, Ortolani C, Oesterballe M, Poulsen LK, Vieths S & Bindslev-Jensen C (2007) Clinical characteristics of soybean allergy in Europe: a double-blind, placebo-controlled food challenge study. J Allergy Clin Immunol, **119**(6): 1489–1496.

Barlow SM, Greig JB, Bridges JW, Carere A, Carpy AJ, Galli CL, Kleiner J, Knudsen I, Koeter HB, Levy LS, Madsen C, Mayer S, Narbonne JF, Pfannkuch F, Prodanchuk MG, Smith MR & Steinberg P (2002) Hazard identification by methods of animal-based toxicology. Food Chem Toxicol, **40**: 145–191.

Basketter DA, Evans P, Fielder RJ, Gerberick GF, Dearman RJ & Kimber I (2002) Local lymph node assay—validation, conduct and use in practice. Food Chem Toxicol, **40**(5): 593–598.

Becker WM & Reese G (2001) Immunological identification and characterization of individual food allergens. J Chromatogr B, **756**: 131–140.

Bindslev-Jensen C, Ballmer-Weber BK, Bengtsson U, Blanco C, Ebner C, Hourihane J, Knulst AC, Moneret-Vautrin DA, Nekam K, Niggemann B, Osterballe M, Ortolani C, Ring J, Schnopp C & Werfel T (2004) Standardization of food challenges in patients with immediate reactions to foods—Position paper from the European Academy of Allergology and Clinical Immunology. Allergy, **59**: 690–697.

Bock SA, Sampson HA, Atkins FM, Zeigler RS, Lehrer S, Sachs M, Bush RK & Metcalfe DD (1988) Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual. J Allergy Clin Immunol, **82**: 986–997.

Bøgh KL, Kroghsbo S, Dahl L, Barkholt V, Rigby NM, Mills ENC & Madsen CB (2008) Digested Ara h 1 retains its sensitising capacity in Brown Norway rats. Presented at the Nordic Symposium on Allergy, Sønderborg (abstract).

Bonita R, Beaglehole R & Kjellström T (2006) Basic epidemiology, 2nd ed. Geneva, World Health Organization.

Boobis AR, Cohen SM, Dellarco V, McGregor D, Meek ME, Vickers C, Willcocks D & Farland W (2006) IPCS framework for analyzing the relevance of a cancer mode of action for humans. Crit Rev Toxicol, **36**: 781–792.

Boyano Martínez T, García-Ara C, Diaz-Pena JM, Muñoz FM, García Sánchez G & Esteban MM (2001) Validity of specific IgE antibodies in children with egg allergy. Clin Exp Allergy, **31**: 1464–1469.

Bradford Hill A (1965) The environment and disease: association or causation? Proc R Soc Med, **58**: 295–300.

Brandtzaeg P (2002) Current understanding of gastrointestinal immunoregulation and its relation to food allergy. Ann NY Acad Sci, **964**: 13–45.

Bredehorst R & David K (2001) What establishes a protein as an allergen? J Chromatogr B, **756**: 33–40.

Breiteneder H & Ebner C (2000) Molecular and biochemical classification of plant-derived food allergens. J Allergy Clin Immunol, **106**: 27–36.

Breiteneder H & Mills ENC (2005) Molecular properties of food allergens. J Allergy Clin Immunol, **115**: 14–23.

Broxup B (1991) Neuropathology as a screen for neurotoxicity assessment. J Am Coll Toxicol, **10**: 689–695.

Buchanan R, Dennis S, Gendel S, Acheson D, Assimon SA, Beru N, Bolger P, Carlson D, Carvajal R, Copp C, Falci K, Garber E, Harden E, Kane R, Kvenberg J, Luccioli S, Park D, Raybourne R, Troxell T & Vierk K; Threshold Working Group (2008) Approaches to establish thresholds for major food allergens and for gluten in food. J Food Prot, **71**: 1043–1088.

Buelke-Sam J, Kimmel CA & Adams J eds (1985) Design considerations in screening for behavioural teratogens: results of the collaborative behavioural teratology study. Neurobehav Toxicol Teratol, **7**(6): 537–789.

Burchiel SW, Kerkvliet NL, Gerberick GF, Lawrence DA & Ladics GS (1997) Assessment of immunotoxicity by multiparameter flow cytometry. Fundam Appl Toxicol, **38**(1): 38–54.

Burleson GR, Dean JH & Munson AE (1995) Methods in immunotoxicology. New York, NY, Wiley-Liss.

Bushnell P, Padilla S, Ward T, Pope C & Olszyk V (1991) Behavioral and neurochemical changes in rats dosed repeatedly with diisopropylfluorophosphate (DFP). J Pharmacol Exp Ther, **256**: 741–750.

Capen CC, Dybing E, Rice JM & Wilbourn JD (1999) Species differences in thyroid, kidney and urinary bladder carcinogenesis. Lyon, International Agency for Research on Cancer (IARC Scientific Publications, No. 147).

Catalano PJ, McDaniel KL & Moser VC (1997) The IPCS Collaborative Study on Neurobehavioral Screening Methods. VI. Agreement and reliability of the data. Neurotoxicology, **18**: 1057–1064.

Catassi C, Fabiani E, Iacono G, D'Agate C, Francavilla R, Biagi F, Volta U, Accomando S, Picarelli A, De Vitis I, Pianelli G, Gesuita R, Carle F, Mandolesi A, Bearzi I & Fasano A (2007) A prospective, double-blind, placebo-controlled trial to establish a safe gluten threshold for patients with celiac disease. Am J Clin Nutr, **85**(1): 160–166.

Chang LW & Slikker W Jr eds (1995) Neurotoxicology: approaches and methods. San Diego, CA, Academic Press.

Chapin RE & Sloane RA (1997) Reproductive assessment by continuous breeding: evolving study design and summaries of ninety studies. Environ Health Perspect, **105**(Suppl 1): 199–205.

Chernoff N, Roger JM & Kavlock RJ (1989) An overview of maternal toxicity and prenatal development: considerations for developmental toxicity hazard assessments. Toxicology, **59**: 111–125.

Chung K, Baker JR Jr, Baldwin JL & Chou A (2001) Identification of carmine allergens among three carmine allergy patients. Allergy, **56**(1): 73–77.

Coggon D, Rose G & Barker DJM (1997) Epidemiology for the uninitiated, 4th ed. London, BMJ Publishing Group (http://www.bmj.com/epidem/epid.html).

Cohen SM, Robinson D & MacDonald J (2001) Alternative models for carcinogenicity testing. Toxicol Sci, **64**(1): 14–19.

Coligan JE, Bierer BE, Margulies DH, Shevach EM, Strober W & Coico R (2005) Current protocols in immunology. Winston-Salem, NC, John Wiley & Sons.

Cooper JA, Saracci R & Cole P (1979) Describing the validity of carcinogen screening tests. Br J Cancer, **39**: 87–89.

Cooper RL, Lamb JC, Barlow SM, Bentley K, Brady AM, Doerrer NG, Eisenbrandt DL, Fenner-Crisp PA, Hines RN, Irvine LFH, Kimmel CA, Koeter H, Li AA, Makris SL, Sheets LP, Speijers GJA & Whitby KE (2006) A tiered approach to life stages testing for agricultural chemical safety assessment. Crit Rev Toxicol, **36**: 69–98.

Cory-Slechta DA, Crofton KM, Foran JA, Ross JF, Sheets LP, Weiss B & Mileson B (2001) Methods to identify and characterize developmental neurotoxicity for human health risk assessment. I: Behavioral effects. Environ Health Perspect, **109**: 79–91.

Crevel RWR, Briggs D, Hefle SL, Knulst AC & Taylor SL (2007) Hazard characterisation in food allergen risk assessment: the application of statistical approaches and the use of clinical data. Food Chem Toxicol, **45**: 691–701.

Crevel RW, Ballmer-Weber BK, Holzhauser T, Hourihane JO, Knulst AC, Mackie AR, Timmermans F & Taylor SL (2008) Thresholds for food allergens and their value to different stakeholders. Allergy, **63**: 597–609.

Crow JF (1968) Rate of genetic change under selection. Proc Natl Acad Sci USA, **59**: 655–668.

Dalal I, Binson I, Reifen R, Amitai Z, Shohat T, Rahmani S, Levine A, Ballin A & Somekh E (2002) Food allergy is a matter of geography after all: sesame as a major cause of severe IgE-mediated food allergic reactions among infants and young children in Israel. Allergy, **57**(4): 362–365.

Damstra T, Barlow S, Bergman A, Kavlock R & Van Der Kraak G eds (2002) Global assessment of the state-of-the-science of endocrine disruptors. Geneva, World Health Organization, International Programme on Chemical Safety, 180 pp (WHO/IPCS/EDC/02.2; http://www.who.int/ipcs/publications/new_issues/endocrine_disruptors/en/).

Daston GP (1994) Relationships between maternal and developmental toxicity. In: Kimmel CA & Buelke-Sam J eds. Developmental toxicology, 2nd ed. New York, NY, Raven Press, pp 189–214.

Dean JH, Twerdok LE, Tice RR, Sailstad DM, Hattan DG & Stokes WS (2001) ICCVAM evaluation of the murine local lymph node assay. Conclusions and

recommendations of an independent scientific peer review panel. Regul Toxicol Pharmacol, **34**(3): 258–273.

Descotes J (2003) From clinical to human toxicology: linking animal research and risk assessment in man. Toxicol Lett, **140–141**: 3–10.

Deuel RK (1977) Determining sensory deficits in animals. In: Myers R ed. Methods in psychobiology. New York, NY, Academic Press, pp 99–125.

Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA Jr, Butel JS & Bradley A (1992) Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature, **356**: 215–221.

Dorman DC, Allen SL, Byczkowski JZ, Claudio L, Fisher JE, Fisher JW, Harry GJ, Li AA, Makris SL, Padilla S, Sultatos LG & Mileson BE (2001) Methods to identify and characterize developmental neurotoxicity for human health risk assessment. III: Pharmacokinetic and pharmacodynamic considerations. Environ Health Perspect, **109**: 101–111.

Duffus JH & Worth HGJ (2006) Fundamental toxicology. London, Royal Society of Chemistry.

Duggan C, Gannon J & Walker WA (2002) Protective nutrients and functional foods for the gastrointestinal tract. Am J Clin Nutr, **75**: 789–908.

Dybing E, Doe J, Groten J, Kleiner J, O'Brien J, Renwick AG, Schlatter J, Steinberg P, Tritscher A, Walker R & Younes M (2002) Hazard characterization of chemicals in food and diet: dose response, mechanisms and extrapolation issues. Food Chem Toxicol, **40**: 237–282.

Ebo DG & Stevens WJ (2001) IgE-mediated food allergy—Extensive review of the literature. Acta Clin Belg, **56**(4): 234–247.

EC (1996) Technical guidance document in support of Commission Directive 93/67/EEC on risk assessment for new notified substances and Commission Regulation EC/1488/94 on risk assessment for existing substances. Luxembourg, European Commission.

EC (1997) Criteria for the qualitative evaluation of human neurobehavioral studies of neurotoxicity. Luxembourg, European Commission, Office for Official Publications of the European Communities.

EC (1999) Report of the Working Group on Endocrine Disrupters of the Scientific Committee on Toxicity, Ecotoxicity and the Environment, Directorate-General for Consumer Policy and Consumer Health Protection. Brussels, European Commission.

EC (2006) European Union risk assessment report on phenol. CAS No. 108-95-2. EINECS No. 203-632-7. 1st Priority List, Volume 64. Revised edition. EUR 22522 EN/1. Luxembourg, European Commission, Directorate-General Joint Research Centre, European Chemicals Bureau; Office for Official Publications

of the European Communities (http://ecb.jrc.it/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/phenolreport060.pdf).

ECETOC (1992) Evaluation of the neurotoxic potential of chemicals. Brussels, European Centre for Ecotoxicology and Toxicology of Chemicals (Monograph No. 18).

ECETOC (1998) Organophosphorous pesticides and long-term effects on the nervous system. Brussels, European Centre for Ecotoxicology and Toxicology of Chemicals (Technical Report No. 75).

EDSTAC (1998) The Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) final report. Washington, DC, United States Environmental Protection Agency (http://www.epa.gov/endo/pubs/edspoverview/finalrpt.htm).

EFSA (2004) Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission relating to the evaluation of allergenic foods for labelling purposes. EFSA J, **32**: 1–197.

EFSA (2009) Draft scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. Parma, European Food Safety Authority, Panel on Genetically Modified Organisms (http://www.efsa.europa.eu/en/consultations/call/gmo091201-ax1.pdf).

Eisenbrandt DL, Allen SL, Berry PH, Classen W, Bury D, Mellert W, Millischer RJ, Schuh W & Bontinck WJ (1994) Evaluation of the neurotoxic potential of chemicals in animals. Food Chem Toxicol, **32**: 655–669.

Eiwegger T, Rigby N, Mondoulet L, Bernard H, Krauth MT, Boehm A, Dehlink E, Valent P, Wal JM, Mills ENC & Szepfalusi Z (2006) Gastro-duodenal digestion products of the major peanut allergen Ara h 1 retain an allergenic potential. Clin Exp Allergy, **36**: 1281–1288.

Enzmann H, Bomhard E, Iatropoulos M, Ahr HJ, Schlueter G & Williams GM (1998a) Short- and intermediate-term carcinogenicity testing—a review. Part 1: The prototypes mouse skin tumour assay and rat liver focus assay. Food Chem Toxicol, **36**: 979–995.

Enzmann H, Iatropoulos M, Brunnemann KD, Bomhard E, Ahr HJ, Schlueter G & Williams GM (1998b) Short- and intermediate-term carcinogenicity testing—a review. Part 2: Available experimental models. Food Chem Toxicol, **36**: 997–1013.

Faccini JM, Butler WR, Friedmann JC, Hess R, Reznik GK, Ito N, Hayashi Y & Williams GM (1992) IFSTP guidelines for the design and interpretation of the chronic rodent carcinogenicity bioassay. Exp Toxicol Pathol, **44**: 443–456.

FAO (1995) Food allergies. Report of the FAO Technical Consultation on Food Allergies, Rome, 13–14 November 1995. Rome, Food and Agriculture Organization of the United Nations.

FAO/WHO (1971) Evaluation of food additives: specifications for the identity and purity of food additives and their toxicological evaluation: some extraction solvents and certain other substances; and a review of the technological efficacy of some antimicrobial agents. Fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Nutrition Meetings Series, No. 48; WHO Technical Report Series, No. 462; http://whqlibdoc.who.int/trs/WHO_TRS_462.pdf).

FAO/WHO (1995) Evaluation of certain food additives and contaminants. Forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 859; http://whqlibdoc.who.int/trs/WHO_TRS_859.pdf).

FAO/WHO (1997) Evaluation of certain food additives and contaminants. Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 868; http://whqlibdoc.who.int/trs/WHO_TRS_868.pdf).

FAO/WHO (1998) Abamectin (addendum). In: Pesticide residues in food—1997 evaluations. Geneva, World Health Organization (WHO/PCS/98.6; http://www.inchem.org/documents/jmpr/jmpmono/v097pr02.htm).

FAO/WHO (1999a) Procedures for estimating acute reference dose. In: Pesticide residues in food—1998. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations, pp 14–17 (FAO Plant Production and Protection Paper, No. 148).

FAO/WHO (1999b) Evaluation of certain veterinary drug residues in food. Fiftieth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 888; http://whqlibdoc.who.int/trs/WHO_TRS_888.pdf).

FAO/WHO (2000a) Evaluation of certain veterinary drug residues in food. Fifty-second report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 893; http://whqlibdoc.who.int/trs/WHO_TRS_893.pdf).

FAO/WHO (2000b) Evaluation of certain food additives and contaminants. Fifty-third report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 896; http://whqlibdoc.who.int/trs/WHO_TRS_896.pdf).

FAO/WHO (2001a) Evaluation of certain veterinary drug residues in food. Fifty-fourth report of the Joint FAO/WHO Meeting on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 900; http://whqlibdoc.who.int/trs/WHO_TRS_900.pdf).

FAO/WHO (2001b) Evaluation of allergenicity of genetically modified foods. Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology, Rome, 22–25 January 2001. Geneva, World Health Organization (http://www.who.int/foodsafety/publications/biotech/ec_jan2001/en/index.html).

FAO/WHO (2002a) Pesticide residues in food—2002. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 172; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/2002_rep/2002JMPRReport2.pdf).

FAO/WHO (2002b) Evaluation of certain veterinary drug residues in food. Fifty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 911; http://whqlibdoc.who.int/trs/WHO_TRS_911.pdf).

FAO/WHO (2003) Appendix III, Draft guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants; Appendix IV, Proposed draft annex on the assessment of possible allergenicity of the draft guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants. In: Report of the third session of the Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology, Yokohama, 4–8 March 2002. Rome, Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission, pp 47–60 (ALINORM 03/34; http://www.codexalimentarius.net/web/archives.jsp?year=03).

FAO/WHO (2004) Evaluation of certain veterinary drug residues in food. Sixty-second report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 925; http://whqlibdoc.who.int/trs/WHO_TRS_925.pdf).

FAO/WHO (2006) Evaluation of certain veterinary drug residues in food. Sixty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 939; http://whqlibdoc.who.int/publications/2006/9241209399_eng.pdf).

FAO/WHO (2008) Appendix III, Draft revised Codex standard for special dietary use for persons intolerant to gluten. In: Report of the twenty-ninth session of the Codex Committee on Nutrition and Foods for Special Dietary Purposes, Bad Neuenahr-Ahrweiler, 12–16 November 2007. Rome, Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission (ALINORM 08/31/26; ftp://ftp.fao.org/codex/Alinorm08/al31_26e.pdf).

Fernandez-Rivas M, Bolhaar S, Gonzalez-Mancebo E, Asero R, Van Leeuwen A, Bohle B, Ma Y, Ebner C, Rigby N, Sancho AI, Miles S, Zuidmeer L, Knulst A, Breiteneder H, Mills C, Hoffmann-Sommergruber K & Van Ree R (2006) Apple allergy across Europe: how allergen sensitization profiles determine the clinical expression of allergies to plant foods. J Allergy Clin Immunol, **118**: 481–488.

Flammang TJ, Tungeln LS, Kadlubar FF & Fu PP (1997) Neonatal mouse assay for tumorigenicity: alternative to the chronic rodent bioassay. Regul Toxicol Pharmacol, **26**: 230–240.

Flinterman AE, Pasmans SG, Hoekstra MO, Meijer Y, Hoffen EV, Knol EF, Hefle SL, Bruijnzeel-Koomen CA & Knulst AC (2006) Determination of no-observed-adverse-effect levels and eliciting doses in a representative group of peanut-sensitized children. J Allergy Clin Immunol, **117**: 448–454.

Foote RH & Carney EW (2000) The rabbit as a model for reproductive and developmental toxicity studies. Reprod Toxicol, **14**: 77–93.

French J, Storer RD & Donehower LA (2001) The nature of the heterozygous Trp53 knockout model for identification of mutagenic carcinogens. Toxicol Pathol, **29** (Suppl): 24–29.

FSA (2002) Guidelines on the use of human studies in the pre-market safety assessment of novel foods. London, United Kingdom Food Standards Agency, Advisory Committee on Novel Foods and Processes (http://www.acnfp.gov.uk/acnfppapers/inforelatass/guidetastehuman/guidlinespre-market).

Fu TT, Abbott UR & Hatzos C (2002) Digestibility of food allergens and non-allergenic proteins in simulated gastric fluid and simulated intestinal fluid—A comparative study. J Agric Food Chem, **50**: 7154–7160.

Gad SC (1982) A neuromuscular screen for use in industrial toxicology. J Toxicol Environ Health, **9**: 691–704.

Gad SC (1989) Principles of screening in toxicology with special emphasis on applications to neurotoxicology. J Am Coll Toxicol, **8**: 21–27.

Garman RH, Fix AS, Jortner BS, Jensen KF, Hardisty JF, Claudio L & Ferenc S (2001) Methods to identify and characterize developmental neurotoxicity for human health risk assessment. II: Neuropathology. Environ Health Perspect, **109**: 93–100.

Gatzidou ET, Zira AN & Theocharis SE (2007) Toxicogenomics: a pivotal piece in the puzzle of toxicological research. J Appl Toxicol, **27**: 302–309.

Gerberick GF, Ryan CA, Dearman RJ & Kimber I (2007) Local lymph node assay (LLNA) for detection of sensitization capacity of chemicals. Methods, **41**(1): 54–60.

Germolec DR, Nyska A, Kashon M, Kuper CF, Portier C, Kommineni C, Johnson KA & Luster MI (2004a) Extended histopathology in immunotoxicity testing: interlaboratory validation studies. Toxicol Sci, **78**(1): 107–115.

Germolec DR, Kashon M, Nyska A, Kuper CF, Portier C, Kommineni C, Johnson KA & Luster MI (2004b) The accuracy of extended histopathology to detect immunotoxic chemicals. Toxicol Sci, **82**(2): 504–514.

Gold LS & Zeiger E (1996) Handbook of carcinogenic potency and genotoxicity data bases. Boca Raton, FL, CRC Press.

Goodman JI (2001) A perspective on current and future uses of alternative models for carcinogenicity testing. Toxicol Pathol, **29**(Suppl): 173–176.

Goodman RE, Vieths S, Sampson HA, Hill D, Ebisawa M, Taylor SL & Ree R (2008) Allergenicity assessment of genetically modified crops—what makes sense? Nat Biotechnol, **26**: 74–81.

Gray LE Jr, Ostby J, Wilson V, Lambright C, Bobseine K, Hartig P, Hotchkiss A, Wolf C, Furr J, Price M, Parks L, Cooper RL, Stoker TE, Laws SC, Degitz SJ, Jensen KM, Kahl MD, Korte JJ, Makynen EA, Tietge JE & Ankley GT (2002) Xenoendocrine disrupters—tiered screening and testing: filling key data gaps. Toxicology, **181–182**: 371–382.

Griffin JW (1990) Basic pathologic processes in the nervous system. Toxicol Pathol, **18**: 83–88.

Gulezian D, Jacobson-Kram D, McCullough CB, Olson HM, Recio L, Robinson DE, Storer RD, Tennant RW, Ward JM & Neumann DA (2000) Use of transgenic animals for carcinogenicity testing: consideration and implications for risk assessment. Toxicol Pathol, **28**: 482–499.

Gundert-Remy U, Dahl SG, Boobis A, Kremers P, Kopp-Schneider A, Oberemm A, Renwick A & Pelkonen O (2005) Molecular approaches to the identification of biomarkers of exposure and effect—report of an expert meeting organized by COST Action B15. November 28, 2003. Toxicol Lett, **156**(2): 227–240.

Haley P, Perry R, Ennulat D, Frame S, Johnson C, Lapointe JM, Nyska A, Snyder P, Walker D, Walter G; STP Immunotoxicology Working Group (2005) Best practice guideline for the routine pathology evaluation of the immune system. Toxicol Pathol, **33**(3): 404–406 (http://www.toxpath.org/Position_Papers/Immune_System.pdf).

Harleman JH (2000) Approaches to the identification and recording of findings in the lymphoreticular organs indicative for immunotoxicity in regulatory type toxicity studies. Toxicology, **142**(3): 213–219.

Harris MW, Chapin RE, Lockhart AC & Jokinen MP (1992) Assessment of short-term reproductive and development toxicity screen. Fundam Appl Toxicol, **19**: 186–196.

Harry GJ (1998) Developmental neurotoxicity. In: Korach KS ed. Reproductive and developmental toxicology. New York, NY, Marcel Dekker, pp 211–249.

Health Canada (1994) Human health risk assessment for priority substances. Ottawa, Ontario, Health Canada, Environmental Health Directorate (Publication No. En40-215/41E).

Hefle SL, Nordlee JA & Taylor SL (1996) Allergenic foods. Crit Rev Food Sci Nutr, **36**(Suppl): S69–S89.

Heilmann C, Grandjean P, Weihe P, Nielsen F & Budtz-Jørgensen E (2006) Reduced antibody responses to vaccinations in children exposed to polychlorinated biphenyls. PLoS Med, **3**(8): 1352–1359.

Heinje WH, Keinhuis AS, Van Ommen B, Stierum RH & Groten JP (2005) Systems toxicology: applications of toxicogenomics, transcriptomics, proteomics and metabolomics in toxicology. Expert Rev Proteomics, **2**: 767–780.

Hertel RF (1996) Outline on risk assessment of existing substances in the European Union. Environ Toxicol Pharmacol, **2**: 93–96.

Hill DJ, Hosking CS, Zhie CY, Leung R, Baratwidjaja K, Iikura Y, Iyngkaran N, Gonzalez-Andaya A, Wah LB & Hsieh KH (1997) The frequency of food allergy in Australia and Asia. Environ Toxicol Pharmacol, **4**: 101–110.

Hill RN, Crisp TM, Hurley PM, Rosenthal SL & Singh DV (1998) Risk assessment of thyroid follicular cell tumors. Environ Health Perspect, **106**: 447–457.

Hischenhuber C, Crevel R, Jarry B, Mäki M, Moneret-Vautrin DA, Romano A, Troncone R & Ward R (2006) Review article: Safe amounts of gluten for patients with wheat allergy or coeliac disease. Aliment Pharmacol Ther, **23**(5): 559–575.

Holden HE (1982) Comparison of somatic and germ cell models for cytogenetic screening. J Appl Toxicol, **2**: 196–200.

Holladay SD & Luster MI (1994) Developmental immunotoxicology. In: Kimmel CA & Buelke-Sam J eds. Developmental toxicology, 2nd ed. New York, NY, Raven Press, pp 93–118.

Hood R (2006) Developmental and reproductive toxicology—A practical approach. Boca Raton, FL, CRC Taylor & Francis.

Host A, Halken S, Jacobsen HP, Christensen AE, Herskind AM & Plesner K (2002) Clinical course of cow's milk protein allergy/intolerance and atopic diseases in childhood. Pediatr Allergy Immunol, **13**(Suppl 15): 23–28.

Hourihane JO (2002) Recent advances in peanut allergy. Curr Opin Allergy Clin Immunol, **2**: 227–231.

Hourihane JO, Kilburn SA, Nordlee JA, Hefle SL, Taylor SL & Warner JO (1997) An evaluation of sensitivity of subjects with peanut allergy to very low doses of peanut protein: a randomized, double-blind, placebo-controlled food challenge study. J Allergy Clin Immunol, **100**: 596–600.

Huby RDJ, Dearman RJ & Kimber I (2000) Why are some proteins allergens? Toxicol Sci, **55**: 235–246.

IARC (1995) Peroxisome proliferation and its role in carcinogenesis. Views and expert opinions of an IARC working group. Lyon, International Agency for Research on Cancer (IARC Technical Report No. 24).

Iatropoulos MJ & Williams GM (1996) Proliferation markers. Exp Toxicol Pathol, **48**: 175–181.

ICH (1994a) Note for guidance on toxicokinetics: The assessment of systemic exposure in toxicity studies. S3A. Geneva, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Harmonised Tripartite Guideline; http://www.ich.org/LOB/media/MEDIA495.pdf).

ICH (1994b) Pharmacokinetics: Guidance for repeated dose tissue distribution studies. S3B. Geneva, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Harmonised Tripartite Guideline; http://www.ich.org/LOB/media/MEDIA496.pdf).

ICH (1994c) Guideline for industry: Detection of toxicity to reproduction for medicinal products. ICH-S5A. Geneva, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm074950.pdf).

ICH (1997) Testing for carcinogenicity of pharmaceuticals. S1B. Geneva, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Harmonised Tripartite Guideline; http://www.ich.org/LOB/media/MEDIA490.pdf).

ICH (2005) Immunotoxicity studies for human pharmaceuticals. S8. Geneva, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Harmonised Tripartite Guideline; http://www.ich.org/LOB/media/MEDIA1706.pdf).

ICICIS Group Investigators (1998) Report of validation study of assessment of direct immunotoxicity in the rat. Toxicology, **125**(2–3): 183–201.

ICME (1994) A guide to neurotoxicity: international regulation and testing strategies. Ottawa, Ontario, International Council on Metals and the Environment.

ILSI (2001) ILSI/HESI alternatives to carcinogenicity testing project. Washington, DC, International Life Sciences Institute. Toxicol Pathol, **29**(Suppl): 1–322.

Immamura T, Kanagawa Y & Ebisawa M (2008) A survey of patients with self-reported severe food allergies in Japan. Pediatr Allergy Immunol, **19**: 270–274.

IPCS (1986a) Principles of toxicokinetic studies. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 57; http://www.inchem.org/documents/ehc/ehc/ehc57.htm).

IPCS (1986b) Principles and methods for the assessment of neurotoxicity associated with exposure to chemicals. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 60; http://www.inchem.org/documents/ehc/ehc/ehc060.htm).

IPCS (1987) Principles for the safety assessment of food additives and contaminants in food. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 70; http://www.inchem.org/documents/ehc/ehc/ehc70.htm).

IPCS (1990) Principles for the toxicological assessment of pesticide residues in food. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 104; http://www.inchem.org/documents/ehc/ehc/ehc104.htm).

IPCS (1996) Principles and methods for assessing direct immunotoxicity associated with exposure to chemicals. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 180; http://www.inchem.org/documents/ehc/ehc/ehc180.htm).

IPCS (1999) Principles and methods for assessing allergic hypersensitization associated with exposure to chemicals. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 212; http://www.inchem.org/documents/ehc/ehc/ehc212.htm).

IPCS (2001a) Neurotoxicity risk assessment for human health: principles and approaches. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 223; http://www.inchem.org/documents/ehc/ehc/ehc223.htm).

IPCS (2001b) Principles for evaluating health risks to reproduction associated with exposure to chemicals. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 225; http://www.inchem.org/documents/ehc/ehc/ehc225.htm).

IPCS (2001c) Biomarkers in risk assessment: validity and validation. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 222; http://www.inchem.org/documents/ehc/ehc/ehc222.htm).

IPCS (2003) Toxicogenomics and the risk assessment of chemicals for the protection of human health. Workshop report. Summary. Geneva, World Health Organization (IPCS/Toxicogenomics/03/1; http://www.who.int/ipcs/methods/en/toxicogenomicssummaryreport.pdf).

IPCS (2004) IPCS risk assessment terminology. Geneva, World Health Organization, International Programme on Chemical Safety (Harmonization Project Document, No. 1; http://www.who.int/ipcs/methods/harmonization/areas/ipcsterminologyparts1and2.pdf).

IPCS (2005) Chemical-specific adjustment factors for interspecies differences and human variability: guidance document for use of data in dose/concentration–response assessment (Harmonization Project Document, No. 2; http://whqlibdoc.who.int/publications/2005/9241546786_eng.pdf).

IPCS (2006a) Principles and methods for assessing autoimmunity associated with exposure to chemicals. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 236; http://www.who.int/ipcs/publications/ehc/ehc236.pdf).

IPCS (2006b) Principles for evaluating health risks in children associated with exposure to chemicals. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 237; http://www.who.int/ipcs/publications/ehc/ehc237.pdf).

IPCS (2009) Principles for modelling dose–response for the risk assessment of chemicals. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 239; http://whqlibdoc.who.int/publications/2009/9789241572392_eng.pdf).

Isaacson RL & Jensen KF (1992a) The vulnerable brain and environmental risks. Vol. 1. Malnutrition and hazard assessment. New York, NY, Plenum Press.

Isaacson RL & Jensen KF (1992b) The vulnerable brain and environmental risks. Vol. 2. Toxins in food. New York, NY, Plenum Press.

Ivarsson A, Persson LA & Nystrom L (2000) Epidemic of celiac disease in Swedish children. Acta Paediatr, **89**: 165–171.

Jacobson-Kram D & Keller KA eds (2006) Toxicological testing handbook: Principles, applications, and data interpretation, 2nd ed. New York, NY, Informa Healthcare USA, Inc.

Jenkins JA, Griffiths-Jones S, Shewry PR, Breiteneder H & Mills ENC (2005) Structural relatedness of plant food allergens with specific reference to cross-reactive allergens: an in silico analysis. J Allergy Clin Immunol, **115**: 163–170.

Jenkins JA, Breiteneder H & Mills EN (2007) Evolutionary distance from human homologs reflects allergenicity of animal food proteins. J Allergy Clin Immunol, **120**: 1399–1405.

Johansson SGO, Hourihane JO, Bousquet J, Bruijnzeel-Koomen C, Dreborg S, Haahtela T, Kowalski ML, Mygind N, Ring J, Van Cauwenberge P, Van Hage-Hamsten M, Wüthrich B; EAACI (the European Academy of Allergology and Clinical Immunology) nomenclature task force (2001) A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. Allergy, **56**: 813–824 [cited in Asero et al., 2007].

Johansson SGO, Bieber T, Dahl R, Friedmann PS, Lanier BQ, Lockey RF, Motala C, Ortega Martell JA, Platts-Mills TA, Ring J, Thien F, Van Cauwenberge

P & Williams HC (2004) Revised nomenclature for allergy for global use: report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. J Allergy Clin Immunol, **113**(5): 832–836.

Johnsen H, Lund SP, Matikainen E, Midtgard U, Simonsen L & Wennberg A (1992) Occupational neurotoxicity: criteria document for evaluation of existing data. Copenhagen, Nordic Council of Ministers and National Institute of Occupational Health.

Johnson MK (1990) Organophosphates and delayed neuropathy. Is NTE alive and well? Toxicol Appl Pharmacol, **102**: 385–399.

Kägi MK, Wüthrich B & Johansson SG (1994) Campari-Orange anaphylaxis due to carmine allergy. Lancet, **344**: 60–61.

Kanny G, Moneret-Vautrin D-A, Flabbee J, Beaudouin E, Morisset M & Thevenin F (2001) Population study of food allergy in France. Allergy, **108**: 133–140.

Kavlock RJ, Logsdon T & Gray JA (1993) Fetal development in the rat following disruption of maternal renal function during pregnancy. Teratology, **48**: 247–258.

Keen CL, Clegg MS, Hanna LA, Lanoue L, Rogers JM, Daston GP, Oteiza P & Uriu-Adams JY (2003) The plausibility of micronutrient deficiencies being a significant contributing factor to the occurrence of pregnancy complications. J Nutr, **133**(5 Suppl 2): 1597S–1605S.

Kemper RA, Hayes JA & Bogdanffy MS (2007) Metabolism: a determinant of toxicity. In: Hayes AW ed. Principles and methods of toxicology, 5th ed. Boca Raton, FL, CRC Press, pp 103–178.

Kimmel CA & Makris S (2001) Recent developments in regulatory requirements for developmental toxicity. Toxicol Lett, **120**: 73–82.

Kimmel CA, Rees DC & Francis EZ (1990) Qualitative and quantitative comparability of human and animal developmental neurotoxicology. Teratology, **12**: 175–292.

Kirkland D, Aardema M, Henderson L & Müller L (2005) Evaluation of the ability of a battery of three in vitro genotoxicity tests to discriminate rodent carcinogens and non-carcinogens. I. Sensitivity, specificity and relative predictivity. Mutat Res, **584**: 1–256.

Kitchin KT (1999) Carcinogenicity: testing, predicting, and interpreting chemical effects. New York, NY, Marcel Dekker.

Kitchin KT & Brown JL (1994) Dose–response relationship for rat liver DNA damage caused by 49 rodent carcinogens. Toxicology, **88**: 31–49.

Klaunig JE, Babich MA, Baetcke KP, Cook JC, Corton JC, David RM, DeLuca JG, Lai DY, McKee RH, Peters JM, Roberts RA & Fenner-Crisp PA (2003)

PPARα agonist-induced rodent tumors: modes of action and human relevance. Crit Rev Toxicol, **33**(6): 655–780.

Krinke GJ (1989) Neuropathologic screening in rodent and other species. J Am Coll Toxicol, **8**: 141–155.

Krishnan K & Andersen ME (2007) Physiologically-based pharmacokinetic and toxicokinetic modeling. In: Wallace Hayes A ed. Principles and methods in toxicology. New York, NY, Taylor & Francis, pp 232–291.

Kroes R, Renwick AG, Cheeseman M, Kleiner J, Mangelsdorf I, Piersma A, Schilter B, Schlatter J, Van Schothorst F, Vos JG & Wurtzen G (2004) Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. Food Chem Toxicol, **42**: 65–83.

Kulig BM (1996) Comprehensive neurotoxicity assessment. Environ Health Perspect, **104**(Suppl 2): 317–322.

Kuper CF, Harleman JH, Richter-Reichelm HB & Vos JG (2000) Histopathologic approaches to detect changes indicative of immunotoxicity. Toxicol Pathol, **28**(3): 454–466.

Lack G, Fox D, Northstone K & Golding J; Avon Longitudinal Study of Parents and Children Study Team (2003) Factors associated with the development of peanut allergy in childhood. New Engl J Med, **348**: 977–985.

Ladefoged O, Lam HR, Ostergaard G & Nielsen E (1995) Neurotoxicology: review of definitions, methodology and criteria. Copenhagen, National Food Agency of Denmark and Danish Environmental Protection Agency (Miljoproject nr. 282).

Lau C & Kavlock RJ (1994) Functional toxicity of the heart, lungs and kidneys. In: Kimmel CA & Buelke-Sam J eds. Developmental toxicology, 2nd ed. New York, NY, Raven Press, pp 119–188.

LeBoeuf RA, Kerckaert KA, Aardema MJ & Isfort RJ (1999) Use of Syrian hamster embryo and BALB/c 3T3 cell transformation for assessing the carcinogenic potential of chemicals. In McGregor DB, Rice JM & Venitt S eds. The use of short- and medium-term tests for carcinogens and data on genetic effects in carcinogenic hazard evaluation. Lyon, International Agency for Research on Cancer, pp 409–425 (IARC Scientific Publications, No. 146).

Leder A, Kuo A, Cardiff RD, Sinn E & Leder P (1990) v-Ha-*ras* transgene abrogates the initiation step in mouse skin tumorigenesis: effects of phorbol esters and retinoic acid. Proc Natl Acad Sci USA, **87**: 9178–9182.

Lee W & Kim RB (2004) Transporters and renal drug elimination. Annu Rev Pharmacol Toxicol, **44**: 137–166.

Lehrer SB, Horner WE & Reese G (1996) Why are some proteins allergenic? Implications for biotechnology. Crit Rev Food Sci Nutr, **36**(6): 553–564.

Leon AS, Hunninghake DB, Bell C, Rassin DK & Tephly TR (1989) Safety of long-term large doses of aspartame. Arch Intern Med, **149**: 2318–2324.

Lipscomb JC & Ohanian EV eds (2007) Toxicokinetics and risk assessment. New York, NY, Informa Healthcare USA, Inc., 361 pp.

Luebke RW, Chen D, Dietert R, Yang Y, King M & Luster MI; Immunotoxicology Working Group (2006) The comparative immunotoxicity of five selected compounds following developmental or adult exposure. J Toxicol Environ Health B Crit Rev, **9**(1): 1–26.

Luster MI, Portier C, Pait DG, White KL Jr, Gennings C, Munson AE & Rosenthal GJ (1992) Risk assessment in immunotoxicology. I. Sensitivity and predictability of immune tests. Fundam Appl Toxicol, **18**(2): 200–210.

Luster MI, Portier C, Pait DG, Rosenthal GJ, Germolec DR, Corsini E, Blaylock BL, Pollock P, Kouchi Y, Craig W, White KL, Munson AE & Comment CE (1993) Risk assessment in immunotoxicology. II. Relationships between immune and host resistance tests. Fundam Appl Toxicol, **21**(1): 71–82.

Luster MI, Germolec DR, Parks C, Blanciforti L, Kashon M & Luebke RW (2005) Associating changes in the immune system with clinical diseases for interpretation in risk assessment. In: Tryphonas H, Fournier M, Blakley BR, Smits JEG & Brousseau P eds. Investigative immunotoxicology. New York, NY, Taylor & Francis, pp 165–182.

Luster MI, Dietert RR, Germolec DR, Luebke RW & Makris SL (2008) Developmental immunotoxicology. In: Sonawane B & Brown R eds. Encyclopedia of environmental health. Oxford, Elsevier.

Madsen CB, Hattersley S, Buck J, Gendel SM, Houben GF, Hourihane JO, Mackie A, Mills EN, Nørhede P, Taylor SL & Crevel RW (2009) Approaches to risk assessment in food allergy: report from a workshop "Developing a framework for assessing the risk from allergenic foods". Food Chem Toxicol, **47**(2): 480–489.

Mahler JF, Flagler ND, Malarkey DE, Mann PC, Haseman JK & Eastin W (1998) Spontaneous and chemically induced proliferative lesions in Tg.AC transgenic and p53-heterozygous mice. Toxicol Pathol, **26**: 501–511.

Matthews EJ, Spalding JW & Tennant RW (1993) Transformation of BALB/c 3T3 cells: transformation responses of 168 chemicals compared with mutagenicity in *Salmonella* and carcinogenicity in rodent bioassays. Environ Health Perspect, **101**(Suppl 2): 347–482.

McClain RM (1993) Growth factors and tumor promotion, implications for risk assessment. Proceedings of the seventh conference on carcinogenesis and risk assessment. Austin, TX, Wiley-Liss.

McClain RM, Keller D, Casciano D, Fu P, MacDonald J, Popp J & Sagartz J (2001) Neonatal mouse model: review of methods and results. Toxicol Pathol, **29**(Suppl): 128–137.

McGregor DB (1996) A review of some properties of ethylene glycol ethers relevant to their carcinogenic evaluation. Occup Hyg, **2**: 213–235.

McGregor D (2006) Genetic toxicology. In: Duffus JH, Worth HGJ eds. Fundamental toxicology. Cambridge, RSC Publishing.

McGregor D & Anderson D (1999) DNA damage and repair in mammalian cells in vitro and in vivo as indicators of exposure to carcinogens. In: McGregor DB, Rice JM & Venitt S eds. The use of short- and medium-term tests for carcinogens and data on genetic effects in carcinogenic hazard evaluation. Lyon, International Agency for Research on Cancer, pp 309–354 (IARC Scientific Publications, No. 146).

McGregor DB, Rice JM & Venitt S eds (1999) Consensus report. In: The use of short- and medium-term tests for carcinogens and data on genetic effects in carcinogenic hazard evaluation. Lyon, International Agency for Research on Cancer, pp 1–18 (IARC Scientific Publications, No. 146).

McMillan DE (1987) Risk assessment for neurobehavioral toxicity. Environ Health Perspect, **76**: 155–161.

Mendez E, Vela C, Immer U & Janssen FW (2005) Report of a collaborative trial to investigate the performance of the R5 enzyme linked immunoassay to determine gliadin in gluten-free food. Eur J Gastroenterol Hepatol, **17**: 1053–1063.

Metcalfe DD, Astwood JD, Townsend R, Sampson HA, Taylor SL & Fuchs RL (1996) Assessment of the allergenic potential of foods derived from genetically engineered crop plants. Crit Rev Food Sci Nutr, **36**(Suppl): S165–S186.

Mileson BE & Ferenc SA (2001) Methods to identify and characterize developmental neurotoxicity for human health risk assessment: overview. Environ Health Perspect, **109**: 77–78.

Miller EC & Miller JA (1979) Milestones in chemical carcinogenesis. Semin Oncol, **6**: 445–460.

Miller TJ, Honchel R, Espandiari P, Knapton A, Zhang J, Sistare FD & Hanig JP (2008) The utility of the K6/ODC transgenic mouse as an alternative short term dermal model for carcinogenicity testing of pharmaceuticals. Regul Toxicol Pharmacol, **50**(1): 87–97.

Moreno FJ, Mellon FA, Wickham MSJ, Bottrill AR & Mills ENC (2005) Stability of the major allergen Brazil nut 2S albumin (Ber e 1) to physiologically relevant in vitro gastrointestinal digestion. FEBS J, **272**: 341–352.

Morton D, Alden CL, Roth AJ & Usui T (2002) The Tg rasH2 mouse in cancer hazard identification. Toxicol Pathol, **30**(1): 139–146.

Moser VC (1989) Screening approaches to neurotoxicity: a functional observational battery. J Am Coll Toxicol, **8**: 85–93.

Moser VC (1995) Comparisons of the acute effects of cholinesterase inhibitors using a neurobehavioural screening battery in rats. Neurotoxicol Teratol, **17**: 617–625.

Moser VC (1997) Behavioural screening for neurotoxicity. In: Lowndes HE & Reuhl KR eds. Comprehensive toxicology. Vol. 11. Nervous system and behavioural toxicology. New York, NY, Pergamon Press, pp 351–362.

Moser VC, Tilson HA, MacPhail RC, Becking GC, Cuomo V, Frantik E, Kulig BM & Winneke G (1997a) The IPCS collaborative study on neurobehavioral screening methods: II. Protocol design and testing procedures. Neurotoxicology, **18**: 929–938.

Moser VC, Becking GC, Cuomo V, Frantik E, Kulig BM, MacPhail RC, Tilson HA, Winneke G, Brightwell WS, Cagiano R, Gill MW, Haggerty GC, Hornychova M, Lammers J, Larsen J-J, McDaniel KL, Nelson BK & Ostergaard G (1997b) The IPCS collaborative study on neurobehavioral screening methods: III. Results of proficiency studies. Neurotoxicology, **18**: 939–946.

Moser VC, Becking GC, Cuomo V, Frantik E, Kulig BM, MacPhail RC, Tilson HA, Winneke G, Brightwell WS, De Salvia MA, Gill MW, Haggerty GC, Hornychova M, Lammers J, Larsen J-J, McDaniel KL, Nelson BK & Ostergaard G (1997c) The IPCS collaborative study on neurobehavioral screening methods: IV. Control data. Neurotoxicology, **18**: 947–967.

Moser VC, Becking GC, Cuomo V, Frantik E, Kulig BM, MacPhail RC, Tilson HA, Winneke G, Brightwell WS, De Salvia MA, Gill MW, Haggerty GC, Hornychova M, Lammers J, Larsen J-J, McDaniel KL, Nelson BK & Ostergaard G (1997d) The IPCS collaborative study on neurobehavioral screening methods: V. Results of chemical testing. Neurotoxicology, **18**: 969–1055.

Müller HJ (1927) Artificial transmutation of the gene. Science, **66**: 84–87.

Munro IC, Ford RA, Kennepohl E & Sprenger JG (1996) Correlation of structural class with no-observed-effect levels: a proposal for establishing a threshold of concern. Food Chem Toxicol, **34**(9): 829–867.

Nelson BK (1991) Selecting exposure parameters in developmental neurotoxicity assessments. Neurotoxicol Teratol, **13**: 569–573.

O'Donoghue JL (1989) Screening for neurotoxicity using a neurologically based examination and neuropathology. J Am Coll Toxicol, **8**: 97–115.

O'Donoghue JL (1994) Defining what is neurotoxic. In: Weiss B & O'Donoghue JL eds. Neurobehavioural toxicity: analysis and interpretation. New York, NY, Raven Press, pp 19–33.

OECD (1981a) Carcinogenicity studies. OECD Guidelines for Testing of Chemicals: Test Guideline No. 451. Paris, Organisation for Economic Co-operation and Development (http://masetto.sourceoecd.org/vl=1992568/cl=12/nw=1/rpsv/ij/oecdjournals/1607310x/v1n4/s36/p1).

OECD (1981b) Chronic toxicity studies. OECD Guidelines for Testing of Chemicals: Test Guideline No. 452. Paris, Organisation for Economic Co-operation and Development (http://masetto.sourceoecd.org/vl=1992568/cl=12/nw=1/rpsv/ij/oecdjournals/1607310x/v1n4/s37/p1).

OECD (1981c) Combined chronic toxicity/carcinogenicity studies. OECD Guidelines for Testing of Chemicals: Test Guideline No. 453. Paris, Organisation for Economic Co-operation and Development (http://masetto.sourceoecd.org/vl=1992568/cl=12/nw=1/rpsv/ij/oecdjournals/1607310x/v1n4/s38/p1).

OECD (1983) One-generation reproduction toxicity study. OECD Guidelines for Testing of Chemicals: Test Guideline No. 415. Paris, Organization for Economic Co-operation and Development (http://masetto.sourceoecd.org/vl=1992568/cl=12/nw=1/rpsv/ij/oecdjournals/1607310x/v1n4/s16/p1).

OECD (1995a) Repeated dose 28-day oral toxicity study in rodents. OECD Guidelines for Testing of Chemicals: Test Guideline No. 407. Paris, Organisation for Economic Co-operation and Development.

OECD (1995b) Delayed neurotoxicity of organophosphorus substances following acute exposure. OECD Guidelines for Testing of Chemicals: Test Guideline No. 418. Paris, Organisation for Economic Co-operation and Development (http://masetto.sourceoecd.org/vl=1992568/cl=12/nw=1/rpsv/ij/oecdjournals/1607310x/v1n4/s19/p1).

OECD (1995c) Delayed neurotoxicity of organophosphorus substances: 28-day repeated dose study. OECD Guidelines for Testing of Chemicals: Test Guideline No. 419. Paris, Organisation for Economic Co-operation and Development (http://masetto.sourceoecd.org/vl=1992568/cl=12/nw=1/rpsv/ij/oecdjournals/1607310x/v1n4/s20/p1).

OECD (1995d) Reproduction/developmental toxicity screening test. OECD Guidelines for Testing of Chemicals: Test Guideline No. 421. Paris, Organisation for Economic Co-operation and Development (http://masetto.sourceoecd.org/vl=1992568/cl=12/nw=1/rpsv/ij/oecdjournals/1607310x/v1n4/s22/p1).

OECD (1996) Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test. OECD Guidelines for Testing of Chemicals: Test Guideline No. 422. Paris, Organisation for Economic Co-operation and Development (http://masetto.sourceoecd.org/vl=1992568/cl=12/nw=1/rpsv/ij/oecdjournals/1607310x/v1n4/s23/p1).

OECD (1997) Neurotoxicity study in rodents. OECD Guidelines for Testing of Chemicals: Test Guideline No. 424. Paris, Organisation for Economic Co-operation and Development (http://masetto.sourceoecd.org/vl=1992568/cl=12/nw=1/rpsv/ij/oecdjournals/1607310x/v1n4/s25/p1).

OECD (1998a) Repeated dose 90-day oral toxicity study in rodents. OECD Guidelines for Testing of Chemicals: Test Guideline No. 408. Paris, Organisation for Economic Co-operation and Development (http://masetto.sourceoecd.org/vl=1992568/cl=12/nw=1/rpsv/ij/oecdjournals/1607310x/v1n4/s9/p1).

OECD (1998b) Repeated dose 90-day oral toxicity study in non-rodents. OECD Guidelines for Testing of Chemicals: Test Guideline No. 409. Paris, Organisation for Economic Co-operation and Development (http://masetto.sourceoecd.org/vl=1992568/cl=12/nw=1/rpsv/ij/oecdjournals/1607310x/v1n4/s10/p1).

OECD (2001a) Prenatal developmental toxicity study. OECD Guidelines for Testing of Chemicals: Test Guideline No. 414. Paris, Organisation for Economic Co-operation and Development (http://masetto.sourceoecd.org/vl=1992568/cl=12/nw=1/rpsv/ij/oecdjournals/1607310x/v1n4/s15/p1).

OECD (2001b) Two-generation reproduction toxicity study. OECD Guidelines for Testing of Chemicals: Test Guideline No. 416. Paris, Organisation for Economic Co-operation and Development (http://masetto.sourceoecd.org/vl=1992568/cl=12/nw=1/rpsv/ij/oecdjournals/1607310x/v1n4/s17/p1).

OECD (2007) Developmental neurotoxicity study. OECD Guidelines for Testing of Chemicals: Test Guideline No. 426. Paris, Organisation for Economic Co-operation and Development (http://masetto.sourceoecd.org/vl=1992568/cl=12/nw=1/rpsv/ij/oecdjournals/1607310x/v1n4/s27/p1).

OECD (2008) Repeated dose 28-day oral toxicity study in rodents. OECD Guidelines for Testing of Chemicals: Test Guideline No. 407. Paris, Organisation for Economic Co-operation and Development (http://masetto.sourceoecd.org/vl=1992568/cl=12/nw=1/rpsv/ij/oecdjournals/1607310x/v1n4/s8/p1).

Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, Keogh JP, Meyskens FL Jr, Valanis B, Williams JH Jr, Barnhart S, Cherniack MG, Brodkin CA & Hammar S (1996) Risk factors for lung cancer and for intervention effects in CARET, the Beta-Carotene and Retinol Efficacy Trial. J Natl Cancer Inst, **88**: 1550–1559.

Osterballe M, Hansen TK, Mortz CG, Host A & Bindslev-Jensen C (2005) The prevalence of food hypersensitivity in an unselected population of children and adults. Pediatr Allergy Immunol, **16**: 567–573.

Padilla S, Wilson VZ & Bushnell PJ (1994) Studies on the correlation between blood cholinesterase inhibition and “tissue” inhibition in pesticide-treated rats. Toxicology, **92**: 11–25.

Parry JM, Fielder RJ & McDonald A (1994) Thresholds for aneuploidy-inducing chemicals. Advisory Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment of the UK Department of Health. Mutagenesis, **9**: 503–504.

Pepelko WE (1987) Feasibility of route extrapolation in risk assessment. Br J Ind Med, **44**(10): 649–651.

Peto R, Pike MC, Day NE, Gray RG, Lee PN, Parish S, Peto J, Richards S & Wahrendorf J (1980) Guidelines for simple, sensitive significance tests for

carcinogenic effects in long-term animal experiments. Lyon, International Agency for Research on Cancer.

Pumphrey RSH & Gowland H (2007) Further fatal allergic reactions to foods in the United Kingdom, 1999–2006. J Allergy Clin Immuol, **119**: 1018–1019.

Quillardet P & McGregor D (1999) Identification of carcinogenic substances by means of short-term tests in bacteria. In: McGregor DB, Rice JM & Venitt S eds. The use of short- and medium-term tests for carcinogens and data on genetic effects in carcinogenic hazard evaluation. Lyon, International Agency for Research on Cancer, pp 487–498 (IARC Scientific Publications, No. 146).

Ray DE (1999) Chronic effects of low level exposure to anticholinesterases—a mechanistic review. Toxicol Lett, **103**: 527–533.

Rees DC, Francis EZ & Kimmel CA (1990) Qualitative and quantitative comparability of human and animal developmental neurotoxicity. Neurotoxicol Teratol, **12**: 171–292.

Renwick AG (2008) Toxicokinetics. In: Hayes AW ed. Principles and methods of toxicology, 5th ed. Boca Raton, FL, CRC Press, pp 179–230.

Rice JM, Baan RA, Blettner M, Genevois-Charmeau C, Grosse Y, McGregor DB, Partensky C & Wilbourn JD (1999) Rodent tumors of urinary bladder, renal cortex, and thyroid gland in IARC Monographs evaluations of carcinogenic risk to humans. Toxicol Sci, **49**: 166–171.

Richardson RJ (1995) Assessment of the neurotoxic potential of chlorpyrifos relative to other organophosphorous compounds: a critical review of the literature. J Toxicol Environ Health, **44**: 135–165.

Robaire B & Hales BF eds (2003) Advances in male mediated developmental toxicity. In: Advances in experimental medicine and biology. Vol. 518. New York, NY, Kluwer Academic/Plenum Publishers, 300 pp.

Robinson DE & MacDonald JS (2001) Background and framework for ILSI's collaborative evaluation program on alternative models for carcinogenicity assessment. International Life Sciences Institute. Toxicol Pathol, **29**(Suppl): 13–19.

Rodier PM (1990) Developmental neurotoxicology. Toxicol Pathol, **18**: 89–95.

Rodier PM, Cohen IP & Buelke-Sam J (1994). Developmental neurotoxicity: neuroendocrine manifestations of CNS insults. In: Kimmel CA & Buelke-Sam J eds. Developmental toxicology, 2nd ed. New York, NY, Raven Press, pp 65–92.

Rodricks JV, Gaylor DW & Turnbull D (2007) Quantitative extrapolations in toxicology. In: Hayes AW ed. Principles and methods of toxicology, 5th ed. Boca Raton, FL, CRC Press, pp 453–474.

Rona RJ, Keil T, Summers C, Gislason D, Zuidmeer L, Sodergren E, Sigurdardottir ST, Lindner T, Goldhahn K, Dahlstrom J, McBride D & Madsen C (2007)

The prevalence of food allergy: a meta-analysis. J Allergy Clin Immunol, **120**(3): 638–646.

Royal College of Physicians (1990a) Guidelines on the practice of ethics committees in medical research involving human subjects, 2nd ed. London, Royal College of Physicians.

Royal College of Physicians (1990b) Research involving patients. Summary and recommendations of a report of the UK Royal College of Physicians. J R Coll Physicians Lond, **24**: 10–14.

Sampson HA (1999) Food allergy. Part 1: Immunopathogenesis and clinical disorders. J Allergy Clin Immunol, **103**: 717–728.

Sampson HA (2004) Update on food allergy. J Allergy Clin Immunol, **113**: 805–819.

Schardein JL (2000) Chemically induced birth defects, 3rd ed. New York, NY, Marcel Dekker, pp 1–65.

Scheurer S, Lauer I, Foetisch K, Moncin MSM, Retzek M, Hartz C, Enrique E, Lidholm J, Cistero-Bahima A & Vieths S (2004) Strong allergenicity of Pru av 3, the lipid transfer protein from cherry, is related to high stability against thermal processing and digestion. J Allergy Clin Immunol, **114**: 900–907.

Schulze GE & Boysen BG (1991) A neurotoxicity screening battery for use in safety evaluation: effects of acrylamide and 3,3-iminodipropionitrile. Fundam Appl Toxicol, **16**: 602–615.

Sen M, Kopper R, Pons L, Abraham EC, Burks AW & Bannon GA (2002) Protein structure plays a critical role in peanut allergen stability and may determine immunodominant IgE-binding epitopes. J Immunol, **169**: 882–887.

Sette WF & MacPhail RC (1992) Qualitative and quantitative issues in assessment of neurotoxic effects. In: Tilson H & Mitchel C eds. Neurotoxicology. New York, NY, Raven Press, pp 345–361.

SGOMSEC (1996) Risk assessment for neurobehavioural toxicity. Scientific Group on Methodologies for the Safety Evaluation of Chemicals. Environ Health Perspect, **104**(Suppl 4): 171–212.

Shah E & Pongracic J (2008) Food-induced anaphylaxis: who, what, why, and where? Pediatr Ann, **37**: 536–541.

Shelby MD, Bishop JB, Mason JM & Tindall KR (1993) Fertility, reproduction, and genetic disease: studies on the mutagenic effects of environmental agents on mammalian germ cells. Environ Health Perspect, **100**: 283–291.

Skolnick HS, Conover-Walker MK, Koerner CB, Sampson HA, Burks W & Wood RA (2001) The natural history of peanut allergy. J Allergy Clin Immunol, **107**: 367–374.

Sleijffers A, Yucesoy B, Kashon M, Garssen J, De Gruijl FR, Boland GJ, Van Hattum J, Luster MI & Van Loveren H (2003) Cytokine polymorphisms play a role in susceptibility to ultraviolet B–induced modulation of immune responses after hepatitis B vaccination. J Immunol, **170**(6): 3423–3428.

Slikker W (1997) The developing nervous system. In: Lowndes HE & Reuhl KR eds. Comprehensive toxicology. Vol. 11. Nervous system and behavioural toxicology. New York, NY, Pergamon Press, pp 295–308.

Solecki R, Bürgin H, Buschmann J, Clark R, Duverger M, Fialkowski O, Guittin P, Hazelden K, Hellwig J, Hoffmann E, Hofmann T, Hübel U, Khalil S, Lingk W, Mantovani A, Moxon M, Müller S, Parkinson M, Paul M, Paumgartten F & Pfeil R (2001) Harmonisation of rat fetal skeletal terminology and classification. Report of the third workshop on the terminology in developmental toxicology: Berlin, 14–16 September 2000. Reprod Toxicol, **15**: 713–721.

Solecki R, Davies L, Dellarco V, Dewhurst I, Van Raaij M & Tritscher A (2005) Guidance on setting of acute reference dose (ARfD) for pesticides. Food Chem Toxicol, **43**: 1569–1593.

Sonich-Mullin C, Fielder R, Wiltse K, Baetcke K, Dempsey J, Fenner-Crisp P, Grant D, Hartley M, Knaap A, Kroese D, Mangelsdorf I, Meek E, Rice JM & Younes M (2001) IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. Regul Toxicol Pharmacol, **34**: 146–152.

Spanjersberg MQ, Kruizinga AG, Rennen MA & Houben GF (2007) Risk assessment and food allergy: the probabilistic model applied to allergens. Food Chem Toxicol, **45**(1): 49–54.

Spencer PS & Schaumburg HH (1985) Organic solvent neurotoxicity. Facts and research needs. Scand J Work Environ Health, **11**(Suppl 1): 53–60.

Spencer PS, Bischoff MC & Schaumberg HH (1980) Neuropathological methods for the detection of neurotoxic disease. In: Spencer PS & Schaumberg HH eds. Experimental and clinical neurotoxicology. Baltimore, MD, Williams and Wilkins Press, pp 743–757.

Spencer PS, Schaumburg HH & Ludolph AC (2000) Experimental and clinical neurotoxicology, 2nd ed. New York, NY, Oxford University Press.

Stadler MB & Stadler BM (2003) Allergenicity prediction by protein sequence. FASEB J, **17**: 1141–1143 [cited in Goodman et al., 2008].

Stevenson DE (1990) Mouse liver carcinogenesis: mechanisms and species comparisons. Austin, TX, Wiley-Liss.

Storer RD, McKelvey TW, Kraynak AR, Elia MC, Barnum JE, Harmon LS, Nichols WW & DeLuca JG (1996) Revalidation of the in vitro alkaline elution/rat hepatocytes assay for DNA damage: improved criteria for assessment of cytotoxicity and genotoxicity and results for 81 compounds. Mutat Res, **368**: 59–101.

Storer RD, French JE, Haseman J, Hajian G, LeGrand EK, Long GG, Mixson LA, Ochoa R, Sagartz JE & Soper KA (2001) P53+/− hemizygous knockout mouse: overview of available data. Toxicol Pathol, **29**(Suppl): 30–50.

Strahl BD & Allis CD (2000) The language of covalent histone modifications. Nature, **403**: 41–45.

Tandon P, Padilla S, Barone S, Pope CN & Tilson HA (1994) Fenthion produces a persistent decrease in muscarinic receptor function in the adult rat retina. Toxicol Appl Pharmacol, **125**: 271–280.

Taylor SL & Hefle SL (2001) Food allergies and other food sensitivities. Food Technol, **55**(9): 68–83.

Taylor SL & Lehrer SB (1996) Principles and characteristics of food allergens. Crit Rev Food Sci Nutr, **36**(Suppl): S91–S118.

Taylor SL, Bush RK & Nordlee JA (1997) Sulfites. In: Metcalfe DD, Sampson HA & Simon RA eds. Food allergy, 2nd ed. Cambridge, MA, Blackwell Science.

Taylor SL, Hefle SL, Bindslev-Jensen C, Burks AW, Christie L, Hill DJ, Host A, Hourihane JO, Lack G, Metcalfe DD, Moneret-Vautrin DA, Vadas PA, Rance F, Skrypec DJ, Trautman TA, Yman IM & Zeigler RS (2002) Factors affecting the determination of threshold doses for allergenic foods: how much is too much? J Allergy Clin Immunol, **109**: 24–30.

Taylor SL, Hefle SL, Bindslev-Jensen C, Atkins FM, Andre C, Bruijnzeel-Koomen C, Burks AW, Bush RK, Ebisawa M, Eigenmann PA, Host A, Hourihane JO, Isolauri E, Hill DJ, Knulst A, Lack G, Sampson HA, Moneret-Vautrin DA, Rance F, Vadas PA, Yunginger JW, Zeigler RS, Salminen JW, Madsen C & Abbott P (2004) A consensus protocol for the determination of the threshold doses for allergenic foods: how much is too much? Clin Exp Allergy, **34**: 689–695.

Taylor SL, Crevel RWR, Sheffield D, Kabourek J & Baumert J (2009) Threshold dose for peanut: risk characterization based upon published results from challenges of peanut-allergic individuals. Food Chem Toxicol, **47**(6): 1198–1204.

Tennant RW, Margolin BH, Shelby MD, Zeiger E, Haseman JK, Spalding J, Caspary W, Resnick M, Stasiewicz S, Anderson B & Minor B (1987) Prediction of chemical carcinogenicity in rodents from in vitro genetic toxicity assays. Science, **236**: 933–941.

Tennant RW, Stasiewicz S, Eastin WC, Mennear JH & Spalding JW (2001) The Tg.AC (v-Ha-ras) transgenic mouse: nature of the model. Toxicol Pathol, **29**(Suppl): 51–59.

Thake DC, Iatropoulos MJ, Hard GC, Hotz KJ, Wang CX, Williams GM & Wilson AG (1995) A study of the mechanism of butachlor-associated gastric neoplasms in Sprague-Dawley rats. Exp Toxicol Pathol, **47**: 107–116.

Thomas GA & Williams ED (1991) Evidence for and possible mechanisms of non-genotoxic carcinogenesis in the rodent thyroid. Mutat Res, **248**: 357–370.

Thomas K, Herouet-Guicheney C, Ladics G, Bannon G, Cockburn A, Crevel R, Fitzpatrick J, Mills C, Privalle L & Vieths S (2007) Evaluating the effect of food processing on the potential human allergenicity of novel proteins: international workshop report. Food Chem Toxicol, **45**: 1116–1122.

Thompson ED (1986) Comparison of in vivo and in vitro cytogenetic assay results. Environ Mutagen, **8**: 753–768.

Thorgeirsson UP, Dalgard DW, Reeves J & Adamson RH (1994) Tumor incidence in a chemical carcinogenesis study of nonhuman primates. Regul Toxicol Pharmacol, **19**: 130–151.

Tilson HA (1990a) Behavioural indices of neurotoxicity. Toxicol Pathol, **18**: 96–104.

Tilson HA (1990b) Neurotoxicology in the 1990s. Neurotoxicol Teratol, **12**: 293–300.

Tilson HA (1998) Developmental neurotoxicology of endocrine disruptors and pesticides: identification of information gaps and research needs. Environ Health Perspect, **106**: 807–811.

Tilson HA & Moser VC (1992) Comparison of screening approaches. Neurotoxicology, **13**: 1–14.

Tilson HA, MacPhail RC, Moser VC, Becking GC, Cuomo V, Frantik E, Kulig BM & Winneke G (1997) The IPCS collaborative study on neurobehavioural screening methods: VII. Summary and conclusions. Neurotoxicology, **18**: 1065–1070.

Toews AD & Morell P (1999) Molecular biological approaches in neurotoxicology. In: Tilson HA & Harry GJ eds. Neurotoxicology. New York, NY, Taylor & Francis, pp 1–35.

Troncone R, Ivarsson A, Szajewska H & Mearin ML; Members of European Multistakeholder Platform on CD (CDEUSSA) (2008) Review article: Future research on coeliac disease—a position report from the European multistakeholder platform on coeliac disease (CDEUSSA). Aliment Pharmacol Ther, **27**: 1030–1043.

Tuch K, Ockert D, Hauschke D & Christ B (1992) Comparison of the ECL-cell frequency in the stomachs of 3 different rat strains. Pathol Res Pract, **188**: 672–675.

Tugwood JD & Elcombe CR (1999) Predicting carcinogenicity: peroxisome proliferators. In: Kitchin KT ed. Carcinogenicity. New York, NY, Marcel Dekker, pp 337–360.

Tupper DE & Wallace RB (1980) Utility of the neurological examination in rats. Acta Neurobiol Exp, **40**: 999–1003.

United States Congress, Office of Technology Assessment (1990) Neurotoxicity: identifying and controlling poisons of the nervous system. Washington, DC, United States Government Printing Office (OTA-BA-436).

USEPA (1991a) Pesticide assessment guidelines, subdivision F, hazard evaluation: human and domestic animals. Addendum 10: Neurotoxicity, series 81, 82, and 83. Washington, DC, United States Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances (EPA No. 540/09-91-123).

USEPA (1991b) Guidelines for developmental toxicity risk assessment. Washington, DC, United States Environmental Protection Agency. Fed Regist, **56**: 63798–63826 (EPA/600/FR-91/001; http://www.epa.gov/NCEA/raf/pdfs/devtox.pdf).

USEPA (1991c) Neurotoxicity testing guidelines. Washington, DC, United States Environmental Protection Agency.

USEPA (1991d) Alpha 2µ-globulin: association with chemically induced renal toxicity and neoplasia in the male rat. Washington, DC, United States Environmental Protection Agency, Risk Assessment Forum.

USEPA (1996) Guidelines for reproductive toxicity risk assessment. Washington, DC, United States Environmental Protection Agency (http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=2838).

USEPA (1998a) Guidelines for neurotoxicity risk assessment. Washington, DC, United States Environmental Protection Agency. Fed Regist, **63**: 26926–26951.

USEPA (1998b) Health effects test guidelines. OPPTS 870.3800. Reproduction and fertility effects. Washington, DC, United States Environmental Protection Agency (Document No. EPA 712-C-98-208; http://www.epa.gov/opptsfrs/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Series/870-3800.pdf).

USEPA (1998c) Health effects test guidelines. OPPTS 870.3700. Prenatal developmental toxicity study. Washington, DC, United States Environmental Protection Agency (Document No. EPA 712-C-98-207; http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Series/870-3700.pdf).

USEPA (1998d) Health effects test guidelines. OPPTS 870.3150. 90-day oral toxicity in nonrodents. Washington, DC, United States Environmental Protection Agency (Document No. EPA 712-C-98-2200; http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Series/870-3150.pdf).

USEPA (1998e) Health effects test guidelines. OPPTS 870.3100. 90-day oral toxicity in rodents. Washington, DC, United States Environmental Protection Agency (Document No. EPA 712-C-98-199; http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Series/870-3100.pdf).

USEPA (1998f) Health effects test guidelines. OPPTS 870.4100. Chronic toxicity. Washington, DC, United States Environmental Protection Agency (Document No. EPA 712-C-98-210; http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Series/870-4100.pdf).

USEPA (2000) Health effects test guidelines. OPPTS 870.3050. Repeated dose 28-day oral toxicity in rodents. Washington, DC, United States Environmental Protection Agency (Document No. EPA 712-C-00-366; http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Series/870-3050.pdf).

USEPA (2002) A review of the reference dose and reference concentration processes. May 2002 external review draft. Washington, DC, United States Environmental Protection Agency (EPA/630/P-02/002A; http://www.epa.gov/ncea/raf/pdfs/RfDRfC/rfdrfcextrevdrft.pdf).

USFDA (2000) Toxicological principles for the safety assessment of direct food additives and color additives used in food (Redbook 2000). Washington, DC, United States Food and Drug Administration, Center for Food Safety and Applied Nutrition (http://www.cfsan.fda.gov/~redbook/red-toca.html).

USNRC (1992) Environmental neurotoxicology. Prepared by the Committee on Neurotoxicology and Models for Assessing Risk, Board on Environmental Studies and Toxicology, Commission on Life Sciences, National Research Council. Washington, DC, National Academy Press (http://books.nap.edu/openbook.php?record_id=1801&page=9).

USNRC (1993) Pesticides in the diets of infants and children. Prepared by the Committee on Pesticides in the Diets of Infants and Children, Board on Agriculture and Board on Environmental Studies and Toxicology, Commission on Life Sciences, National Research Council. Washington, DC, National Academy Press (http://www.nap.edu/openbook.php?isbn=0309048753).

USNRC (1999) Hormonally active agents in the environment. Prepared by the Committee on Hormonally Active Agents in the Environment, Board on Environmental Studies and Toxicology, Commission on Life Sciences, National Research Council. Washington, DC, National Academy Press (http://www.nap.edu/openbook.php?isbn=0309064198).

USNRC (2004) Intentional human dosing studies for EPA regulatory purposes: scientific and ethical issues. Prepared by the Committee on the Use of Third Party Toxicity Research with Human Research Participants, Science and Technology Law Program, Policy and Global Affairs Division, National Research Council. Washington, DC, National Academies Press (http://www.nap.edu/openbook.php?isbn=0309091721).

USNRC (2006) Health risks from dioxin and related compounds: evaluation of the EPA reassessment. Prepared by the Committee on EPA's Exposure and Human Health Reassessment of TCDD and Related Compounds, Board on

Environmental Studies and Toxicology, National Research Council of the National Academies. Washington, DC, National Academies Press (http://www.nap.edu/catalog.php?record_id=11688#toc).

Vaino H, Magee PN, McGregor DB & McMichael AJ eds (1992) Consensus report. In: Mechanisms of carcinogenesis in risk identification. Lyon, International Agency for Research on Cancer, pp 1–14 (IARC Scientific Publications, No. 116).

Van den Brandt P, Voorrips L, Hertz-Picciotto I, Shuker D, Boeing H, Speijers G, Guittard C, Kleiner J, Knowles M, Wolk A & Goldbohm A (2002) The contribution of epidemiology to risk assessment of chemicals in food and diet. Food Chem Toxicol, **40**: 387–424.

Van Loveren H (1995) Host resistance models. Hum Exp Toxicol, **14**: 137–140.

Van Loveren H, Cockshott A, Gebel T, Gundert-Remy U, de Jong WH, Matheson J, McGarry H, Musset L, Selgrade MK & Vickers C (2008) Skin sensitization in chemical risk assessment: report of a WHO/IPCS international workshop focusing on dose–response assessment. Regul Toxicol Pharmacol, **50**(2): 155–199.

Van Oosterhout JP, Van der Laan JW, De Waal EJ, Olejniczak K, Hilgenfeld M, Schmidt V & Bass R (1997) The utility of two rodent species in carcinogenic risk assessment of pharmaceuticals in Europe. Regul Toxicol Pharmacol, **25**: 6–17.

Van Steeg H, de Vries A, Van Oostrom CT, Van Benthem J, Beems RB & Van Kreijl CF (2001) DNA repair-deficient Xpa and Xpa/p53+/− knock-out mice: nature of the models. Toxicol Pathol, **29**(Suppl): 109–116.

Vassilopoulou E, Rigby N, Moreno FJ, Zuidmeer L, Akkerdaas J, Tassios I, Papadopoulos NG, Saxoni-Papageorgiou P, Van Ree R & Mills C (2006) Effect of in vitro gastric and duodenal digestion on the allergenicity of grape lipid transfer protein. J Allergy Clin Immunol, **118**: 473–480.

VICH (2000) Good Clinical Practice. Brussels, International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH GL9; http://www.vichsec.org/pdf/2000/Gl09_st7.pdf).

VICH (2002) Studies to evaluate the safety of residues of veterinary drugs in human food: carcinogenicity testing. Brussels, International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH GL28; http://www.vichsec.org/pdf/11_2002/Gl28_st7.pdf).

VICH (2004) Studies to evaluate the safety of residues of veterinary drugs in human food: general approach to establish a microbiological ADI. Brussels, International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH GL36; http://www.vichsec.org/pdf/05_2004/Gl36_st7_F_rev.pdf).

Vohr H-W (2005) Encyclopedic reference of immunotoxicology. Berlin, Springer.

Vorhees CV (1987) Reliability, sensitivity and validity of indices of neurotoxicity. Neurotoxicol Teratol, **9**: 445–464.

Vos JG & Van Loveren H (1998) Experimental studies on immunosuppression: how do they predict for man? Toxicology, **129**(1): 13–26.

Wal JM (2001) Structure and function of milk allergens. Allergy, **56**: 35–38.

Weisglas-Kuperus N, Sas TC, Koopman-Esseboom C, Van der Zwan CW, De Ridder MA, Beishuizen A, Hooijkaas H & Sauer PJ (1995) Immunologic effects of background prenatal and postnatal exposure to dioxins and polychlorinated biphenyls in Dutch infants. Pediatr Res, **38**(3): 404–410.

Weisglas-Kuperus N, Patandin S, Berbers GA, Sas TC, Mulder PG, Sauer PJ & Hooijkaas H (2000) Immunologic effects of background exposure to polychlorinated biphenyls and dioxins in Dutch preschool children. Environ Health Perspect, **108**(12): 1203–1207.

Wensing M, Penninks AH, Hefle SL, Koppelman SJ, Bruijinzeel-Koomen CAFM & Knulst AC (2002) The distribution of individual threshold doses eliciting allergic reactions in a population with peanut allergy. J Allergy Clin Immunol, **110**: 915–920.

WHO (1967) Procedures for investigating intentional and unintentional food additives. Report of a WHO Scientific Group. Geneva, World Health Organization (WHO Technical Report Series, No. 348).

Wier PJ, Guerriero FJ & Walker RF (1989) Implantation of a primary screen for developmental neurotoxicity. Fundam Appl Toxicol, **13**: 118–136.

Wilks MF (2001) Human in vivo studies of non-pharmaceutical products. Toxicol Lett, **120**: 125–130.

Williams GM (1992) DNA reactive and epigenetic carcinogens. Exp Toxicol Pathol, **44**: 457–464.

Williams GM (1997) Chemicals with carcinogenic activity in the rodent liver; mechanistic evaluation of human risk. Cancer Lett, **117**: 175–188.

Williams GM (1999) Chemically induced preneoplastic lesions in rodents as indicators of carcinogenic activity. Lyon, International Agency for Research on Cancer, pp 185–202 (IARC Scientific Publications, No. 146).

Williams GM & Iatropoulos MJ (2001) Principles of testing for carcinogenic activity. In: Hayes W ed. Principles & methods of toxicology. Philadelphia, PA, Taylor & Francis, pp 959–1000.

Williams GM & Iatropoulos MJ (2002) Alteration of liver cell function and proliferation: differentiation between adaptation and toxicity. Toxicol Pathol, **30**: 41–53.

Williams GM & Perrone C (1996) Mechanism-based risk assessment of peroxisome proliferating rodent hepatocarcinogens. In: Reddy JK, Suga T, Mannaerts GP, Lazarow PB & Subramani S eds. Peroxisomes: biology and role in toxicology and disease. Ann NY Acad Sci, **804**: 554–572.

Wise LD, Beck SL, Beltrame D, Beyer BK, Chahoud I, Clark RL, Clark R, Druga AM, Feuston MH, Guittin P, Henwood SM, Kimmel CA, Lindstrom P, Palmer AK, Petrere JA, Solomon HM, Yasuda M & York RG (1997) Terminology of developmental abnormalities in common laboratory mammals (version 1). Teratology, **55**: 249–292.

World Medical Association (1997) World Medical Association Declaration of Helsinki: Recommendations guiding medical doctors in biomedical research involving human subjects. As revised at the 48th General Assembly of the World Medical Association, Somerset West, South Africa, 1996. JAMA, **277**: 925–926.

Wüthrich B (1993) Adverse reaction to food additives. Ann Allergy, **71**: 379–384.

Wüthrich B, Kägi MK & Stücker W (1997) Anaphylactic reactions to ingested carmine (E120). Allergy, **52**: 1133–1137.

Youssef AF, Borellini F, Jacobson-Kram D & Fort FL (2001) Analysis for loss of heterozygosity (LOH) of *p53* allele in tumors derived from p53+/− and CD-1 mice following repeated subcutaneous injections of solutions containing antioxidants. Environ Mol Mutagen, **37**: 27–30.

Zuberbier T, Edenharter G, Worm M, Ehlers I, Reimann S, Hantke T, Roehr CC, Bergmann KE & Niggemann B (2004) Prevalence of adverse reactions to food in Germany—a population study. Allergy, **59**: 338–345.

Zuidmeer L, Goldhahn K, Rona RJ, Gislason D, Madsen C, Summers C, Sodergren E, Dahlstrom J, Lindner T, Sigurdardottir ST, McBride D & Keil T (2008) The prevalence of plant food allergies: a systematic review. J Allergy Clin Immunol, **121**: 1210–1218.

5. DOSE–RESPONSE ASSESSMENT AND DERIVATION OF HEALTH-BASED GUIDANCE VALUES

For acronyms and abbreviations used in the text, the reader may refer to the list of acronyms and abbreviations at the front of this monograph. Definitions of select terms may be found in the glossary at the end of the monograph.

5.1 Dose–response assessment

5.1.1 Basic concepts of dose–response assessment

Dose–response assessment approaches generally take one of two forms: 1) analyses that provide a quantitative (or sometimes just qualitative) estimation of risk and 2) analyses that establish health-based guidance values, such as an acceptable daily intake (ADI) or tolerable daily intake (TDI), which are levels of human exposure considered to be without appreciable health risk. The latter approach, which is often described as "safety assessment", is used more often in cases where exposure can be controlled, such as for food additives and residues of pesticides and veterinary drugs in foods.

One of the primary criteria of a risk assessment is determination of the presence or absence of a cause–effect relationship. If there is sufficient plausibility for the presence of such a relationship, then dose–response data are essential, and dose–response analysis is a major part of the hazard characterization within the risk assessment paradigm.

Dose–response data may be derived from in vivo studies in laboratory animals or humans, which usually provide the basis for risk characterization, and in vitro studies, which are often related to investigations of mode of action. In each case, interpretation of the data on effects usually requires recognition of the levels of exposure that do not produce a measurable effect and the relationship between the increase in incidence, severity or nature of the effect with increase in exposure.

Toxicological or epidemiological data have been used in hazard characterization by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in three main ways (see chapter 7):

1) derivation of a health-based guidance value, such as an ADI, TDI or acute reference dose (ARfD);
2) estimation of the margin of exposure (MOE) between a defined point on the dose–response curve and the level of human exposure; and
3) quantification of the magnitude of the risk at specified levels of human exposure.

In addition, it is possible to use dose–response data to define the exposure that theoretically would be associated with some specified level of risk, such as a 1 in a million increase in lifetime risk of cancer.

Having established that there is a statistically significant treatment-related or exposure-related effect that is relevant to human health, the calculation of a health-based guidance value or MOE requires definition of a reference point or point of departure (POD) on the dose–response curve. There have been two basic approaches to dose–response assessment applied to data from studies in animals:

1) Pairwise comparisons of the findings in different groups in order to define experimental doses that cause statistically significant effects and the highest experimental dose that does not produce an observed adverse effect in that study, the no-observed-adverse-effect level (NOAEL). The NOAEL is then used as the POD to estimate a health-based guidance value, after allowing for uncertainties such as species differences and human variability.

2) Fitting a model or models to the dose–response data for all groups in order to define the relationship in the observed range; the model can then be used to define the exposure associated with a specified level of response. This value can then be used as the POD to estimate a health-based guidance value or calculate an MOE or extrapolated to estimate the risk at the levels of human exposure that are relevant to problem formulation and risk characterization.

These approaches and variants on them are discussed in this chapter, which is based on an Environmental Health Criteria (EHC) document on Principles for Modelling Dose–Response for the Risk Assessment of Chemicals, developed as part of the International Programme on Chemical Safety (IPCS) Harmonization

Project on Approaches to the Assessment of Risk from Exposure to Chemicals. EHC 239 (IPCS, 2009) covers toxicants with threshold effects and those for which there may be no practical threshold, such as substances that are genotoxic and carcinogenic. It focuses primarily on experimental animal studies, but dose–response relationships are also critical to the assessment of human experimental studies and epidemiological data. Dose–response assessment is also important for studies that attempt to define the relationships of different steps in a postulated mode of action. EHC 239 also includes areas that are not of direct relevance to this chapter, such as the basic risk analysis paradigm and the consequences of dose–response modelling (DRM) for the advice provided by risk assessors to risk managers.

5.1.1.1 *Dose*

It is critical when performing dose–response analyses to have a clear concept of what type of "dose" has been used in the available dose–response data. There are three basic types of dose that arise from scientific investigations; they are inter-related, and each of them can be used to express dose–response relationships. They are 1) the administered or external dose, 2) the internal (absorbed) dose and 3) the target or tissue dose.

External dose denotes the amount of an agent or chemical administered to an experimental animal or human in a controlled experimental setting by some specific route at some specific frequency. In the terminology used by JECFA, the external dose is often referred to as exposure or intake (see chapter 6). External dose, or external exposure, is frequently the dose metric that is used in observational epidemiological studies.

Internal dose is the amount that is systemically available and can be regarded as the fraction of the external dose that is absorbed and enters the general circulation. It is affected by absorption, metabolism and excretion of the chemical and can be derived from suitable toxicokinetic mass balance studies. The analytical method used in the toxicokinetic studies will determine whether the dose refers to the parent compound alone or the parent compound plus first-pass metabolites (see chapter 4, section 4.2). Biomarkers of body burden, such as plasma concentrations or urinary excretion, are sometimes available in epidemiological studies.

The tissue dose is the amount that is distributed to and present in a specific tissue of interest. As for internal dose, the analytical method used in the toxicokinetic studies will determine whether the dose refers to the toxic entity, whether it be the parent compound alone or the parent compound plus first-pass metabolites (see chapter 4, section 4.2). An additional consideration for tissue dose is whether the dose metric is the peak concentration or a time-weighted average, such as the area under the concentration–time curve (AUC).

Two temporal parameters are important determinants of dose: the dose frequency and the duration of dosing. Dosing can be acute, subchronic or chronic; the term dose can apply to any of these, and the principles of dose–response assessment apply to all three forms. The description of dose should reflect the magnitude, frequency and duration over which it applies. Dose can be expressed in a variety of metrics, including a simple single external dose (e.g. mg/kg body weight), daily intake (e.g. mg/kg body weight per day),[1] peak body burden or body burden averaged over a given period of time (e.g. ng/kg body weight) or tissue concentration (e.g. ng/kg).

In epidemiological studies, exposure (the external dose) is rarely known precisely, and its estimation often requires various assumptions. Sometimes exposure is measured by the biomonitoring of blood or tissue concentrations; dose–response assessment for such data usually raises the issue of conversion of the biomarker of internal exposure into an external dose. An additional problem that has arisen (e.g. with the dioxin database) is that measurements of the biomarker were made many years after what was believed to be the period of highest exposure (FAO/WHO, 2002a).

Sometimes the doses used in an experimental animal study are transformed to the equivalent human exposures prior to DRM. In this situation, models of internal exposure linked to the response data may be used to develop a dose–response model. However, such models need knowledge, for both experimental animals and humans, of the events controlling absorption, tissue distribution, metabolism, excretion and the other molecular and biochemical processes that ultimately

[1] In animal studies, exposure is often measured as concentration in feed only. For conversion from feed concentration to external dose, refer to Annex 2.

lead to particular responses. Interspecies extrapolation of such a dose metric may be possible by the use of a physiologically based toxicokinetic (PBTK) model. Although this more sophisticated approach can refine DRM, incomplete data will add uncertainty to the output of the modelling. The issue of interspecies extrapolation is usually addressed separately and subsequent to DRM using the unadjusted animal data and application of an uncertainty factor (section 5.2.3).

5.1.1.2 *Response*

Response, in this context, generally relates to an observation or effect seen following exposure in vivo or in vitro. Possible end-points cover a broad range of observations, from early responses such as biochemical alterations to more complicated responses such as cancer and developmental defects.

Responses can be either adaptive or adverse. Adverse effects are defined as a change in the morphology, physiology, growth, development, reproduction or lifespan of an organism or subsystem (e.g. subpopulation of cells) that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences (IPCS, 2004). The responses are sometimes species or tissue specific and have different degrees of variation across individuals. DRM can address each response, provide insight into their quantitative similarities across species and tissues and link responses in a mechanistically reasonable manner.

Response is generally considered to vary across experimental units (experimental animals, humans, cell cultures) in the same dose group in a random fashion. This random variation is usually assumed to follow some statistical distribution describing the frequency of any given response for a population. In general, statistical distributions are characterized by their central tendency (usually the mean or median) and their effective range (usually based on the standard deviation or geometric standard deviation).

Most responses of interest in the context of dose–response assessment fall into one of four basic categories:

1) *Quantal responses*: Also referred to as binary or categorical responses, these generally relate to an effect that is either observed

or not observed in each individual subject (laboratory animal or human); for each dose, the number of subjects responding out of the number of subjects available is reported (e.g. the proportion of animals with a tumour in a cancer bioassay).

2) *Counts*: These generally relate to a discrete number of items measured in a single experimental unit (e.g. number of papillomas on the skin).

3) *Continuous measures*: These generally relate to a quantitative measurement that is associated with each individual subject and can take on any value within a defined range (e.g. body weight).

4) *Ordinal categorical measures*: These generally take on one value from a small set of ordered values (e.g. tumour severity grades); ordinal data are an intermediate type of data and reflect (ordered) severity categories—i.e. they are qualitative data but with a rank order (e.g. histopathological severity data) in each individual. When the categories are non-ordered, they are called categorical data, but these are rare for response data.

Sometimes it is useful for DRM purposes to convert continuous data into proportions (e.g. number of animals outside a clinically relevant range for an immune system marker) or categories (e.g. measured degree of liver necrosis converted to minimal, moderate or extensive).

There are some differences in how each of these different types of data are handled for DRM, but as a general rule, the goal of DRM is to describe the mean and variance of the response as a function of exposure or time.

5.1.2 Dose–response modelling (DRM)

5.1.2.1 Overview

DRM can be described by six basic steps, with a variety of options at each step (Table 5.1). The first four steps relate to the analysis of the dose–response data, which is referred to as dose–response analysis (IPCS, 2009). Dose–response analysis provides the linkage of a model to dose–response data for the purposes of predicting response to a

Table 5.1. Basic steps in dose–response assessment/modelling (adapted from IPCS, 2009)

Step	Description	Options
1. Data selection	Determine the response to be modelled, and select appropriate data	End-point, quality, sample size, utility, availability
2. Model selection	Choose the type of model to be applied to the data	End-point, data availability, purpose
3. Statistical linkage	Choose statistical distributions to describe the variability in response	End-point, data type, model choice, software availability
4. Parameter estimation	Combine the first three steps in an appropriate computer program to obtain estimates of the model parameters	Linkage function, software availability, variance
5. Implementation	Use the estimated model parameters and the model formula to predict response/dose as needed	Outputs, target selection, model predictions, BMD, direct extrapolation
6. Evaluation	Examine the sensitivity of the resulting predictions to the assumptions used in the analysis ("model validation")	Model comparison, uncertainty

BMD, benchmark dose.

given dose or predicting the dose causing a given level of response. The last two steps deal with implementation and evaluation of the results of the analysis.

Step 1 involves selection of appropriate data for dose–response assessment. The criteria applied to assess whether the data are suitable for risk characterization purposes are similar whether hazard characterization is based on pairwise analyses of groups or modelling using all dose groups.

Step 2 involves the choice of an appropriate model. The type of data available can have a marked impact on the complexity of the model that can be used. For example, whereas two points can be used to identify the slope of a line, it takes at least three points to identify the shape of a more complex dose–response relationship. The issue of whether there are enough data to support a given model is

complex (see IPCS, 2009). Models may be divided into two categories: empirical and biologically based models. Most DRM to date has used empirical models—i.e. mathematical descriptions of the data that are not based on a mechanism of action. Biologically based models are generally based on basic principles about the onset and progression of disease in a biological system, are functionally complex and have far greater data requirements than do empirical models.

Step 3 requires the choice of a statistical linkage between the data and the model. The most common linkage method is to assume a statistical distribution for the response and use that distribution to derive a mathematical function describing the quality of the fit of the model to the data. The advantage of choosing a formal statistical linkage is the ability to test hypotheses and derive confidence intervals for model predictions.

Step 4 is the fitting of the selected model to the data. As the primary components of a model are the parameters that define the model, curve fitting simply involves choosing values for the parameters in the model. If a formal statistical function has been developed for linking the data to the model, then the parameters are chosen such that they "optimize" the value of the linkage function. A common choice is to link the data to the model by minimizing the sum of the squares of the differences between the predicted value from the model and the observed value. Simpler methods can also be used to estimate model parameters. Formal optimization is a better choice for modelling than ad hoc procedures, which lack transparency.

Step 5 is to make the inferences necessary to address the risk assessment questions developed at the problem formulation stage. The different types of data (quantal, count, continuous, categorical) require different methods for predicting changes in response beyond the normal response. In general, treatment-related responses may be described by added response (treated minus control response), relative response (fold change relative to control response) and extra response (added response scaled to range from zero to the maximum possible response). Each of these choices can have an impact on the final decision, so care should be taken to understand why a specific choice is made. Development of risk assessment advice usually requires extrapolation of results from the specific responses seen for the experiment

being modelled to other exposure scenarios and other doses. This step can also involve an extrapolation from a laboratory species to humans.

Step 6, uncertainty analysis, can be used to show the impact of sampling error and model selection on the model estimates. Sensitivity analysis can be used to evaluate the impact of a particular model choice on the estimate.

Dose–response assessment may be used to develop risk assessment advice in a variety of ways:

1) Simple pairwise comparisons of the data for different dose levels can be used to define the NOAEL or sometimes a lowest-observed-adverse-effect level (LOAEL), which is used as a POD for the observed dose–response data.

2) The dose–response model may be used to identify a dose with a known level of response at or slightly below the observable range. A specified response or level of effect for quantal and continuous data, respectively, is known as the benchmark response (BMR), and the dose associated with that response, the benchmark dose (BMD). The lower one-sided confidence limit of the BMD (the BMDL) can be used as the POD for the derivation of a health-based guidance value or for calculation of an MOE. Alternatively, the BMDL may be the starting point for linear low-dose extrapolation (see below).

3) The model may be used to find the dose associated with a negligible (e.g. 1 in a million) response over control. In general, this requires extrapolation far beyond the range of the data, which creates considerable uncertainty.

In addition, the model may be used to estimate the magnitude of effect associated with current levels of exposure for chemicals where exposure is ongoing and the dose–response data are derived from human studies.

Approach 1 is currently used by JECFA and JMPR to derive health-based guidance values in order to protect against effects that are considered to show a threshold.

Approach 2 was used by JECFA at its sixty-fourth meeting (FAO/WHO, 2006) to define MOEs for a number of genotoxic carcinogens. The same meeting also considered the use of linear extrapolation from the BMDL to estimate the risk of cancer at relevant levels of human exposure and concluded that

> calculation of the intake associated with an incidence of 1 in 1 million from the BMDL for a 10% incidence using linear extrapolation is simply equivalent to dividing the BMDL by 100 000, and this approach is therefore no more informative than calculation of a MOE.

Approach 3 was considered by JECFA at its sixty-fourth meeting (FAO/WHO, 2006), and the Committee concluded that:

> In order to provide realistic estimates of the possible carcinogenic effect at the estimated exposure for humans, mathematical modelling would need to take into account the shape of the dose–response relationship for the high doses used in the bioassay for cancer and for the much lower intakes by humans. Such information cannot be derived from the available data on cancer incidence from studies in animals. In the future, it may be possible to incorporate data on dose–response or concentration–response relationships for the critical biological activities involved in the generation of cancer (e.g. metabolic bioactivation and detoxification processes, DNA [deoxyribonucleic acid] binding, DNA repair, rates of cell proliferation and apoptosis) into a biologically based dose–response model for cancer that would also incorporate data on species differences in these processes. However, such data are not currently available. At present, any estimate of the possible incidence of cancer in experimental animals at intakes equal to those for humans has to be based on empirical mathematical equations that may not reflect the complexity of the underlying biology. A number of mathematical equations have been proposed for extrapolation to low doses. The resulting risk estimates are dependent on the mathematical model used; the divergence increases as the dose decreases and the output by different equations can differ by orders of magnitude at very low incidences.

In step 6, the basic steps of DRM shown in Table 5.1 are repeated to consider other options in the process in order to understand the impact of choices on the health-based measures derived from DRM. This final step is aimed at understanding the sensitivity of the analysis to specific choices and to judge the overall quality of the final predictions. Depending on the degree of difference between choices, there could be value in performing a formal analysis of the quality of the fit of the model to the data. Other methods can also be used to assess the

impact of choices used in the modelling on the eventual outcome, such as uncertainty analysis and Bayesian mixing.

5.1.2.2 *Mathematical models*

A number of mathematical models have been or can be used to describe dose–response data. Their application and interpretation require specialized expertise. The main models are outlined below, and further details are provided in the report of the sixty-fourth meeting of JECFA (FAO/WHO, 2006) and in EHC 239 (IPCS, 2009).

Dose–response models are mathematical expressions fitted to scientific data that characterize the relationship between dose and response. Mathematical models consist of three basic components: 1) assumptions used to derive the model, 2) a functional form for the model and 3) parameters that are components of the functional form.

Dose–response models range from very simple models, such as the linear model described above, to extremely complicated models for which the eventual functional form cannot easily be expressed as a single equation (e.g. biologically based dose–response models).

Models can also be linked, meaning that one model could describe part of the dose–response process while another describes the remainder of the process. For example, for chemical carcinogenesis, in most cases tissue concentration is more closely linked to cancer risk than is administered dose. Given data on dose, tissue concentration and tumour response, a toxicokinetic model may be able to relate external dose to tissue concentration, and a multistage cancer model may be able to relate tissue concentration to response. The two models need to be combined in order to describe the dose–response relationship.

Dose–response models may incorporate other information into the model form. Age and time on study are commonly used in DRM, but other factors, such as species/strain/human ethnicity, sex and body weight, have also been used to expand the utility of dose–response models.

5.1.2.3 *Dose–response models for continuous data*

The models listed in Table 5.2 are some of the forms that may be used to describe the relationship between dose and the magnitude of

Table 5.2. Dose–response models for continuous data

Name	Notes	Equation for response	Parameter explanations
Hill equation log-logistic	A modification of the Michaelis-Menten equation that supposes that the occupation of multiple sites or receptors is required for the production of an effect.	$= \text{RMax} \dfrac{D^n}{K_D^n + D^n}$	RMax is the maximum response, D is the dose, K_D is the reaction constant for the drug–receptor interaction and n is the number of (hypothetical) binding sites.
Exponential	If the interaction between a chemical and a target site is irreversible, then the rate of the reaction is determined by the rate of association (ka) only.	$= \text{RMax}\,(1 - e^{-rD})$ The above is an equation for a first-order exponential model.	RMax is the maximum response, D is the dose and r is the exponential rate constant.
Power	Simple exponential model.	$= \beta D^{\alpha}$	D is the dose, α is the shape parameter and β is the scale parameter.
Linear	Although there is usually no biological theory to suggest it, linear models are often justified by their simplicity; linear models have but a single parameter.	$= mD$	D is the dose, and m is the slope.

a response on a continuous scale in an individual. When combined with a statistical distribution (e.g. normal or lognormal), these equations can also be used to describe the relationship between dose and a continuous response in a population, where the continuous model corresponds to the central estimate.

Dose–response data are often adjusted by subtracting the (mean) control value from each individual observation. However, this procedure does not account for the fact that the background response level in the controls is, as in the experimental groups, subject to sampling error and individual variability. A better approach is to account for the background response in the model with a parameter that needs to be estimated from the data (see IPCS, 2009).

5.1.2.4 *Dose–response models for quantal data*

Quantal dose–response functions describe the relationship between dose and the frequency of a particular outcome in a population (see Table 5.3). For a group of homogeneous or nearly identical individuals, the relationship between dose and frequency could be described with a step function, where all subjects either respond or fail to respond at any given dose. However, because variability is ubiquitous in living organisms, quantal dose–response data typically show gradually increasing incidence with dose. One interpretation of this is that individual subjects differ in tolerance to the agent, which can be described by a statistical tolerance distribution. Hence, any cumulative distribution function (CDF) may be used as a quantal dose–response function. Other models have been derived from statistical assumptions about how the agent might exert its effect in an organism, such as the gamma multi-hit model.

Background response rates should be accounted for by incorporating an additional parameter in the dose–response model (see IPCS, 2009).

5.1.2.5 *Model fitting and estimation of parameters*

Two basic methodologies are available for model fitting: conventional, in which parameters are selected to minimize or maximize an objective function, and Bayesian, in which information in a data set is

Table 5.3. Dose–response models for quantal data

Name	Theoretical basis	Equation for frequency (*F*)	Parameter explanations
Step function	No variability.	If $D < T$, $F = 0$ If $D \geq T$, $F = 1$	*D* is the dose, and *T* is the threshold parameter.
One-hit (single-hit)	Hit theory models employ the use of a rate to describe the interaction between a group of causal agents (e.g. molecules) and a group of targets (e.g. a human population).	$= 1 - e^{-(\alpha + D^*\beta)}$	*D* is the dose, α is a location parameter and β is the slope parameter.
Gamma multi-hit	An expansion of the one-hit model, which is based on the notion that multiple hits or events are required to produce a particular effect.	= Γ(gamma**D*, *k*)	Γ() is the incomplete gamma CDF, *D* is the dose, gamma is a rate parameter and *k* is the number of hits required to produce the effect.
Probit normal	A descriptive model based on a normal or Gaussian distribution.	$= \Phi(\alpha + D^*\beta)$	Φ() is the normal CDF, *D* is the dose, α is a location parameter and β is the slope parameter.
Log-probit	An expansion of the probit model.	$= \Phi(\alpha + \ln D^*\beta)$	Φ() is the normal CDF, *D* is the dose, α is a location parameter and β is the slope parameter.
Logistic	The statistical logistic model is also a descriptive tool with no theoretical basis.	$= 1/(1 + e^{-(\alpha + D^*\beta)})$	*D* is the dose, α is a location parameter and β is the slope parameter.
Log-logistic	An expansion of the logistic model.	$= 1/(1+ e^{-(\alpha + \ln D^*\beta)})$	*D* is the dose, α is a location parameter and β is the slope parameter.
Weibull	A flexible descriptive model originally developed to describe survival data in demography.	$= e^{-(\alpha + (D^*\beta)^{\gamma})}$	*D* is the dose, α is the background parameter, β is the slope parameter and γ is an exponent.

combined with prior information about model parameters, resulting in a posterior distribution for those parameters that reflects the degree of uncertainty about the parameters. For historical and computational reasons, "user-friendly" software designed for carrying out dose–response analysis and non-linear modelling in general has been restricted to using conventional methodologies, whereas Bayesian methods are implemented in packages that require more extensive programming and substantially greater understanding of the statistical details (for further details on Bayesian approaches, see Hasselblad & Jarabek, 1995; Gelman et al., 2004). Whereas current software requires substantial statistical understanding for successful use of Bayesian methods and is thus beyond the reach of this document, even conventional methods require an understanding of some basic principles before outcomes from applying the software can be properly interpreted. Some general remarks may be helpful here.

The general approach of fitting a model is to find parameter values for the model that optimize the fit of the model to the data. To that end, a criterion function is defined, reflecting what is considered to be a good fit of the model. The goal is to find the parameter values that optimize the value of the criterion. For many models typically used, this can be achieved only by an iterative "trial and error" approach (see below). In many applications, the logarithm of the likelihood function is used as the criterion. The likelihood derives directly from the distribution assumed for the scatter in the data. For quantal data, the binomial likelihood is typically used. For continuous data, the normal likelihood is often used, be it for the observed responses themselves or for the log-transformed responses. Note that maximizing the normal likelihood function is in fact equivalent to minimizing the sum of squares.

Computer software uses algorithms to find parameter values that optimize the fit of the model to the data, and the user does not need to worry about the exact nature of the calculations. However, some basic understanding of the search process is required in order to interpret the outcomes. An iterative search algorithm tries to find "better" parameter values in a process by evaluating whether the fit can be improved by changing the parameter values through a trial and error process. More advanced algorithms operate by evaluating the slope at which the fit is improved for one or more parameter value changes. The algorithm

can start searching only when the parameters have values to start with. Although the software often gives a reasonable first guess for the starting values, the user may have to change these. It is not unusual (in particular when the information in the data is hardly sufficient to estimate the intended parameters) that the end result depends on the starting values chosen, and the user should be aware of that.

5.1.3 Modelling with covariates

In some circumstances, it is desirable to include variables in addition to just an exposure variable in dose–response models. For example, in epidemiological studies, it is common to model disease risk in terms of not only exposure, but also age, sex, socioeconomic status, smoking status and other measurements that may be relevant to the disease state. These other factors may not themselves be directly affected by the exposure, but they may be correlated with exposure status because of the way in which the sample was taken. Unless the proper covariates are included in a model for the relationship between exposure and the health end-point, the effect of exposure will be incorrectly estimated.

In principle, this sort of confounding cannot occur in bioassay studies in which animals are randomized to treatment groups, but it may be useful to include a covariate such as sex or body weight to account for some of the variability in a related measure.

5.1.4 Biologically based dose–response models

Although biological considerations may motivate the choice of one or several empirical models, the level of biological detail in such models is minimal. Thus, their credibility for interpolating and extrapolating a data set derives mainly from their fit to the data, as evaluated statistically. The biologically based dose–response models, another class of model, are much more complicated and are explicitly designed to model the biological details that lead from initial exposure to a toxicant to the ultimate pathological outcome. Typically, such a model includes a PBTK model to describe the distribution and metabolism of the parent compound and toxic metabolites, as well as other mechanistic or toxicodynamic models that link target tissue concentration to the ultimate response. The toxicodynamic part of the model may be relatively simple or may be as complicated as a fully elaborated stochastic model for carcinogenesis.

Such a model is really a quantitative expression of a set of biological hypotheses and, when rigorously tested against critical experiments, becomes a credible tool for extrapolating from experimental results into exposure realms that are difficult or expensive to reproduce in controlled experiments. Such models are quite expensive to construct in terms of both resources and time and thus would be expected to be developed fully only for exposures and toxicities of the highest concern.

5.1.5 Uncertainty

Any parameters or predictions estimated from a given model are only point estimates and, to a larger or smaller extent, uncertain. This uncertainty arises from at least three sources: 1) the sampling error arising from inferences about a larger population from a single experiment; 2) the reality that dose–response estimates often differ among experiments with different experimental design, protocol or uncontrolled circumstances; and 3) the fact that the "true" model is not known, which results in additional uncertainty when interpolating between doses, but even more so when extrapolating outside the dose range containing observations. These uncertainties may all be represented in a dose–response assessment through the use of probability distributions or probability trees. The latter technique involves using multiple alternative plausible assumptions about what data sets or models are to be used to produce an estimate, which results in a range of plausible estimates.

5.1.6 Issues of extrapolation

Extrapolation is a necessary part of all risk assessments, except in those rare cases where DRM uses data from studies in sufficient numbers of humans who are representative of the potential exposed population and who have had a level of exposure similar to that which is of concern.

Most of the methods used to implement the results of a dose–response analysis (step 5) address these extrapolation issues. The strategies used for extrapolation basically fall into two categories: 1) those aimed at providing estimates of risk for exposures outside of the range of the data used in the dose–response analysis and 2) those

aimed at establishing health-based guidance values, such as the ADI, without quantification of risk. The methods that have been used for extrapolation are diverse and sometimes contentious, with different countries, and even different agencies within a given country, using different approaches.

Even when human data are available and suitable for dose–response analysis, they are generally from selected populations or study groups, such as workers in occupational settings, whose exposures differ from those of the general population. Thus, dose–response analyses normally need to be extrapolated from the observed conditions where scientific support is available to conditions where scientific support is weaker or non-existent. For dose–response analyses based on human studies, extrapolation is generally a downward extrapolation to different levels of exposure, but can also be to different life stages (e.g. fetus, child) or different populations with different environmental factors that might affect exposure (e.g. dietary differences).

In most cases considered by JECFA and JMPR, the data used for DRM come from experiments in laboratory animals administered doses significantly exceeding the potential human exposure. For such dose–response analyses, there are two issues of extrapolation: 1) extrapolating from the test species to humans and 2) allowing for possible human differences in response. The methods employed for these extrapolation issues are varied, ranging from the use of uncertainty factors (see section 5.2.3) to more complicated modelling schemes based upon differences in toxicokinetics and toxicodynamics between humans and experimental animals and variability between different human individuals.

5.2 Setting health-based guidance values

5.2.1 Introduction

The setting of health-based guidance values provides quantitative information from risk assessment for risk managers, enabling them to make decisions concerning the protection of human health. Health-based guidance values developed by JECFA and JMPR for substances found in food and also drinking-water are the quantitative expression of the range of oral exposure (either acute or chronic) that would be expected to be without appreciable health risk.

For substances intentionally added to food, such as food additives, and for residues of pesticides and veterinary drugs in food, the health-based guidance value is termed the ADI. JECFA and JMPR determine ADIs based on all the known facts at the time of the evaluation.

Substances that have long half-lives and accumulate in the body are not suitable for use as food additives (FAO/WHO, 1962a). Data packages should include metabolism and excretion studies designed to provide information on the cumulative properties of food additives.

At the time of its first meeting, JECFA recognized that the amount of an additive used in food should be established with due attention to "an adequate margin of safety to reduce to a minimum any hazard to health in all groups of consumers" (FAO/WHO, 1957). The second JECFA meeting (FAO/WHO, 1958), in outlining procedures for the testing of intentional food additives to establish their safety for use, concluded that the results of laboratory animal studies can be extrapolated to humans, and that

> some margin of safety is desirable to allow for any species difference in susceptibility, the numerical differences between the test animals and the human population exposed to the hazard, the greater variety of complicating disease processes in the human population, the difficulty of estimating the human intake, and the possibility of synergistic action among food additives.

This conclusion formed the basis for establishing the ADI, which is defined as an estimate of the amount of a food additive, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risk.

JECFA generally sets the ADI on the basis of the lowest relevant NOAEL in the most sensitive species.

The ADI is expressed in amount (e.g. mg) per kilogram of body weight, usually as a range from 0 to an upper limit. ADIs are normally expressed numerically using only one significant figure. The use of more than one significant figure might be taken to imply a greater degree of accuracy than that which can be achieved when assessing the hazard from the wide range of factors that influence toxicity.

When appropriate, JMPR and JECFA develop ARfDs (see section 5.2.9). The ARfD is defined as (FAO/WHO, 2002a):

> an estimate of the amount of a substance in food and/or drinking-water, normally expressed on a body-weight basis, that can be ingested in a period of 24 h or less, without appreciable health risk to the consumer, on the basis of all the known facts at the time of the evaluation.

For food contaminants that are generally unavoidable, JECFA has used the term "tolerable" for health-based guidance values. This term was considered more appropriate than "acceptable", as it signifies permissibility for the intake of contaminants associated with the consumption of otherwise wholesome and nutritious food. These have included TDI, provisional maximum tolerable daily intake (PMTDI), provisional tolerable weekly intake (PTWI) and provisional tolerable monthly intake (PTMI). The use of the term "provisional" expresses the tentative nature of the evaluation, in view of the paucity of reliable data on the consequences of human exposure at levels approaching those with which JECFA is concerned.

Health-based guidance values may be derived from either NOAELs or BMDs (BMDLs), often called the POD or reference point. The NOAEL approach has been used for over 50 years, and testing guidelines (chapter 4) have been developed to ensure that toxicological data are suitable to identify the adverse effect of concern and also to define a NOAEL. In the BMD approach, a NOAEL does not have to be identified, but doses with graded responses are needed to provide optimum model output.

Calculation of the health-based guidance value (HBGV) can be described as follows:

$$\text{HBGV} = \frac{\text{POD}}{\text{UFs}}$$

where UF is the uncertainty factor, a term often used synonymously with safety factor.

When relevant, JMPR and JECFA use an overall NOAEL as a basis for the ADI, considering the most relevant studies together. JMPR

made the following comment with regard to an overall NOAEL (FAO/WHO, 2004b):

> During the toxicological evaluation of a compound, the Meeting often has available more than one study in which the same end-points have been addressed. In such situations, the dose spacing may be different, resulting in different NOAELs and lowest-observed-adverse-effect levels (LOAELs). The Meeting agreed that in such circumstances it might be appropriate to consider the studies together. When they are comparable, including consideration of study design, end-points addressed, and strain of animal, the "overall NOAEL" should be the highest value identified in the available studies that provides a reasonable margin (≥ 2) over the lowest LOAEL, provided that due consideration is given to the shape of the dose–response curve.

JECFA subsequently applied this approach in the evaluation of phytosterols, phytostanols and their esters (FAO/WHO, 2009b).

Calculations of a health-based guidance value based on the NOAEL or BMD approach for the example of quantal response data are summarized in Table 5.4.

The table shows calculation of an ADI, but the methods are applicable to any health-based guidance value.

5.2.2 Data

In selecting an experimental animal study for use in risk assessment, due consideration needs to be given to matching, as far as is possible, the pattern of potential human exposure—i.e. the route and duration of exposure (as a fraction of lifetime) and the pattern of exposure (e.g. intermittent bolus dosing or dietary administration).

When considering which data to use from a set of available toxicity studies on a particular compound, it is not necessary to undertake DRM for each observed end-point in each study. Whether the NOAEL or BMD approach is used for risk assessment, the aim is to define the adverse effect that is produced at the lowest levels of exposure. Therefore, a first step would be to exclude studies that have NOAELs that are obviously larger than those from the other studies. In addition, end-points clearly not showing a dose–response on visual and

Table 5.4. Comparison of methods used to derive health-based guidance values based on NOAEL and BMD approaches (using the Weibull model for illustrative purposes) for the case of quantal data (adapted from IPCS, 2009)

Step	NOAEL-derived ADI	BMD-derived ADI
1. Data selection	Sufficient sample sizes, at least one dose with no statistically significant effect. Relevant end-points in a relevant species are important for any approach.	Sufficient number of doses with different response levels and a sufficient total number of subjects.
2. Model selection	None.	Fit dose–response model (e.g. Weibull model).
3. Statistical linkage	Pairwise statistical tests between dose groups and control group.	Predicted fractions are linked to observed fractions, and their "distance" is minimized by optimizing some fit criteria function (e.g. likelihood function based on assumed distribution).
4. Parameter estimation	No parameter; the NOAEL corresponds to one of the dose levels in the study.	Choose an appropriate response, p, in the range of experimental response. Estimate $BMDL_p$, the 95% lower confidence bound on the BMD_p, where $\frac{R(BMD_p) - R(0)}{1 - R(0)} = p$
5. Implementation	ADI = NOAEL / UFs where UF is uncertainty factor.[a]	ADI = $BMDL_p$ / UFs
6. Evaluation	Statistical power analysis can be performed to check if the test was sensitive enough to detect relevant effects.	Sensitivity of BMD to model choice can be checked by fitting various models.

[a] The term "uncertainty factor" (UF) is used synonymously with the term "safety factor".

statistical inspection of the data can be omitted. Then, based on the toxicological impact together with the apparent magnitude of the response, a selection of end-points can be made as candidates for DRM. After selecting the potentially relevant end-points, the suitability of each dose–response data set for dose–response analysis is considered. For the BMD approach, it is generally desirable to have at least three or four different doses (including controls) and different levels of effect associated with different doses.

A design optimal for the NOAEL approach could limit the use of DRM, and vice versa. Whereas the NOAEL approach requires sufficient sample sizes within dose groups (to provide statistical power), the BMD approach requires a sufficient number of dose groups (to provide a description of the whole dose–response).

The BMD approach can be used to analyse data from studies carried out in the past and based on the traditional designs (with three dose groups and a control). Although these may not be optimal for model fitting, the BMD approach retains the advantages outlined above. The BMD approach can also be used for combined analysis of multiple similar studies.

Both the BMD and NOAEL approaches may prove inadequate when the number of animals per dose group is too small. For example, when the critical effect is seen in an experimental animal such as the dog, with few animals per dose group, the NOAEL may be high because of the insensitivity of the test. Although the BMD approach is better for evaluating sparse dose–response data, it may also provide very uncertain estimates; unlike the NOAEL approach, however, the inherent uncertainty is more explicit.

5.2.3 Safety/uncertainty factors

The terms “safety factor” and “uncertainty factor” are often used interchangeably, “safety factor” having been used historically, but the preference now is to use “uncertainty factor”. Comparable terms used by other bodies are “adjustment factor” and “assessment factor”. Application of the factors is intended to provide an adequate margin of safety for the consumer, considering sensitive human population subgroups.

Uncertainty factors are default factors used to account for both uncertainty and variability. Historically, an uncertainty factor of 100 has been used to convert the NOAEL from a study in experimental animals into a health-based guidance value for human exposure (IPCS, 1987). Additional uncertainty factors may be used to allow for important database deficiencies, such as the absence of a chronic study or when effects are detected at all experimental dose levels and a NOAEL has not been defined. In such cases, a LOAEL might be used for establishing a health-based guidance value (IPCS, 1994).

The default 100-fold uncertainty factor may be seen to represent the product of two separate 10-fold factors that allow for 1) differences between the average responses in the experimental animals used in the study identified to derive the POD and those in average humans and 2) the variability in responses between average humans and those who are highly sensitive (IPCS, 1987). The recognition that the original 100-fold uncertainty factor could be considered to represent two 10-fold factors allowed some flexibility, because different factors could be applied to the NOAEL from a study in humans and the NOAEL from a study in experimental animals.

Although uncertainty factors were to some degree determined arbitrarily and validated subsequently by scientific data and practical experience, they are dependent on the nature of the compound, the amount, nature and quality of the toxicological data available, and the nature of the toxic effects of the compound. When considering appropriate uncertainty factors, a number of aspects have to be taken into account, such as species differences, individual variations and incompleteness of available data.

A number of basic principles have been developed for considering appropriate uncertainty factors (adapted from EHC 104; IPCS, 1990), as described below.

When determining health-based guidance values, the 100-fold default factor is used as the starting point for extrapolating animal data to humans and may be modified in the light of the data that are available and the various concerns that arise when considering these data. Some of these are given below:

1) When relevant human data are available, the 10-fold factor for interspecies variability may not be necessary or may be reduced, depending on whether the available data represent the most susceptible part of the population as well as representing a sufficiently large group of individuals. Recommendations on numbers required can be found in IPCS (2005).

2) The quality of the data supporting the NOAELs or BMDLs determined in the animal experiments (and also in human experiments) influences the choice of the uncertainty factor. An increased factor may be appropriate to account for deficiencies in the studies.

3) The quality of the total database may affect the choice of uncertainty factor. Significant data deficiencies may warrant an increased factor due to increased uncertainty. A clear explanation needs to be given as to the exact nature of the deficiency.

4) The type and significance of the initial toxic response may alter the uncertainty factor. Thus, a response that is marginal and reversible may result in a reduced safety factor, if the effect is still considered relevant for human health.

5) The shape of the dose–response curve (in those cases where data are adequate to permit derivation of such a curve) may also be considered in assessing uncertainty factors. An increased factor can be considered when the dose–response curve is very steep, particularly when the NOAEL is close to the LOAEL.

6) Metabolic considerations may influence the choice of uncertainty factor. Thus, saturation of metabolic pathways resulting in toxic manifestations, biphasic metabolic patterns and data on comparative metabolism may all affect the magnitude of the uncertainty factor. Suitable toxicokinetic data may be used to derive chemical-specific adjustment factors (CSAFs) (see below).

7) Knowledge of the comparative mechanism or mode of toxic action in experimental animals and humans may influence the choice of uncertainty factor. More broadly, information on the dose–response relationships for one or more key events comprising a mode of action, in experimental animals or humans, can be

invaluable in informing the choice of uncertainty factors. Suitable toxicodynamic data may be used to derive CSAFs (see below).

Some experimental support for the default uncertainty factors was published by Dourson & Stara (1983). This paper also proposed additional factors for extrapolating from subchronic data (10-fold) and for converting LOAELs to NOAELs (1- to 10-fold, depending upon the severity and concern raised by the observed effect). Reviews (Renwick & Lazarus, 1998; Dorne & Renwick, 2005; Dorne et al., 2005) of clinical data on human variation in the major pathways of foreign compound metabolism and pharmacological sensitivity have shown that the 10-fold factor is a reasonable default value. In addition, clinical and/or epidemiological research in humans may provide information on variation in response within the human population to a chemical and hence allow a more accurate determination of uncertainty factors.

The concept of CSAFs (IPCS, 1994, 2005) has been introduced to allow appropriate data on species differences or human variability in either toxicokinetics (fate of the chemical in the body) or toxicodynamics (actions of the chemical within the body) to modify the relevant default 10-fold uncertainty factor (Table 5.5). The strategy proposed by IPCS involves replacing the original 100-fold uncertainty factor with CSAFs.

The CSAFs enable the incorporation in risk assessment of specific quantitative data on species differences or human variability in either toxicokinetics or toxicodynamics to replace part of the default uncertainty factor. Although such information is often not available, information on pathways of elimination or mode of action may be available. For example, JECFA used comparative body burden data rather than external dose data in its calculation of a PTMI for dioxin-like substances, allowing the usual 100-fold uncertainty factor to be subdivided and replaced with chemical-specific lower values, as there was no need for interspecies differences in toxicokinetics or for toxicodynamic differences between species (FAO/WHO, 2002b). Detailed guidance on the application of CSAFs in risk assessment has been published (Meek et al., 2002; IPCS, 2005).

As information is available on the extent to which some of these pathways or processes vary between experimental animals and humans

Table 5.5. Values for default uncertainty subfactors that can be replaced by CSAFs to derive composite uncertainty factors (from IPCS, 2005)

	Default subfactor		
Source of uncertainty	Toxicokinetic	Toxicodynamic	Combined
Interspecies variation	4.0	2.5	10
Human interindividual variation	3.16	3.16	10

or within humans, an approach has been proposed to enable this information to be used to inform the choice of uncertainty factors. This approach therefore lies somewhere between the normal default situation (100-fold uncertainty factor) and the derivation of CSAFs on the basis of quantitative chemical-specific data. Such factors have been termed "categorical factors" (Walton et al., 2001) or pathway-related factors (Dorne et al., 2005). This concept is applied by JMPR for pesticides where the effect (mostly acute) is dependent on the peak plasma concentrations (C_{max}) rather than the plasma concentration integrated over time (area under the curve, or AUC) in order to derive a combined uncertainty factor based on categorical and default factors. This would lead, for example, to a factor of 25 instead of the default of 100 for use with carbamates (for details, refer to section 2.5 of FAO/WHO, 2009a).

Several of the factors cited above may apply in the consideration of any one compound. Certain factors may serve to increase and others to decrease the choice of the final uncertainty factor. Therefore, it must be stressed that the total weight of evidence has to be considered in determining the appropriate uncertainty factor to be used and that the determination of uncertainty factors must be considered on a case-by-case basis.

5.2.4 The NOAEL approach to deriving health-based guidance values

The critical steps in this approach are selection of the appropriate data and determination of the NOAEL. Historically, JECFA has used the term no-observed-effect level (NOEL), which was defined in EHC 70 (IPCS, 1987) as "The greatest concentration or amount of an agent, found by study or observation, that causes no detectable, usually

adverse, alteration of morphology, functional capacity, growth, development, or lifespan of the target". In contrast, JMPR has used the term NOAEL, which was defined in EHC 104 (IPCS, 1990) as "The highest dose of a substance at which no toxic effects are observed". In reality, both terms have similar meaning, and the NOAEL has been used similarly to set health-based guidance values by both JECFA and JMPR.

For the purpose of this monograph, NOAEL will be used, defined as follows:

- *No-observed-adverse-effect level (NOAEL)*: Greatest concentration or amount of a substance, found by experiment or observation, which causes no detectable adverse alteration of morphology, functional capacity, growth, development, or lifespan of the target organism under defined conditions of exposure.

The main difficulty with this approach is that it is critically dependent on the sensitivity of the test method. The statistical linkage (step 3) determines whether or not there is a statistically significant effect (e.g. at the 5% level) compared with background (e.g. the control group) for each dose level separately. When the response is not statistically significant, it is generally considered that this level of intake is without biologically significant adverse health effects, but the power of the study to detect an adverse effect is not analysed. Given the typical animal studies used in toxicology, the effect size that can be detected by a statistical test may be larger than 10% (additional risk). Therefore, the NOAEL may be expected to be a dose at which the effect is in reality somewhere between 0% and 10% or more. The selection of the NOAEL (step 4) identifies the highest dose level that does not produce a statistically significant effect. The NOAEL approach tends to give lower health-based guidance values for studies with a higher power to detect adverse effects, which in effect "penalizes" better-designed studies. This emphasizes the importance of adherence to testing guidelines in order to ensure that the data are suitable for risk assessment purposes.

The value of the NOAEL depends strongly on the following characteristics of the study design:

- *Group size*. The power to detect a NOAEL at some dose level is directly dependent on the sample sizes chosen at those dose

levels. The larger the group size, the smaller the possible undetected effect size at the NOAEL.

- *Dose selection*. The NOAEL must be one of the doses actually applied in the study. If the true threshold is higher than the NOAEL, the distance between the two can be expected to be limited (related to the dose spacing used), whereas if the true threshold is lower than the NOAEL, the distance between the two is potentially unlimited.

- *Experimental variation*. Experimental variation comprises biological (e.g. genetic) variation between subjects, variation in experimental conditions (e.g. time of feeding, location in experimental room, time of section or interim measurements) and measurement errors. Larger experimental variation between subjects will result in lower statistical power, and hence higher NOAELs.

Calculation of the health-based guidance value (HBGV) from NOAEL-based DRM (step 5 above) is given by the equation:

$$\text{HBGV} = \frac{\text{NOAEL}}{\text{UFs}}$$

Step 6 could be undertaken to analyse the power of the dose group identified as representing the NOAEL to detect the adverse effect found at higher dose levels. For example, DRM could be used to determine, with 95% confidence intervals, the magnitude of effect that would be predicted to occur in the NOAEL group. In addition, step 6 could be used for both the NOAEL and BMD approaches to evaluate the sensitivity of the calculated health-based guidance value to the values of the uncertainty factors chosen.

5.2.5 Benchmark dose approach to deriving health-based guidance values

As an alternative to the NOAEL approach, the BMD concept has been introduced (Crump, 1984; Kimmel & Gaylor, 1988). In contrast to the NOAEL approach, this method defines a level of exposure producing a non-zero effect size or level of response as the POD for risk assessment. The BMD method has a number of advantages,

including the use of the full dose–response data in the statistical analysis, which allows the quantification of the uncertainty in the data. Higher uncertainty in the data—for example, due to small group sizes or high variation within a group—would be reflected in lower health-based guidance values.

In choosing the data (step 1) for BMD modelling, similar basic considerations apply as for the NOAEL method. Group sizes are less critical, because the POD is not based on identifying a level of exposure at which the adverse effect was not detected. Studies showing a graded monotonic response with a significant dose-related trend provide the best experimental data for modelling.

The main difficulty with this approach is that it requires the selection of a level of response, the BMR. In general, the level chosen is such that it is close to the limit of detection of the study, or a level that would generally be considered as representing a negligible health effect. A generic form of the BMD and BMDL is presented in Table 5.4 for the example of quantal data. A variety of response levels, such as 1%, 5% and 10%, may be selected as the BMR; differences in selection of the BMR could lead to discrepancies in health-based guidance values between different regulatory bodies.

Choosing a model (step 2) for the BMD method is dependent upon the types of data available and the characteristics of the response being modelled. Complicated models will require a larger number of dose groups than simpler models, and several models have been proposed for each type of data. In the United States Environmental Protection Agency BMD software program (http://www.epa.gov/NCEA/bmds/), a number of routinely used models are cited. If widely varying estimates are given when multiple models are applied to the same data, it may be necessary to select a particular model to calculate the BMDL. Strategies that have been suggested include using a criterion for goodness of fit (e.g. the Akaike Information Criterion), model averaging or the model that yields the lowest BMDL (IPCS, 2009).

At its sixty-fourth meeting, JECFA calculated the MOEs for a number of genotoxic and carcinogenic food contaminants using BMDL values derived by fitting a range of models to the available experimental dose–response data (FAO/WHO, 2006). Annex 3 of the

report of that meeting provides useful background information on the use of the BMD approach for risk assessment purposes.

The statistical linkage (step 3) between the data and the model can assume a number of different forms. For quantal data, it is appropriate to assume that the data are binomially distributed for each dose group.

Selection of the POD (step 4) for the BMD method is in reality selection of the BMR, because the model outputs simply report the BMD and BMDL values for the selected BMR. It is often not clear what level of response (BMR) can be considered as representing a negligible health effect. Selection of the BMR requires discussion among toxicologists and clinicians. Although an explicit statement on the BMR is an improvement compared with the generally unknown response level that may be associated with a NOAEL, choices of a BMR need consensus building and will remain a subjective "expert" judgement in what is essentially a mathematical approach. An alternative approach to selection of the BMR is to choose an excess response, often 10%, that is close to the limit of detection of the study, below which there was insufficient support from the experimental data; however, this simply leaves open the issue of the possible health consequences of the resulting level of response at that BMR. Further information on the selection of the BMR is given in IPCS (2009).

The health-based guidance value (HBGV) can be calculated as follows:

$$\text{HBGV} = \frac{\text{BMDL}}{\text{UFs}}$$

In this calculation, the values of the uncertainty factors could be the same as those used for the NOAEL or adjusted to account for a slightly different interpretation of the BMDL relative to the NOAEL. Unlike with the NOAEL approach, an extra uncertainty factor would not be necessary if all dose levels resulted in significant levels of adverse effect (indeed, such data would be more suitable for modelling). Empirical investigations showed for a large and representative set of compounds that the BMDL may be regarded as an analogue to

a NOAEL, and substituting one for the other would result in similar health-based guidance values (Crump, 1984; Barnes et al., 1995).

Unlike the NOAEL approach, the BMD method includes the determination of the response at a given dose, the magnitude of the dose at a given response and their confidence limits. By extrapolation of the dose–response model below the biologically observable dose range, the response at specified (lower) dose levels can be estimated, as well as the dose corresponding to a specific response level. It should be noted, however, that extrapolation from a single model that fits the data in the observed range cannot be justified, as other models fitting the data equally well may result in substantially different estimates of low-dose risk. Linear extrapolation from a BMD for a 10% response (BMD_{10}) has been applied as a simple method for low-dose extrapolation, but the sixty-fourth meeting of JECFA (FAO/WHO, 2006) concluded that "Linear extrapolation from a point of departure offers no advantages over an MOE and the results are open to misinterpretation because the numerical estimates may be regarded as quantification of the actual risk."

5.2.6 Acceptable daily intakes

5.2.6.1 Food additives

In calculating the ADI, an uncertainty factor is applied to the NOAEL to provide a conservative margin of safety on account of the inherent uncertainties in extrapolating toxicity data from experimental animal studies to potential effects in humans as well as variation within the human species. When results from two or more animal studies are available, the ADI is based on the most sensitive animal species—i.e. the species that displayed the toxic effect at the lowest dose, unless metabolic or pharmacokinetic data are available establishing that the test in the other species is more appropriate for humans.

Generally, the ADI is established on the basis of toxicological information and provides a useful assessment of safety without the need for data on intended or actual use or dietary exposure. However, in setting ADIs, it may be necessary to know whether particular subpopulations are exposed, as the ADI applies to the whole population. Therefore, general information about exposure patterns should be known at the time of the safety assessment (see chapter 6). For example, if a food

additive is to be used in infant formulas, the safety assessment is not complete without looking carefully at safety studies involving exposure of very young animals.

There are occasions when JECFA considers the setting of an ADI in numerical terms not to be appropriate. This situation arises when the estimated consumption of the additive is expected to be well below any numerical value that would ordinarily be assigned to it. Under such circumstances, JECFA uses the term ADI "not specified". The Committee defines this term to mean that, on the basis of available data (chemical, biochemical, toxicological and other), the total daily intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background in food does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for the reasons stated in the individual evaluations, the establishment of an ADI in numerical form is not deemed necessary (e.g. FAO/WHO, 1984, Annex II). An additive meeting this criterion must be used within the bounds of Good Manufacturing Practice (GMP)—i.e. it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal inferior food quality or adulteration, and it should not create a nutritional imbalance (FAO/WHO, 1974). That the background occurrence of the chemical must be taken into account in the evaluation of its safety was articulated by the WHO Scientific Group on Procedures for Investigating Intentional and Unintentional Food Additives (WHO, 1967).

JECFA has encountered several situations in which either the body of available data on a new additive had some limitations or the safety of a food additive for which the Committee had previously assigned an ADI was brought into question by new data. When the Committee feels confident that the use of the substance is safe over the relatively short period of time required to generate and evaluate further safety data, but is not confident that its use is safe over a lifetime, it often establishes a "temporary" ADI, pending the submission of appropriate data to resolve the safety issue on a timetable established by JECFA. When establishing a temporary (numerical) ADI, the Committee always uses a higher than usual safety factor, usually increasing it by a factor of 2. The additional biochemical or toxicological data required for the establishment of an ADI are clearly stated,

and a review of these new data is conducted before expiry of the provisional period. In many cases, long-term studies are requested, but timetables are not met, which means that JECFA has had to extend temporary ADIs for further periods of time. In instances where data have not been forthcoming, JECFA has withdrawn temporary ADIs as a safety precaution.

5.2.6.2 *Pesticides*

The Joint FAO/WHO Meeting on Principles Governing Consumer Safety in Relation to Pesticide Residues indicated that the assessment of the amount of pesticide to which humans can be exposed daily for a lifetime, without injury, was the primary aim of toxicological investigations. The meeting indicated that "when the (toxicological) investigations are completed, it is possible, by the use of scientific judgement, to name the acceptable daily intake" (FAO/WHO, 1962b).

JMPR stated that the following information should be available in order to arrive at an ADI (FAO/WHO, 1964):

- The chemical nature of the residue. Pesticides may undergo chemical changes and are frequently metabolized by the tissues of plants and animals that have been treated with them. Even when a single chemical has been applied, the residue may consist of a number of derivatives with distinct properties, the exact nature of which may differ in animals and plants and in different crops and products.

- The toxicities of the chemicals forming the residue from acute, short-term and long-term studies in animals. In addition, knowledge is required of the metabolism, mechanism of action and possible carcinogenicity of residue chemicals when consumed.

- A sufficient knowledge of the effects of these chemicals in humans.

The principles discussed above were adopted by subsequent Meetings but, as would be expected, have been further developed with time. Thus, the 1968 JMPR (FAO/WHO, 1968) indicated that metabolites would, under certain conditions, be considered to be included in the ADI. Generally, if the metabolites in food commodities are qualitatively and quantitatively the same as those observed in

laboratory test species, the ADI would apply to the parent compound as well as to metabolites. If the metabolites are not identical or not present at the same order of magnitude, separate studies on the metabolites may be necessary. When one or several pesticides are degradation products of another pesticide, a single ADI may be appropriate for the pesticide and its metabolites (e.g. oxydemeton-methyl, demeton-*S*-methyl sulfone and demeton-*S*-methyl) (FAO/WHO, 1989).

The use of the temporary ADI, first proposed by the WHO Scientific Group on Procedures for Investigating Intentional and Unintentional Food Additives (WHO, 1967), was adopted by JMPR. Criteria were set that had to be met prior to the establishment of the temporary ADI. These included the consideration of each chemical on its own merits, the establishment of the temporary ADI for a fixed period (usually 3–5 years) and the subsequent review of original and new data prior to expiry of the provisional period.

The establishment of a temporary ADI has always been accompanied by a requirement for further work by a specified date and, for numerical ADIs, by the application of an increased safety factor. The 1972 JMPR (FAO/WHO, 1973) considered the course of action to be taken if requested data were not forthcoming and indicated that, under these circumstances, the temporary ADI would be withdrawn. It emphasized, however, that such an action

> did not necessarily indicate a potential health hazard, but only that insufficient information is available at the time of review to permit the Meeting to state with reasonable certainty that there is no likelihood of adverse effects on health resulting from ingestion over a prolonged period.

In 1986, JMPR (FAO/WHO, 1986) indicated that the previously utilized terms "Further work or information required" or "Further work or information desirable" were being replaced, the former by the statement "Studies without which the determination of an ADI is impracticable" and the latter by the statement "Studies which will provide information valuable to the continued evaluation of the compound." Not only do these new statements reflect the actual work performed by JMPR much more clearly than the previous terms "required" and "desirable", but they also reflect the Meeting's increasing reluctance to allocate temporary ADIs as well as the desire to continue the evaluation of a compound even after an ADI has been allocated.

In 1988, JMPR (FAO/WHO, 1988a) recommended that temporary ADIs should not be allocated for new compounds and that an ADI should not be allocated in the absence of an adequate database. The Meeting intended that monographs would be published for all chemicals that are reviewed, regardless of whether an ADI is allocated, and that data requirements would be clearly specified for those chemicals with an inadequate database.

5.2.6.3 *Veterinary drug residues*

Recognizing the principles applied in evaluating a substance for the purposes of establishing an ADI in the Principles for the Safety Assessment of Food Additives and Contaminants in Food (IPCS, 1987), the thirty-second JECFA meeting elaborated many of these principles as a framework for the specific assessment of residues of veterinary drugs in food (FAO/WHO, 1988b). Most importantly, where possible and appropriate, they affirmed that an ADI based on determination of a NOAEL from experimental animal or human toxicological data should be used as the end-point of the safety evaluation with use of an appropriate safety factor. The thirty-second meeting of the Committee recognized that in some instances it might be inappropriate to establish an ADI. When it has been determined that establishing an ADI is unnecessary because of a large margin of safety, the recommendation of a maximum residue limit (MRL) is also unnecessary. For example, at the fortieth meeting, an ADI "not specified" was established for the bovine somatotropins (FAO/WHO, 1993). The Committee noted the lack of activity of the recombinant somatotropins and insulin-like growth factor-1 after oral dosing as well as the low amounts and non-toxic nature of the residues of these compounds even at exaggerated doses. The Committee concluded that these results provide an extremely large margin of safety for humans consuming dairy products from animals treated with the recombinant somatotropins and therefore warranted the establishment of an ADI "not specified".

The Committee has noted that an ADI for a drug is usually based on the toxicity of the parent drug rather than on its metabolite or metabolites. However, it may sometimes be necessary to calculate an ADI for individual metabolites. Although most compounds have been evaluated as individual substances, there are instances where an ADI has been established as a group ADI (e.g. streptomycin/

dihydrostreptomycin, enrofloxacin/ciprofloxacin; see section 5.2.8) and where an ADI has been established on a microbiological end-point rather than a toxicological end-point (e.g. spiramycin and spectinomycin). The thirty-eighth meeting of the Committee (FAO/WHO, 1991) noted that if the pharmacological effects are more relevant and sensitive than the toxicological effects, the ADI should be established on the basis of pharmacology.

There have been a limited number of situations where an ADI numerical value or range was not identified. For allergenic considerations, the Committee did not establish an ADI for benzylpenicillin, as there were insufficient data with which to establish a NOEL (FAO/WHO, 1990). The Committee recommended that the daily intake from food should be kept as low as possible (below 0.03 mg/person per day).

The thirty-eighth meeting of the Committee also addressed the issue of establishing ADIs and MRLs for those substances that are rapidly converted to their metabolites when they are administered to the target animal or host (FAO/WHO, 1991). The Committee recognized that there may be occasions when drug metabolites present as residues are responsible for the specific activity of concern possessed by the parent drug. In these situations, the activity of the parent drug would be discounted in establishing the ADI on which to base the MRL; the ADI would instead be based on a toxicological property of the metabolites with an appropriate safety factor applied. In the case of febantel, an ADI was established for febantel per se, based on a study in animals administered the parent compound, but the MRL was established for metabolites, measured as oxfendazole sulfone, using an ADI established for oxfendazole.

The fortieth meeting of the Committee noted that certain conditions apply regarding the identity and quality of veterinary drugs subject to Committee review (FAO/WHO, 1993). The Committee evaluations depend on studies performed with a chemical substance of defined identity, purity and physical form. In particular, the ADI is valid only for substances that do not differ significantly in identity and quality from the material used to generate the data on which the Committee's evaluation is based (see chapter 3).

The thirty-eighth meeting of the Committee (FAO/WHO, 1991) affirmed that in calculating the ADI, the Committee has usually

followed the procedures described in Principles for the Safety Assessment of Food Additives and Contaminants in Food (IPCS, 1987), applying a safety factor to the NOAEL derived from the most relevant and appropriate toxicological, microbiological or pharmacological end-point study. The safety factor usually chosen is 100 in the situation where a NOAEL is derived from a long-term animal study, on the assumptions that humans are 10 times as sensitive as the test animals used in such studies and that a 10-fold range of sensitivity within the human population may exist. When no adverse health effects are seen in long-term studies, an uncertainty factor of 100 may be applied to the NOAEL derived from short-term studies where higher dose levels have been used and an effect has been noted. Typically, acceptable short-term studies need to be at least 3-month studies. The Committee noted, however, that, depending on the quantity, quality and nature of the available data, a safety factor of 100 might be insufficient. This may occur when the required data are incomplete, when the study from which the NOAEL is established is inadequate (e.g. insufficient numbers of animals per test group or when no individual animal data are reported) or when irreversible effects such as teratogenicity or non-genotoxic carcinogenicity are noted. The Committee may employ, and on limited occasions has employed, higher safety factors (e.g. 200, 500 and 1000), depending on the quality and quantity of relevant data. The Committee noted that safety factors are usually not appropriate for genotoxic carcinogens. When the only noteworthy toxicological effects are observed in human studies, a lower safety factor (e.g. 10) may be applied. The Committee stressed that the safety factor applied with each drug would be assessed on its own merits, considering all the above factors.

A different approach is used for the establishment of an ADI based on an effect on the human gut microflora. A decision tree approach that complies with Guideline 36 of the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH, 2004) has been developed by JECFA. It is used to determine the need to establish a microbiological ADI for the compound under review. The decision tree approach initially seeks to determine if there may be microbiologically active residues entering the human colon. This is done in three steps, in which the questions are:

1) Step 1: Are residues of the drug, and (or) its metabolites, microbiologically active against representatives of the human intestinal flora?

2) Step 2: Do residues enter the human colon?

3) Step 3: Do the residues entering the human colon remain microbiologically active?

If the answer is "no" to any of the first three steps, then no microbiological ADI is necessary. However, should such residues be present, then two end-points of public health concern are to be considered: 1) disruption of the colonization barrier and 2) increase of the population(s) of resistant bacteria.

At Step 4 of the decision tree process, it is possible to provide scientific justification to eliminate testing (i.e. the need for a microbiological ADI) for either one or both end-points.

Step 5 is where a microbiological ADI is determined. Should a microbiological ADI not be necessary, then the toxicological or pharmacological ADI would be used.

The decision tree approach makes use of observations in humans if such data are available. If this is the case, it is reflected in the uncertainty factor used by the Committee. However, the typical situation is that the ADI is based on in vitro minimum inhibitory concentration (MIC) data. The following formula is used to derive a microbiological ADI from in vitro MIC data:

$$ADI = \frac{MIC_{calc} \times \text{Mass of colon content}}{\text{Fraction of oral dose available to microorganisms} \times UF \times 60\ kg}$$

where:

- The MIC_{calc} represents the lower 90% confidence limit for the mean MIC_{50} (the minimum inhibitory concentration for 50% of strains of the most sensitive relevant organism) of the relevant genera for which the drug is active.

- The mass of colon content is assumed to be 220 g/day.
- The fraction of an oral dose available to microorganisms is ideally based on in vivo measurements for the drug administered orally. Alternatively, if sufficient data are available, the fraction of the dose available for colonic microorganisms can be calculated as 1 minus the fraction of an oral dose excreted in urine. Human data are encouraged; in their absence, non-ruminant animal data are recommended. In the absence of data to the contrary, it should be assumed that metabolites have antimicrobial activity equal to that of the parent compound. The fraction may be lowered if the applicant provides quantitative in vitro or in vivo data to show that the drug is inactivated during transit through the intestine.
- UF is the uncertainty factor.
- 60 kg is the standard human body weight used by JECFA.

In these cases, the uncertainty factor is used in an entirely different way than when applied to an ADI based on toxicological data. When establishing a microbiologically based ADI, the safety factor is used to account for uncertainty about the amount and relevance of the MIC data available for review. For example, where microbiological effects were studied directly in humans or in a sufficient number of microorganisms representative of the potentially susceptible fraction of the human gut microflora, an uncertainty factor of 1 may be used. Generally, uncertainty factors considered appropriate for microbiological end-points are 1–10, depending on the quantity and quality of the data.

Several meetings of the Committee on residues of veterinary drugs in food have had substances with limited toxicological data available upon which to establish an ADI. The thirty-sixth meeting of the Committee (FAO/WHO, 1990) noted that when the Committee, in its scientific judgement, is confident that the consumption of residues of the veterinary drug is without toxicological hazard over a limited amount of time (e.g. the amount of time required to generate and evaluate further data for toxicological assessment), but not sufficiently confident that consumption of these residues over a lifetime may not pose a health concern, it may establish a temporary ADI. In applying this approach, the Committee considers whether those data might be made available to the Committee within a relatively short period of time. As is noted below, temporary MRLs may be recommended for similar

or additional reasons, such as the availability of reliable analytical methods or additional information on the nature of the quantification of residues.

Where the Committee has established temporary ADIs, it specifies what information is required to resolve the data needs and sets a date by which the data are requested for re-evaluation by the Committee. The same approach is applied with MRLs. At the reassessment, if one is done, the Committee has the option to 1) establish a full ADI, 2) extend the temporary ADI or 3) not extend the temporary ADI (i.e. the ADI is withdrawn). The same options are available with temporary MRLs. The thirty-sixth meeting of the Committee established a temporary ADI and temporary MRLs for levamisole and requested additional toxicological and residue data for re-evaluation by the Committee (FAO/WHO, 1990). Based on the additional data provided, the forty-second meeting of the Committee established an ADI; however, it withdrew the temporary MRL for levamisole in milk, as no additional data were made available. Similarly, the Committee withdrew the MRL in eggs because of high amounts of residues (FAO/WHO, 1995).

5.2.7 Tolerable intakes

JECFA has considered the presence of food contaminants on many occasions since 1972, when mercury, lead and cadmium were first assessed (FAO/WHO, 1972). These food contaminants have included, in addition to heavy metals, environmental contaminants such as dioxins, mycotoxins, impurities arising in food additives, solvents used in food processing, packaging material migrants and residues arising from the use of animal feed additives or the non-active components of veterinary drug formulations. Each of these classes of food contaminants possesses its own unique characteristics and evaluation requirements. Thus, JECFA has recognized through the years that evaluation principles should pertain to classes or groups of contaminants rather than to food contaminants in toto. Guidelines for the evaluation of classes of contaminants are provided in various sections of this report.

When JECFA considered mercury, cadmium and lead in 1972, it established the concept of a PTWI, which was a departure from the traditional ADI concept (FAO/WHO, 1972). JECFA has continued to use this concept, with some modifications, ever since for

contaminants with cumulative properties. The use of the term "provisional" expresses the tentative nature of the evaluation, in view of the paucity of reliable data on the consequences of human exposure at levels approaching those with which JECFA is concerned.

PMTDIs are established for food contaminants that are known not to accumulate in the body. The value assigned to a PMTDI represents permissible human exposure as a result of the background occurrence of the substance in food and also in drinking-water.

For contaminants that may accumulate within the body over a period of time, JECFA has used the PTWI and PTMI. On any particular day, consumption of food containing above-average levels of the contaminant may exceed the proportionate share of its weekly or monthly tolerable intake (TI). JECFA's assessment takes into account such daily variations, its real concern being prolonged exposure to the contaminant, because of its ability to accumulate within the body over a period of time.

The principles for establishing tolerable intakes are the same as for acceptable intakes as described above. For contaminants, there are often epidemiological studies available that can form the basis for derivation of tolerable intakes. If sufficient information is available to perform a dose–response assessment, the POD can be defined from epidemiological studies, and uncertainty factors can then be applied according to the principles outlined above. JECFA often applies the concept of CSAFs when deriving tolerable intakes for contaminants.

5.2.8 Group ADIs/TIs

If several substances that produce similar toxic effects are to be considered for use as food additives, pesticides or veterinary drugs or occur as contaminants (e.g. dioxins), it may be appropriate in establishing an ADI or TI to consider the group of substances in order to limit their overall intake. For this procedure to be feasible, the substances should have a similar mode of action and a similar range of toxic potency. Flexibility should be used in determining which NOAEL is to be used in calculating the ADI or TI. In some cases, the average NOAEL for all the substances in the group may be used for calculating the group ADI. A more conservative approach is to base

the group ADI or TI on the substance with the lowest NOAEL. The relative quality and length of studies on the various substances should be considered when setting the group ADI or TI. When the NOAEL for one of the substances is out of line with the others in the group, it should be treated separately.

When considering a substance that is a member of a series of substances that are very closely related chemically (e.g. fatty acids), but for which toxicological information is limited, it may be possible to base its evaluation on the group ADI or TI established for the series of substances. This procedure can be followed only if a great deal of toxicological information is available on at least one member of the series and if the known toxic properties of the various substances fall along a well-defined continuum. Interpolation, but not extrapolation, can be performed. The use of this procedure by JECFA represents one of the few situations in which the Committee has used structure–activity relationships in its safety assessments.

In some instances, group ADIs can be established primarily on the basis of metabolic information. For example, the safety of esters used as food flavours could be assessed on the basis of toxicological information on their constituent acids and alcohols, provided that it is shown that they are quantitatively hydrolysed in the gut.

The calculation of a group ADI is also appropriate for substances that cause additive physiological or toxic effects, even if they are not closely related chemically. For example, it may be appropriate to establish a group ADI for additives such as bulk sweeteners that are poorly absorbed and cause a laxative effect.

5.2.9 Setting of acute reference doses (ARfDs)

5.2.9.1 General considerations

JMPR routinely evaluates the acute and chronic effects of pesticide residues in food and has developed guidance on the setting of ARfDs for pesticides (FAO/WHO, 1999, 2001a,b, 2002c, 2004a; Solecki et al., 2005). The guidance provided in these documents for agricultural pesticides should be of value in general considerations of the necessity of establishing an ARfD, as well as in the specific end-point considerations in the derivation of an ARfD. The text that

follows relates mainly to pesticide residues, but JECFA may apply similar principles to other types of compounds when the establishment of an ARfD is needed.

The ARfD of a chemical refers to the amount of a substance that can be ingested in a period of 24 h or less (see section 5.2.1). Because the ARfD is compared with exposure data for a 24 h period, this will provide a conservative risk assessment for rapidly reversible effects (e.g. cholinesterase inhibition by carbamates) where the ARfD would be applicable to a shorter time period.

The decision as to whether the setting of an ARfD is necessary should be based on the hazard profile of a pesticide, as well as on specific end-points that may be particularly relevant to acute effects. Most of the scientific concepts applying to the setting of ADIs apply equally to the setting of ARfDs (e.g. consideration of the scientific quality of studies, selection of the critical effect). When assessing the need for an ARfD, the entire database should be reviewed using a weight of evidence approach to determine whether adverse effects seen in repeated-dose toxicity studies might be relevant to single exposures. Usually a single ARfD is set, but two values may be required (e.g. one for the general population and one for a subgroup of the population) in exceptional cases. In some cases, it may also be necessary to set an additional ARfD for main metabolites if they occur on crops and are therefore included in the residue definition (e.g. if these metabolites are likely to show an acute toxicity profile that is different from that of the parent compound) or when metabolites formed in humans are not observed in experimental animal metabolism studies.

5.2.9.2 *Practical cut-off value for ARfDs*

Bearing in mind practical considerations, such as the maximum quantity of a particular food likely to be consumed in a single sitting, a value above which the formal setting of an ARfD is unnecessary can be proposed. This practical cut-off value (upper limit) for an ARfD should be considered with reference to the potential range of dietary exposures to an acutely toxic pesticide. For example, the acute exposure to a pesticide used on fruit, for which there is an MRL set according to Good Agricultural Practice (GAP), may be calculated as follows:

- A 50 kg person consumes 500 g of fruit in a single sitting. The fruit consists of a single large item (e.g. a melon) and has been treated with a pesticide having an MRL of, for example, 20 mg/kg. Trial data show that a variability factor[1] of 5 is applicable.

- The estimated maximum exposure could be [20 mg/kg (MRL) × 5 (variability factor) × 0.5 kg (mass)] / 50 kg body weight = 1 mg/kg body weight.

However, further issues need consideration when deciding on a practical cut-off value for ARfDs:

- A small number of pesticide/commodity combinations have MRLs in excess of 20 mg/kg, although they might not have a toxicity profile indicating acute toxicity concern.

- Infants and small children might have a higher rate of consumption relative to body weight.

- For certain commodities, a variability factor greater than 5 might be applicable.

This estimate indicates that any general cut-off for ARfDs should be at a value greater than 1 mg/kg body weight. A value of 5 mg/kg body weight is proposed as a conservative value to cover all eventualities for agricultural pesticides, based on practical considerations on consumption and maximum residue levels in foods. An ARfD cut-off at 5 mg/kg body weight would equate to a NOAEL of 500 mg/kg body weight per day in an animal study, when default uncertainty factors are applied. Thus, if acute toxicity were seen only at doses greater than 500 mg/kg body weight, then there would be no necessity to set an ARfD.

By analogy, relevant upper limits might be considered for other chemicals (e.g. for non-agricultural pesticides).

[1] The variability factor is defined as the ratio of the 97.5th percentile of the distribution of pesticide residue per unit to the mean residue for the lot (ν = 97.5th percentile divided by the mean) (FAO, 2002).

If, during the derivation of an ARfD, it becomes apparent that a previously derived ADI is higher than the ARfD, the ADI should be reconsidered. Such a situation can occur for a number of reasons (e.g. the availability of additional studies, or compounds producing more severe effects when given by gavage than when administered in the diet) (FAO/WHO, 2001b). Even when there is no obvious basis to revise the ADI, it is recommended that the lower of the ARfD and the ADI be used as the ADI.

5.2.9.3 Biological and toxicological considerations

The following are key points for consideration when evaluating the database regarding the potential for acute toxicity:

- In the absence of data to the contrary, all indications of acute toxicity observed in repeated-dose studies should be considered as potentially relevant to setting an ARfD.

- Particular weight should be given to observations and investigations at the beginning of repeated-dose studies.

- The NOAEL from the most sensitive species should be used unless there is evidence to demonstrate that it is not appropriate for a human risk assessment.

- Isolated findings showing no specificity or clear pattern are not necessarily indications of acute toxicity.

In determining the appropriateness of using doses and end-points from subchronic or chronic toxicity studies to establish an ARfD, a weight of evidence evaluation should be conducted that considers all relevant data. This includes what is known about the toxic mode of action and the pertinent biology of the system that is affected. One of the main challenges is to evaluate whether those effects are also likely to occur at the same observed dose levels following an acute exposure.

Toxicological information from interim results or consideration of progression of a lesion in repeated-dose studies may provide insights into the relevance of end-points for setting ARfDs. For example, if interim data indicate that the response is minimal and

becomes pronounced or severe after increasing exposure duration, then repeated exposures are probably the determining factor in the response. Interpretation of the relevance of end-points should also consider toxicokinetic information that would raise concern for acute toxicity, such as slow elimination kinetics or toxicities dependent on the maximum plasma concentrations (C_{max}) achieved, as well as information on the acute toxicity of chemicals with a similar structure.

5.2.9.4 *Stepwise process for setting ARfDs*

The following stepwise process for setting ARfDs for agricultural pesticides is recommended:

- Evaluate the total database for the pesticide, and establish a toxicological profile for the active substance.
- Consider the principles for not needing to set an ARfD:
  - No findings indicative of effects elicited by an acute exposure are observed at doses up to about 500 mg/kg body weight per day; and/or
  - No substance-related mortalities are observed at doses up to 1000 mg/kg body weight in single-dose oral studies; and/or
  - If mortality is the only trigger, the cause of death should be confirmed as being relevant to human exposures.

If a decision is taken at this stage not to set an ARfD, the reasons should be clearly explained.

If the above criteria do not exclude the setting of an ARfD, then one should be set as follows using the most appropriate end-point and safety factors:

- Select the toxicological end-points most relevant for a single (day) exposure (see section 5.2.9.5).
- Select the most relevant study in which these end-points have been adequately investigated.
- Identify the NOAELs for these end-points.
- Select the most relevant end-point providing the lowest NOAEL.

- Derive the ARfD using the most appropriate safety factors[1] (see section 5.2.9.6).

An end-point from a repeated-dose toxicity study should be used if the critical effect of the compound has not been adequately evaluated in a single-dose study. This is likely to be a more conservative approach and should be stated as such. This does not mean that a safety factor other than the default value should be applied. A refinement of such a NOAEL (e.g. in a special single-dose study) may be necessary if the acute intake estimation (see section 5.2.9.9) exceeds such a potentially conservatively established ARfD. This will be necessary only for a very limited number of substances, according to a retrospective analysis (Moeller et al., 2009). Under the Organisation for Economic Co-operation and Development test guidelines programme, a document is under development on "Guidance for a single-dose study" (OECD, 2009), based on the guidance developed by JMPR, to inform investigators should a specific study be necessary as a basis for derivation of the ARfD.

If at this stage, after consideration of all the end-points, an ARfD is not set, then the reasons should be clearly explained.

5.2.9.5 *Toxicological end-points relevant for ARfD derivation*

A number of effects could be caused by a single exposure. The relevance of these effects for ARfD derivation should be considered on a case-by-case basis. The route of substance administration should be considered carefully with regard to available toxicokinetic data, in order to minimize influences that are not relevant for the intake of residues (e.g. effects induced by gavage or by a specific vehicle or formulation used).

The following list of target effects is not an exhaustive list of all possible relevant end-points (FAO/WHO, 2004a), but these toxic mechanisms are regarded as alerts for acute toxicity, relevant for the consideration of the need to set an ARfD:

[1] The term "safety factor" is based on current JMPR terminology and applied as a synonym for the terms "uncertainty factor", "adjustment factor" and "assessment factor" used by other bodies.

- *Haematotoxicity*: The induction of methaemoglobinaemia is regarded as a critical effect in consideration of acute responses to chemical exposure. Haemolytic anaemia is considered to be less relevant for ARfD derivation, as the severity of such an effect generally appears to depend on prolonged exposure.

- *Immunotoxicity*: Immunotoxicity data derived from subchronic studies are not likely to be appropriate for setting an ARfD for acute adult exposure limits, because immune system cells are constantly replaced and because of inherent redundancy in the system.

- *Neurotoxicity*: Any neurotoxicity seen in repeated-dose studies could be the result of a single exposure that is not repairable; thus, any evidence of neurotoxicity should be considered relevant to the setting of an ARfD, unless it can be demonstrated that the effects are produced only after repeated exposures.

- *Kidney and liver effects*: If the effects on these organs cannot be discounted as being either adaptive or the result of prolonged exposure, an ARfD can be derived on the basis of such effects. Such an ARfD is likely to be conservative, and it may be possible to subsequently refine it using an appropriately designed single-dose study.

- *Endocrine effects*: In general, adverse effects on the endocrine system observed in routine toxicological testing for regulatory purposes—other than those affecting female reproduction and development of the offspring—are considered to be unlikely to arise as a consequence of acute exposure. However, exceptions may occur, and a case-by-case analysis is required.

- *Developmental effects*: Any treatment-related adverse effect on embryos, fetuses or offspring that has resulted from exposure during any phase of development should be considered as potentially appropriate to use in acute dietary risk assessment, despite the fact that the treatment period typically consists of repeated dosing, as it could be the result of a single exposure during a critical window of development.

Direct effects on the gastrointestinal tract or stomach should be assessed carefully to determine their relevance to human exposure.

Considerations would include whether they are due to irritation or a pharmacological action or whether they are related to the method of administration (e.g. occur with bolus dosing but not with incorporation into the diet). Similarly, diarrhoea and vomiting in dogs should be considered as not relevant for setting an ARfD if these effects are related to high concentrations following specific dosing methods (e.g. capsule administration or gavage) and local (irritant) effects.

Other findings relevant for setting an ARfD, such as clinical signs, changes in body weight/body weight gain, changes in food and/or water intake and mortalities observed after one or several doses in repeat oral exposure toxicity studies, may suggest the need to establish an ARfD.

5.2.9.6 *Uncertainty factors for ARfDs*

The process for deriving an ARfD is essentially the same as that for deriving an ADI, involving the identification of the most appropriate NOAEL (or BMDL) and application of safety factors, usually 100-fold or 10-fold for data from studies in experimental animals or humans, respectively. Safety factors are used to extrapolate from animals to the average human and to allow for variation in sensitivity within the human population. The default factor of 10 for extrapolating from laboratory animals to humans can be subdivided into 2.5 for toxicodynamics and 4 for toxicokinetics, whereas the default human variability factor of 10 can be subdivided into identical factors of 3.2 for both toxicokinetics and toxicodynamics (IPCS, 2005), as described above under the concept of CSAFs (section 5.2.3).

A number of other situations may justify the use of safety factors higher or lower than the default values of 100 or 10 that are conventionally used on the basis of experimental animal or human data, respectively (FAO/WHO, 2001a). Such situations may arise when certain types of data are available. For example, data on the mode of toxic action are often available for chemicals such as veterinary medicines and pesticides that have a common mechanism against both the target species and non-target mammals. These data, together with information on the time course of effects, can provide an indication as to whether the action is reversible. Data on absorption, excretion and toxicokinetics, together with information on the mode of action, may

help to assess whether effects are likely to be related to C_{max} or AUC. Human toxicity data are available for a small number of chemicals and can be used either directly to derive ARfDs or as part of the overall consideration of interspecies sensitivity.

When the effect under consideration is due to reversible interaction of the substance with a pharmacological target (e.g. a receptor or ion channel), then the concentration of the substance rather than total exposure should determine the magnitude of the effect (i.e. the C_{max} is likely to be more relevant than the AUC). Similarly, if the effect of concern is due to direct irritation, then the concentration at the site of action is more relevant than the total exposure expressed on a body weight basis. In such cases, there will be less interspecies and interindividual variation in toxicokinetics; this would justify a 2-fold reduction in the respective safety factors, leading to an overall composite factor of 25 for extrapolation from animal studies (i.e. 5 × 5 instead of 10 × 10 for interspecies and intraspecies factors) and 5 (instead of 10) for human studies.

JMPR has used such categorical factors in the derivation of ARfDs for several carbamate insecticides that inhibit acetylcholinesterase (FAO/WHO, 2009a). These compounds do not require metabolic activation, they react reversibly with a pharmacological target (acetylcholinesterase), the magnitude of the pharmacological effect is proportional to the C_{max} rather than the AUC and the excretion is rapid. In such circumstances, the determining factor is the C_{max}, which has been shown to have lower variability than clearance, as it depends mainly on the rate and extent of gastrointestinal absorption. This reduced variability in toxicokinetics is used by JMPR to derive a composite factor that is 50% of the default value.

If human data are available but are not used directly to derive the ARfD, they might be sufficient to demonstrate that the findings in experimental animals are qualitatively and quantitatively similar to those in humans, thereby supporting the use of a reduced, data-derived factor (e.g. data on the production and degradation of a toxic metabolite). Similarly, if data show that a wide range of species exhibit similar qualitative and quantitative effects, it could be possible to conclude that the variation between the most sensitive of these and humans would be less than 10.

A reduced safety factor might also be appropriate if the end-point used to derive an ARfD is of minimal adversity and the critical NOAEL is from a repeated-dose study (e.g. increased organ weight with minimal pathological change, or reduced food consumption and body weight gain observed in the first days of dosing). When considering whether body weight changes are relevant for setting of an ARfD, consideration should be given to potential problems of palatability of the feed.

When a NOAEL has not been identified for the most appropriate end-point, the LOAEL can be used in exceptional cases as the basis for the ARfD. In such a situation, the selection of an additional safety factor up to 10 will depend upon the magnitude of the effect and the steepness of the dose–response curve. If dose spacing results in a LOAEL that is markedly higher than the NOAEL, then the BMD approach, with the usual safety factor, would be a better alternative for defining the ARfD.

An extra uncertainty factor has sometimes been adopted for the severity of the effect. However, judging the degree of severity of an effect may be somewhat subjective, and it would not be feasible to grade all possible toxicological effects by their severity. Therefore, if a toxicological effect is judged to be irreversible or particularly severe, this should be a trigger to consider the finding in more detail before choosing an appropriate uncertainty factor. The following considerations may be helpful:

- Has the study shown an adequate margin between the NOAEL and the LOAEL?
- Is the finding supported by data from other studies or by knowledge of the mode of action of the compound?
- Is there a high level of uncertainty in the database?
- Have measurements been taken at appropriate times, and have they used appropriately sensitive methods?
- Has the study on which it is proposed to base the ARfD used adequate group sizes?

In determining the appropriate uncertainty factors for deriving an ARfD, a stepwise approach is proposed:

- Determine if the data are adequate to support the derivation of scientifically based assessment factors (i.e. CSAFs).

- If CSAFs cannot be derived, consider if there is any other information to indicate reduced or increased uncertainty. If not, the 10-fold or 100-fold default should be used.

- Whenever an uncertainty factor other than the default is used, a clear explanation of the derivation of the factor should be provided.

5.2.9.7 *Different ARfDs for population subgroups*

It is preferable, especially for clarity of subsequent risk management and enforcement, to set a single ARfD to cover the whole population. It is important to ensure that any ARfDs established are adequate to protect the embryo or fetus from possible in utero effects. Although an ARfD based on developmental (embryo/fetal) effects would necessarily apply to women of childbearing age, it is recognized that such an ARfD may be conservative and not relevant to other population subgroups. This may also be the case for children 1–6 years of age for whom specific acute consumption data are available and thus can be separately modelled with respect to acute dietary intake of pesticide residues. The use of an ARfD for a sensitive end-point in pregnant women could lead to an unreasonably conservative short-term dietary risk assessment for the population as a whole. Thus, in those situations in which a developmental end-point drives an ARfD for a compound exhibiting no other toxicity at the developmental NOAEL, consideration could be given to setting a second value based on another (non-developmental) end-point for the rest of the population.

5.2.9.8 *Use of human data in setting ARfDs*

Human data on a pesticide can be extremely valuable in setting the laboratory animal data into context and, when available, should always be evaluated, even if they are not used to derive an ARfD. Not only may a human study sometimes allow identification of end-points (NOAELs/LOAELs) for use in risk assessment, other important information may be gained, such as the nature of the adverse effect and its pattern of onset and duration.

Human data may be available from a number of sources, including epidemiological studies of acute effects in human populations exposed to the chemical, direct administration to volunteers, monitoring of those exposed following normal use of the chemical, exposures from accidental or deliberate poisonings, and exposures from use of the same substances as human pharmaceuticals. Such studies often involve single or short-term exposures that can be of relevance, directly or indirectly, to the derivation of ARfDs.

Further guidance from JMPR on the use of human data for setting ARfDs can be found in the review by Solecki et al. (2005).

5.2.9.9 Intake considerations in relation to ARfDs

For risk characterization purposes, the ARfD of a compound is compared with the estimated acute intake of a pesticide through various foods. This allows risk managers to identify for which crops and pesticide applications regulatory actions may be necessary for public health protection. The methodology for estimating acute dietary intakes for pesticides is described in detail in chapter 6.

5.2.9.10 Specific guidance on the derivation of ARfDs

JMPR has given more detailed consideration to the use of particular toxicological end-points (as outlined in section 5.2.9.5) that are relevant to the establishing of ARfDs. This guidance can be found in the review by Solecki et al. (2005). The guidance is not intended to cover all potentially relevant end-points comprehensively but focuses on the interpretation of those that have proved to be problematic in reaching a decision as to whether an effect is relevant to an acute exposure to residues of agricultural pesticides in foods.

5.3 References[1]

Barnes DG, Daston GP, Evans JS, Jarabek AM, Kavlock RJ, Kimmel CA, Park C & Spitzer HL (1995) Benchmark dose workshop: criteria for use of a benchmark dose to estimate a reference dose. Regul Toxicol Pharmacol, **21**: 296–306.

[1] Internet links provided in these references were active as of the date of final editing.

Crump KS (1984) A new method for determining allowable daily intakes. Fundam Appl Toxicol, **4**: 854–871.

Dorne JLCM & Renwick AG (2005) Refinement of uncertainty/safety factors in risk assessment by the incorporation of data on toxicokinetic variability in humans. Toxicol Sci, **86**: 20–26.

Dorne JLCM, Walton K & Renwick AG (2005) Human variability in xenobiotic metabolism and pathway-related uncertainty factors for chemical risk assessment: a review. Food Chem Toxicol, **43**: 203–216.

Dourson ML & Stara JF (1983) Regulatory history and experimental support of uncertainty (safety) factors. Regul Toxicol Pharmacol, **3**: 224–238.

FAO (2002) Submission and evaluation of pesticide residues data for the estimation of maximum residue levels in food and feed, 1st ed. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 170; http://www.fao.org/ag/agp/agpp/pesticid/JMPR/Download/FAOM2002.pdf).

FAO/WHO (1957) General principles governing the use of food additives. First report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Nutrition Meetings Report Series, No. 15; WHO Technical Report Series, No. 129; http://whqlibdoc.who.int/trs/WHO_TRS_129.pdf).

FAO/WHO (1958) Procedures for the testing of intentional food additives to establish their safety for use. Second report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Nutrition Meetings Report Series, No. 17; WHO Technical Report Series, No. 144; http://whqlibdoc.who.int/trs/WHO_TRS_144.pdf).

FAO/WHO (1962a) Evaluation of the toxicity of a number of antimicrobials and antioxidants. Sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Nutrition Meetings Report Series, No. 31; WHO Technical Report Series, No. 228; http://whqlibdoc.who.int/trs/WHO_TRS_228.pdf).

FAO/WHO (1962b) Principles governing consumer safety in relation to pesticide residues. Report of a meeting of a WHO Expert Committee on Pesticide Residues held jointly with the FAO Panel of Experts on the Use of Pesticides in Agriculture. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Plant Production and Protection Division Report, No. PL/1961/11; WHO Technical Report Series, No. 240).

FAO/WHO (1964) Evaluation of the toxicity of pesticide residues in food. Report of a Joint Meeting of the FAO Committee on Pesticides in Agriculture and the WHO Expert Committee on Pesticide Residues. Rome, Food and Agriculture

Organization of the United Nations; Geneva, World Health Organization (FAO Meeting Report, No. PL/1963/13; WHO/Food Add./23).

FAO/WHO (1968) Pesticide residues in food—1968. Report of the Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Agricultural Studies, No. 78; WHO Technical Report Series, No. 417; http://whqlibdoc.who.int/trs/WHO_TRS_417.pdf).

FAO/WHO (1972) Evaluation of certain food additives and the contaminants mercury, lead, and cadmium. Sixteenth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 505 and corrigendum; http://whqlibdoc.who.int/trs/WHO_TRS_505.pdf).

FAO/WHO (1973) Pesticide residues in food—1972. Report of the Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Agricultural Studies, No. 90; WHO Technical Report Series, No. 525; http://whqlibdoc.who.int/trs/WHO_TRS_525.pdf).

FAO/WHO (1974) Toxicological evaluation of certain food additives with a review of general principles and of specifications. Seventeenth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (FAO Nutrition Meetings Report Series, No. 53; WHO Technical Report Series, No. 539 and corrigendum; http://whqlibdoc.who.int/trs/WHO_TRS_539.pdf).

FAO/WHO (1984) Evaluation of certain food additives and contaminants. Twenty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 710; http://whqlibdoc.who.int/trs/WHO_TRS_710.pdf).

FAO/WHO (1986) Pesticide residues in food—1986. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 77).

FAO/WHO (1988a) Pesticide residues in food—1988. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 92).

FAO/WHO (1988b) Evaluation of certain veterinary drug residues in food. Thirty-second report of the Joint FAO/WHO Expert Committee on Food Additives.

Geneva, World Health Organization (WHO Technical Report Series, No. 763; http://whqlibdoc.who.int/trs/WHO_TRS_763.pdf).

FAO/WHO (1989) Pesticide residues in food—1989. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 99).

FAO/WHO (1990) Evaluation of certain veterinary drug residues in food. Thirty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 799; http://whqlibdoc.who.int/trs/WHO_TRS_799.pdf).

FAO/WHO (1991) Evaluation of certain veterinary drug residues in food. Thirty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 815; http://whqlibdoc.who.int/trs/WHO_TRS_815.pdf).

FAO/WHO (1993) Evaluation of certain veterinary drug residues in food. Fortieth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 832; http://whqlibdoc.who.int/trs/WHO_TRS_832.pdf).

FAO/WHO (1995) Evaluation of certain veterinary drug residues in food. Forty-second report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 851; http://whqlibdoc.who.int/trs/WHO_TRS_851.pdf).

FAO/WHO) (1999) Procedures for estimating acute reference dose. In: Pesticide residues in food—1998. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 148; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/98_rep/REPORT1998.pdf).

FAO/WHO (2001a) Proposed guidance for interpretation of data generated in studies with single oral doses. In: Pesticide residues in food—2000. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 163; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/2000_rep/cont.pdf).

FAO/WHO (2001b) Further issues related to establishing the acute reference dose. In: Pesticide residues in food—2001. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food

and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 167; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/2001_rep/REPORT2001.pdf).

FAO/WHO (2002a) Pesticide residues in food—2002. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 172; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/2002_rep/2002JMPRReport2.pdf).

FAO/WHO (2002b) Evaluation of certain food additives and contaminants. Fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 909; http://whqlibdoc.who.int/trs/WHO_TRS_909.pdf).

FAO/WHO (2002c) Further guidance on derivation of the ARfD. In: Pesticide residues in food—2002. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 172; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/2002_rep/2002JMPRReport2.pdf).

FAO/WHO (2004a) Guidance on the establishment of acute reference doses. In: Pesticide residues in food—2004. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 178; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/DOWNLOAD/2004_rep/report2004jmpr.pdf).

FAO/WHO (2004b) Pesticide residues in food—2004. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 178; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/DOWNLOAD/2004_rep/report2004jmpr.pdf).

FAO/WHO (2006) Evaluation of certain food contaminants. Sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 930; http://whqlibdoc.who.int/trs/WHO_TRS_930_eng.pdf).

FAO/WHO (2009a) Pesticide residues in food—2008. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 193; http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/JMPRReport08.pdf).

FAO/WHO (2009b) Evaluation of certain food additives and contaminants. Sixty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 952; http://whqlibdoc.who.int/trs/WHO_TRS_952_eng.pdf).

Gelman A, Carlin JB, Stern HS & Rubin DB (2004) Bayesian data analysis, 2nd ed. Boca Raton, FL, Chapman and Hall/CRC.

Hasselblad V & Jarabek AM (1995) Dose–response analysis of toxic chemicals. In: Berry DA & Stangl DK eds. Bayesian biostatistics. New York, NY, Marcel Dekker.

IPCS (1987) Principles for the safety assessment of food additives and contaminants in food. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 70; http://www.inchem.org/documents/ehc/ehc/ehc70.htm).

IPCS (1990) Principles for the toxicological assessment of pesticide residues in food. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 104; http://www.inchem.org/documents/ehc/ehc/ehc104.htm).

IPCS (1994) Assessing human health risks of chemicals: derivation of guidance values for health-based exposure limits. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 170; http://www.inchem.org/documents/ehc/ehc/ehc170.htm).

IPCS (2004) IPCS risk assessment terminology. Geneva, World Health Organization, International Programme on Chemical Safety (Harmonization Project Document, No. 1; http://www.who.int/ipcs/methods/harmonization/areas/ipcsterminologyparts1and2.pdf).

IPCS (2005) Chemical-specific adjustment factors for interspecies differences and human variability: guidance document for use of data in dose/concentration–response assessment. Geneva, World Health Organization, International Programme on Chemical Safety (Harmonization Project Document, No. 2; http://whqlibdoc.who.int/publications/2005/9241546786_eng.pdf).

IPCS (2009) Principles for modelling dose–response for the risk assessment of chemicals. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 239; http://whqlibdoc.who.int/publications/2009/9789241572392_eng.pdf).

Kimmel CA & Gaylor DW (1988) Issues in qualitative and quantitative risk analysis for developmental toxicology. Risk Anal, **8**: 15–20.

Meek ME, Renwick A, Ohanian E, Dourson M, Lake B, Naumann BD & Vu V (2002) Guidelines for application of chemical-specific adjustment factors in dose/concentration–response assessment. Toxicology, **181–182**: 115–120.

Moeller T, Stein B & Solecki R (2009) Retrospective evaluation of acute reference doses (ARfD) for pesticides in the European Union. Toxicol Lett, **189**(Suppl 1): S209–S210.

OECD (2009) Project 4.27: GD for a single dose study for the derivation of an acute reference dose (ARfD). In: Work plan for the Test Guidelines Programme (TGP). Paris, Organisation for Economic Co-operation and Development, Environment Health and Safety Division, Environment Directorate (http://www.oecd.org/dataoecd/50/63/43723559.pdf).

Renwick AG & Lazarus NR (1998) Human variability and noncancer risk assessment—an analysis of the default uncertainty factor. Regul Toxicol Pharmacol, **27**: 3–20.

Solecki R, Davies L, Dellarco V, Dewhurst I, Van Raaij M & Tritscher A (2005) Guidance on setting of acute reference dose (ARfD) for pesticides. Food Chem Toxicol, **43**: 1569–1593.

VICH (2004) Studies to evaluate the safety of residues of veterinary drugs in human food: general approach to establish a microbiological ADI. Brussels, International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH GL36; http://www.vichsec.org/pdf/05_2004/Gl36_st7_F_rev.pdf).

Walton K, Dorne JL & Renwick AG (2001) Categorical default factors for interspecies differences in the major routes of xenobiotic elimination. Hum Ecol Risk Assess, **7**: 181–201.

WHO (1967) Procedures for investigating intentional and unintentional food additives. Report of a WHO Scientific Group. Geneva, World Health Organization (WHO Technical Report Series, No. 348).

6. DIETARY EXPOSURE ASSESSMENT OF CHEMICALS IN FOOD

For acronyms and abbreviations used in the text, the reader may refer to the list of acronyms and abbreviations at the front of this monograph. Definitions of select terms may be found in the glossary at the end of the monograph.

6.1 Introduction

Exposure assessment is an essential element for quantifying risk. The role of dietary exposure assessment has been central to the work of the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in performing risk assessments on chemicals in foods.

The Codex Alimentarius Commission's (CAC) Procedural Manual (FAO/WHO, 2008a) defines exposure assessment as "the qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food as well as exposures from other sources if relevant". This chapter deals with the assessment of dietary exposure to chemicals present in food (i.e. food additives, contaminants, processing aids, nutrients and residues of pesticides and veterinary drugs). However, some of the principles and approaches described here are also applicable to biological agents in food.

Dietary exposure assessment combines food consumption data with data on the concentration of chemicals in food. The resulting dietary exposure estimate may then be compared with the relevant health-based guidance value for the food chemical of concern, if available, as part of the risk characterization. Assessments may be undertaken for acute or chronic exposures, where acute exposure covers a period of up to 24 h and long-term exposure covers average daily exposure over

the entire lifetime. Dietary exposure assessments of nutrients have default assumptions that are different from those for other food chemicals owing to the specific need to look at both nutrient adequacy and potential to exceed upper safety levels (see chapter 9, section 9.2.2).

The general equation for both acute and chronic dietary exposure is:

$$\text{Dietary exposure} = \frac{\Sigma\ (\text{Concentration of chemical in food} \times \text{Food consumption})}{\text{Body weight (kg)}}$$

The use of standard terminology is recommended to ensure consistent application and understanding. It is recommended that "consumption" be used to refer to the amount of food consumed and "dietary exposure" to the amount of chemical ingested via food. The term "dietary exposure" is used synonymously with the term "dietary intake", depending upon existing regulatory frameworks or other related considerations. In this chapter, the term "food" also includes beverages, drinking-water and food supplements.

This chapter updates and expands the report of the FAO/WHO Consultation on Food Consumption and Exposure Assessment of Chemicals (FAO/WHO, 1997). It was developed by an FAO/WHO Workshop on Exposure Assessment for Chemicals in Food held in May 2005 (FAO/WHO, 2008b). Its aim was to provide guidance to WHO and FAO and their expert advisory bodies, CAC, national governments and the risk analysis community at large on how to perform and interpret dietary exposure assessments at the international, regional, national and local levels.

6.1.1 General considerations

The following points are basic general principles and considerations when undertaking dietary exposure assessments:

- The objective of the dietary exposure assessment must be clearly identified before the appropriate food consumption and concentration data may be selected. For example, preregulation (i.e. before approval for use) and post-regulation (i.e. after approval for use)

dietary exposure assessments are undertaken for different purposes and may have different data sources and default assumptions.

- As stated in the FAO/WHO consultation on risk assessment analysis (FAO/WHO, 1995a), CAC should ensure harmonized approaches to the risk assessment of food chemicals. In this chapter, harmonization is understood to result in equivalence, which does not necessarily mean that all dietary exposure assessment procedures across food chemicals need to be the same. Rather, such procedures should aim at providing equivalent levels of consumer protection.

- Irrespective of the severity of toxicological end-point, type of chemical in food, possible population subgroups of concern or reasons for performing the dietary exposure assessment, the most appropriate data and method should be used, harmonizing the approach to dietary exposure assessments where possible.

- International dietary exposure assessments should provide exposure estimates that are equal to or greater than (or lower than, in the case of nutrient deficiency) the best available estimates carried out at the national level. It is assumed that the international estimate covers potential dietary exposure in countries for which no data were available.

- Dietary exposure assessments should cover the general population, as well as critical groups that are vulnerable or are expected to have exposures that are significantly different from those of the general population (e.g. infants, children, pregnant women or elderly).

- If international dietary exposure assessments exceed a health-based guidance value, then national authorities should be asked to submit their national exposure estimates through CAC or its technical committees or directly to JMPR or JECFA.

- It is recommended that national authorities that wish to perform their own dietary exposure assessments use national food consumption and concentration data, but international nutritional and toxicological reference values. It would be helpful for the

Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food), JECFA and JMPR to receive data from national and regional authorities on food consumption and chemical concentrations, as well as the results of their dietary exposure assessments.

- If the estimated international dietary exposure to a chemical does not exceed its relevant health-based guidance value (or is not below the nutritional reference value), then the level of exposure should be acceptable at the national level, because the level of overestimation for international dietary exposure assessments for any region would tend to be greater than that for national estimates. This applies to both acute and chronic exposure assessments.

6.1.2 Dietary exposure assessment methods

The following points are basic general principles and considerations with respect to the methods used for dietary exposure assessment:

- In principle, international dietary exposure assessments need to be performed for all identified chemicals present in the diet that are subject to risk assessment. Similar methods are appropriate for contaminants, pesticide and veterinary drug residues, food additives (including flavourings), processing aids and other chemicals in foods. The methods used may also be applied to estimating nutrient intakes, noting that these assessments are more often undertaken at a national rather than at an international level (see chapter 9, section 9.2.2).

- A stepwise approach is recommended, in which screening methods can be applied to identify, among the large number of chemicals that may be present, those of no safety concern, using minimal resources in the shortest possible time. A refined exposure assessment is not needed for such substances.

- Screening methods, if used, need to overestimate exposure of high consumers using conservative assumptions in terms of food consumption and chemical concentration (see section 6.3.4.1). This is to avoid situations where the exposure estimated with

the screening would erroneously indicate that no safety concern existed (i.e. exposure is below a health-based guidance value) and that no further refined dietary exposure assessment is necessary.

- In order to effectively screen chemical substances and establish risk assessment priorities, the screening procedure should not use unsustainable diets to estimate consumption. Rather, physiological limits of consumption should be taken into account.

- Further steps to allow the refinement of the dietary exposure assessment should be designed in such a way that potential high dietary exposure to a specific chemical is not underestimated. The methodologies should take into consideration non-average individuals, such as those who consume large portions of specific food items. Some consumers may also be loyal to those foods or brands of food containing the highest concentrations of the chemical of interest or may occasionally consume foods with very high concentrations of the chemical.

6.1.3 *Presentation of results of dietary exposure assessment*

The following points are general considerations with respect to the presentation of the results of the dietary exposure assessment:

- The method applied should be clearly described. Information about the model and data sources used, assumptions, limitations and uncertainties should also be documented (see section 6.3.3).

- Any assumptions concerning concentrations of the chemical in foods and food consumption patterns upon which dietary exposure estimates are based need to be transparent (see sections 6.2.1 and 6.2.2).

- The percentiles (e.g. 90th, 95th or 97.5th) used to represent highly exposed consumers should be clearly stated and their derivation described (see section 6.2.2.3).

6.2 Data sources

The data required for assessing dietary exposure are determined by the objective of the assessment. Dietary exposure can be assessed for a chemical 1) before it has been approved for use (preregulation), 2) after

it has potentially been in the food supply for years (post-regulation) or 3) that is present naturally in foods or as a result of contamination. In the first case, chemical concentration data are available or estimated from the manufacturer or food processor. In the other two cases, additional chemical concentration data could be obtained from food in the marketplace. For each assessment, the suitability of the available data should be assessed (e.g. some market data may not be sufficient for acute exposure assessments).

6.2.1 Data on concentrations of chemicals in food, including water

In dietary exposure assessments, it is important to obtain accurate information on both the concentrations of chemicals in food and food consumption. The selection of the sampling, analysis and reporting procedures is critical for obtaining consistent and comparable data on chemical concentrations in food (WHO, 1985; Petersen et al., 1994). The selection of data based on consistent procedures is particularly important at the international level, where data from several countries may be compared or combined. Possible sources of chemical concentration data are summarized in Table 6.1.

Appropriate data sources and levels of food chemicals to use in dietary exposure assessments at an international level may be determined by the relevant Codex committee based on the advice of JECFA or JMPR.

6.2.1.1 *Use of maximum levels (MLs) or maximum residue limits (MRLs) in dietary exposure assessments (preregulation)*

It is important to understand the method of derivation of Codex MLs or MRLs for various food chemicals when considering the potential uncertainties in the data if they are to be used in dietary exposure assessments. In the case of pesticide residues, MRLs are proposed by JMPR based on field trial studies performed under Good Agricultural Practice (GAP), then considered and recommended to CAC by the Codex Committee on Pesticide Residues (CCPR). For veterinary drugs, the MRLs are derived by JECFA from controlled residue depletion studies carried out in compliance with Good Practice in the Use of Veterinary Drugs (GPVD), then considered and recommended to CAC by the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF).

Table 6.1. Sources of chemical concentration data

Chemical type	Preregulation dietary exposure assessments	Post-regulation dietary exposure assessments[a]
Food additives Packaging materials	Proposed MLs Proposed manufacturers' use levels Migration data (for packaging materials)	Reported manufacturers' use levels Food industry surveys Monitoring and surveillance data TDS Scientific literature
Contaminants, including natural toxicants	Proposed MLs Monitoring and surveillance data TDS GEMS/Food database (see section 6.2.1.8) Scientific literature	
Pesticide residues	Proposed MRLs HR STMR	Monitoring and surveillance data TDS GEMS/Food database on chemical concentrations Scientific literature
Veterinary drug residues	Residue depletion studies	Monitoring and surveillance data TDS Scientific literature
Nutrients	Proposed MLs for fortification Food composition data	Monitoring and surveillance data TDS Scientific literature

HR, highest residue level from trial; ML, maximum level; MRL, maximum residue limit; STMR, supervised trial median residue level; TDS, total diet study.

[a] In addition to all preregulation data sources.

In the case of pesticide residues and food additives, maximum levels/limits (i.e. MRLs and MLs) are usually based on good practice considerations, even if a consideration of consumer safety might allow higher levels than these. For veterinary drugs, good practice considerations are also taken into account. However, the determining criterion is that dietary exposure estimates should be below the acceptable daily intake (ADI). In the preregulation phase when proposed maximum levels/limits based on good practice result in potential chronic or acute

dietary exposures that exceed relevant health-based guidance values, the refinement of dietary exposure estimates with more accurate data may be possible before a final decision on the MRL or ML is taken. For veterinary drug residues, the current practice by JECFA is to use a set "food basket" to derive an estimate of potential dietary exposure; at an international level, this estimate cannot be refined, although at a national level, further refinement may be possible.

In the case of chemical contaminants, MLs are established by the Codex Committee on Contaminants in Food (CCCF), following advice from JECFA. MLs need to be compatible with tolerable intake levels and are based on the lowest level of contamination that can be reasonably achieved without removing the food from the food supply. For contaminants having a chronic toxic effect, the setting of an ML for the chemical in the food in which it occurs is unlikely to have direct and immediate impact on the exposure of the population unless a significant proportion of the food is withdrawn from the market. In addition, when the overall exposure to a chemical is below the health-based guidance value, MLs in food contributing to the exposure are unlikely to have any impact in terms of public health.

Codex standards for nutrients may reflect typical levels in foods. Sometimes these levels apply to raw commodities, which require processing before being consumed.

6.2.1.2 Use of other concentration data sources for dietary exposure assessments (preregulation and post-regulation)

Maximum levels/limits are convenient values to use to assess dietary exposure for preregulation purposes, but it is recognized that a person would not always consume foods containing chemicals at their corresponding maximum levels/limits. Analytical data on concentrations of chemicals in food are needed to more accurately estimate the levels likely to be found in the diet as consumed. These data can be derived from crop and animal trial data (pesticide and veterinary drug residues) or monitoring and surveillance data on food (all chemicals). It may be appropriate to select different data sources in international and national assessments. Certain foods are widely blended across many individual units (e.g. orange juice); in these cases, it may be appropriate to estimate concentrations in blended commodities by

using the arithmetic mean of the concentrations in the individual or composite samples.

When using data provided by national governments as well as other sources in international exposure assessments, it is important, wherever possible, to have detailed information on the data source, survey type or design, sampling procedures, sample preparation, analytical method, limit of detection (LOD) or limit of quantification (LOQ), and quality assurance procedures.

For acute dietary exposure assessments, it should be recognized that although aggregated monitoring data may provide a reliable estimate of mean residue level, such data do not provide reliable estimates of the highest residue levels in single units, as required for these estimates.

6.2.1.3 *Approaches for obtaining food chemical concentration data*

(a) Supervised trials and residue depletion studies (pesticide and veterinary drug residues only)

Traditionally, the primary source of preregulation residue data in foods has been supervised trial data for pesticides and residue depletion studies for veterinary drugs that must be submitted in support of the registration of a pesticide or veterinary drug, respectively.

For pesticides, the trials are usually performed by a manufacturer or other parties. In the trials, a maximum registered use scenario (with respect to application rates, number of applications, preharvest or withdrawal intervals, etc.) is simulated. The trials are designed to determine the maximum residue concentrations that may be present in food and feed of animal or plant origin at the earliest point at which these food commodities could enter commerce and are used to establish legally enforceable residue limits. These data often overestimate the residue concentrations that are likely to occur in food as actually consumed, because they reflect the maximum application rate and shortest preharvest interval. Therefore, these data should not be the first choice when assessing actual dietary exposure, but are the first choice for assessing the safety implications for consumers of a proposed MRL calculated on the basis of GAP.

For veterinary drugs, the residue depletion studies are usually performed by the manufacturer or other commercial entities, using the commercial formulation and recommended dose regimens in the target animal species. The doses chosen should represent the upper end of registered doses. The studies are designed to estimate the formation and depletion of residues (determined as the marker residue) of the veterinary drug in edible tissues and products and serve as the basis for the derivation of the MRLs and estimation of exposure (see chapter 8). MRLs are derived to represent the upper 95th confidence limit of the 95th percentile of the residue concentrations at the chosen time point on the residue depletion curve. Using the MRLs for estimation of exposure would overestimate the residue concentrations that are likely to occur in food products of animal origin, as it would assume that all animals of a target species would be treated and that the products are obtained exactly when 95% of the residue concentrations had depleted to the MRL. Therefore, the MRL values should not be considered as a first choice when assessing dietary exposure. However, the MRLs may be used for a conservative assessment of exposure in the case where low or non-detectable residue levels are measured in the depletion studies or when the MRLs are based on other considerations, such as the LOQ of the analytical method.

Supervised trial data and the results of residue depletion studies do not account for residue degradation that sometimes occurs during the interval between the farm and the market or the home or subsequent residue losses when food is processed and prepared for consumption.

(b) Monitoring and surveillance data

Data that reflect concentrations of chemicals in food are often available from monitoring and surveillance programmes in which food samples are obtained closer to the point of consumption in the chain of commerce. These data generally provide a better characterization of chemicals in foods as purchased by consumers (EC, 2004; USFDA, 2004b; USDA, 2008).

There are two types of monitoring and surveillance data: random and targeted. Targeted data are often collected for enforcement purposes in response to specific problems and should be used with caution in dietary exposure assessments, as they may not be representative

of all the food available for sale. Truly representative residue data are scarce, and the source of residue data used in dietary exposure assessments should always be carefully described and evaluated.

For post-regulation chronic dietary exposure assessments of pesticide and veterinary drug residues, suitable monitoring and surveillance data are preferred over data from supervised trials and depletion studies, as these in principle more closely represent what is consumed. The samples are usually collected on a random basis close to the point of consumption, at terminal markets and large-chain store distribution centres immediately prior to distribution to supermarkets and grocery stores. Such sampling therefore accounts for residue degradation during transit and storage and, in the case of pesticides, may also provide data on residues resulting from post-harvest applications of fungicides and growth regulators used as preservatives during food delivery. However, some monitoring programmes are designed to measure compliance with a given standard and may not use the most sensitive methods of analysis or may not describe concentrations in the food as consumed because marker organs have been used—for example, levels of heavy metal contamination only in the liver may be analysed.

For acute dietary exposure assessments, the fact that only a small proportion of any commodity entering the food-chain is monitored means that there are significant limitations in using monitoring data.

(c) Refinement of concentration data by use of correction factors

Concentration data for food chemicals may be refined by applying correction factors to the concentration data when based on raw commodities to reflect changes due to processing or to account for the portion that is actually consumed. Processing factors can be routinely incorporated into dietary exposure assessments to make the results more reflective of actual exposures. Specifically, processing of agricultural commodities can increase or decrease chemical concentrations or alter the nature of chemicals in foods. Processing studies are usually regarded as specific for the food, the active substance and the process. In cases where processing studies are not available, standard mass balance assumptions, based on general information on the effects of some processing operations, such as drying of grapes to make raisins, may sometimes be used (USEPA, 1996).

In some cases, the risk assessor may refine estimates of dietary exposure to pesticide residues by taking into account the proportions of crop or food commodity produced domestically and imported. In many cases, only a fraction of the total food or crop supply may be anticipated to contain the substance being evaluated. Where data exist to quantify the percentage affected, these values can be incorporated as an adjustment factor to be applied to concentration data in order to more accurately estimate chronic dietary exposures. There is no international consensus on using this type of information in the context of dietary exposure estimates in the process for setting MRLs for pesticide residues. Some of these factors are country or region specific and may be appropriate to use only when undertaking national dietary exposure assessments.

(d) Total diet studies

Total diet studies (TDSs) in principle provide the most accurate measure of the average concentrations of pesticide residues, contaminants, nutrients and other chemicals actually ingested in foods by the population living in a country and, if possible, population subgroups. However, the accuracy of some TDSs is lowered by using limited sample sizes and survey durations. Therefore, when using a TDS in a dietary exposure assessment, it should be checked whether the TDS is fit for purpose.

Concentration data from TDSs differ from data obtained from other chemical surveillance or monitoring programmes, because concentrations of chemicals are measured in foods after they have been prepared for normal consumption. Concentration data in a TDS are not based on historical composition data, and processing factors for raw food commodities (FAO/WHO, 1997) do not need to be applied, because estimated dietary exposures are based on the edible portions of the food—for example, bananas are peeled and the skin discarded along with any associated chemical residues. A TDS also incorporates the impact of cooking on less stable chemicals and on the formation of new ones.

Analytical methods used in a TDS should be capable of measuring concentrations of chemicals in foods at appropriate levels. Typically, methods with LODs or LOQs 10–1000 times lower than those needed for enforcement purposes are used for TDSs.

The broad scope of a TDS may necessitate significant compositing of samples if resources are limited (see also section 6.2.1.4). Compositing may be on either an individual food basis or a food group basis. Such compositing will not prevent the estimation of total exposure but will limit the ability to identify the specific sources of the food chemical. Owing to resource considerations, TDSs usually have a small number of mean concentration data (usually n = 1–8) for each individual food or food group, in contrast to data usually generated through surveillance or monitoring of individual food commodities (where n = 30–50 or more).

6.2.1.4 *Sampling*

(a) Sample collection

When undertaking programmes to generate data on concentrations of chemicals in food, the sampling procedure selected and how it is carried out are critical to the validity of the results obtained. Different sampling plans and methods are required, depending on the objectives of the studies.

The following questions should be addressed when the sampling plan is designed (WHO, 1985, 2002a,b, 2005a; Kroes et al., 2002):

- Is the food list representative of the foods normally consumed by the population or the specific age/sex groups to be investigated?
- Are foods with very low consumption but of potential concern regarding chemical content included?
- How many sampling sites are involved, and are they representative?
- Should the sampling be representative of commercial food processing or of homemade foodstuffs?
- Does sampling account for regional differences in soil content, climate, pest vectors and GAP, as well as those foods extensively distributed on a national basis, including imported foods?
- Are seasonal differences also considered?

- Are the main brands/cultivars covered for each food?
- Is sample size sufficient to cope with localized analytes, such as aflatoxins?
- Have standard operating procedures (SOPs) been established to standardize sampling?

For an acute exposure assessment, additional information is required on residues in single samples or individual unit crops. If such detailed data are not available, concentrations in single samples can also be derived from composite samples taken from a lot by applying a variability factor (see sections 6.2.1.5 and 6.3.6.2) to take into account the differences in chemical concentrations in sample increments or unit crops.

(b) Sample preparation and processing

Sample preparation includes actions taken to prepare the analytical sample from the laboratory (bulk) sample—for example, reducing the size of a large bulk sample by subsampling and removing foreign materials or parts of the sample material that are not analysed (e.g. stones, withered leaves, stone of fruits, bones of meat). For generating data to be used in dietary exposure assessment, the chemical concentrations in the edible portion of the commodities are of interest; for enforcement, the portion of the commodity specified in the relevant regulation should be prepared for analysis. Sample preparation may include, for instance, washing, peeling, cooking, etc., so that foods are prepared as for normal consumption (i.e. table ready). In such cases, cooking of foods needs to be based on one or more recipes or methods for each food item, in order to account for food habits. Sample preparation might also involve compositing of food samples taken from different regions, brands and even food types (e.g. milks and milk products), before homogenization and analysis. Such preparation will provide an estimate closer to the true average.

Sample processing includes physical operations performed to prepare a well-mixed or homogeneous matrix to form the analytical sample, from which the test portions for the analysis are taken. Some labile and volatile compounds may be lost during these processes, so special handling, including cryogenic processing, may be required.

Special care should also be taken to ensure that the size of the test portion is representative and sufficient for the accurate and reproducible determination of the average chemical or residue content of the analytical sample (FAO/WHO, 2003).

(c) Specific design approaches for generating concentration data

A good study design is the most important element of any exposure study (FAO/WHO, 2000). There are two main approaches to analysing foods when generating analytical data from surveys, including TDSs, and both can impact significantly, but differently, on the estimated dietary exposures. These two approaches are 1) analysis of food group composites and 2) analysis of individual foods (either as single samples or as composites).

In the *food group composite approach*, samples of similar foods (e.g. milk, cheese, butter, cream) are prepared and then combined to form a composite for a food group (e.g. dairy products). The basis for the relative proportions of foods contributing to the food group composite needs to be defined, but the proportions are generally based on food consumption data for an average consumer in the population.

The advantage of the food group composite approach relates primarily to the ability to determine the approximate dietary exposure to chemicals by analysis of a relatively small number of samples. By analysis of perhaps 10–20 representative food group composites that are carefully prepared to represent the national, socioeconomic, regional or ethnic dietary habits of a population, an approximation of chemical dietary exposure can be obtained.

The main disadvantage of the food group composite approach is that it restricts calculating chemical exposures to only that segment of the population upon which the proportional contribution of foods was based. If, for example, it was based on an adult male diet, this can only roughly approximate an adolescent or child or adult female diet, as types of foods and proportions of each consumed may differ substantially between age/sex groups.

The food group composites approach is often used when undertaking a TDS. As an example, the United Kingdom TDS has 20 food group composites (Ysart et al., 1999; FSA, 2004). Separate groups

have been established for foods consumed in large amounts (e.g. staples, such as bread, milk and potatoes) and also for food groups that may make a significant contribution to dietary exposure because they are known to be susceptible to contamination (e.g. offal and fish). This combined approach can facilitate the identification of sources of exposure while conserving resources.

In the *individual food approach*, each food is prepared and analysed separately. Often multiple samples of the same food purchased across the country are composited so as to get as representative a sample across the diet as possible. Each individual food composite may, depending on available resources, be composited in a targeted manner across brands, retail outlets, cities/regions or seasons for that food.

The major advantages of the individual food approach over the food group composite approach for analyses are the ability to estimate the contribution of individual foods to exposures as well as the greater flexibility in calculating dietary exposures for various segments of the population, provided appropriate food consumption information is available (WHO, 1985). The major disadvantage of the individual foods approach is the larger number of samples that need to be analysed in order to represent all foods consumed by the population. If the individual foods are also composited, then the principal disadvantage, which also applies to food group composites, is the so-called "dilution effect" inherent in the use of composites. For example, the concentration of one food in the composite may well be significantly in excess of the LOD or LOQ, but diluted to below the LOD or LOQ by other foods in the composite, such that the overall composite has a "not detected" (ND) result. This dilution effect can lead to significant underestimation or overestimation of dietary exposures, depending on the protocol used for assigning values to the samples with ND or "not quantified" (NQ) results (see section 6.2.1.5). In addition, unusual sources of elevated concentrations could be masked in the composite.

Some countries have used the individual foods approach in their TDSs. The associated number of individual foods specified are as follows: Canada, 135 foods (Dabeka et al., 2003); the Czech Republic, 220 foods (WHO, 2005a); France, 338 foods (Leblanc et al., 2005); Ireland, 107 foods (WHO, 2005a); New Zealand, 121

foods (Vannoort, 2003, 2004a,b,c); and the United States of America (USA), 286 foods (USFDA, 2004a). Australia has tended to use a more limited range of individual foods (70 foods; FSANZ, 2003), but this has occasionally presented problems for dietary exposure estimates (e.g. when lead was detected in honey, and honey was mapped to represent sugar-containing products, including highly consumed soft drinks that were not likely to contain lead) (FSANZ, 2001). Such grouping or mapping can lead to significant overestimation of actual dietary exposure and illustrates the need for a full description of any assumptions inherent in a dietary exposure assessment.

6.2.1.5 *Analysis*

There are a number of important differences in analytical methodology depending on whether the samples are analysed to provide data for dietary exposure assessment (e.g. TDSs) or for enforcement of MRLs or MLs. For instance, some veterinary drug residue metabolites that are of toxicological concern and are important for dietary exposure assessment are not analysed in monitoring programmes for enforcement purposes, as they are not part of the relevant residue definition. Method sensitivity can also differ. Generally, for accurate dietary exposure assessments, the LOD or LOQ should be as low as technically possible, because most foods will not contain detectable residues, and the value assigned to those samples will affect the estimated dietary exposures (see below). Most TDSs utilize sensitive methods, whereas monitoring or surveillance programmes typically use less sensitive methods, if the purpose is to confirm that residue concentrations are below the legal limits. In any case, residue data generated for enforcement purposes can be used for dietary exposure assessment provided the appropriate assumptions for samples below the LOD or LOQ are applied and numerical data are reported, not just pass or fail results.

(a) Quality assurance

Obtaining best estimates for dietary exposure is critically dependent on the quality of the concentration data. Concentration data should be obtained using validated methods where possible (see chapter 3) that are fit for the purpose of the assessment. Key aspects of data quality include:

- suitability of the sampling plan in order to obtain representative samples of food (e.g. early identification of the foods contributing most to the estimated dietary exposures can assist in directing resources to the most important foods);
- basing the number of samples determined on the statistical characteristics of each data set;
- appropriateness of sample handling procedures;
- selection and validation of the analytical method; and
- use of analytical quality control programmes.

Analytical quality control programmes include employing properly trained personnel familiar with the specific objectives of the tasks performed, regular testing of the performance parameters of the analytical methods by use of reference materials where available and applicable, and testing the bias/accuracy, reproducibility and sensitivity of the procedures. Participation in proficiency tests provides objective means to verify the capability of the laboratory and comparability of the results obtained in different laboratories. The established quality system and capability of the laboratory should be demonstrated by appropriate accreditation. Relevant detailed information can be obtained from a number of sources (Keith et al., 1983; USNRC, 1993; Hughes in WHO, 2002a; Kroes et al., 2002; Sack in WHO, 2002a; Vannoort in WHO, 2002b; FAO/WHO, 2003; IANZ, 2004).

(b) Handling non-detects or non-quantified results

The protocol for assigning concentration values to ND or NQ results is critical to the dietary exposure estimate. Concentrations should err on the side of nutritional or toxicological caution, while remaining scientifically defensible. This issue has been extensively considered (USNRC, 1993; WHO, 1994, 1995c; USEPA, 2000b; Vannoort et al., 2000; Egan et al., 2002; Kroes et al., 2002; Renwick et al., 2003; Tressou et al., 2004; Counil et al., 2005; Sinha et al., 2006; Jain et al., 2008). There are no international guidelines on the need to report both the LOD and LOQ in a standardized manner. Inconsistent reporting of LODs or LOQs may lead to differences in the numerical value that should be assigned to ND or NQ results for use in dietary exposure estimates. It is therefore important to recognize that this is currently considered on a case-by-case basis, so all assumptions made need to be recorded.

Unless there is reason to assume that a food does not contain a chemical of interest (e.g. foods for which a pesticide is not registered for use or for which a food additive is not permitted, or foods that undergo extensive processing during which a chemical is likely to be completely removed), it should be assumed that samples without detectable (or quantifiable) concentrations may contain the chemical below the LOD or LOQ. The risk assessor must decide what value to assign to such samples. One common, albeit arbitrary, option is to assign a value of one half the LOD or LOQ to these samples. If the number of samples with ND or NQ residues is large, such replacement would distort the calculated mean and chemical variability values. It should be noted that the median concentration derived from data sets with over 50% of results below the LOD or LOQ will not be influenced at all by the magnitude of the positive results, whereas the mean can be heavily influenced by a cluster of very high results.

Another option is to use lower-bound or upper-bound values (e.g. zero and the LOD). In general, for chemicals likely to be present in the food (e.g. naturally occurring contaminants, nutrients and mycotoxins), both lower and upper bounds should be calculated for the mean food concentration. The lower bound is obtained by assigning a zero value to those samples in which the chemical was ND or NQ and using these values to estimate dietary exposure. An upper-bound dietary exposure is estimated by assigning the LOD to all samples with ND results and the LOQ to all samples with less than the LOQ but more than the LOD. In some cases, the LOD may equal the LOQ.

In cases where different chemicals are considered as a group for dietary exposure assessment purposes (e.g. dioxins or aflatoxins), the assignment of numerical values to ND or NQ results can be complex when different LODs or LOQs were used for the analysis of each individual chemical in the group. The simple summation of the LODs or LOQs is not feasible, as this will tend to result in an overestimation of dietary exposure, and rules for how to deal with these results need to be developed and recorded.

The impact of these assumptions on the concentration selected for the dietary exposure estimate should be presented in the dietary exposure assessment and also in any associated risk assessment. Some

guidance has been provided (Helsel, 1990; WHO, 1995). For example, GEMS/Food Europe has suggested that if fewer than 60% of results are less than the LOD or LOQ, then a reasonable estimate of the mean can probably be obtained by setting all ND or NQ results to LOD/2 or LOQ/2, respectively (WHO, 1995). Some experts have suggested that additional considerations should be undertaken if more than 10–15% of the samples are below the LOD. In general, when data sets have a large number of samples that are less than the LOD or LOQ, it may be advisable to perform sensitivity analyses by first assigning all ND or NQ results to zero, setting these values to the full LOD or LOQ and then evaluating how the exposure estimates change. The assignment of different values to ND results may have a significant impact on estimated dietary exposures, the effect being greater for less sensitive analytical methods with higher LODs. Alternatively, more sophisticated methods such as maximum likelihood estimation or regression on order statistics can be used to evaluate the impact of the values assigned to ND or NQ results. For chemicals unlikely to be present unless specifically added (i.e. pesticide and veterinary drug residues, additives), using a lower-bound mean concentration only is generally the norm.

In field trial residue data, the occurrence of samples in which no pesticide residue was detected requires a decision about how to include a precise quantitative value in the residue data file if it is to be used for probabilistic analysis. Unlike non-treated crops, it can be assumed that there is a finite residue present, but that it is merely below the LOD. The USEPA (1998) has chosen to use a value of LOD/2 or LOQ/2 as a reasonable means to address such findings. When residues from a set of supervised trials are all below the LOQ, JMPR assumes that the median and high residues are equal to the LOQ unless there is scientific evidence that residues are "essentially zero". This is clearly distinguished from consideration of non-treated crops (above), in which the pesticide residue is properly assigned as "zero".

6.2.1.6 *Deriving concentration data for use in estimating dietary exposures*

This is an important issue, where the choice of concentration data to use in a dietary exposure estimate depends on the purpose of the modelling exercise. For a probabilistic approach, an empirical, parametric or non-parametric distribution of available concentration data is used (see section 6.3.5.2). For a deterministic or point estimate approach, a statistic such as the mean or median may be derived

from the whole data set. The approach taken and underlying reasoning should be clearly stated in the dietary exposure assessment.

For contaminants, the mean food concentration value derived from monitoring or surveillance data is often used in dietary exposure estimates. However, depending upon the anticipated profiles of contamination or the sampling design, in some situations a median or geometric mean may be a more appropriate measure of the concentration—for example, when there is a highly skewed distribution of concentration data or where a significant proportion of results are below the LOD or LOQ (WHO, 1994, 1995; FAO/WHO, 2000). For TDSs and nutrients, the mean is generally used, as there are usually insufficient concentration data to justify use of the median, especially for the individual food composite approach, where often only a few results for each food may be available. For chemicals that are intentionally added to foods, the mean concentration is often used to reflect the expected concentration in food over time and may be derived from manufacturers' use data (food additives, including flavours) and monitoring or surveillance data (food additives, including flavours, pesticide and veterinary drug residues). The highest or median residue levels from supervised trials (highest residue level found in trials [HR]; supervised trials median residue [STMR]) or the MRL may be used to represent pesticide and veterinary drug residue levels, depending on the dietary exposure scenario and whether an acute or chronic dietary exposure estimate is required.

6.2.1.7 *Uncertainty in food chemical concentration data*

The use of maximum food chemical concentrations (MLs and MRLs) in dietary exposure estimates substantially overestimates the amount of chemicals present, and these data therefore have the greatest uncertainty if used other than for a worst-case analysis. Data from direct measurements after use of or treatment with pesticides or veterinary drugs, from a supervised field trial or manufacturer use levels for food additives, have less associated uncertainty. Although these data provide a more accurate estimate of exposure compared with maximum concentrations of the chemical in or on the food commodity as it enters the food distribution system, they do not reflect the impact of storage, transportation or preparation of the food. Still more accurate information on concentrations of chemicals in food is available from national monitoring and surveillance data. The most accurate

data are obtained from the measurement of chemical concentrations in foods as consumed. Although this approach would provide the least uncertainty, it is typically the most resource intensive.

A common method for describing uncertainty in food chemical concentration data is to repeat the analysis using 1) bounding "high-end" estimates for all parameters, 2) bounding "low-end" estimates for all parameters and 3) central tendency estimates (mean or median) for all parameters. Based on the implied uncertainty, the risk manager can then determine if the expenditure of time and resources necessary to gather additional information about these parameters to further refine the dietary exposure estimate is warranted. The handling of non-detects in the data set of chemical concentrations is of importance in determining the high-end and low-end estimates, as is the treatment of censored values, as assumptions about those values and their treatment may influence the result of the assessment.

Uncertainties in data on concentrations of chemicals in food can be reduced by improving the quality of the data available (see section 6.2.1.5). Uncertainty in dietary exposure assessments has been discussed elsewhere (EFSA, 2006; IPCS, 2008; see also chapter 7, section 7.2.2).

Indicators of data quality need to be clearly defined and provided to users of the data. This information should be sufficiently complete to enable critical decisions to be made concerning the appropriateness of the available data for the specific use.

(a) Errors in analytical measurements

Three types of error can be distinguished in most measurements:

- *Gross errors* refer to unintentional or unpredictable errors that occur while generating the analytical result. Errors of this type invalidate the measurement. It is not possible or desirable to statistically evaluate and include the data with gross errors in the estimation of uncertainty. Laboratory quality assurance procedures should minimize gross errors.

- *Random errors* are present in all measurements and cause replicate results to fall on either side of the mean value. The random

error of a measurement cannot be compensated for, but increasing the number of observations and training of the analyst may reduce such errors.

- *Systematic errors* occur in most experiments, but their effects are quite different. The sum of all the systematic errors in an experiment is referred to as the bias. As they do not sum to zero over a large number of measurements, individual systematic errors cannot be detected directly by replicate analyses. The problem with systematic errors is that they may go undetected unless appropriate precautions are taken. For example, systematic errors in an analysis can be identified only if the analytical technique is applied to a reference material, the sample is analysed by another analyst or preferably in another laboratory, or the sample is reanalysed by another analytical method. However, only if the reference material matches identically in terms of analyte, matrix and concentration does it meet the ideal conditions for determining the bias of the method. The bias of a method may also be investigated by recovery studies. However, recovery studies assess only the effects of analysis and do not necessarily apply to naturally incurred samples or components of the bias that may be introduced prior to the analytical step. In pesticide residue analysis, results are not normally corrected for recovery. If the result has been corrected for recovery, the uncertainty associated with recovery should be incorporated in the uncertainty estimation of the measurement.

Some examples of sources of errors are illustrated in Table 6.2. It should be noted that not all sources mentioned have to be evaluated in the uncertainty estimation. Some sources are already incorporated in the overall uncertainty, whereas others are negligible and may be disregarded. However, it is important to recognize and assess all sources before elimination. Further information may be obtained from published documents (Eurachem, 1999; FAO, 2002).

(b) Procedures for estimating measurement uncertainty

Although there are a number of options available to laboratories for the estimation of measurement uncertainty, there are two preferred procedures, commonly described as the "bottom up" approach and the

Table 6.2. Sources of error in sampling, sample preparation and analysis

Procedure	Sources of systematic error	Sources of random error
Sampling	Selection of sampling position	Large variation of food chemical concentration in food or on treated crops
		Small number of primary samples taken (sample size)
	Incorrect labelling	
	Contamination of sample	
Shipping and storage	Decomposition of analytes	
Sample preparation	The portion of sample to be analysed (analytical sample) may be incorrectly selected	The analytical sample is in contact with and contaminated by other portions of the sample
		Rinsing and brushing are performed to varying extents, stalks and stones may be differentially removed
		Is food for analysis raw or cooked? If cooked, how is it cooked?
Sample processing	Decomposition of analyte during sample processing, cross-contamination of the samples	Non-homogeneity of the analyte in single units of the analytical sample
		Non-homogeneity of the analyte in the ground or chopped analytical sample
		Variation of temperature during the homogenization process
		Texture (maturity) of foods or plant materials affecting the efficiency of the homogenization process
Extraction/ cleanup	Incomplete recovery of analyte	Variation in the composition (e.g. water, fat and sugar content) of sample materials taken from a commodity
	Interference of co-extracted materials (load of the adsorbent)	Temperature and composition of sample/solvent matrix

Table 6.2. ***(Continued)***

Procedure	Sources of systematic error	Sources of random error
Quantitative determination	Interference of co-extracted compounds	Variation of nominal volume of devices within the permitted tolerance intervals
	Incorrect purity of analytical standard	Precision and linearity of balances
	Biased weight/volume measurements	Incomplete and variable derivatization reactions
	Operator bias in reading analogue instruments, equipment	Changing of laboratory environmental conditions during analysis
	Determination of substance that does not originate from the sample (e.g. contamination from the packing material)	Varying injection, chromatographic and detection conditions (matrix effect, system inertness, detector response, signal to noise variation, etc.)
	Determination of substance differing from the residue definition	Operator effects (lack of attention)
	Biased calibration	Calibration

"top down" approach. The bottom up or component-by-component approach breaks down all the analytical operations into primary activities. These are then combined or grouped into common activities, and an estimate is made of the contribution of these activities to the combined uncertainty value of the measurement process. The top down approach is based on method validation and long-term precision data derived from laboratory control samples, proficiency testing results, published literature data and interlaboratory collaborative trials. Uncertainty estimates based on interlaboratory studies may also take into account the between-laboratory variability of the data and provide a reliable estimate of the method performance and the uncertainty associated with its application. It is important to acknowledge, however, that collaborative studies are designed to evaluate the performance of a specific method and participating laboratories. They normally do not evaluate imprecision due to sample preparation or processing, as the samples generally tend to be highly homogenized.

6.2.1.8 Available food composition databases

(a) Food composition data for nutrients

Food composition databases contain information on the nutrient content of various foods and beverages. They are based on chemical analysis of nutrients in foods, which are complemented with calculated and imputed values. Most food composition databases are compiled at a national level, whereas some exist at a regional level. Most national databases report nutrient values that are not readily comparable at an international level owing to differences in foods from different countries (e.g. variety, soil, processing and fortification), but also artificial differences as a result of component identification, food description and nomenclature, analytical methods, mode of expression and units used (Deharveng et al., 1999).

International efforts are under way to harmonize these issues under the International Network of Food Data Systems (INFOODS) (http://www.fao.org/infoods/index_en.stm) of the United Nations University or, at the European level, under the European Food Information Resource Network (EuroFIR) (http://www.EuroFir.net), in order to be able to generate and compile high-quality nutrient values that are more comparable among countries. Generally, the exchange of nutrient values on the basis of food names alone is not sufficient to use and evaluate these data. Standardized vocabularies for foods and components will facilitate international use of the data. Some work has already been completed, including standardized vocabulary (http://www.fao.org/infoods/nomenclature_en.stm), component identification (Klensin et al., 1989; http://www.fao.org/infoods/tagnames_en.stm) and interchange formats and procedures (Klensin, 1992; http://www.fao.org/infoods/interchange_en.stm). Guidelines on interchange of food composition data have been proposed since 1992 and have been enlarged or updated since (see above web pages plus http://www.fao.org/infoods/index_en.stm).

Increasingly, in many nations, voluntary fortification of a wide array of foods creates an almost insurmountable challenge to managers of food composition databases. To portray the nutrient content in foods accurately, food composition databases should be updated frequently and be specific enough to accommodate many different formulations of the same foods. To improve the accuracy of estimates of nutrient intake, food consumption assessments should include the

collection of sufficient information for processed foods to ensure that food composition data match the foods consumed.

(b) GEMS/Food database

One of the activities of the WHO GEMS/Food Programme is the maintenance of databases of information collected by contributing institutions on contaminant and pesticide residue levels in foods and estimated dietary exposures to food chemicals from TDSs and duplicate diet studies based on internationally recommended procedures (WHO, 1979, 1985, 1997; FAO/WHO, 1997).

GEMS/Food international databases include individual and aggregated data on contaminants and pesticide residues in foods. GEMS/Food has provided information to assist in understanding the terminology used and how to submit data (EC, 2004; WHO, 2005b). GEMS/Food has also developed core, intermediate and comprehensive lists of priority contaminant/commodity combinations that should be considered for monitoring for public health reasons. These lists are periodically updated (see Annex V of WHO, 2002a).

In addition to protocols for electronic data submission, WHO has developed a computer system to allow the direct entry of data into the GEMS/Food database as well as the retrieval of data and creation of reports from the database. The system, Operating Program for Analytical Laboratories for data on individual and aggregate contaminant levels in foods (OPAL I), is available on request (foodsafety@who.int). OPAL II, for submitting data on dietary exposures to contaminants from TDSs and duplicate diet studies, is also available.

The GEMS/Food database is accessible through the Internet at http://www.who.int/foodsafety/chem/gems/en/. In this regard, data deemed confidential by the data submitter will not be made public without the expressed permission of the data submitter. In these cases, the database will display only the name of the country, the contaminant and the number of records.

Examples of national food chemical concentration data can be accessed on the Internet from various sources, including Australia (FSANZ, 2003), New Zealand (Vannoort, 2003, 2004a,b,c), the USA (USFDA, 2004a,b; USDA, 2008) and Europe (EC, 2004).

6.2.2 Food consumption data

Food consumption data reflect what individuals or groups consume in terms of solid foods, beverages, including drinking-water, and dietary supplements. Food consumption can be estimated through food consumption surveys at an individual or household level or approximated through food production statistics. Food consumption surveys include records/diaries, food frequency questionnaires (FFQs), dietary recall and TDSs. The quality of data from food consumption surveys depends on the survey design, the method and tools used, the motivation and memory of the respondents, the statistical treatment and the presentation (foods as purchased versus as consumed) of the data. Food production statistics by definition represent foods available for consumption by the whole population, typically in the raw form as produced.

6.2.2.1 Food consumption data requirements

Ideally, food consumption data used at the international level should take into account the differences in food consumption patterns in different regions. To the extent possible, consumption data used in dietary exposure assessments should include information on factors that may influence dietary exposure (those that may either increase or decrease risk). Such factors include demographic characteristics of the population sampled (age, sex, ethnicity, socioeconomic group), body weight, the geographic region, day of the week and the season in which the data are collected. Consideration of food consumption patterns for sensitive subpopulations (e.g. young children, women of childbearing age, the elderly) and consumption patterns for individuals at the extreme ends of the distributions is also important. Given that the design of consumption studies can have a critical impact on the results of any dietary exposure assessment, harmonization of study design should be achieved to the extent possible. All food consumption surveys should preferably include data on foods, beverages (including drinking-water) and food supplements. Ideally, all countries, including developing countries, should conduct food consumption surveys on a periodic basis, preferably with individual dietary records.

Individual record data will generally provide the most precise estimates of food consumption. Broad surveys, covering the food consumption patterns of the whole population, may not be needed if the food in which the chemical of interest is found is consumed by

only a subset of the population. If resources are limited, small-scale studies are appropriate and may cover specific foods or target population subgroups (e.g. children, nursing women, ethnic minorities or vegetarians). This approach can improve the precision of estimates of dietary exposure for specific population subgroups or specific food chemicals.

6.2.2.2 *Approaches for food consumption data collection*

(a) Population-based methods

Food supply data at the national level, such as food balance sheets or food disappearance data, provide gross annual estimates of the national availability of food commodities. These data may also be used to calculate the average per capita availability of energy and macronutrients and exposure to chemicals (e.g. pesticides and contaminants). Because consumption is expressed in terms of raw and semiprocessed commodities, these data are not generally useful for estimating dietary exposure to food additives. The major limitation of national food supply data is that they reflect food availability rather than food consumption. Losses due to cooking or processing, spoilage and other sources of waste and additions from subsistence practices cannot easily be assessed. According to FAO/WHO (1997), food balance sheet consumption estimates tend to be about 15% higher than the consumption estimates derived from household surveys or national dietary surveys. These data do not include water consumption. Where water consumption data are not available, a default water consumption value of 2 litres per adult may be used as per the WHO drinking-water guidelines (WHO, 2008).

Despite these limitations, food balance sheet data are useful for tracking trends in the food supply, for determining the availability of foods that are potentially important sources of nutrients or chemicals and for monitoring food groups targeted for control. Food supply data are not useful for either evaluating individual nutritional intake or food chemical dietary exposure or identifying subgroups of the population at risk.

(b) Household-based methods

A variety of information regarding food availability or consumption at the household level may be collected, including data on foodstuffs purchased by a household, follow-up of consumed foods or

changes in food stocks. Such data are useful for comparing food availability among different communities, geographic areas and socioeconomic groups and for tracking dietary changes in the total population. However, these data do not provide information on the distribution of food consumption among individual members of the household.

(c) Individual-based methods

Data collected by individual-based methods provide detailed information on food consumption patterns; however, as with other food consumption surveys, they may be prone to bias. For instance, several studies have found that nutrient intakes derived from 24 h recalls tend to underestimate true intakes of some macronutrients for some subjects (Madden et al., 1976; Carter et al., 1981; Karvetti & Knutts, 1985). Regression analyses between recall and actual intakes exhibited the "flat-slope syndrome", whereby individuals tend to overestimate food amounts when consumption is low and underestimate food amounts when consumption is high. In some cases, individuals may overestimate consumption of foods perceived as "good foods" and underestimate consumption of foods perceived as "bad foods".

The *food record*, or food diary, requires that the subject (or observer) report all foods consumed during a specified period (usually 7 days or less). These surveys generally collect information not only about the types of food consumed, but also about the source of the foods and the time of day when and place where foods are consumed. The amounts consumed should be measured as accurately as possible. Amounts may be determined by weighing or measuring volume.

The *24 h dietary recall* consists of listing of foods and beverages (including drinking-water and sometimes dietary supplements) consumed during the previous day or during the 24 h prior to the recall interview. Such surveys generally collect information not only about the types and amounts of food consumed, but also about the source of the foods and the time of day when and place where foods are consumed. Foods and drinks are recalled from memory with the aid of an interviewer who has been trained in methods for soliciting dietary information, without the introduction of interviewer bias. The interview is usually conducted in person, but may be conducted by telephone or via the Internet. In some situations, the recall is self-administered by the subject, but this approach results in less reliable

data. Researchers have developed multipass methods that guide the respondent through the 24 h reference period several times, providing opportunity for the respondent to remember food details and additional foods (Slimani et al., 1999; Raper et al., 2004).

The *FFQ*, sometimes referred to as a "list-based diet history", consists of a structured listing of individual foods or food groups. For each item on the food list, the respondent is asked to estimate the number of times the food is usually consumed per day, week, month or year. The number and types of food items may vary, as well as the number and types of frequency categories. FFQs may be unquantified, semiquantified or completely quantified. The unquantified questionnaire does not specify serving sizes, whereas the semiquantified tool provides a typical serving size. A completely quantified FFQ allows the respondent to indicate any amount of food typically consumed. Some FFQs include questions regarding the usual food preparation methods, trimming of meats, use of dietary supplements and identification of the most common brand of certain types of foods consumed.

The validity of dietary patterns assessed with FFQs depends on the representativeness of the foods listed in the questionnaire. Whereas some authors (Rimm et al., 1992; Green et al., 1998; Thompson et al., 2000; Brunner et al., 2001) have concluded that FFQs produce valid data for dietary exposure assessments, others (Kroke et al., 1999; Schaefer et al., 2000) have found that FFQs do not produce reliable estimates of some macronutrients.

FFQs are commonly used to rank individuals by consumption of selected foods or nutrients. Although FFQs are not designed to be used to measure absolute dietary exposure, the method may be more accurate than other methods for use in estimating average dietary exposure to those chemicals having large day-to-day variability and for which there are relatively few significant food sources. Brief FFQs may focus on one or several specific nutrients or food chemicals and include a limited number of food items. In addition, FFQs can be used in the identification of absolute non-consumers of certain foods.

The meal-based *diet history survey* is designed to assess usual individual food consumption. It consists of a detailed listing of the types of foods and beverages commonly consumed at each eating occasion

over a defined time period, which is often a "typical week". A trained interviewer probes for the respondent's customary pattern of food consumption on each day of the typical week and may use software designed for this type of interview (e.g. Mensink et al., 2001). The reference time frame is often over the past month or the past several months or may reflect seasonal differences if the reference time frame is the past year.

The *food habit questionnaire* may be designed to collect either general or specific types of information, such as food perceptions and beliefs, food likes and dislikes, methods of preparing foods, use of dietary supplements and social settings surrounding eating occasions. These types of information are frequently included along with the other four methods, but may also be used as the sole basis for data collection. These approaches are commonly used in rapid assessment procedures. The questionnaire may be open-ended or structured and self-administered or interviewer-administered and may include any number of questions, depending on the information desired.

(d) Combined methods

Consumption data obtained by different collection methods may be combined to improve accuracy and facilitate validity of the dietary data and for other practical reasons. For example, the food record has been combined with the 24 h recall. The FFQ that focused on selected nutrients has been used in addition to the 24 h recall. The 24 h recall is frequently used to help establish the typical meal plan. This information can be used to obtain better information from the diet history method. The FFQ may also be used as a cross-check for the other three types of methods.

An example of a recommendation to use two methods of collecting food consumption data is that of the European Food Consumption Survey Method (EFCOSUM) project, where the most cost-effective method for harmonizing food consumption data between European Union (EU) member countries was determined as follows: at least two 24 h recalls should be performed for each subject on non-consecutive days taking working and non-working days into account, in combination with a questionnaire on habitual consumption of infrequently consumed foods, to get insights into the proportion of non-consumers

(Brussard et al., 2002). The collection of repeated non-consecutive recalls allows for the estimation of usual food consumption by a modelling technique that separates intraindividual and interindividual differences in consumption (see section 6.2.2.4). Other combinations of consumption data from different sources may be appropriate, depending on the purpose of the dietary exposure assessment.

6.2.2.3 *Data reporting and use*

(a) Mapping

Food consumption data should be available in a format that allows matching of the consumption data with the concentration data used in the dietary exposure assessment. For example, for raw agricultural commodities and some semiprocessed commodities (e.g. polished rice and flour), the GEMS/Food format (see section 6.2.1.8) uses the Codex Classification System for Food and Feeds. This system was established by CCPR to specify foods for which pesticide MRLs are applicable. The system includes the common name of the food in English, French and Spanish, as well as the Latin name or names. This coding is also used by CCCF for identifying foods subject to MLs for contaminants. The system is being revised and expanded to include more foods, including processed foods. In the case of acrylamide, which occurs only in processed foods, additional fields have been included to more accurately describe the analysed food. These include four fields for ingredients (in order of predominance), the Codex code for processed foods, the method of heating and the processing method (FAO/WHO Acrylamide in Food Network: http://www.acrylamide-food.org/).

Foods may be consumed as such or as an ingredient as part of a recipe or food mixtures. For example, ground beef may be consumed as a single food item or as a component of a beef casserole. When modelling food consumption, it is important to know whether the consumption estimate includes all sources of the food. Recipes can be broken down into their ingredients, which can then be mapped to the corresponding individual food and added to the total consumption of that food from all sources (e.g. whether “apples” includes the apple in a baked apple pie and apple juice; whether “potatoes” includes potatoes fried as in french fries or potato chips/crisps: if potatoes and french fries are considered separate foods, then this should be stated). The recipe mapping approach needs to be documented.

The use of standard recipes and the attribution of the ingredients to individual foods introduce some uncertainty into consumption data (e.g. assuming that, on average, 70% of bread is flour). The error would be significantly higher if the contribution of mixed foods were omitted. Using standardized recipes results in reduced variability that may underestimate or overestimate the amount of individual foods or food ingredients consumed for high-percentile consumers, depending on the relative quantity of the ingredient in the recipe. Another potential source of error lies in the decisions taken in mapping foods from food consumption surveys to foods with concentration data, because in many cases the food and the food description do not correspond exactly (Slimani et al., 2000).

(b) Data format/modelling

Data collected using *population-based methods* are generally compiled and reported for raw or semiprocessed agricultural commodities, and they represent the total annual amount of a commodity available for domestic consumption per year. The amount may be for the entire population or at the per capita level. A daily consumption amount may be estimated by dividing the total annual amount by 365. It is not possible to estimate the consumption amount per eating occasion or only for consumers of the foods from these data alone.

Data from *individual food consumption surveys* are often not publicly available in raw format (i.e. at the individual respondent level), and risk assessors have to rely on published summary statistics. When the raw data are available, they can be used to estimate dietary exposures from multiple foods, to estimate dietary exposures by specific population subgroups or to estimate distributions of food consumption, rather than just mean consumption.

When only summary data are available, it is important to know and document the commodity, the type of commodity (e.g. raw juice, juice concentrate), how the statistics are aggregated and whether they refer to typical or high-end consumers, how a typical consumer is defined (e.g. median or mean food consumption or dietary exposure level), whether they refer to consumers only or to the total population (all survey respondents, per capita estimates), whether they represent daily consumption, consumption per eating occasion or per meal or averages across survey days (in the case of multiday surveys), as well

as the data requirements listed in section 6.2.2.1. When comparing food consumption data among countries or surveys, caution should be exercised even if the same methods are used, because the results may not be readily comparable owing to differences in study design, tools, statistical analysis and reporting of results (Slimani et al., 2000; Brussard et al., 2002).

Market share corrections can be applied to food consumption data for processed foods or percentage of treated crops. The approach is used mainly when the substance being evaluated has been deliberately added to the food. The maximum or mean concentration of a chemical is assigned only to the proportion of the market in which the additive is used or the proportion of the crop in which a pesticide is used, not to the consumption data for the whole food category. This technique may refine the estimate of mean dietary exposure, but it does not refine the dietary exposure estimate for the most exposed section of the population (i.e. consumers who are loyal to the food products containing the additive or the pesticide), as it may underestimate their actual dietary exposure. When assessing dietary exposure to additives or flavourings, market share data should consider brand loyalty, where feasible. For pesticides, correction for the percentage of crop treated can be taken into account when setting MRLs; in post-regulation situations, however, at a national level, consideration should be given to the possibility that a section of the population may systematically consume foods derived from treated crops only.

(c) Food portion sizes

Unit weights represent weights of typical commodity units (e.g. a single apple or a single banana) and are used in the calculation of acute dietary exposure estimates, such as the international estimated short-term intake (IESTI). Unit weights may also be used to convert reports of food consumption by single units in an FFQ or 24 h recall survey to gram weights. Estimates of mean or median unit weights of raw agricultural commodities and the per cent edible portion (e.g. one orange and the percentage of orange pulp) have been provided by France, Japan, Sweden, the United Kingdom and the USA and compiled by GEMS/Food (http://www.who.int/foodsafety/chem/acute_data/en/).

Standard portion sizes are used to assess the consumption of foods and beverages in a large number of food surveys. That is, a standard

weight will be assigned to a banana, a cookie or a glass of soft drink. These portions can be more or less detailed (with, for example, differing weights for different glass sizes). However, standard portion sizes do not usually describe the full variability in the weights of portions as consumed in the population. Their use can lead to an overestimate of low portions and an underestimate of high portions and thus to overestimates and underestimates of dietary exposure. They are a very useful and pragmatic tool, but the uncertainty that they introduce in food consumption data must be kept in mind—specifically, the impact on the estimate of high levels of dietary exposure to food chemicals and low levels of intake for nutrients.

Large portion (LP) sizes have been used for a variety of risk assessments in Europe and by JMPR. For these purposes, the LP values have been based on the 97.5th percentile of food consumption derived from records of individual consumer days (i.e. survey days on which the food or foods of interest were consumed). For use in an acute dietary exposure assessment for pesticide residues (see section 6.3.6.2), the LP value should be matched to the raw Codex commodity to which the residue data relate. In the case of commodities that are eaten predominantly fresh, such as fruits and vegetables, the LP value should be derived for the raw commodity. When a high proportion of the commodity, such as cereal grains, is consumed in a processed form, the LP value should relate to the processed commodity (e.g. bread, flour), provided matching residue concentration data are also available for the processed food.

Upper-percentile and lower-percentile food consumption amounts should be defined based on individual consumer days. For surveys collecting multiple days of consumption data per person, the individual consumer days are assumed to be independent observations in the derivation of upper and lower percentiles as follows:

- If the survey includes multiple days per participant, only the valid consumer days on which consumption of the food of interest occurs should be used.

- If a survey participant has multiple valid consumer days, these consumer days should be considered as independent observations in the database and not averaged.

- The number of consumer days on which the percentile is based should be explicitly stated, as the purpose of the assessment may determine how these records are treated. For example, multiple consumer days for each participant would be treated separately in an acute dietary exposure estimate, but may be combined or adjusted by a mathematical formula to represent "usual" consumption in a chronic dietary exposure estimate (see section 6.2.2.4).

In estimating acute dietary exposures to chemical residues in a single commodity or food, it is appropriate to use food consumption data for only those people who consume the single food (consumers only). Estimations of acute dietary exposures to chemical residues in multiple commodities or foods should be conducted for both consumers only and all respondents in the survey (total survey population).

LP (97.5th percentile) consumption values as well as body weights and ages are compiled by GEMS/Food and are available at http://www.who.int/foodsafety/chem/acute_data/en/. These data were provided by Australia, France, the Netherlands, Japan, South Africa, the United Kingdom and the USA, along with body weights of the general population and children aged 6 years and under.

Ideally, the food consumption values in the GEMS/Food LP database should be based on the 97.5th percentile of individual consumer days from national surveys. This database needs to be expanded to include data from additional countries to better represent all member countries. When data are provided, additional information is desirable that fully describes the underlying data, food groups used and assumptions that were made in preparing the estimates of the LP values.

If individual records are not available, the risk assessor can estimate a high-percentile food consumption value by multiplying a central estimate by an inflation factor. If the approximate shape of the distribution for a particular parameter is known, better high-percentile estimates can be developed.

6.2.2.4 *Usual food consumption patterns*

For a probabilistic exposure assessment, the readily available distributions of food consumption data are not representative of true long-term consumption; for example, consumption data are usually

collected over a period of a few days, but are often used to represent food consumption during a lifetime. It is difficult from the methodological point of view to obtain representative data from single subjects to represent the lifetime exposure of consumers. Nevertheless, food consumption data on a national or group level reported across a range of age groups at one point in time or over a short time period can be used to model lifetime consumption.

Approaches that have been used to estimate long-term consumption have included methods combining food frequency data with consumption amount information (e.g. IEFS, 1998; Tran et al., 2004) and statistical models that use the correlations among the days of consumption to estimate the "usual" intake of nutrients or contaminants using short-term consumption data (e.g. USNRC, 1986; Slob, 1993, 1996; Carriquiry et al., 1995; Nusser et al., 1996). These models are most appropriate when the chemical of interest occurs in various basic food products, resulting in a nutrient intake or chemical dietary exposure different from zero for virtually every individual each day. Parametric and non-parametric methods are needed in order to better simulate the frequency of consumption for occasionally eaten food on a long-term basis.

Application of such methods results in a distribution of long-term nutrient intakes or food chemical dietary exposures that shows less variability than the distribution of dietary exposures directly derived from short-term food consumption data (Carriquiry, 2003).

Lambe & Kearney (1999) warned against using short-term consumption data for estimating long-term or usual consumption and showed that survey duration affects estimates of the per cent consumers, the mean and high consumption of foods and the classification of individuals as high or low consumers of foods or nutrients. Thus, data from such surveys need to be adjusted for use in the estimation of long-term consumption for chronic dietary exposure assessments.

6.2.2.5 Food consumption databases

(a) Databases collected through population-based methods

Food balance sheet data include the amounts of foods available for human consumption derived from national statistics on food

production, disappearance or utilization. They are generally available for most countries. Examples include those compiled by the United States Department of Agriculture's (USDA) Economic Research Service (Putnam & Allshouse, 1999) and the Australian Bureau of Statistics (2000). The FAO's statistical database (FAOSTAT) is a compilation of similar statistics for more than 250 countries. When official data from Member countries are missing, the data are estimated from national food production and utilization statistics (http://faostat.fao.org/).

The GEMS/Food consumption cluster diets developed by WHO are based on selected FAO food balance sheets and represent average per capita food consumption. Using a cluster analysis approach where countries with similar patterns of consumption of 20 key foods were grouped together and then sorted by geographic location, 13 consumption cluster diets were produced based on all available FAO food balance sheet data for the period 1997–2001 (http://www.who.int/foodsafety/chem/gems/en/index1.html). The consumption cluster diets were last revised in 2006, incorporating country comments on the first version; although they are still based on the 1997–2001 data, identified data gaps were filled where possible. Further details on these diets are available on the WHO web site (http://www.who.int/foodsafety/chem/ClusterDietsAug06.xls). The consumption cluster diets are expected to be updated every 10 years. The 13 GEMS/Food consumption cluster diets are now used as a tool for international chronic dietary exposure assessments by JMPR and JECFA. The consumption cluster diets replace the five regional diets previously developed by WHO (1998, 2003).

(b) Databases collected through individual-based methods

Many countries now collect food consumption data at an individual level. Some examples of these food consumption databases are listed below:

- The 1994–1996 and 1998 USDA Continuing Survey of Food Intakes by Individuals (CSFII) (USDA, 2000) and, since 1999, the National Health and Nutrition Examination Survey (NHANES) (http://www.cdc.gov/nchs/nhanes.htm) provide 2-day (CSFII) and 1- or 2-day (NHANES) food consumption data for individuals in

the USA, along with corresponding demographic and anthropometric data (age, sex, race, ethnicity, body weight and height, etc.) for each individual.

- Many European countries have national dietary surveys (Verger et al., 2002). Data from 17 European food consumption surveys were published in 2008 in the European Food Safety Authority's (EFSA) Concise European Food Consumption Database (http://www.efsa.europa.eu/en/datex/datexfooddb.htm).

- The 1995 Australian National Nutrition Survey collected data from one 24 h food recall for 13 858 individuals aged 2 years and older (McLennan & Podger, 1997, 1998, 1999), and the Australian National Children's Nutrition and Physical Activity Survey collected data from two 24 h recalls for children 5–16 years of age (Commonwealth of Australia, 2008).

- The 1997 New Zealand National Nutrition Survey collected data on one 24 h food recall for 4636 individuals aged 15 years and older (New Zealand Ministry of Health, 1999), and the 2002 National Children's Nutrition Survey collected data from two 24 h recalls for individuals aged 5–14 years (New Zealand Ministry of Health, 2003).

- The 2002–2003 Brazilian Household Budget Survey (Pesquisa de Orcamentos Familiares) provides the amount of food acquired during 7 consecutive days by 48 470 households in all 27 Brazilian states (http://www.ibge.gov.br).

6.3 Estimating dietary exposure

6.3.1 Introduction

The most appropriate method to use in estimating dietary exposure will depend upon a variety of factors. The following sections discuss the range of options, highlight some methods that are currently used and summarize the advantages and disadvantages of those methods.

The method applied in any dietary exposure assessment should be clearly stated and reproducible. Information about the model and data

sources used, assumptions, limitations and uncertainties should also be documented.

A framework for conducting exposure assessments should be established that will allow the analyst to select the most appropriate method for the intended use of the assessment. A framework that includes a stepwise approach is recommended, noting that the "best estimate" in terms of the "most realistic" dietary exposure assessment may not always be the "best estimate" in terms of the "most appropriate" one to suit the purpose of the dietary exposure exercise. In general, the early steps of the framework will include screening methods that use minimal resources and the shortest possible time (see Figure 6.1) to identify, among the large number of chemicals, those of no safety concern. No further (refined) exposure assessment is needed for substances that do not present safety concerns when analysed using screening methods that include conservative assumptions.

For the purposes of dietary exposure estimates, food consumption data should be presented such that individual consumer body weights are applied to the consumption figures for each consumer. If individual body weight data are not available or if the individual body weights have not been correlated to the food consumption figures, average body weights for the target population should be used. Average body weights of 60 kg for adults and 15 kg for children are assumed for most populations in the world; however, for certain regions, the average body weight of the population may differ significantly from 60 kg. For the adult Asian population, an average body weight of 55 kg is assumed. Actual average body weights in a country may vary significantly from 60 kg. If the default 60 kg adult body weight underestimates the actual individual body weights, the dietary exposure estimate on a per kilogram body weight basis will be overestimated. Likewise, if the default 60 kg adult body weight overestimates the actual individual body weights, the dietary exposure estimate on a per kilogram body weight basis will be underestimated.

6.3.2 Considerations when undertaking an exposure assessment

The specific approach that is most appropriate for estimating dietary exposure depends on several considerations, including 1) the type of substance being evaluated (food additive, including flavouring,

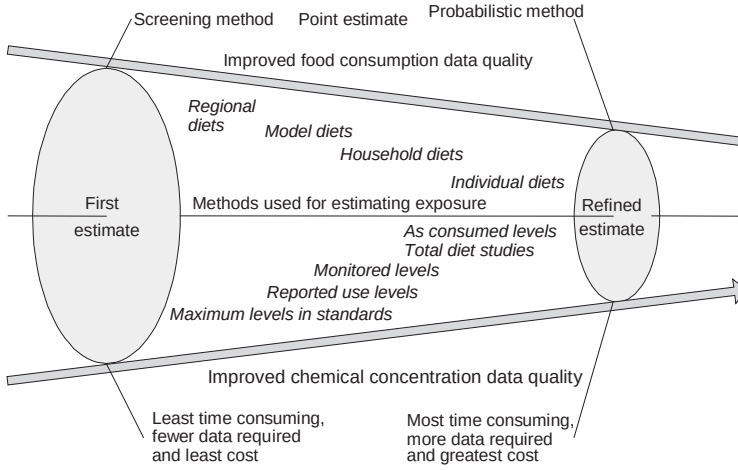

Fig. 6.1. Stepwise approach to obtaining realistic dietary exposure assessments

pesticide, veterinary drug, contaminant or nutrient) and whether the concern is the potential for too much or, for nutrients, too little intake, 2) the duration of exposure required to produce the toxic or beneficial effect, 3) the potential for different exposures in different subgroups or individuals within the population of consumers and 4) the type of estimate needed (point estimate or probabilistic characterization of the distribution of exposures). These considerations are further elaborated below in conjunction with each of the methods discussed.

6.3.3 Stepwise approach to exposure assessment

Ideally, exposure assessments should aim to identify substances that may be of safety concern with the minimum expenditure of resources. Therefore, most exposure assessment frameworks employ a stepwise or tiered approach in which the initial steps rely on conservative screening methods. If no safety concerns are identified, no additional exposure assessment is required. Where potential safety concerns are identified, the subsequent steps of the framework provide methods that incorporate increasingly specific or refined data (and require more resources).

At step (tier) 1, dietary exposure can be assessed by using screening methods based on conservative assumptions. If the estimated dietary

exposure to a given chemical substance exceeds its health-based guidance value (e.g. ADI, provisional maximum tolerable daily intake [PMTDI] or, for nutrients, the upper level of intake [UL]; see FAO/WHO, 2006b), a more accurate method of dietary exposure assessment should be applied. A stepwise approach is being used by JECFA for additives (including flavourings), contaminants and nutrients.

In the sections that follow, examples of the available methods have been organized (somewhat arbitrarily) into categories to assist the reader in selecting the most appropriate framework and the desired methods for each step of the framework. The methods are divided into those that provide single (point) estimates and those that characterize the full distribution of consumer exposures.

Point estimates include 1) screening methods, 2) exposure methods that rely on crude estimates of consumption (default factors based on physiological limits, food production data or usage/poundage data), such as the theoretical added maximum daily intake (TAMDI) and other model diets (for veterinary drug residues and packaging materials), and 3) more refined exposure methods based on actual consumption data and chemical concentration data, such as TDSs, selective studies of individual foods and duplicate portion diets (see sections 6.3.4.1 and 6.3.4.2).

Characterizing the full distribution of consumer exposures is the most resource-intensive assessment, as data are required that characterize the range of food consumption practices as well as the range of chemical concentrations in the foods that are eaten. Therefore, such methods are usually reserved for later steps. When such methods are employed, appropriate statistical models are used to evaluate the data and to describe the range of consumer exposures and the associated probabilities of consumers having each level of exposure. These exposure assessments are generally referred to as probabilistic exposure estimates. Examples of probabilistic assessments are the Monte Carlo assessments that have been conducted to assess consumer exposure to acrylamide (FAO/WHO Acrylamide in Food Network: http://www.acrylamide-food.org). The possibility of using probabilistic modelling has also been discussed at meetings of JMPR and CCPR, and some preliminary investigations of its use at an international level have been undertaken.

6.3.4 Deterministic/point estimates of dietary exposure

A deterministic or point estimate of dietary exposure is simply a single value that describes some parameter of consumer exposure (e.g. the average exposure of a population). For example, an average dietary exposure is calculated as the product of the average consumption of the foods of interest and the average residues of the substance of interest in those foods. A point estimate of a high-consumer exposure (e.g. the upper 90th-percentile consumer) can also be calculated, provided the appropriate data are available.

A point estimate is not inherently "conservative" or "realistic". The conservatism incorporated into the analysis is determined by the data and assumptions that are used in calculating the estimate. Point estimates can range from initial screening methods that use very few data and generally include very conservative assumptions to refined exposure assessments that include extensive underlying data in order to realistically calculate the desired exposure estimates.

6.3.4.1 Screening methods

Screening methods should be designed to reflect the particulars of the exposures that are to be considered. The screening assessments currently performed by international organizations, such as those conducted by JECFA and JMPR, are different for food additives, pesticides and veterinary drugs.

The screening method that is selected should be easy to use and pragmatic. Screening methods should overestimate dietary exposure of high consumers using conservative assumptions in terms of food consumption and chemical concentration (e.g. budget methods). This will avoid situations where the dietary exposure estimated by the screening process would erroneously indicate no safety concern (i.e. understate exposure). However, in order to effectively screen chemical substances and establish risk assessment priorities, the first steps of the procedure should not consider unsustainable diets, or the results will be too unrealistic to be useful. At a minimum, physiological limits of consumption should be taken into account.

Although screening methods are sometimes criticized as being "too conservative", it must be borne in mind that their aim is not to

assess true dietary exposure but to identify food chemicals for which a more comprehensive dietary exposure assessment is necessary. This must be made clear when results are presented, as should all assumptions made. For example, the budget method (see below) was used to screen intakes of 58 additives in Europe. For 22 of the additives, the potential dietary exposure calculated with the budget method was lower than the relevant ADI (EC, 1998), whereas 36 of these additives did not "pass" the budget method. For the 36 that did not pass, it was recommended that more refined exposure assessments be conducted.

Different screening methods are described below, together with a critical analysis of the assumptions on which they are based and of their fitness for purpose. There is a need for harmonization, where possible, of these methods.

Screening methods can be created that are appropriate for a worst-case assessment of compounds that are toxic for both acute and chronic exposures, as well as for specific subpopulations of interest.

(a) Poundage data (food additives, including flavours)

Poundage data provide estimates of the amount of a chemical substance available per capita for use in food manufacturing in a country during a period of time, usually over 1 year. The estimated dietary exposure that is provided with such a calculation is based neither on observed consumption patterns nor on data on the actual concentration of the chemical substance in foods. These estimates may take into account the import or export of the chemical and of foods containing it. They may also include non-food uses. Surveys of poundage data are usually performed by producer associations that ask single producers to report their volumes of production. A very large year-to-year variability in poundage data may occur, especially for substances produced in low quantities. This limits the usefulness of poundage data surveyed on a single-year basis.

Exposure estimates based on poundage data may be adjusted by the proportion of the population likely to consume the food (per cent consumers) in which the chemical may be present, as well as for under-reporting of the amount of chemical produced. Nonetheless, there is a very large uncertainty in a mean dietary exposure derived from

poundage data, as typically no information is available that allows the user to identify the precise foods in which the substance is consumed, who is consuming the food or how much of the substance is discarded without being consumed. Poundage data and derivative methods do not adequately describe highly exposed consumers and are therefore not sufficient to determine if their dietary exposure is within health-based guidance values. Additional methods based on use level data should be used in the first step of the screening (e.g. budget method). Poundage data can be used to provide an indication of the historical and geographical trends in the use of a substance or as a comparative measure of overall population dietary exposure relative to other substances.

(b) Budget method

A screening method referred to as the "budget method" has been used to assess the theoretical maximum daily dietary exposure to some food additives. The results are compared with the ADI for the substance. The budget method has been used at an early stage in assessing additives by JECFA (FAO/WHO, 2001) and for assessments within the EU.

The method relies on assumptions regarding 1) the level of consumption of foods and of non-milk beverages, 2) the concentration of the additive in foods and in non-milk beverages and 3) the proportion of foods and of non-milk beverages that may contain it. More specifically, the levels of consumption of foods and beverages considered are maximum physiological levels of consumption—i.e. the daily consumption of 0.1 litre of non-milk beverages per kilogram of body weight and the daily consumption of 100 kcal/kg body weight from foods (equivalent to 0.05 kg/kg body weight based on an estimated energy density of 2 kcal/g) (Hansen, 1979). In a 60 kg person, these levels correspond to the daily consumption of 6 litres of non-milk beverages and 3 kg of food.

The levels contained in foods and beverages are assumed to be the highest maximum levels of the additive reported in any category for foods and for beverages, respectively. When the level of an additive is particularly high in a very specific category of food or beverage (e.g. chewing gum), the additive level considered is the highest maximum

level among the other categories that are more "representative", in order to provide somewhat more realistic estimates. The proportions of solid foods and beverages that may contain the substance are set arbitrarily. In the case of food additives, a default proportion that is often used for European assessments is 12.5% for solid foods and 25% for beverages (EC, 1998). For additives used in a wide range of foods, the proportion of solid foods may be set at 25%.

The overall theoretical maximum daily exposure to an additive is calculated by summing the potential exposure from beverages and from foods, as shown below:

Overall theoretical maximum daily exposure	=	[maximum level of the additive in beverages (mg/l) × 0.1 (litre/kg body weight) × percentage of beverages that may contain the substance] + [maximum level of the chemical in solid foods (mg/kg) × 0.05 (kg/kg body weight) × percentage of solid foods that may contain the substance]

The potential dietary exposure to the additive is expressed in milligrams per kilogram body weight per day.

For example, if an additive may be present at up to 350 mg/l in beverages and up to 1000 mg/kg in foods and if the proportions of beverages and foods that may contain it are assumed to be, respectively, 25% and 12.5%, the theoretical maximum daily exposure to this substance will be:

$$[350 \times 0.1 \times 0.25] + [1000 \times 0.05 \times 0.125] = 15 \text{ mg/kg body weight}$$

In a 60 kg person, this daily exposure corresponds to 900 mg of the food additive deriving from the consumption of 1.5 litres of beverages and 375 g of food containing the substance at the maximum level.

The budget method may need to be applied to different food consumption levels to provide similar levels of conservatism for adults and for children. For example, when the budget method was applied to consider exposures to food additives authorized for use in the EU (EC, 1998), a specific budget calculation was performed for children by setting the proportion of beverages that could contain the additives

at 100%. The level of consumption of beverages considered was therefore 0.1 litre/kg body weight (i.e. 1.5 litres in a typical 3-year-old child weighing 15 kg). This is a conservative assumption according to the results of a survey in the United Kingdom, which reported that the 97.5th percentile of consumption of beverages containing additives was 0.07–0.08 litre/kg body weight in children aged 1.5–4.5 years (Gregory et al., 1995).

The budget method has the advantage of requiring virtually no product-specific data and of being very simple and rapid to perform. A disadvantage of the budget method is that the results depend largely on the proportions of foods and beverages that are assumed to contain the substance, and typically those proportions are set arbitrarily. The usefulness of the method can be improved if the proportions are chosen with an understanding of the impact on the conservativeness of the method.

Another arbitrary assumption of the budget method is the identification of categories of foods and beverages with very high use levels that are considered not "representative", such as chewing gums. When such items are identified, assessment of the quantity of the specific food item that would lead to exposure in excess of the toxicity reference value should be performed in parallel with the budget method in order to determine if the consumption of the specific item can lead to exposure in excess of the health-based guidance value.

The assumptions of the budget method with respect to energy have been examined in a case-study of food additives, applying the assumptions used for EU assessments (Douglass et al., 1997). The assumptions for the energy density of foods were found to be only a slight overestimate, which would detract from the overall conservatism of the method. On the other hand, the assumptions regarding energy intake and beverage consumption were overestimates of even high levels of consumption. Overall, the exposure to additives estimated with the budget method was found to be higher than the survey-based 95th-percentile exposure to additives (Douglass et al., 1997).

In summary, the budget method is a simple, inexpensive and conservative screening method that can easily be applied to all chemicals intentionally added to food (additives, including flavourings,

processing aids, etc.) for comparison with their relevant toxicological reference values, provided the maximum concentrations of the chemical in foods and beverages can be ascertained.

(c) Model diets

Model diets are constructed from available information on food consumption and are designed to represent a typical diet for the population whose exposure is to be considered. A model diet can be constructed that reflects the diet of the general population or a specified subpopulation. For example, it may be of interest to evaluate the subgroup of the population that has the highest consumption of foods of interest or high consumption in relation to body weight.

Although model diets can be extremely useful, the models are only as good as the underlying data and assumptions, which should be stated for each model. Some examples of model diets that have been used to evaluate consumer exposure are summarized below.

TAMDI model diet for flavourings. The TAMDI model diet was designed to provide a conservative estimate of potential exposure to specific flavouring substances on the basis of allowed maximum (upper use) levels (UUL) in the different categories of foods and beverages that could be flavoured. The resulting exposure estimate is for a hypothetical consumer who consumes a fixed amount of flavoured foods and beverages every day, and those foods always contain the specific flavouring at its specified UUL (Cadby, 1996). The TAMDI is calculated by summing the exposures estimated for each individual food category (see Table 6.3).

The consumption levels considered are aimed at representing typical portions of flavoured foods and beverages (e.g. a glass of non-alcoholic beverage, a piece of bakery ware). The portion sizes are twice those that were used by CAC to estimate exposure to intense sweeteners in the absence of sufficient data relevant to the consumption of sugar-free products (FAO/WHO, 1989a).

The TAMDI was used by the European Scientific Committee on Food (SCF) to assess potential exposure to single flavourings (EC, 2003). A modified TAMDI, in which typical use levels have been used instead of UULs, has been applied in the evaluations of groups of

Table 6.3. Food consumption and concentration levels used in the TAMDI calculations[a]

Foods and beverages	Consumption (g/day)	Concentration (mg/g)
Beverages (not alcoholic)	324	UUL1
Foods	133	UUL2
Exceptions:		
- Candy, confectionery	27	UUL3
- Condiments, seasonings	20	UUL4
- Alcoholic beverages	20	UUL5
- Soups, savouries	20	UUL6
- Other exceptions (e.g. chewing gum)	2	UUL7

[a] TAMDI (mg/day) = (324 × UUL1) + (133 × UUL2) + (27 × UUL3) + (20 × UUL4) + (20 × UUL5) + (20 × UUL6) + (2 × UUL7).

chemically defined flavourings published by EFSA since 2004 (EFSA, 2004). The selection of a typical use level instead of a UUL, as a general principle in a screening process, may not be representative of the highest daily intakes, as consumers could be loyal to flavoured products containing a UUL.

The consumption levels considered in the TAMDI calculation may underestimate the average consumption of flavoured foods by some consumers. On the other hand, the assumption that all flavoured foods consumed each day will contain the same flavouring at its UUL is obviously conservative.

A major disadvantage of the TAMDI model is the arbitrary choice of food categories and portion size. The method cannot differentiate between different types of products that are grouped in the same category in Table 6.3. Also, the TAMDI model does not specify whether it is assessing the exposure at the upper 90th, 95th or some other percentile of exposure.

The advantages of TAMDI are that it is very easy to apply and that the hypotheses on which it is based are transparent in terms of consumption levels and concentrations. On the basis of some limited case-studies, the TAMDI appears to provide a conservative estimate

of high exposure to flavourings (Lambe et al., 2002). It can therefore be considered as a tool to prioritize dietary exposure assessments provided the underlying assumptions are clearly delineated. The TAMDI method may need to be supplemented with dietary exposure assessments targeted to high consumers of single categories of flavoured foods and beverages.

An alternative, less conservative, estimate for dietary exposures to flavouring agents was recently developed by JECFA (FAO/WHO, 2009a), using the single portion exposure technique (SPET). The use of the SPET estimate in the JECFA screening procedure for flavouring agents is further described in chapter 9.

Model diet for veterinary drug residues. A model diet intended to cover high consumers of animal products is used by JECFA to check that proposed MRLs for veterinary drug residues in foods of animal origin would not result in the ADI being exceeded. The model assumes that the amounts of foods are consumed daily by a person weighing 60 kg, and it is intended to cover the consumption of all processed foods with these foods as ingredients (Table 6.4). The consumption of meat and fish in 1 day is considered mutually exclusive. As the skin of pigs, poultry and certain fish species may be consumed, the residues in this associated tissue also have to be taken into account.

JECFA considered the consumption estimate for honey to be used in the model diet at its seventieth meeting (FAO, 2009; FAO/WHO, 2009b). It was noted that honey is widely used as a sweetener and glazing agent in confectionery products, breakfast cereal and baked goods, in addition to direct consumption of liquid and set honey, and that such uses must be taken into account for dietary exposure estimates. Based on limited data from two European countries, the Committee concluded that a consumption amount of 20 g per day was between the median and up to the 95th percentile of daily consumption for honey eaters. Based on the limited data, consumption of 50 g honey per person per day would be expected to cover all consumers of honey, but further data are necessary to determine the accuracy of this figure, in particular whether this figure would also cover consumption of products containing honey. In the case where residues are found in both honey and wax, this would need to be considered in dietary exposure estimates, where a ratio of honey to wax of 9:1 will be used.

Table 6.4. Model diet for exposure assessment of veterinary drug residues (FAO/WHO, 1989b, 1995c, 2009b)

Category of food of animal origin	Tissue or product	Consumption (g/day)	Remarks
Meat tissues (500 g in total)	Muscle	300	a) For definitions of meat and muscle, see chapter 8, section 8.4.1
	Liver	100	
	Kidney	50	b) For pigs and poultry, muscle may be replaced by fat and skin in natural proportions
	Fat	50	
Fish	Muscle	300	May be replaced by muscle and skin in natural proportions
Milk	Whole milk	1500	
Eggs	Egg content, excluding shell	100	
Honey		20	

JECFA has in the past calculated MRLs such that the dietary exposure estimated was lower than the relevant ADI, using the MRL as the point estimate of concentration for the exposure estimate. The MRL is a point concentration of the marker residue on the residue depletion curve describing the upper one-sided 95% confidence limit over the 95th percentile (see section 6.2.1.3 for derivation and food amounts from the model diet). Such a model clearly corresponds to a non-sustainable diet but was used to provide a conservative dietary exposure estimate, known as the theoretical maximum daily intake (TMDI).

For estimating chronic dietary exposures to veterinary drug residues, JECFA decided in 2006 to use the median of the residue distribution to substitute for the MRL in the dietary exposure estimate (FAO/WHO, 2006a). The new estimate of dietary exposure is called the estimated daily intake (EDI). In calculating the median from an array of results, results below the LOQ or LOD are assigned a value of half of the respective limit for the calculation of the median concentration of residues. Definitions of the foods in the model were also revised. The contribution to the EDI due to the consumption of the individual

tissues is calculated by multiplying the amount of tissue in the model diet by the median concentration of marker residue corresponding to the MRL of the tissue and by the ratio of the concentrations of the total residue of concern and the marker residue. The dietary exposure resulting from consuming 100 g (0.1 kg) of liver would, for example, be calculated as follows:

$$\text{Intake}_{\text{total residue from liver}} \text{ (mg/person per day)} = 0.1 \text{ (kg)} \times \text{median residue}_{\text{liver}} \text{ (mg/kg)} \times \text{ratio}_{\text{liver}}$$

The EDI itself is then the sum of the individual intakes resulting from similar calculations for all tissues.

Model diet for chemical substances migrating from packaging materials. Currently, the EU and the USA each have methods for assessing substances migrating from food packaging materials. The models are described below.

The *EU model diet* for chemical substances migrating from packaging materials is used to establish a maximum limit of migration, the so-called specific migration limit, or SML (Barlow, 1994; EC, 2002).

The maximum limit of migration is determined by assuming that a person weighing 60 kg could ingest daily up to 1 kg of foodstuffs in contact with a plastic article (600 cm^2 contact surface) that would always contain the substance under consideration at a concentration corresponding to the SML without exceeding the relevant health-based guidance value (i.e. TDI).

The assumption of repeated daily exposure to the same type of packaging material is conservative, but in some cases the other assumptions are not. For example, individuals may consume daily more than 1 kg of packaged food, especially if beverages are considered. Moreover, the default ratio of surface to mass (600 cm^2/1 kg) is that of a cube of 10 cm side width (total area 6 × 100 cm^2) containing 1 kg food; this ratio is low in comparison with that of foods in small packages (e.g. single portions, food in slices, some baby foods).

The *United States model diet* used to evaluate food contact substances assumes a consumption of 3 kg of packaged foods and beverages and employs consumption factors that describe the fraction of

the daily diet expected to be in contact with specific packaging material types (e.g. glass, plastic, paper) (http://www.cfsan.fda.gov/~lrd/foodadd.html). Migration levels are then assigned according to the nature of the food likely to be in contact with the packaging material (aqueous, acidic, alcoholic and fatty).

6.3.4.2 *More refined deterministic/point estimates*

Point estimate modelling may also be appropriate as a second step in a tiered approach. The model selected can be more or less conservative, depending upon the purpose and the available information.

As noted above, deterministic models use a single point estimate for each model parameter. For concentration data, the point estimate typically consists of the mean, the median, a high percentile of all observed values or even the ML proposed by national or international food authorities. Concentrations can be further modified using additional correction factors as appropriate (see section 6.2.1.2). For food consumption data, the point estimate typically consists of the mean or a high percentile of all the consumption values of a considered food in a population of interest.

This type of deterministic modelling has the advantage of being relatively simple to implement. Models can often be "developed" by using tools such as spreadsheet or database programs. However, because such models generally contain limited information, interpretation of the results can be problematic. The results are dependent on the input data and their appropriate treatment, but the impact may not be readily apparent (e.g. if the chosen input value used is not representative of the underlying distribution, then the result is likewise not representative). If "conservative" values (e.g. high concentration and high consumption values) are used in the model, the resulting exposure estimates will overstate typical exposures. For this reason, use of point estimate modelling with conservative parameter values may be appropriate for screening-level assessments. Nonetheless, it is important to keep in mind that it is difficult to know just how conservative the result will be.

When high-percentile values for either food consumption or food concentration levels are not known, there are default procedures that can be used to develop proxies for these points.

(a) Modelling high consumers

Model diets for high consumers can be developed on the basis of published data from food consumption surveys as an alternative to the budget method or as an additional step in the screening process. For example, a model diet has been used in Europe to estimate chronic dietary exposure based on the assumption that a person might consume average amounts of several different foods but only one or two at high levels (EC, 1998). The behaviour of such a consumer in the European model is determined by adding up potential dietary exposure to a food chemical at the 97.5th percentile of consumers of the two food categories that lead to the highest dietary exposure with the mean potential exposure for all other food categories (EFSA, 2008). The choice of the upper percentile of dietary exposure that represents a high consumer is, however, dependent on the purpose of the dietary exposure and the data available to the risk assessor and risk manager. The European high-consumer model has the advantage of being applicable to surveys for which only data on mean and high consumption of large food groups are available, without the need to have access to the raw data of individual dietary records. It can therefore be used on the basis of published data. This approach has usually been used by EFSA and more recently by JECFA for chronic dietary exposure assessments for additives where the food consumption data have been aggregated into fewer than 20 large food categories. The basic assumption of this model diet is considered valid if the number of food groups is limited.

Food consumption amounts and dietary exposures for high consumers can also be derived from distributional data. The percentile of distribution selected to represent a high consumer depends on the purpose of the dietary exposure assessment and the type of food consumption data available. For example, for chronic dietary exposure estimates based on 1 or 2 days of food consumption data per individual, the 90th percentile of dietary exposure for consumers (eaters) only is often used to represent a high consumer. Where more survey days of food consumption data are available such that average (mean) daily food consumption amounts over a period of time can be derived for each individual, the use of a higher percentile may be appropriate. For acute dietary exposure estimates for consumers of foods containing the food chemical, the 97.5th percentile is derived from multiple consumer days with no averaging across survey days for individuals (see section 6.2.2.3).

The derivation of high-percentile values needs to be undertaken with caution, first checking that there are a sufficient number of consumers of the foods containing the chemical to make the derivation valid. This can be a problem for infrequently consumed foods or where dietary exposure estimates for subpopulation groups are undertaken. In cases where the high-percentile value cannot be derived, food consumption data for the parent food group can be used instead of that for a single food, providing they are generally consumed in a similar way. For example, a 97.5th-percentile consumption of all root vegetables could be used for carrots in an acute dietary exposure assessment, if there were not enough carrot consumers. Alternatively, statistical methods can be used to construct a distribution curve from summary food consumption data (e.g. mean, standard deviation), from which a high percentile of food consumption can then be derived (Cullen & Frey, 1999).

Modelling dietary exposures for high consumers of a food chemical can be accomplished by conducting a full distributional analysis using Monte Carlo techniques (see section 6.3.5). Where adequate data are not available to conduct a distributional analysis, arbitrary factors may be incorporated in a point estimate to simulate the upper end of the distribution of food chemical exposure (e.g. by assuming that the distribution is lognormal, a factor of 2 or 3 might be applied to the mean to roughly estimate the dietary exposure of high consumers). Different assumptions may be appropriate when modelling acute and chronic dietary exposures, as the concentrations of the substances will not always be high.

(b) Regular consumers

The tendency of consumers to repeatedly purchase and consume the same food products, sometimes termed consumer loyalty, may need to be considered and a range of concentrations may need to be used to generate dietary exposure estimates to cover various scenarios of consumer behaviour. Thus, if a specific brand of processed food contains a high concentration of a substance, regular consumers of that brand would have higher dietary exposure to the substance than those consuming brands without, or with lower amounts of, the substance. Consideration of regular consumers may be relevant when assessing high chronic dietary exposure to food chemicals present in processed

foods, such as additives, including flavouring agents, processing aids or chemicals migrating from packaging (Arcella et al., 2003). The impact of regular consumption of a certain food is likely to be less important in the case of residues of pesticides or veterinary drugs, as there is frequent mixing of raw agricultural commodities before purchase by consumers. However, consumer behaviour in relation to food purchases may need to be taken into account in relation to the selection of organic versus non-organic foods or regional foods if pesticide and veterinary drug use varies. Consumer behaviour towards fortified and non-fortified foods may also need to be considered when assessing nutrient intakes.

6.3.4.3 *Further examples of point estimates using model diets*

Some examples of more refined point estimate models are summarized below.

(a) GEMS/Food consumption cluster diets

Data submitted on the priority contaminants/commodities in GEMS/Food (section 6.2.1.8) have been used to assess the potential risk to human health from such exposures (UNEP/FAO/WHO, 1988; WHO, 1989b; UNEP, 1992; Bhat & Moy, 1997; Schutz et al., 1998). In these assessments, the estimated dietary exposures determined for each country were compared, when possible, with relevant ADIs or provisional tolerable weekly intakes (PTWIs) established by JMPR and JECFA. GEMS/Food provides relevant information to JMPR, JECFA and CAC and its subsidiary bodies as appropriate.

The GEMS/Food consumption cluster diets are used as model diets by both JMPR and JECFA in chronic dietary exposure assessments (see section 6.2.2.5 for more detailed information on the diets; WHO, 1989a). Since 1996, following the recommendations of a Joint FAO/WHO Consultation held in York, England (FAO/WHO, 1995b), the dietary exposure estimates of pesticide residues undertaken by JMPR use STMR levels in the calculation of international estimated daily intakes (IEDIs). JMPR uses this procedure in a single-step approach, using the best available information, rather than the stepwise approach adopted for some other food chemicals. Whenever possible, residues are estimated for the edible portion. This may require the use of processing

factors and data on consumption of processed food. Although it is appropriate to correct for the edible portion if the commodity is always prepared in the same way, care should be taken with processes such as peeling, where it is often assumed that the commodity is always peeled before consumption, whereas in reality this is not true.

One of the principles for international exposure assessment is that the underlying data should be conservative. The GEMS/Food diets fulfil these requirements as long as a significant proportion of the commodities containing the food chemical is included in the diets. The FAO food balance sheet data, which form the basis of the consumption cluster diets, tend to overestimate mean food consumption for the population, as they report food available for consumption. However, because the calculation of per capita mean food consumption divides the amount of food available for consumption in a country or region by the whole population (consumers of foods and non-consumers), the consumption cluster diets tend to underestimate food consumption for consumers of specific foods. The consumption cluster diets were not intended to represent high consumers, although a correction factor can be applied to mean consumption amounts to approximate the high percentiles of dietary exposure (WHO, 1985).

(b) Total diet studies (TDSs)

TDSs are designed to assess chronic dietary exposure to food chemicals using the amounts of chemicals in food actually ingested by the population living in a country and, if possible, population subgroups (WHO, 1992). This is accomplished by measuring chemical concentrations in food "as consumed", including drinking-water. Although the traditional focus of TDSs has been on assessing dietary exposure to pesticide residues and contaminants, the advent of multielement analyses has seen TDSs increasingly include selected nutrients. TDSs have also been used for estimating dietary exposure to food additives. TDSs differ from other chemical surveillance or monitoring programmes because they aim to assess dietary exposure to food chemicals across the total diet in one study. If conducted on a regular basis, TDS results can provide a continuous means of checking the effectiveness of regulatory measures that have been established to control the levels of chemicals in the food supply, as well as monitor trends in dietary exposures.

The majority of TDSs worldwide use the point estimate (deterministic) approach to assess mean dietary exposure for a whole population. In some studies, high-consumer dietary exposures are estimated by applying specified factors to mean consumption data (WHO, 1985). Estimates for specific population subgroups (e.g. infants or young children) can also be determined if food consumption data are available. Some countries combine distribution of food consumption data at an individual level with one fixed value for the concentration of the chemical in the TDS foods or food groups (FSANZ, 2003; FSA, 2004; Leblanc et al., 2005). TDSs are not suitable for the assessment of acute dietary exposures because of the high degree of compositing of samples.

6.3.4.4 Specialized studies designed to answer specific questions

If necessary, studies may be designed to answer specific questions about consumer dietary exposure. The study may measure exposure directly or may provide additional information about one or more parameters of the exposure assessment algorithm. Examples of specialized studies are given below.

(a) Selective studies of individual foods

In some cases, surveys that encompass the whole diet, such as a TDS, may not be necessary. Surveys of specific foods are particularly useful if the dietary exposure to a chemical is predominantly influenced by one, two or a limited range of foods or when food surveillance or monitoring has already established average chemical concentrations in the foods (WHO, 1985). For example, mercury in fish and seafood, persistent organic pollutants (POPs) in fat-containing foods (van Zoonen in WHO, 2002a; Baars et al., 2004), mycotoxins (Leblanc et al., 2005), additives (Chen in WHO, 2002a; Yoon in WHO, 2005a) and veterinary drugs would all generally be best approached via a selected individual foods approach.

(b) Duplicate portion studies

Duplicate portion studies may also be used to assess dietary exposures for population subgroups, as they provide dietary exposure information at the individual level, based on the diet "as consumed". This can be especially useful for well-defined population subgroups, such

as vegetarians (MAFF, 2000; Clarke et al., 2003), children (Wilhelm et al., 2002; Murakami et al., 2003), breastfeeding mothers (Gulson et al., 2001), adult women (Tsuda et al., 1995) or people who consume catering establishment meals (Leblanc et al., 2000). However, such studies are very costly in terms of participant involvement and management and are used for small groups of people only (IPCS, 2000). Nonetheless, such a study can be very useful, in that it can provide an estimate of total dietary exposure that can be used as a benchmark for estimating the degree of overestimation or underestimation of exposure when assessments are conducted with more limited data. For example, in the early evaluations of dietary exposure to acrylamide, a TDS conducted by the Swiss government (Swiss Federal Office of Public Health, 2002) provided an estimate of total exposure that was used to assess whether the foods that had already been analysed were those that represented the most important sources of acrylamide or whether other significant sources remained to be identified.

6.3.5 Refined dietary exposure assessments (probabilistic distributional analyses)

If the existence of a safety concern cannot be ruled out on the basis of dietary exposure assessed at the initial steps, more accurate assessments of dietary exposure may be needed. It should be emphasized that the consumer exposures are not altered; rather, the accuracy with which those exposures are estimated is improved by using more refined methods. Probabilistic analysis gives more information on the variability in dietary exposure estimates across the population of interest for use by risk assessors and risk managers. It is noted that a probabilistic approach would not necessarily give a lower dietary exposure estimate than the deterministic approach.

Refinements could include more defined information about the foods that are consumed (less conservative assumptions about the amounts consumed, the concentrations of the chemical in the foods, impact of processing and food preparation, etc.), or more complex exposure assessment models can be employed that allow more realistic simulation of consumer practices.

Nonetheless, further steps to allow the refinement of the dietary exposure assessment should be designed in such a way that potential

high dietary exposures to a specific chemical are not underestimated. The methods should take into consideration non-average individuals, in particular those who consume large portions of specific food items or are loyal to those foods containing the highest concentration of the chemical of interest and those who have low or infrequent consumption of foods with very high concentrations of the chemical of concern.

For the models to be accurate, the food consumption data and food chemical concentration data should be for the same food products (see section 6.2.2.3). Good estimates are derived from good data, and a complex or complete model will not transform insufficient or deficient data into good data. Additional data may need to be collected to adequately represent the actual exposure situations.

6.3.5.1 *Overview of probabilistic estimates of exposure*

For substances requiring further refinement beyond screening methods or point estimates of exposure (as described above), a probabilistic analysis of exposure variability can be conducted. Conceptually, population exposure must be thought of as a range of values, rather than a single value, because individual members of the population experience different levels of exposure. Factors that contribute to this variability include age (due to differences in body weight and the type and amount of food consumed), sex, ethnicity, nationality and region, and personal preferences, among others. Variability in dietary exposure is often described using a frequency plot (see Figure 6.2). Sometimes, the frequency distribution is approximated as a continuous probability distribution (see Figure 6.3). In both cases, the horizontal axis corresponds to the level of exposure, and the vertical axis corresponds to the relative proportion of the population.

The variability distribution can be characterized by referring to representative members of the population. For example, the median individual has an exposure at the middle of the distribution (i.e. half of the population has exposures that are less than that of the median individual, whereas the other half has exposure levels exceeding that of the median individual). The 95th-percentile individual has an exposure that exceeds the levels experienced by 95% of the population. The average or mean exposure does not necessarily represent any particular individual. Instead, it is computed by summing the exposures of all individuals and dividing by the size of the population.

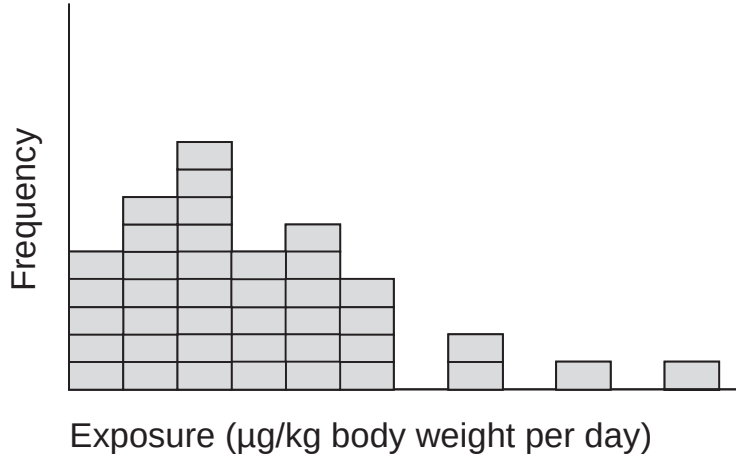

Fig. 6.2. Frequency distribution

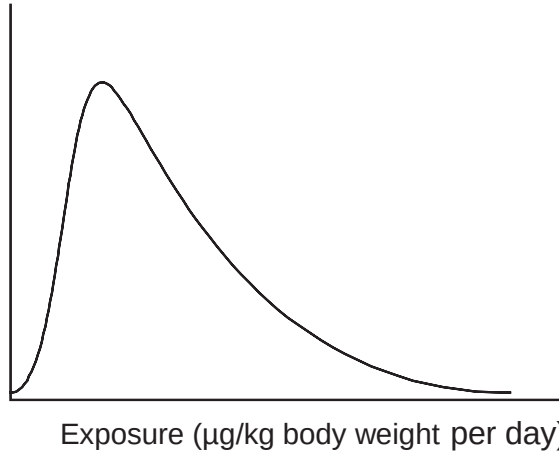

Fig. 6.3. Continuous probability distribution

Section 6.3.5.3 discusses some of the models that are available for conducting probabilistic assessments. Finally, in those cases warranting the greatest level of scrutiny, so-called two-stage simulation techniques can be used to characterize both uncertainty and variability (see chapter 7, section 7.2.2). In all instances, adequate data must be available to allow meaningful assessment.

6.3.5.2 *Probabilistic models*

The structure of a probabilistic model is similar to that of the deterministic models described previously in section 6.3.4, in that it is based on the same basic equations whereby food consumption data are combined with concentration data to estimate dietary exposure. The fundamental difference is that at least one variable is represented by a distribution function instead of a single value and the model sample from each distribution is a distribution of potential dietary exposures generated using several thousand iterations. As for point estimate models, it may be possible to further refine probabilistic models by taking account of factors such as edible portion, percentage crop treated or consumer behaviour, where appropriate to do so (see section 6.3.4.2). Simple probabilistic models may account for the food chemical in only a single food, but more complex models can include the possibility that a person may consume several foods containing the food chemical in a single meal or day. The following text is a discussion of approaches to developing probabilistic models for dietary exposure assessments.

(a) Simple empirical distribution estimate

Dietary exposure assessments can be based on a food consumption distribution determined empirically from a food consumption survey and a single point estimate to represent the chemical concentration in the relevant food product. Each point of the distribution curve of food consumption can be multiplied by the concentration in the relevant food commodity. Conversely, it is possible to have a single point estimate for consumption and an empirical distribution of chemical concentrations in that food.

(b) Developing probabilistic models from data sets

This approach requires data sets representing the distribution of concentrations in each relevant food category and also distributions of consumption for the same food categories for the population of interest. It explicitly takes into account the variability of input data, providing a more realistic result than that produced by simple deterministic or simple empirical distribution scenarios, which generally are constrained by conservative default assumptions when a single value is selected to represent the entire distribution.

There are two general approaches to developing distributions for use in a probabilistic assessment. Non-parametric techniques can be used when actual data sets are available for a parameter. In these cases, the data sets can be assumed to represent the distribution of interest. The probabilistic assessment is implemented by randomly selecting one of the values from the data set for each iteration of the simulation. For example, if a data set with 100 concentration measurements contains two observations of 5 mg/kg, then the probabilistic assessment will effectively assume that there is a 2% frequency of the concentration being equal to this value.

Parametric techniques interpolate among the data points and extrapolate beyond them by assuming a particular distributional form. For example, standard techniques can be used to fit a normal, lognormal or any other type of distribution to a data set. Although the extrapolation "fills in" gaps that may be particular to a specific data set, the elimination of these gaps comes at the cost of requiring an assumption to be made as to the functional form of the distribution. The assessor can evaluate the impact of the assumption by repeating the analysis assuming alternative (but plausible) functional forms.

Other methods, including iterative simulation methods, have been used in exposure assessment modelling but are beyond the scope of this chapter. In general, the primary differences in the techniques are the methods that are employed to draw values from the data and in the evaluation of uncertainty and variability. Simple risk assessment models of the multiplicative form may be appropriate for a variety of exposure assessments (Slob, 1994).

(c) Stratified sampling

A stratified sampling method is a way of selecting data to ensure that the probabilistic model selects values at regular intervals throughout each distribution of the food consumption and concentration data. For example, the mean or median of each quartile of each distribution may be determined. The primary disadvantage of the single-stratum calculation is that it produces no estimates for extreme values. This problem may be ameliorated, but never entirely overcome, by using more strata (e.g. estimating the mean of each decile instead of estimating a value for each quartile). Detailed, accurate and reproducible characterizations of the output distributions may be obtained by using many strata. The difficulty with stratified sampling is that the number

of iterations required may become very large and may require additional computer software or computer expertise.

(d) Random sampling (Monte Carlo simulation)

Monte Carlo simulation involves the use of random numbers to select values from the input distributions. The technique has been applied to a wide variety of modelling scenarios. As a result, it can be concluded that when conducted appropriately (e.g. with appropriate data and when the simulation is conducted with a sufficiently large number of "iterations"), the results will simulate the actual situation, because the technique utilizes values throughout the range of each input distribution. Because the sampling is random, there is the possibility that the Monte Carlo simulation will be inaccurate at the extreme (upper, lower) ends of a distribution, which is particularly true if using parametric distribution rather than non-parametric (empirical) distribution data. In such a case, when using a parametric approach for contamination data, a cut-off limit in the distribution tail in regard to a "realistic" maximum observed value in selected foods may be introduced to avoid taking "unrealistic" contamination events that would never occur in real life into account in the model.

(e) Latin hypercube

Latin hypercube is a statistical method that is essentially a hybrid of the stratified and random sampling methods. Distributions are divided into strata, and then random samples are drawn from each stratum in order to ensure that the iterations are balanced throughout the range of each concentration and food consumption data distribution. This method also allows for some samples to be drawn at the extremes of the distributions.

6.3.5.3 Applicability of a probabilistic approach at the international level

Probabilistic models are increasingly being considered at national and international levels. For example, the United States Environmental Protection Agency uses this approach for acute dietary exposure estimates for pesticide residues (USEPA, 1998, 2000a). In Europe, there have been projects that outline potential models (EU Monte Carlo project, http://montecarlo.tchpc.tcd.ie/, and the data sets available for use in the models; SAFE FOODS, http://www.safefoods.nl/default.aspx).

At an international level, time and resources should be dedicated to the application of probabilistic methodology only when there is a dietary exposure concern that cannot be refined using simpler and less resource-intensive methods. Where this is the situation, it may be useful to evaluate probabilistic exposure estimates derived for a representative selection of national populations to arrive at an understanding of the international situation.

It may be more feasible in many cases to refine the point estimate of dietary exposure than to use a probabilistic method as described in section 6.3.4.2. For example, for contaminants and pesticide and veterinary drug residues, the dietary exposure assessment may be refined by incorporating processing factors that adjust the initial concentration data to reflect the impact of processing (rice → polished rice; fruit → peeled fruit; potato → cooked potato). Likewise, the consumption data can be refined to provide estimates of dietary exposure of different forms of the food (raw, processed).

6.3.6 Specific considerations for modelling approaches for acute and chronic dietary exposure assessments

Different methods for conducting dietary exposure assessments may need to be selected based on the length of exposure times required to elicit the toxic or beneficial effects. Two time frames—chronic (long-term) and acute (a single meal or over a whole day)—have been considered for some assessments at the international level and by some national governments. These time frames are discussed below; however, it should be noted that these are arbitrary, and other lengths of time may be more appropriate for some chemical substances. Different assumptions will be appropriate when modelling acute and chronic exposures.

6.3.6.1 *Chronic dietary exposure assessments*

Typically, toxicological studies carried out to examine the adverse health effects resulting from consumption of a chemical substance in the diet are completed over a long period of time (e.g. several months or a substantial portion of the lifespan of test animals). Adverse effects generally arise at lower dose levels following long-term exposure to the substance being studied. Exposure assessments conducted to be comparable have been termed chronic dietary exposure assessments.

Typically, a mean dietary exposure will be compared with a chronic (long-term) health-based guidance value (e.g. ADI, PTWI). The mean dietary exposure may be calculated by applying a deterministic model using average food consumption levels and the average concentrations in the relevant food products. Where desired, it is possible to also conduct this assessment using parameters that will compute the dietary exposure of consumers with high exposure. Where data are not available, as a rough approximation, exposures of individuals with high consumption can be estimated by using a fixed factor of multiplication to simulate an upper percentile.

For a chemical with long-term effects, the mean chemical concentration is typically used, assuming that this value represents the long-term average of truly encountered concentrations. In some cases, the median concentration may be selected (see section 6.2.1.4). This value (mean or median) is combined with high percentiles or with the full distribution of food consumption. In the case of a non-staple food (i.e. a food not typically consumed every day by most consumers), high-percentile estimates assessed for the whole population may be low owing to the fact that a large number of non-consumers are included. In this case, high-percentile estimates should be assessed in consumers only rather than in the whole population, in order to avoid underestimation of high levels of exposure. However, one must bear in mind that high levels of exposure assessed on the basis of a short-duration survey in consumers provide an overestimate of high levels of exposure over the long term (IEFS, 1998; Tran et al., 2004; see section 6.2.2.4 for details on how statistical adjustments can be made to correct the food consumption data for "usual" consumption patterns).

If this first point estimate for dietary exposure is below the health-based guidance value, further refinement steps are not necessary, and the chemical is unlikely to be of safety concern. However, when the initial screening results in an estimate of dietary exposure close to or above the health-based guidance value, a more accurate assessment will usually be necessary.

6.3.6.2 *Acute dietary exposure assessments*

In the early 1990s, it became apparent that, in some cases, residues of a chemical substance could pose risks due to a single or at most a few days of exposure.

Two developments focused attention on acute dietary exposure assessments. First, as chronic dietary exposure methodology has improved, there has been a move away from "worst-case" estimates of chronic dietary exposures. Whereas in the past there were always large conservative assumptions to account for lack of data, now, with more data available, the chronic dietary exposure assessments are more realistic. This has directed more attention to a greater need for an explicit consideration of acute dietary exposure. Secondly, research on residues of acutely toxic pesticides (organophosphates and carbamates) in individual fruits and vegetables revealed random occurrences of comparatively high residue levels. Some individuals who consume significant amounts of such foods will occasionally eat the "hot" commodity unit. Acute dietary exposure assessments may be deterministic (point values) or distributional (probabilistic or stochastic). At an international level, a deterministic methodology was developed to address the calculation of the acute dietary exposure (Hamilton & Crossley, 2004).

(a) Pesticide residues

The FAO/WHO Consultation held in Geneva in 1997 (FAO/WHO, 1997) recommended a procedure for performing acute dietary exposure assessment for compounds for which an acute reference dose (ARfD) was established (see chapter 4, section 4.4). This was followed by the International York Consultation (MAFF, 1999) and the ad hoc Expert Meeting held before the 1999 CCPR session (see Annex V of FAO/WHO, 1999b) that further developed the method. Although it was recognized that probabilistic modelling would provide the most refined estimate, it was also recognized that this would be difficult at the international level, and a simpler method was developed. At its 1999 meeting (FAO/WHO, 1999b), JMPR performed acute dietary exposure assessments for the first time, by calculating IESTI. For compounds with low acute toxicity, JMPR concluded that "an ARfD is unnecessary" and that assessing the acute exposure is irrelevant. In the IESTI method, the estimates are performed for each crop separately, as it is considered that it would be unlikely that an individual will consume, within a meal or 24 h, two different commodities of LP weights that contain the same pesticide at the highest residue level. This methodology has been further refined by subsequent JMPR meetings, and the equations used by JMPR are shown in appendix 6.1. Figure 6.4 shows the decision tree for acute dietary exposure assessment, which could be applied to any food chemical with an ARfD.

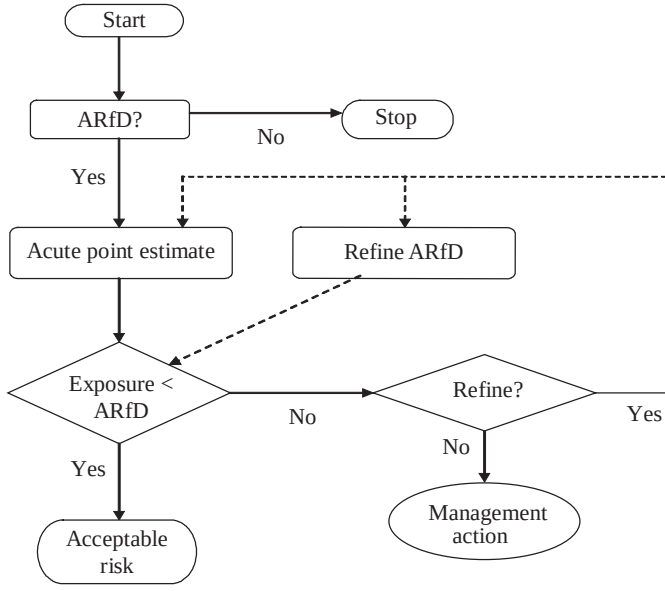

Fig. 6.4. Decision tree for acute dietary exposure assessment

(b) Veterinary drug residues

For veterinary drug residues, some of which may also represent an acute hazard, the manner in which MRLs are established ensures that the ADI (which may be based on an acute effect if it is produced at lower doses than are chronic effects) in general is not exceeded. Substances with acute pharmacological or toxicological properties are of concern and include classes such as beta-blockers, beta-agonists, anaesthetics, tranquillizers, vasodilators and compounds that may trigger acute hypersensitivity reactions (e.g. penicillins).

There is also a potential concern that even though the model diet used by JECFA (see section 6.3.4.1) is considered to be rather conservative and would therefore be sufficient to use for an acute dietary exposure, in some cases it may not be adequate. For example, when these daily food consumption amounts were compared with the values that JMPR uses in its acute dietary exposure assessments, based on the highest available 97.5th percentile of consumption from six countries (WHO, 2004), it was found that in some cases the food consump-

tion amounts in the model diet were lower than the 97.5th percentile amount, and hence use of the diet may in fact underestimate the acute dietary exposure for that food. In cases where an ARfD for a veterinary drug residue has been set, specific exposure scenarios are used instead of the model diet (e.g. for assessment of injection site residues).

Although the procedures for establishing MRLs appear to deal adequately with drug residues of the acutely toxic compounds in the principal edible tissues noted previously (see section 6.3.4.1), JECFA and CCRVDF are developing guidelines for injection site residues. These residues pose the potential problem of exceeding the health-based guidance value even when residues in other tissues are at or below their MRLs.

(c) Contaminants and food additives, including flavourings

For contaminants, when the toxicological evaluation indicates a need for an acute dietary exposure assessment, the case 1 IESTI calculation can be used (see appendix 6.1 for details of the calculation), with the GEMS/Food value for the highest reported 97.5th percentile of consumption (WHO, 2004).

For most food additives and flavourings, no acute toxicity occurs at the doses used as the basis for deriving health-based guidance values for the potential levels of human exposure, and therefore no acute dietary exposure assessments are needed. Occasionally, acute intolerance reactions may be relevant, such as laxation from polyol sweeteners. For some chemicals, allergic reactions may sometimes be of concern, but there are currently no clear health-based guidance values for allergic reactions to use in evaluating the significance of acute exposures. Research is under way to allow the identification of thresholds for allergenicity of a variety of food allergens.

6.3.7 Aggregate/cumulative exposures

Historically, the safety of food additives and residues of pesticides and veterinary drugs and the risk of chemical contaminants have been evaluated on the basis of single-chemical and single-exposure pathway scenarios. That is, risk assessors generally performed risk assessments and risk managers developed management options by examining each chemical exposure scenario separately. In general,

exposures to a chemical through the food, drinking-water and residential/occupational pathways were each assessed independently, and no concerted effort was made to evaluate potential exposures through multiple pathways simultaneously. This problem is often exacerbated because the responsibility for these different routes of exposure resides in different parts of national governments and international organizations.

Although different chemicals may act by the same mechanism and produce the same effect (e.g. organophosphate pesticides and acetylcholinesterase [AChE] inhibition), in the past, consideration was seldom given to the fact that exposure to multiple chemicals could occur and that the toxicological effects might be additive or synergistic (see sections 4.13 and 7.3 in chapters 4 and 7, respectively). For example, although two pesticides might act by a common mechanism of toxicity (e.g. AChE inhibition) and exposure on any given day might result in additive effects, standard or traditional exposure assessment methodologies did not consider this potential.

This concern was recognized in 1993 in a report issued by the United States National Research Council entitled *Pesticides in the Diets of Infants and Children* (USNRC, 1993). Subsequently, similar reports were issued by the United Kingdom Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (FSA, 2002), the Health Council of the Netherlands (2004), Boon et al. (2004) and EFSA (2007). These reports made several recommendations on how to improve the assessment of health risks posed by pesticides in the diets of infants and children. One recommendation was that consideration be given to all sources of dietary and non-dietary exposures to pesticides. Consideration of combined exposures to a single chemical across multiple routes (oral, dermal, inhalation) and across multiple pathways (food, drinking-water, residential) is known as *aggregate exposure*. The reports also recommended that consideration be given to the assessment of risks from exposure to multiple pesticide residues that have a common mechanism of toxicity. This consideration of combined exposures associated with multiple chemicals that act by a common mechanism is termed *cumulative exposure*.

This issue of aggregate and cumulative risk assessments was also recognized and discussed during an FAO/WHO Consultation held in

Table 6.5. Scenarios and the range of exposure assessments[a]

Toxic concern	Exposure route	Assessment type
Single chemical	Single food	Dietary assessment
	Multiple foods	Aggregate dietary assessment
	Multiple media	Aggregate assessment
Multiple chemicals with the same mechanism of action	Single food	Dietary assessment
	Multiple foods	Cumulative dietary assessment
	Multiple media	Cumulative assessment

[a] Table modified from that appearing in the original report (FAO/WHO, 1997) to clarify naming conventions.

Geneva during 1997 (FAO/WHO, 1997). Specifically, the Consultation noted that exposures to food chemicals through other routes may occur and that exposures to chemicals or drugs sharing the same mechanism of action (toxicity) may also be encountered. These scenarios and the range of exposure assessments that can be developed, as summarized at the meeting, are shown in Table 6.5.

The method for estimating cumulative dietary exposure to chemical substances with a common mechanism of toxicity could be considered at the international level regardless of the development of probabilistic methods. One of the approaches in cumulative risk assessment for specific chemicals is to use a toxic equivalency factor (TEF). These factors, representing the toxicities of individual substances relative to an "index compound", are applied to the concentration data of each substance within a group with a common mechanism and a total exposure is calculated, expressed in terms of the index compound. This approach was used by JMPR for dithiocarbamates (FAO/WHO, 1999a) and by JECFA (WHO, 2002d) for chlorinated dibenzo-*p*-dioxin congeners. Different compounds have been used as the index compound for the AChE insecticides, including chlorpyriphos, methamidophos and acephate. The choice of the index compound, however, is not trivial and will greatly depend on the toxicity database available and the toxicological end-point used. Ideally, data on the concentrations of substances in food should be collected in a manner that determines the co-occurrence of residues, but such data may not always be available at the international level.

Guidance for estimating aggregate exposure and for performing cumulative risk assessments has been issued by IPCS (2009), EFSA (2007) and USEPA (2001, 2002).

6.3.8 Biomarkers of exposure

Biomarkers include a broad class of biological changes to the body that are measurable, subclinical and reversible (Grandjean, 1995). These terms are further described by USNRC (1987) and include biomarkers of exposure—i.e. "agents or their metabolites either in tissues, secreta, excreta, expired air or any combination of these" (Berlin et al., 1984) that can be independently used to quantify overall exposure to a substance. Examples of biomarkers of exposure include the concentration of lead in blood (µg/dl blood), the concentration of mercury in either blood (µg/l blood) or hair (µg/g hair) and the concentrations of pesticides or their metabolites in serum, fat, urine, blood or breast milk (Anwar, 1997; USCDC, 2003, 2004).

Biomarkers of exposure do not depend on food consumption and substance concentration data; because they are "downstream" from consumption and hence causally closer to the health effects of interest, they represent a measure of exposure that is potentially more appealing than conventional measures of exposure expressed as estimated dietary exposures or intakes. Perhaps the greatest challenge associated with the use of biomarkers of exposure is interpreting their public health significance and particularly their quantitative relationship to adverse health effects, because data on the same biomarker are rarely available for both toxicity studies and exposure estimations. Biomarkers can be used effectively to evaluate whether a control measure has successfully altered the level of exposure in a population (Schulte & Waters, 1999) or to compare one consumer group with another non-exposed subpopulation. On the other hand, it is often difficult to characterize the relationship between biomarker levels and health risk.

A second challenge associated with the use of biomarkers relates to source attribution. Because biomarkers are integrative measures of exposure, they do not distinguish between alternative sources of exposure (Aitio & Kallio, 1999). For example, exposure to polycyclic aromatic hydrocarbons (PAHs) not only is via the diet but also can result from smoking (or being in the vicinity of smokers), coal tar

treatments and occupational activities (e.g. road paving and work near coke ovens) (Strickland et al., 1996). Even among individuals with no apparent notable exposure to PAHs, PAH metabolites have been detected in urine, albeit at low levels (Strickland et al., 1996).

Relating changes in biomarker levels to changes in exposure is further complicated by analytical considerations (Aitio & Kallio, 1999). With measurement of the parent compound (e.g. benzene or lead in blood, mercury in hair or blood), specificity is precise. However, whereas some metabolic products are relatively specific (e.g. methylhippuric acids in the case of exposure to xylene, or mandelic acid in the case of exposure to styrene or ethylbenzene) (Aitio & Kallio, 1999), in other cases specificity is limited. For example, phenol or hippuric acid concentrations in urine can be used as indicators of exposure to benzene or toluene, respectively, but these metabolites may also be generated by exposure to other parent compounds (Aitio & Kallio, 1999).

Differences in biomarker persistence pose an additional challenge to their use. Although some biomarkers (e.g. bone lead concentrations) have a half-life of many years, others, such as the concentration of contaminants in blood, typically have much shorter half-lives. For example, the half-life of mercury in blood is approximately 60 days (Aitio & Kallio, 1999). In these cases, representative measurements of exposure depend on more frequent monitoring. In some extreme examples, such as urinary iodine, the half-life is in the order of hours (Wild et al., 2001). In these cases, characterizing exposure for an individual would require multiple measurements in a single day. Measurement results for a group of individuals (taken at different times of the day) might be interpreted as representing the distribution of biomarker levels for the population, even though such measurements are not adequate for the purpose of characterizing individual levels of exposure.

Finally, even if a biomarker with a long half-life is available, it is not always the case that it is the most relevant measure of exposure for the purpose of risk assessment. Exposure measured as the product of the average rate of exposure and time is thought to be the most relevant measure of exposure in some cases. The assumption that toxicity depends on this exposure measure is known as Haber's Law

(Weller et al., 1999). On the other hand, some acutely toxic effects may instead depend on the magnitude and frequency of peak exposure levels (Lauwerys et al., 1995). In this case, levels of biomarkers with long half-lives may offer a misleading characterization of risk.

Human milk is a unique biological matrix for monitoring certain environmental contaminants, because it can provide exposure information about both the mother and the breastfed infant through a non-invasive method of collection. For some chemicals, levels in milk can provide an integrated assessment of exposure from multiple foods and multiple media. Although human milk is the natural food for infants, with the optimal composition to meet their nutritional needs in early life and providing associated immunological, psychological and economic advantages (WHO, 2002c), it has been unintentionally compromised by chemicals from our environment. Nevertheless, the mere presence of an environmental chemical in human milk does not necessarily indicate a health risk for breastfed infants.

POPs in human milk are good examples of exposure biomarkers, as POPs are known to accumulate in the food-chain. Consequently, human milk monitoring can yield information about the kinds and quantities of POPs in the environment as well as in our bodies. Better understanding of our exposure to harmful environmental chemicals will help us to better manage them by eliminating or reducing their emissions or by limiting their presence in the food supply.

Over the past several decades, GEMS/Food, whose interest is in international studies on levels of contaminants in food, has collected information on the levels and time trends of many POPs in food, including human milk (e.g. WHO, 1989b, 1996; Van Leeuwen & Malisch, 2002). Results have shown a variety of contamination profiles, indicating different sources of exposure. Consistent with dietary exposure assessments submitted to GEMS/Food prior to 1992 and risk assessments of certain organochlorine compounds in human milk performed in 1998, basic monitoring and assessment programmes in all countries for organochlorine compounds in food and human tissues are essential in order to appropriately protect public health from these risks.

In summary, the use of biomarkers of exposure offers some advantages over conventional estimates of exposure measured in terms of

food consumption and food concentration. Biomarkers integrate exposure over time from multiple sources. Moreover, they can be measured directly and hence do not rely on mathematical models developed using multiple assumptions, with their attendant uncertainties, to estimate exposure. In a causal sense, they are also "closer" to adverse health effects of interest than are other types of exposure estimates. On the other hand, their interpretation is complicated by the fact that data on toxicity end-points related to different levels of the biomarker are generally unavailable. In addition, because of their integrative nature, it can be difficult to attribute changes in biomarker levels to a particular exposure source, or in some cases even to a particular substance. Finally, the use of biomarkers can be complicated if their half-life is short.

6.4 References[1]

Aitio A & Kallio A (1999) Exposure and effect monitoring: a critical appraisal of their practical application. Toxicol Lett, **108**: 137–147.

Anwar WA (1997) Biomarkers of human exposure to pesticides. Environ Health Perspect, **105**(Suppl 4): 801–806.

Arcella D, Soggiu ME & Leclercq C (2003) Probabilistic modelling of human exposure to intense sweeteners in Italian teenagers: validation and sensitivity analysis of a probabilistic model including indicators of market share and brand loyalty. Food Addit Contam, **20**(Suppl): S73–S86.

Australian Bureau of Statistics (2000) Australian apparent consumption of foodstuffs, 1997–98 and 1998–99. Canberra, Australian Bureau of Statistics (http://www.ausstats.abs.gov.au/ausstats/subscriber.nsf/0/3CDE7A3E9BEF68F3CA256982007E8CD6/$File/43060_1997-98%20and%201998-99.pdf).

Baars AJ, Bakker MI, Baumann RA, Boon PE, Freijer JI, Hoogenboom LAP, Hoogerbrugge R, Van Klaveren JD, Liem AKD & de Vries J (2004) Dioxin, dioxin-like PCBs and non-dioxin-like PCBs in food stuffs: occurrence and dietary intake in the Netherlands. Toxicol Lett, **151**: 51–61.

Barlow SM (1994) The role of the Scientific Committee for Food in evaluating plastics for packaging. Food Addit Contam, **11**: 249–259.

Berlin A, Yodaiken RE & Henman BA (1984) Assessment of toxic agents at the workplace: roles of ambient and biological monitoring. The Hague, Marinus Nijhoff.

[1] Internet links provided in these references were active as of the date of final editing.

Bhat RV & Moy GG (1997) Monitoring and assessment of dietary exposure to chemical contaminants. World Health Stat Q, **50**(1–2): 132–149.

Boon PE, Nij EIMT, Koopman N & Van Klaveren JD (2004) Dietary habits and exposure to pesticides in Dutch infants. Wageningen, RIKILT Institute of Food Safety.

Brunner E, Stallone D, Juneja M, Bingham S & Marmot M (2001) Dietary assessment in Whitehall II: comparison of 7d diet diary and food-frequency questionnaire and validity against biomarkers. Br J Nutr, **86**: 405–414.

Brussard HH, Lowik MRH, Steingrimsdottir L, Moller A, Kearny J, De Henauw S & Becker W for the EFCOSUM Group (2002) A European food consumption survey method—conclusions and recommendations. Eur J Clin Nutr, **56**(Suppl 2): S89–S94.

Cadby P (1996) Estimating intakes of flavouring substances. Food Addit Contam, **13**: 453–460.

Carriquiry AL (2003) Estimation of usual intake distributions of nutrients and foods. J Nutr, **133**: 601–608.

Carriquiry AL, Goyeneche JJ, Fuller WA & Jensen HH (1995) Estimated correlations among days for the combined 1989–91 CSFII. Ames, IA, Iowa State University, Center for Agricultural and Rural Development, September (Staff Report 95-SR 77).

Carter RL, Sharaugh CO & Stapell CA (1981) Reliability and validity of the 24-hour recall. J Am Diet Assoc, **79**: 542–547.

Clarke DB, Barnes KA, Castle L, Rose M, Wilson LA, Baxter MJ, Price KR & DuPont MS (2003) Levels of phytoestrogens, inorganic trace-elements, natural toxicants and nitrate in vegetarian duplicate diets. Food Chem, **81**(2): 287–300.

Commonwealth of Australia (2008) 2007 Australian National Children's Nutrition and Physical Activity Survey: main findings. Canberra, Commonwealth of Australia (http://www.health.gov.au/internet/main/publishing.nsf/Content/66596E8FC68FD1A3CA2574D50027DB86/$File/childrens-nut-phys-survey.pdf).

Counil E, Verger P & Volatier J-L (2005) Handling of contamination variability in exposure assessment: a case study with ochratoxin A. Food Chem Toxicol, **43**: 1541–1555.

Cullen AC & Frey HC (1999) Probabilistic techniques in exposure assessment. New York, NY, Plenum Press.

Dabeka RW, McKenzie AD & Bradley P (2003) Survey of total mercury in total diet food composites and an estimation of the dietary intake of mercury by adults and children from two Canadian cities, 1998–2000. Food Addit Contam, **20**: 629–638.

Deharveng G, Charrondiere UR, Slimani N, Southgate DAT & Riboli E (1999) Comparison of nutrients in the food composition tables available in the nine European countries participating in EPIC. Eur J Clin Nutr, **53**: 60–79.

Douglass JS, Barraj LM, Tennant DR & Long WR (1997) Evaluation of the budget method for screening food additive intakes. Food Addit Contam, **14**: 791–802.

EC (1998) Report on methodologies for the monitoring of food additive intake across the European Union. Report of a working group on scientific co-operation on questions relating to food. Task 4.2. Luxembourg, Office of Publications of the European Communities (SCOOP/INT/REPORT/2).

EC (2002) Commission Directive 2002/72/EC of 6 August 2002 relating to plastic materials and articles intended to come into contact with foodstuffs. Off J Eur Union, **L220**: 18 (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2002L0072:20080327:EN:PDF).

EC (2003) Opinion of the Scientific Committee on Food on glycyrrhizinic acid and its ammonium salt (opinion expressed on 4 April 2003). Brussels, European Commission, Scientific Committee on Food (SCF/CS/ADD/EDUL/225 Final; http://ec.europa.eu/food/fs/sc/scf/out186_en.pdf).

EC (2004) Monitoring of pesticide residues in products of plant origin in the European Union, Norway, Iceland and Lichtenstein, 2002 report. Brussels, European Commission, Health & Consumer Protection Directorate-General (SANCO/17/04 final; http://ec.europa.eu/food/fs/inspections/fnaoi/reports/annual_eu/monrep_2002_en.pdf).

EFSA (2004) Statement of the European Food Safety Authority's Scientific Panel on Food Additives, Flavourings, Processing Aids, and Materials in Contact with Food (AFC Panel) on estimation of intakes in the course of the safety assessment of chemically defined flavourings (expressed on 13 July 2004). In: Minutes of the 7th plenary meeting of the AFC Panel held in Brussels on 12–13 July 2004. Parma, European Food Safety Authority, pp 10–11 (EFSA/AFC/FLA/GEN/01-Rev2; http://www.efsa.europa.eu/cs/BlobServer/Event_Meeting/afc_minutes_07_en1.pdf?ssbinary=true).

EFSA (2006) Guidance of the Scientific Committee on a request from EFSA related to uncertainties in dietary exposure assessment. Request No. EFSA-Q-20040-019. Adopted on 14 December 2006. EFSA J, **438**: 1–54 (http://www.efsa.europa.eu/EFSA/Scientific_Opinion/sc_op_uncertainty%20exp_en.pdf).

EFSA (2007) Cumulative risk assessment of pesticides to human health: the way forward. EFSA Scientific Colloquium 7. Summary report. Parma, European Food Safety Authority (http://www.efsa.europa.eu/EFSA/Scientific_Document/comm_colloque_7_en.pdf).

EFSA (2008) Guidance document for the use of the Concise European Food Consumption Database in exposure assessment. Parma, European Food Safety Authority (http://www.efsa.europa.eu/en/datexfooddb/document/Coincise_database_guidance_document_and_annexes,0.pdf).

Egan SK, Tao SSH, Pennington JAT & Bolger PM (2002) US Food and Drug Administration Total Diet Study: intake of nutritional and toxic elements, 1991–1996. Food Addit Contam, **19**: 103–125.

Eurachem (1999) Guide to quantifying uncertainty in analytical measurements, 2nd ed. Athens, Eurachem (http://www.measurementuncertainty.org).

FAO (2002) Submission and evaluation of pesticide residues data for the estimation of maximum residue levels in food and feed, 1st ed. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 170; http://www.fao.org/ag/agp/agpp/pesticid/JMPR/Download/FAOM2002.pdf).

FAO (2009) Residue evaluation of certain veterinary drugs. Rome, Food and Agriculture Organization of the United Nations, pp 1–28 (FAO JECFA Monographs, No. 6; http://www.fao.org/docrep/011/i0659e/i0659e00.htm).

FAO/WHO (1989a) Guidelines for simple evaluation of food additive intake. Rome, Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission (CAC/GL 03-1989; http://std.gdciq.gov.cn/gssw/JiShuFaGui/CAC/CXG_003e.pdf).

FAO/WHO (1989b) Evaluation of certain veterinary drug residues in food. Thirty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 788; http://whqlibdoc.who.int/trs/WHO_TRS_788.pdf).

FAO/WHO (1995a) Application of risk analysis to food standard issues. Report of an FAO/WHO consultation, Geneva, 13–17 March 1995. Geneva, World Health Organization (WHO/FNU/FOS/95.3).

FAO/WHO (1995b) Guidelines for predicting dietary intake of pesticide residues. Report of a Joint FAO/WHO Consultation, York, 2–6 May 1995. Geneva, World Health Organization, Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) (Document No. WHO/FNU/FOS/95.11).

FAO/WHO (1995c) Evaluation of certain veterinary drug residues in food. Forty-third report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 855; http://whqlibdoc.who.int/trs/WHO_TRS_855.pdf).

FAO/WHO (1997) Food consumption and exposure assessment of chemicals. Report of an FAO/WHO Consultation on Food Consumption and Exposure Assessment of Chemicals, Geneva, 10–14 February 1997. Geneva, World Health Organization (WHO/FSF/FOS/97.5).

FAO/WHO (1999a) Pesticide residues in food—1998. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome,

Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 148; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/98_rep/REPORT1998.pdf).

FAO/WHO (1999b) Annex V. In: Pesticide residues in food—1999. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations, pp 289–293 (FAO Plant Production and Protection Paper, No. 153; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/99_rep/REPORT1999.pdf).

FAO/WHO (2000) Methodology for exposure assessment of contaminants and toxins in food. Report of a Joint FAO/WHO Workshop, Geneva, 7–8 June 2000. Geneva, World Health Organization (WHO/SDE/PHE/FOS/00.5; http://www.who.int/foodsafety/publications/chem/en/exposure_june2000.pdf).

FAO/WHO (2001) Guidelines for the preparation of working papers on intake of food additives for the Joint FAO/WHO Expert Committee on Food Additives. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization (http://www.who.int/ipcs/food/jecfa/en/intake_guidelines.pdf).

FAO/WHO (2003) Guidelines on Good Laboratory Practice in residue analysis. Rome, Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission (CAC/GL 40-1993, Rev.1-2003; http://www.codexalimentarius.net/web/standard_list.do?lang=en).

FAO/WHO (2004a) Chapters 2 and 3. In: Pesticide residues in food—2003. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations, pp 3–22 (FAO Plant Production and Protection Paper, No. 176; http://www.fao.org/WAICENT/FAOINFO/AGRICULT/AGP/AGPP/Pesticid/JMPR/DOWNLOAD/2003.pdf).

FAO/WHO (2004b) Pesticide residues in food—2004. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 178; http://www.fao.org/WAICENT/FAOINFO/AGRICULT/AGP/AGPP/Pesticid/JMPR/DOWNLOAD/2004_rep/report2004jmpr.pdf).

FAO/WHO (2006a) Evaluation of certain veterinary drug residues in foods. Sixty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 939; http://whqlibdoc.who.int/publications/2006/9241209399_eng.pdf).

FAO/WHO (2006b) A model for establishing upper levels of intake for nutrients and related substances: report of a Joint FAO/WHO technical workshop on

nutrient risk assessment, 2–6 May 2005. Geneva, World Health Organization (http://www.who.int/ipcs/highlights/full_report.pdf).

FAO/WHO (2008a) Codex Alimentarius Commission procedural manual, 18th ed. Rome, Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission (ftp://ftp.fao.org/codex/Publications/ProcManuals/Manual_18e.pdf).

FAO/WHO (2008b) Dietary exposure assessment of chemicals in food. Report of a Joint FAO/WHO Consultation, Annapolis, MD, 2–6 May 2005. Geneva, World Health Organization (http://whqlibdoc.who.int/publications/2008/9789241597470_eng.pdf).

FAO/WHO (2009a) Evaluation of certain food additives. Sixty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 952; http://whqlibdoc.who.int/trs/WHO_TRS_952_eng.pdf).

FAO/WHO (2009b) Evaluation of certain veterinary drug residues in food. Seventieth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 954; http://who.int/ipcs/publications/jecfa/trs_954.pdf).

FSA (2002) Review of the risk assessment of mixtures of pesticides and similar substances (2002). London, United Kingdom Food Standards Agency, Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (FSA/0691/0902.E231:E244; http://cot.food.gov.uk/cotreports/cotwgreports/cocktailreport).

FSA (2004) 2000 Total Diet Study of 12 elements—aluminium, arsenic, cadmium, chromium, copper, lead, manganese, mercury, nickel, selenium, tin and zinc. London, United Kingdom Food Standards Agency (FSA Food Survey Information Sheet, No. 48/04; http://www.food.gov.uk/science/surveillance/fsis2004branch/fsis4804metals).

FSANZ (2001) The 19th Australian Total Diet Survey—a total diet survey of pesticide residues and contaminants. Canberra and Wellington, Food Standards Australia New Zealand (http://www.foodstandards.gov.au/newsroom/publications/19thaustraliantotaldietsurveyapril2001/19thaustraliantotaldietsurvey/index.cfm).

FSANZ (2003) The 20th Australian Total Diet Survey—a total diet survey of pesticide residues and contaminants. Canberra and Wellington, Food Standards Australia New Zealand (http://www.foodstandards.gov.au/newsroom/publications/20thaustraliantotaldietsurveyjanuary2003/20thaustraliantotaldietsurveyfullreport/index.cfm).

Grandjean P (1995) Biomarkers in epidemiology. Clin Chem, **41**(12 Pt 2): 1800–1803.

Green TJ, Allen OB & O'Connor DL (1998) A three-day weighed food record and a semiquantitative food-frequency questionnaire are valid measures for assessing the folate and vitamin B-12 intakes of women aged 16 to 19 years. J Nutr, **128**: 1665–1671.

Gregory JR, Collins DL, Davies PSW, Hughes JM & Clarke PC (1995) National Diet and Nutrition Survey: children aged 1 ½ to 4 ½ years. Vol. 1. Report of the diet and nutrition survey. London, HMSO.

Gulson BL, Mizon KJ, Korsch MJ, Mahaffey KR & Taylor AJ (2001) Dietary intakes of selected elements from longitudinal 6-day duplicate diets for pregnant and nonpregnant subjects and elemental concentrations of breast milk and infant formula. Environ Res, **87**: 160–174.

Hamilton D & Crossley S eds (2004) Pesticide residues in food and drinking water: human exposure and risks. London, John Wiley & Sons.

Hansen SC (1979) Conditions for use of food additives based on a budget method for an acceptable daily intake. J Food Prot, **42**: 429–434.

Health Council of the Netherlands (2004) Pesticides in food: assessing the risk to children. The Hague, Health Council of the Netherlands (Publication No. 2004/11E; http://www.gr.nl/pdf.php?ID=975).

Helsel DR (1990) Less than obvious—statistical treatment of data below the detection limit. Environ Sci Technol, **24**: 1766–1774.

IANZ (2004) Uncertainty of measurement, precision and limits of detection in chemical and microbiological testing laboratories. Auckland, International Accreditation New Zealand (Technical Guide AS TG5; http://www.ianz.govt.nz/publications2/pdfs/AS_TG5_uncert_of_measure.pdf).

IEFS (1998) The effect of survey duration on the estimation of food chemical intakes. Dublin, Institute of European Food Studies (Food Additive Intake Series, Report No. 3).

IPCS (2000) Human exposure assessment. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 214; http://www.inchem.org/documents/ehc/ehc/ehc214.htm).

IPCS (2008) Part 1: Guidance document on characterizing and communicating uncertainty in exposure assessment. In: Uncertainty and data quality in exposure assessment. Geneva, World Health Organization, International Programme on Chemical Safety (Harmonization Project Document, No. 6; http://www.who.int/ipcs/publications/methods/harmonization/exposure_assessment.pdf).

IPCS (2009) Risk assessment of combined exposures to multiple chemicals: a WHO/IPCS framework (Harmonization Project Draft Document for Public and Peer Review). Geneva, World Health Organization, International Programme on Chemical Safety (http://www.who.int/ipcs/methods/harmonization/areas/combinedexposure.pdf).

Jain RB, Caudill SP, Wang RY & Monsell E (2008) Evaluation of maximum likelihood procedures to estimate left censored observations. Anal Chem, **80**(4): 1124–1132.

Karvetti RL & Knutts LR (1985) Validity of the 24-hour recall. J Am Diet Assoc, **85**: 1437–1442.

Keith LH, Crummett W, Deegan J Jr, Libby RA, Taylor JK & Wentler G (1983) Principles of environmental analysis. Anal Chem, **55**: 2210–2218.

Klensin JC (1992) INFOODS: food composition data interchange handbook. Tokyo, United Nations University Press (http://www.unu.edu/unupress/unupbooks/80774e/80774E00.htm).

Klensin JC, Feskanich D, Lin V, Truswell AS & Southgate DAT (1989) Identification of food components for INFOODS data interchange. Tokyo, United Nations University (http://www.unu.edu/unupress/unupbooks/80734e/80734E00.htm).

Kroes R, Müller D, Lambe J, Löwik MRH, Van Klaveren J, Kleiner J, Massey R, Mayer S, Urieta I, Verger P & Visconti A (2002) Assessment of intake from the diet. Food Chem Toxicol, **40**: 327–385.

Kroke A, Klipstein-Grobusch K, Voss S, Möseneder J, Thielecke F, Noack R & Boeing H (1999) Validation of a self-administered food-frequency questionnaire administered in the European Prospective Investigation into Cancer and Nutrition (EPIC) Study: comparison of energy, protein, and macronutrient intakes estimated with the doubly labeled water, urinary nitrogen, and repeated 24-h dietary recall methods. Am J Clin Nutr, **70**: 439–447.

Lambe J & Kearney J (1999) The influence of survey duration on estimates of food intakes—relevance for food-based dietary guidelines. Br J Nutr, **81**(Suppl 2): S139–S142.

Lambe J, Cadby P & Gibney M (2002) Comparison of stochastic modelling of the intakes of intentionally added flavouring substances with theoretical added maximum daily intakes (TAMDI) and maximized survey-derived daily intakes (MSDI). Food Addit Contam, **19**: 2–14.

Lauwerys RR, Bernard A, Roels H & Buchet JP (1995) Health risk assessment of long-term exposure to non-genotoxic chemicals: application of biological indices. Toxicol Lett, **77**: 39–44.

Leblanc J-C, Malmauret L, Guérin T, Bordet F, Boursier B & Verger P (2000) Estimation of the dietary intakes of pesticide residues, lead, cadmium, arsenic and radionuclides in France. Food Addit Contam, **17**: 925–932.

Leblanc JC, Tard A, Volatier JL & Verger P (2005) Estimated dietary exposure to the principal food mycotoxins from the 1st French Total Diet Study. Food Addit Contam, **22**: 652–672.

Madden JP, Goodman SJ & Guthrie HA (1976) Validity of the 24-hr recall. J Am Diet Assoc, **68**: 143–147.

MAFF (1999) Report of the international conference on pesticide residues variability and acute dietary risk assessment, 1–3 December 1998, York, United Kingdom. London, United Kingdom Ministry of Agriculture, Food and Fisheries, Pesticides Safety Directorate.

MAFF (2000) Duplicate diet study of vegetarians—dietary exposures to 12 metals and other elements. London, United Kingdom Ministry of Agriculture, Food and Fisheries, Joint Food Standards Group (Food Surveillance Information Sheet, No. 193; http://archive.food.gov.uk/maff/archive/food/infsheet/2000/no193/193vege.htm).

McLennan W & Podger A (1997) National Nutrition Survey selected highlights Australia. 1995. Canberra, Commonwealth of Australia, Australian Bureau of Statistics (ABS Catalogue No. 4802.0).

McLennan W & Podger A (1998) National Nutrition Survey. Nutrient intakes and physical measurements. Australia. 1995. Canberra, Commonwealth of Australia, Australian Bureau of Statistics (ABS Catalogue No. 4805.0).

McLennan W & Podger A (1999) National Nutrition Survey. Foods eaten. Australia. 1995. Canberra, Commonwealth of Australia, Australian Bureau of Statistics (ABS Catalogue No. 4804.0).

Mensink GBM, Hatenberger M & Thamm M (2001) Validity of DISHES 98, a computerised dietary history interview: energy and macronutrient intake. Eur J Clin Nutr, **55**: 409–417.

Murakami T, Narita N, Nakagaki H, Shibata T & Robinson C (2003) Fluoride intake in Japanese children aged 3–5 years by the duplicate-diet technique. Fluoride, **36**: 49.

New Zealand Ministry of Health (1999) NZ food: NZ people. Key results of the 1997 National Nutrition Survey. Wellington, New Zealand Ministry of Health (http://www.moh.govt.nz/moh.nsf/49b6bf07a4b7346dcc256fb300005a51/8f1dbeb1e0e1c70c4c2567d80009b770?OpenDocument).

New Zealand Ministry of Health (2003) NZ food, NZ children. Findings of the 2002 National Children's Nutrition Survey. Wellington, New Zealand Ministry of Health (http://www.moh.govt.nz/moh.nsf/49b6bf07a4b7346dcc256fb300005a51/064234a7283a0478cc256dd60000ab4c?OpenDocument).

Nusser SM, Carriquiry AL, Dodd KW & Fuller WA (1996) A semiparametric approach to estimating usual intake distributions. J Am Stat Assoc, **91**: 1440–1449.

Petersen BJ, Chaisson CF & Douglass JS (1994) Use of food-intake surveys to estimate exposures to nonnutrients. Am J Clin Nutr, **59**(Suppl): 240S–243S.

Putnam JJ & Allshouse JE (1999) Food consumption, prices, and expenditures, 1970–97. Washington, DC, United States Department of Agriculture, Economic

Research Service, Food and Rural Economics Division (Statistical Bulletin No. 965; http://www.ers.usda.gov/Publications/SB965/).

Raper N, Perloff B, Ingwersen I, Steinfeldt L & Anand J (2004) An overview of USDA's dietary intake data system. J Food Comp Anal, **17**: 545–555.

Renwick AG, Barlow SM, Hertz-Picciotto I, Boobis AR, Dybing E, Edler L, Eisenbrand G, Greig JB, Kleiner J, Lambe J, Muller DJG, Smith MR, Tritscher A, Tuijtelaars S, Van den Brandt PA, Walker R & Kroes R (2003) Risk characterisation of chemicals in food and diet. Food Chem Toxicol, **41**: 1211–1271.

Rimm EB, Giovannucci EL, Stampfer MJ, Colditz GA, Litin LB & Willett WC (1992) Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. Am J Epidemiol, **135**: 1114–1126.

Schaefer EJ, Augustin JL, Schaefer MM, Rasmussen H, Ordovas JM, Dallal GE & Dwyer JT (2000) Lack of efficacy of a food-frequency questionnaire in assessing dietary macronutrient intakes in subjects consuming diets of known composition. Am J Clin Nutr, **71**: 746–751.

Schulte PA & Waters M (1999) Using molecular epidemiology in assessing exposure for risk assessment. Ann NY Acad Sci, **895**: 101–111.

Schutz D, Moy GG & Käferstein FK (1998) GEMS/Food International Dietary Survey: infant exposure to certain organochlorine contaminants from breast milk—a risk assessment. Geneva, World Health Organization (WHO/FSF/FOS/98.4).

Sinha P, Lambert MB & Trumbull VL (2006) Evaluation of statistical methods for left-censored environmental data with nonuniform detection limits. Environ Toxicol Chem, **25**(9): 2533–2540.

Slimani N, Deharveng G, Charrondiere RU, Van Kappel AL, Ocke MC, Welch A, Lagiou A, Van Liere M, Agudo A, Pala V, Brandstetter B, Andren C, Stripp C, Van Staveren WA & Riboli E (1999) Structure of the standardized computerized 24-h diet recall interview used as reference method in the 22 centers participating in the EPIC project. European Prospective Investigation into Cancer and Nutrition. Comput Methods Programs Biomed, **58**: 251–266.

Slimani N, Ferrari P, Ocke M, Welch A, Boeing H, Van Liere M, Pala V, Amiano P, Lagiou A, Mattison I, Stripp C, Engeset D, Charrondiere UR, Buzzard M, Van Staveren W & Riboli E (2000) Standardization of the 24-hour diet recall calibration method used in the European Prospective Investigation into Cancer and Nutrition (EPIC): general concept and preliminary results. Eur J Clin Nutr, **54**: 900–917.

Slob W (1993) Modeling long-term exposure of the whole population to chemicals in food. Risk Anal, **13**: 525–530.

Slob W (1994) Uncertainty analysis in multiplicative models. Risk Anal, **14**: 571–576.

Slob W (1996) A comparison of two statistical approaches to estimate long-term exposure distributions from short-term measurements. Risk Anal, **16**: 195–200.

Strickland P, Kang D & Sithisarankul P (1996) Polycyclic aromatic hydrocarbon metabolites in urine as biomarkers of exposure and effect. Environ Health Perspect, **104**(Suppl 5): 927–932.

Swiss Federal Office of Public Health (2002) Assessment of acrylamide intake by duplicate diet study. Berne, Swiss Federal Office of Public Health (http://www.bfr.bund.de/cm/208/assessment_of_acrylamide_intake_by_duplicate_diet_study.pdf).

Thompson FE, Kipnis V, Subar AF, Krebs-Smith SM, Kahle LL, Midthune D, Potischman N & Schatzkin A (2000) Evaluation of 2 brief instruments and a food frequency questionnaire to estimate daily number of servings of fruit and vegetables. Am J Clin Nutr, **71**: 1503–1510.

Tran NL, Barraj LM, Smith K, Javier A & Burke TA (2004) Combining food frequency and survey data to quantify long-term dietary exposure; a methyl mercury case study. Risk Anal, **24**: 19–30.

Tressou J, Leblanc C, Feinberg M & Bertail P (2004) Statistical methodology to evaluate food exposure to a contaminant and influence of sanitary limits: application to ochratoxin A. Regul Toxicol Pharmacol, **40**: 252–263.

Tsuda T, Inoue T, Kojima M & Aoki S (1995) Market basket and duplicate portion estimation of dietary intakes of cadmium, mercury, arsenic, copper, manganese, and zinc by Japanese adults. J AOAC Int, **78**: 1363–1368.

UNEP (1992) The contamination of food. Nairobi, United Nations Environment Programme (UNEP/GEMS Environmental Library, No. 5).

UNEP/FAO/WHO (1988) Assessment of chemical contaminants in food. Report on the results of the UNEP/FAO/WHO programme on health-related environmental monitoring (prepared in cooperation with the UNEP Monitoring and Research Centre, London). Geneva, World Health Organization.

USCDC (2003) Second national report on human exposure to environmental chemicals. Atlanta, GA, United States Department of Health and Human Services, National Center for Environmental Health, Division of Laboratory Sciences, Centers for Disease Control and Prevention (NCEH Publication No. 02-0716).

USCDC (2004) A review of evidence of health effects of blood lead levels <10 µg/dL in children. Atlanta, GA, United States Department of Health and Human Services, National Center for Environmental Health, Centers for Disease Control and Prevention, Work Group of the Advisory Committee on Childhood Lead Poisoning Prevention (http://www.cdc.gov/nceh/lead/ACCLPP/meetingMinutes/lessThan10MtgMAR04.pdf).

USDA (2000) 1994–96, 1998 Continuing Survey of Food Intakes by Individuals (CSFII) and the Diet and Health Knowledge Survey (DHKS) (CD-ROM). Washington, DC, United States Department of Agriculture. Available from National Technical Information Service, Springfield, VA (NTIS Accession No. PB2000-500027).

USDA (2008) Pesticide Data Program: annual summary, calendar year 2007. Washington, DC, United States Department of Agriculture, Agricultural Marketing Service (http://www.ams.usda.gov/science/pdp/Summary2007.pdf).

USEPA (1996) Residue chemistry test guidelines. OPPTS 860.1520. Processed food/feed. Washington, DC, United States Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances (EPA 712-C-96-184; http://fedbbs.access.gpo.gov/library/epa_860/860-1520.pdf).

USEPA (1998) Guidance for submission of probabilistic human health exposure assessments to the Office of Pesticide Programs. Washington, DC, United States Environmental Protection Agency, Office of Pesticide Programs (http://www.epa.gov/fedrgstr/EPA-PEST/1998/November/Day-05/6021.pdf).

USEPA (2000a) Guidance for refining anticipated residue estimates for use in acute dietary probabilistic risk assessment. Washington, DC, United States Environmental Protection Agency, Office of Pesticide Programs (http://www.epa.gov/pesticides/trac/science/residues.pdf).

USEPA (2000b) Assigning values to non-detected/non-quantified pesticide residues in human health food exposure assessments. Washington, DC, United States Environmental Protection Agency, Office of Pesticide Programs (http://www.epa.gov/fedrgstr/EPA-PEST/2000/March/Day-31/6047.pdf).

USEPA (2001) General principles for performing aggregate exposure and risk assessments. Washington, DC, United States Environmental Protection Agency, Office of Pesticide Programs (http://www.epa.gov/pesticides/trac/science/aggregate.pdf).

USEPA (2002) Guidance on cumulative risk assessment of pesticide chemicals that have a common mechanism of toxicity. Washington, DC, United States Environmental Protection Agency, Office of Pesticide Programs (http://www.epa.gov/pesticides/trac/science/cumulative_guidance.pdf).

USFDA (2004a) Total diet study. Washington, DC, United States Food and Drug Administration, Center for Food Safety and Applied Nutrition (http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/TotalDietStudy/default.htm).

USFDA (2004b) Pesticide residue monitoring program 2002. Washington, DC, United States Food and Drug Administration, Center for Food Safety and Applied Nutrition (http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/Pesticides/ResidueMonitoringReports/ucm125174.htm).

USNRC (1986) Nutrient adequacy: assessment using food consumption surveys. Prepared by Subcommittee on Criteria for Dietary Evaluation, Coordinating

Committee on Evaluation of Food Consumption Surveys, Food and Nutrition Board, Commission on Life Sciences, National Research Council. Washington, DC, National Academy Press.

USNRC (1987) Biological markers in environmental health research. National Research Council Committee on Biological Markers. Environ Health Perspect, **74**: 3–9.

USNRC (1993) Pesticides in the diets of infants and children. Prepared by Committee on Pesticides in the Diets of Infants and Children, National Research Council. Washington, DC, National Academy Press.

Van Leeuwen FXR & Malisch R (2002) Results of the third round of WHO-coordinated exposure study on the levels of PCBs, PCDDs and PCDFs in human milk. Organohalogen Compounds, **56**: 311–316.

Vannoort RW (2003) 2003/04 New Zealand Total Diet Survey analytical results—Q1. Christchurch, Institute of Environmental Science and Research (ESR Client Report No. FW03/77; http://www.nzfsa.govt.nz/science/research-projects/total-diet-survey/reports/quarter-1/quarter-1-nztds.pdf).

Vannoort RW (2004a) 2003/04 New Zealand Total Diet Survey analytical results—Q2. Christchurch, Institute of Environmental Science and Research (ESR Client Report No. FW04/14; http://www.nzfsa.govt.nz/science/research-projects/total-diet-survey/reports/quarter-2/quarter-2-nztds.pdf).

Vannoort RW (2004b) 2003/04 New Zealand Total Diet Survey analytical results—Q3. Christchurch, Institute of Environmental Science and Research (ESR Client Report No. FW04/47; http://www.nzfsa.govt.nz/science/research-projects/total-diet-survey/reports/quarter-3/quarter-3-nztds.pdf).

Vannoort RW (2004c) 2003/04 New Zealand Total Diet Survey analytical results—Q4. Christchurch, Institute of Environmental Science and Research (ESR Client Report No. FW04/93; http://www.nzfsa.govt.nz/science/research-projects/total-diet-survey/reports/quarter-4/quarter-4-nztds.pdf).

Vannoort RW, Cressey PJ & Silvers K (2000) 1997/98 New Zealand Total Diet Survey, Part 2: Elements. Wellington, Institute of Environmental Science and Research (ESR Client Report No. FW99/47; http://www.moh.govt.nz/moh.nsf/49ba80c00757b8804c256673001d47d0/a48868055568b2814c2568b100823cef?OpenDocument).

Verger P, Ireland J, Moller A, Abravicius JA, de Henauw S & Naska A; for the EFCOSUM Group (2002) Improvement of comparability of dietary intake assessment using currently available individual food consumption surveys. Eur J Clin Nutr, **56**(Suppl 2): S18–S24.

Weller E, Long N, Smith A, Williams P, Ravi S, Gill J, Henessey R, Skornik W, Brain J, Kimmel C, Kimmel G, Holmes L & Ryan L (1999) Dose-rate effects of ethylene oxide exposure on developmental toxicity. Toxicol Sci, **50**: 259–270.

WHO (1979) Guidelines for establishing or strengthening national food contamination monitoring programmes. Prepared under the joint sponsorship of UNEP, FAO and WHO. Geneva, World Health Organization (WHO/HCS/FCM/78.1).

WHO (1985) Guidelines for the study of dietary intakes of chemical contaminants. Geneva, World Health Organization (WHO Offset Publication No. 87).

WHO (1989a) Guidelines for predicting dietary intake of pesticide residues. Geneva, World Health Organization, Joint UNEP/FAO/WHO Food Contamination Monitoring Programme in collaboration with the Codex Committee on Pesticide Residues.

WHO (1989b) Levels of PCBs, PCDDs, and PCDFs in breast milk. Copenhagen, World Health Organization Regional Office for Europe (Environmental Health Series, No. 34).

WHO (1992) Assessment of dietary intake of chemical contaminants. Geneva, World Health Organization, Joint UNEP/FAO/WHO Food Contamination Monitoring and Assessment Programme (GEMS/Food) (WHO/HPP/FOS/92.6).

WHO (1994) GEMS/Food-EURO workshop on reliable evaluation of low-level contamination of food. Report on a workshop in the frame of GEMS/Food-Euro, Kulmbach, 3–5 March 1994. Rome, World Health Organization Regional Office for Europe, GEMS/Food-EURO (EUR/ICP/EHAZ.94.12/WS03).

WHO (1995) Second workshop on reliable evaluation of low-level contamination of food. Report on a workshop in the frame of GEMS/Food-EURO, Kulmbach, 26–27 May 1995. Rome, World Health Organization Regional Office for Europe, GEMS/Food-EURO (EUR/ICP/EHAZ.94.12/WS04; http://www.who.int/foodsafety/publications/chem/lowlevel_may1995/en/).

WHO (1996) Levels of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) in human milk: second round of WHO-coordinated exposure study. Copenhagen, World Health Organization Regional Office for Europe (Environmental Health Series, No. 3).

WHO (1997) Guidelines for predicting dietary intake of pesticide residues (revised). Prepared by the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) in collaboration with the Codex Committee on Pesticide Residues. Geneva, World Health Organization, Programme of Food Safety and Food Aid (WHO/FSF/FOS/97.7; http://www.who.int/foodsafety/publications/chem/en/pesticide_en.pdf).

WHO (1998) GEMS/Food regional diets (regional per capita consumption of raw and semi-processed agricultural commodities). Geneva, World Health Organization (WHO/FSF/FOS/98.3).

WHO (2002a) GEMS/Food Total Diet Studies. Report of the 2nd international workshop on total diet studies, Brisbane, 4–15 February 2002. Geneva, World

Health Organization, Food Safety Programme (http://www.who.int/foodsafety/publications/chem/en/tds_feb2002.pdf).

WHO (2002b) Latin American total diet study workshop, 8–13 July 2002, Buenos Aires. Buenos Aires, Pan American Health Organization (PAHO), World Health Organization (WHO) and Instituto Panamericano de Protección de Alimentos y Zoonosis (INPPAZ) (http://www.who.int/foodsafety/publications/chem/en/tds_july2002.pdf).

WHO (2002c) The optimal duration of exclusive breastfeeding. Report of an expert consultation, Geneva, 28–30 March 2001. Geneva, World Health Organization (WHO/NHD/01.09; http://www.who.int/nutrition/publications/optimal_duration_of_exc_bfeeding_report_eng.pdf).

WHO (2002d) Polychlorinated dibenzodioxins, polychlorinated dibenzofurans, and coplanar polychlorinated biphenyls. In: Safety evaluation of certain food additives and contaminants. Geneva, World Health Organization (WHO Food Additives Series, No. 48; http://www.inchem.org/documents/jecfa/jecmono/v48je20.htm).

WHO (2003) GEMS/Food regional diets: regional per capita consumption of raw and semi-processed agricultural commodities, rev. ed. Geneva, World Health Organization, Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) (http://www.who.int/foodsafety/chem/gems_regional_diet.pdf).

WHO (2004) Highest reported 97.5th percentile consumption figures (eaters only) for various commodities by the general population and children ages 6 and under, January 2004. Geneva, World Health Organization, Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food).

WHO (2005a) GEMS/Food total diet studies. Report of the 3rd international workshop on total diet studies, Paris, 14–21 May 2004. Geneva, World Health Organization (http://www.who.int/foodsafety/publications/chem/TDS_Paris_en.pdf).

WHO (2005b) Instructions for electronic submission of data on chemical contaminants in food and the diet. Geneva, World Health Organization, Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) (http://www.who.int/foodsafety/publications/chem/en/gemsmanual.pdf).

WHO (2008) Guidelines for drinking-water quality, 3rd ed. incorporating 1st and 2nd addenda. Vol. 1. Recommendations. Geneva, World Health Organization (http://www.who.int/water_sanitation_health/dwq/gdwq3rev/en/index.html).

Wild CP, Andersson C, O'Brien NM, Wilson L & Woods JA (2001) A critical evaluation of the application of biomarkers in epidemiological studies on diet and health. Br J Nutr, **86**(Suppl 1): S37–S53.

Wilhelm M, Wittsiepe J, Schrey P, Budde U & Idel H (2002) Dietary intake of cadmium by children and adults from Germany using duplicate portion sampling. Sci Total Environ, **285**(1–3): 11–19.

Ysart G, Miller P, Crews H, Robb P, Baxter M, De L'Argy C, Lofthouse S, Sargent C & Harrison N (1999) Dietary exposure estimates of 30 elements from the UK Total Diet Study. Food Addit Contam, **16**: 391–403.

***

Appendix 6.1: Acute dietary exposure estimates currently used by JMPR

Since its introduction in 1997, the methodology for estimating the acute dietary exposure to pesticide residues has been refined by JMPR (FAO/WHO, 2002, 2004a,b). The calculated exposure is called the international estimated short-term intake (IESTI) or national estimated short-term intake (NESTI).

Calculations of the acute dietary exposure recognize four different cases (1, 2a, 2b and 3). Case 1 is the simple case where the residue in a composite sample reflects the residue level in a meal-sized portion of the commodity. Case 2 is the situation where the meal-sized portion as a single fruit or vegetable unit might have a higher residue than the composite. Case 2 is further divided into case 2a and case 2b, where the unit size is less than or greater than the large portion (LP) size, respectively. Case 3 allows for the likely bulking and blending of processed commodities such as flour, vegetable oils and fruit juices.

The concept of a variability factor (v) was introduced by JMPR to take into account the different concentrations of residues in individual units of a composite sample. JMPR concluded in 2004 that owing to the inevitable random nature of the variability factor derived from the combined uncertainty associated with sampling and analysis, the best estimate of the default variability factor is the mean of the variability factors derived from samples of various crops. The mean variability factor was found to be 3 (FAO/WHO, 2004b) and has been used as a default value by JMPR since 2003. It is important to note that the variability factor as described here can be applied only for samples coming from single lots. Analysts conducting acute exposure assessments for pesticides may want to select an appropriate variability factor for the specific evaluation.

The following definitions apply to all equations:

- LP — Highest large portion reported (97.5th percentile of eaters), kg of food per day.
- HR — Highest residue in composite sample of edible portion found in the supervised trials used for estimating the maximum residue level, mg/kg.
- HR-P — Highest residue in a processed commodity, mg/kg, calculated by multiplying the highest residue in the raw commodity by the processing factor.
- BW — Mean body weight, kg, provided by the country from which the LP was reported.
- U — Unit weight of the edible portion, kg, provided by the country where the trials that gave the highest residue were carried out.
- *v* — Variability factor, the factor applied to the composite residue to estimate the residue level in a high-residue unit.
- STMR — Supervised trials median residue, mg/kg.
- STMR-P — Supervised trials median residue in processed commodity, mg/kg.

Case 1

The residue in a composite sample (raw or processed) reflects the residue level in a meal-sized portion of the commodity (unit weight is below 0.025 kg). Case 1 also applies to meat, liver, kidney, edible offal and eggs, and for grains, oilseed and pulse commodities when the estimates were based on post-harvest use of the pesticide.

$$\text{IESTI} = \frac{\text{LP} \times (\text{HR or HR-P})}{\text{BW}}$$

Case 2

The meal-sized portion, such as a single fruit or vegetable unit, might have a higher residue than the composite (whole fruit or vegetable unit weight is above 0.025 kg).

Case 2a

Unit edible weight of raw commodity is less than large portion weight.

$$\text{IESTI} = \frac{\text{U} \times (\text{HR or HR-P}) \times v + (\text{LP-U}) \times (\text{HR or HR-P})}{\text{BW}}$$

The Case 2a formula is based on the assumption that the first unit contains residues at the [HR × *v*] level and the next ones contain residues at the HR level, which represents the residue in the composite from the same lot as the first one.

Case 2b

Unit edible weight of raw commodity exceeds large portion weight.

$$\text{IESTI} = \frac{\text{LP} \times (\text{HR or HR-P}) \times v}{\text{BW}}$$

The Case 2b formula is based on the assumption that there is only one consumed unit and it contains residues at the [HR × *v*] level.

Case 3

Case 3 is for those processed commodities where bulking or blending means that the STMR-P represents the likely highest residue. Case 3 also applies to milk and to grains, oilseeds and pulses for which the estimates were based on preharvest use of the pesticide.

$$\text{IESTI} = \frac{\text{LP} \times \text{STMR-P}}{\text{BW}}$$

The concept of variability factor was introduced to take into account the different concentrations of residues in individual portions of a composite sample and average residue concentration in the sample lot represented by the composite sample. The variability factor (*v*) was defined as the 97.5th percentile of the residue concentrations

presented in crop units divided by the mean residue concentration of the sample population. The default variability factors of 5 and 10 were replaced by a common default of 3 (FAO/WHO, 2004b).

In this methodology, the estimates are performed for each crop individually, as it is unlikely that an individual will consume, within a meal or 24 h, a large portion of more than one food containing the highest residue level (the one that incorporates the variability factor).

The LP (highest large portion reported [97.5th percentile of eaters], kg of food per day) should be matched to the Codex commodity to which the HR or STMR relates. In the case of commodities that are predominantly eaten as the fresh fruit or vegetable, the LP should relate to the raw agricultural commodity. However, when major portions of the commodity are eaten in a processed way (e.g. grains) and when information on the residue in the processed commodity is available, the LP should relate to the processed commodity (e.g. flour or bread).

7. RISK CHARACTERIZATION

7.1 Introduction

Risk characterization is the fourth step of the risk assessment process, integrating information from the hazard characterization and the exposure assessment to produce scientific advice for risk managers (Renwick et al., 2003). The Codex Alimentarius Commission (CAC) has defined risk characterization as "The qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment" (FAO/WHO, 2008).

Historically, different approaches have been used for the risk characterization of toxic effects considered to have a threshold and for those considered to have no threshold. Health-based guidance values have been used by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) for substances that produce

---

For acronyms and abbreviations used in the text, the reader may refer to the list of acronyms and abbreviations at the front of this monograph. Definitions of select terms may be found in the glossary at the end of the monograph.

threshold effects (see chapter 5). In the risk characterization for these types of substances, the health-based guidance values are compared with estimated or measured human exposure. In circumstances where the data are not sufficient to propose a health-based guidance value for a substance producing threshold effects, JECFA and JMPR may comment on the margin of exposure (MOE) between the doses at which effects are seen in animals and the estimated human dietary exposure.

Substances that are both genotoxic and carcinogenic would generally not be considered acceptable for use as food additives, pesticides or veterinary drugs. For those substances that are genotoxic and carcinogenic, the traditional assumption is that there may not be a threshold dose and that some degree of risk may exist at any level of exposure. Thus, health-based guidance values have not been developed by JECFA for substances, such as certain contaminants, that are known to be both genotoxic and carcinogenic. It should be noted, however, that some chemicals increase the incidence of cancer in experimental animals by non-genotoxic mechanisms, and establishing a health-based guidance value would be appropriate for such chemicals. The types of risk characterization advice that have been developed for substances that are genotoxic and carcinogenic include:

1) a recommendation that the exposure should be as low as reasonably achievable (ALARA);
2) quantification of the risk at different levels of exposure (e.g. aflatoxin) (FAO/WHO, 1999, 2007b); and
3) ranking of compounds producing similar hazards according to their estimated risk (e.g. substances that are genotoxic and carcinogenic) (FAO/WHO, 2006a).

It is recognized that the advice in approach 1 is of limited value, because it does not take into account either human exposure or carcinogenic potency and does not allow risk managers to prioritize different contaminants or to target risk management actions.

While approach 2 can provide advice for risk management of a specific substance, it does not provide the information necessary to prioritize different contaminants.

Approach 3 includes the MOE approach, which is the ratio between an amount of a substance producing a small but measurable effect in

laboratory animals or humans and the estimated human exposure (see section 7.4). For substances that are both genotoxic and carcinogenic, this approach provides advice to inform risk managers of how close human exposures are to those anticipated to produce a measurable effect in laboratory animals or humans. In addition, MOEs for different substances can be compared to assist risk managers in prioritizing risk management actions (EFSA, 2005a; FAO/WHO, 2005; O'Brien et al., 2006).

7.2 Risks at estimated levels of exposure

7.2.1 General considerations

The calculation of health-based guidance values was discussed in chapter 5. In risk characterization of substances exhibiting threshold effects, health-based guidance values are compared with estimates of dietary exposure. If exposures are below the relevant value, then no further information on risk characterization need be provided. However, in cases where exposures exceed health-based guidance values, the values themselves do not provide the risk manager with advice on the possible extent of the risk to those exposed to these higher amounts.

A first consideration should take into account the fact that health-based guidance values themselves incorporate safety or uncertainty factors (see chapter 5). A small or occasional dietary exposure in excess of a health-based guidance value based on a subchronic or chronic study does not necessarily imply that adverse health effects will occur in humans. If further advice is required on the possible health consequences for those exposed to amounts greater than the health-based guidance value, then the toxicity database needs to be considered with respect to the lowest-observed-adverse-effect levels (LOAELs), the nature and severity of the effects observed, the shape of the dose–response curve in the observed range (chapter 5) and whether acute toxicity, including developmental toxicity, is an issue. In the case of acute toxicity, the possible consequences of an estimated dietary exposure in excess of the acute reference dose (ARfD) should also be considered on a case-by-case basis. The option of refining the dietary exposure estimate may also be explored (see chapter 6).

JECFA has taken an MOE approach in characterizing risks associated with certain contaminants in food for which the available data were insufficient to establish a health-based guidance value, such as polybrominated diphenyl ethers (FAO/WHO, 2006a) and temephos (FAO/WHO, 2006b). Consideration of whether the identified MOE presents a concern for human health follows a process similar to selection of appropriate uncertainty factors to be used in establishing a health-based guidance value (e.g. factors of 10 for interspecies differences, 10 for human variability and additional factors for important gaps in the database). Other examples of applying an MOE for effects considered to have a threshold include the JECFA evaluation of the neurotoxic and reproductive effects of acrylamide, for which a health-based guidance value could not be proposed because of its additional genotoxic and carcinogenic properties (FAO/WHO, 2006a). JECFA also applied an MOE approach in considering risks of carrageenan in infant formula (FAO/WHO, 2007b), as a health-based guidance value cannot be assumed to be sufficiently protective for infants under the age of 12 weeks.

Another type of risk characterization output from dose–response modelling is the prediction of risks at specified exposure levels. This output can take the generic form of predicting "X number of health-impacted individuals at exposure Y". An example of such estimates is the case of aflatoxins, where JECFA predicted the additional cancer risk at different levels of exposure (FAO/WHO, 1999, 2007b). In the optimal case, such estimates are supported by parallel assessments that describe the uncertainty in such estimates by providing additional information on the range of estimates, rather than a single value. The risk manager can then make statements such as "Up to X number of individuals may be adversely affected by exposure Y". As discussed in chapters 5 and 6, assumptions inherent in such estimates can influence risk management decisions. These include choice of models, choice of end-points and limitations in initial data sets that were extrapolated.

These types of assessments have also been performed for lead (FAO/WHO, 2000), fumonisins B1 and B2 (Humphreys et al., 2001), methylmercury (Carrington et al., 2004) and cadmium (FAO/WHO, 2006a). In this context, it may be desirable to create a statistical model that estimates the range of effects expected for a population. Availability of such estimates can provide additional information for

risk managers to conduct cost–benefit analyses, risk–benefit assessments and evaluations of public health interventions.

7.2.2 Uncertainty and variability analysis

Uncertainty refers to limitations in the knowledge of the risk assessor about the data and models used. Variability reflects the inherent biological heterogeneity, either in exposure or in response. Thus, although both uncertainty and variability can be characterized using probability distributions, they are different concepts. Uncertainty can be decreased as the quantity or quality of the information available improves. In contrast, modelling variability is an exercise in descriptive statistics that results in a model of a population rather than an individual. Characterization of the variability in dietary exposure in the population, as an example, can be improved by better information, but the variability cannot be eliminated.

Uncertainty analysis can be applied to both exposure data and health effects data, but so far it has been applied mainly to exposure estimates. In an uncertainty analysis (EFSA, 2005b; IPCS, 2008), each component of a model may have its own uncertainties. If the assessor's knowledge were perfect, then the exposure estimates for specific members of the population (e.g. the median individual or the 95th-percentile individual) could be characterized as a single value. This is never the situation, so an uncertainty analysis is an important part of a probabilistic model and should portray the limits of current knowledge by generating a range of estimates that cover the range of plausible interpretation. More typically, knowledge is imprecise, and exposures for representative individuals must be reported as a range of values. The uncertainty analysis is ideally a quantitative exercise where feasible. This serves two basic purposes. First, it gives decision-makers an idea of the overall confidence associated with the estimation process. Second, it facilitates research planning by giving researchers a formal target.

A formal uncertainty analysis is not always necessary. Two good reasons for omitting a formal representation of uncertainty are that 1) the uncertainties involved are relatively small and 2) it is known beforehand that either a most likely case or worst-case scenario will drive the decision process. However, even in these cases, a rationale for determining that these assumptions are true should be given.

The basic notion underlying a "statistical" uncertainty is that the uncertainty about an unidentified (or random) individual or event is characterized by the known frequency distribution of a population or series. Thus, the same distribution may function as either a frequency distribution or an uncertainty distribution, depending on whether it is being used to make a prediction about a population or about an individual.

The concept of statistical sampling error is another important frequency-based uncertainty. Sampling error depends not only on the number of samples taken but also on the variance within the total population from which the sample is taken—that is, the larger the variance, the more samples are required to correctly describe the population. The description of uncertainty involves the use of a statistical distribution to express the doubt that a small sample accurately represents a population. The underlying distribution used is speculative and is usually assumed to be the normal distribution. Confidence intervals for parameter estimates usually reflect sampling error.

Formal representation of uncertainty may utilize statistical concepts of uncertainty, such as measurement and sampling error. In addition, probability trees (Hacking, 1976; Rescher, 1993) may be used to represent uncertainties associated with the use of alternative plausible model forms or alternative surrogate data sets.

For many public health issues, it may be desirable to characterize the uncertainty associated with population estimates for a value that varies among individuals. For example, dietary exposure estimates are often made for a series of individuals in a survey, and hence those population estimates are uncertain. In these circumstances, each inference may have distributions that describe the range of population values and distributions or probability trees that represent uncertainty. An uncertainty analysis may also alleviate concerns over the accuracy of a simulation method for estimating the tails of the frequency distributions by demonstrating that the uncertainties associated with the extreme values are larger than the errors introduced by the simulation method. In order to integrate these different elements into the conclusions, a two-dimensional simulation is useful.

The discussion of variability and uncertainty here is intended to provide a general framework for thinking about the characterization of

population dietary exposure. In practice, the emphasis of public health risk assessments is on the characterization of population variability. Nonetheless, it is useful to keep in mind that the population estimates developed are not certain and that, ideally, the assessor should provide some indication of the plausible range of values for various representative members of the population.

For both exposure and health effects, the risk assessment should include a narrative evaluation of uncertainty. As indicated above, uncertainty can be assessed qualitatively, semiquantitatively or quantitatively. Whereas a complete quantitative assessment would involve probabilistic approaches with sensitivity analysis, this will often not be necessary or even feasible. As a minimum, the major sources of uncertainty in a risk assessment should be identified. Where possible, some idea of their magnitude should be provided, even if only semiquantitatively (e.g. small, moderate, large), together with an indication of whether they tend to increase or decrease the conservatism of the assessment. Such information can provide a guide to which studies would contribute most to helping refine any further risk assessment. Sources of variability should be identified and, where possible, some indication of their magnitude provided.

7.2.3 Sensitivity analysis

Risk assessment models may become very complex. An uncertainty analysis (see above) may reveal that there are substantial uncertainties in an estimate without indicating from where those uncertainties arise. That is, it may not be apparent which of the uncertainties in the assumptions give rise to the uncertainty in the model predictions. Sensitivity analysis refers to quantitative techniques that may be used to identify those aspects of the inputs (concentration or food consumption data) that contribute the greatest extent to the uncertainty. Analyses that evaluate inputs identified as the most important sources of uncertainty may be expected to be the most useful.

There are many different sensitivity analysis techniques (Cullen & Frey, 1999; Frey & Patil, 2002). The simplest of these vary each uncertain input one at a time, with all the other values held at some nominal (i.e. central or most likely) value. The resulting range in the output is then compared for each of the inputs. Although they are invariably

more calculation intensive, the more sophisticated sensitivity analysis methods analyse correlations among input distributions.

Sensitivity analysis is also sometimes used to evaluate frequency distributions (Frey & Patil, 2002). In this case, the relationship of the inputs used to describe population variability and the output distribution for the population estimate are examined. This type of analysis may be useful for identifying food chemical control strategies.

7.3 Risks from exposure to multiple substances

7.3.1 General considerations

There is an increasing awareness by those involved in risk assessment and by the general public of the need to consider any risks associated with combined exposure to mixtures of substances, both human-made and naturally occurring. This has been the focus of considerable risk assessment activity around the world (FSA, 2002; IPCS, 2009b; see also http://www.epa.gov/pesticides/cumulative/).

Given the numbers of human-made and naturally occurring chemical substances to which humans are exposed, there is a very large number of possible binary, tertiary, quaternary, etc. combinations. In consequence, direct experimentation cannot resolve this risk assessment issue, and research has focused on understanding the basic science of combination toxicology. In recent years, there have been major advances in understanding mechanisms of combination toxicology, and a significant theoretical and experimental database has been developed (Ito et al., 1995a,b; Jonker et al., 1996, 2004; Groten et al., 2000, 2001; Feron & Groten, 2002; Feron et al., 2002). In principle, combination effects could occur as a result of different chemicals present in food at the same time or at different times, depending on the rate of clearance of the chemicals from the body. There are four types of combined effect or interaction:

- *Dose addition* occurs when substances produce toxicity via the same mechanism of action. For substances that have a threshold in their dose–response relationships, the total activity of the mixture is the sum of the exposures for each component multiplied by its relative potency. A consequence of this is that a biological effect

may be produced if there is exposure to a mixture that contains a large number of substances that have the same mechanism of action, even though the exposures to each substance are too low to individually elicit a response. This mechanism is the basis for the group acceptable daily intake (ADI) approach for structurally related additives and pesticides (see chapter 5, section 5.2.8) and the use of toxic equivalency factors (TEFs) to derive an overall tolerable intake (TI) for structurally related contaminants (see section 7.3.2). A review of approved food additives with numerical ADI values has shown that dose addition might arise only rarely for structurally unrelated substances (Groten et al., 2000). Dose addition is the basis for recent considerations of pesticides that share the same mode of action by the Pesticide Residues Committee (2007) in the United Kingdom, in which simultaneous exposures to different acetylcholinesterase (AChE) inhibitors are assessed on the basis of summing each exposure as a fraction of the relevant ADI (this method assumes that each ADI is based on inhibition of AChE).

- *Response addition* is possible when two or more substances produce the same response or effect by different mechanisms. If the dose–response models used to estimate effects have thresholds, only those substances present in amounts above the threshold are relevant.

- *Synergism* occurs when the effect of the combination is greater than predicted by the summed activity of each component individually at the same level of exposure that occurs in the mixture. Synergism may arise from either toxicokinetic or toxicodynamic interactions. Toxicokinetic interactions are possible when one compound alters the metabolism of the potentially toxic component to increase the internal dose of or systemic exposure to the active form of the toxic component (parent compound or metabolite). Such an interaction can increase the activity of the toxic component and is the basis for the addition to pesticide formulations of synergistic compounds, which enhance the desired pesticidal activity of the formulation in the target organism. Synergism could result in an otherwise inactive level of exposure to a potential toxicant producing an effect when it is present in combination with sufficient amounts of another component to influence

its activity. Thus, synergism typically occurs when at least one of the components is present in sufficient amounts to affect the biological system in some way. In consequence, synergism is much less likely in an exposure scenario in which the exposure to each component in a mixture is below their respective health-based guidance values.

- *Antagonism* may arise from either toxicokinetic or toxicodynamic interactions, but usually requires that each substance is present at active doses or concentrations. Such an interaction would reduce the toxicity of the active component and therefore would not result in a possible health concern. Antagonism would occur if a substance with a low efficacy, such as a partial agonist, were to compete for a site of action with a high-efficacy compound, such as a full agonist. Such interaction may well occur in the application of TEFs (see section 7.3.2) and would make the assumption of full dose additivity a conservative approach.

One of the major lessons learnt from research to date is that exposure to mixtures of chemicals at levels that are non-toxic for each individual chemical generally will not result in a health risk, but dose addition is an important exception to this.

Evaluations of mixtures have been undertaken by JECFA and JMPR for some food additives, pesticides and veterinary drugs that are produced and tested as mixtures and some co-occurring mixtures of certain contaminants, such as polyhalogenated dibenzodioxins. As the testing of all possible combinations of substances that can occur in food is virtually impossible, substances are usually tested for toxicity singly in order to optimize hazard identification and characterization. Combinations are considered when substances are closely related structurally and co-exposure is likely. Examples are the use of data on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for the risk characterization of mixtures of dioxin-like compounds and the use of data on related substances for flavourings evaluated by the JECFA Procedure for the Safety Evaluation of Flavouring Agents (see JECFA reports from the forty-fourth meeting onwards).

For pesticides and veterinary drugs that are mixtures, JMPR and JECFA, respectively, base the ADI for the residues on the mixture as

tested. In some cases, a group ADI (see chapter 5, section 5.2.8) has been allocated. JECFA has also used the group ADI for certain food additives that are metabolized to a common potentially toxic metabolite and a group tolerable daily intake (TDI) for closely related contaminants that occur as mixtures.

When considering a substance that is a member of a series of compounds that are very closely related chemically (e.g. fatty acids or esters of allyl alcohol), but for which toxicological information is limited, it may be possible to base the safety evaluation on a group ADI established for the series of substances. This procedure can be followed only if a great deal of toxicological information is available on at least one member of the series and if the known toxic properties of the various substances can be predicted to fall along a well-defined continuum. Apart from the evaluation of flavouring substances by JECFA, consideration of mixtures represents one of the few situations in which the Committee has used structure–activity relationships in its safety assessments.

7.3.2 Toxic equivalency factor (TEF) approach

An approach that takes account of dose additivity is the TEF approach. The strategy of the TEF approach is to scale the exposure for each component of a mixture relative to the potency of an index chemical. In principle, TEFs can be used for a toxic end-point or a readily measured biomarker of a toxic response, such as binding affinity to the aryl hydrocarbon receptor or induction of cytochrome P-450 1A1. The biochemical effects used as an index of potency should be associated with subsequent toxic responses. The TEF estimates can be based on the results of in vivo and in vitro studies or a combination of both. The scaled concentrations are added, and the dose–response curve of the index chemical is used to generate a health-based guidance value, which is used as the response estimate for the sum of scaled concentrations. For this dose addition, the same mode of action and similarly shaped dose–response curves across the components are assumed. This method requires both toxicity and exposure data on the components of the mixture and sufficient data on one well-studied component to estimate a health-based guidance value. This component is typically chosen because it has a high relative potency or has been best characterized with respect to its effects and dose–response relationship.

The TEF approach is often complicated to use, is data intensive and requires some statistical modelling and expert judgement. A major disadvantage in the TEF approach is that the use of single point estimates for TEFs incompletely addresses the temporal issues when half-lives of the compounds in question differ considerably and there is a large degree of variability in the time intervals between exposure to the various compounds in the mixture (Mileson et al., 1999). A TEF method may not be appropriate when there are significant non-additive interactions among chemicals within the mixture (Krishnan et al., 1997).

The TEF approach has been developed by WHO (Van den Berg et al., 1998, 2006) and used by JECFA for the evaluation of polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (PCBs) (FAO/WHO, 2002) and has also been considered for possible application in the evaluation of polycyclic aromatic hydrocarbons (PAHs) (FAO/WHO, 2006a).

7.3.3 Surrogate approach

The surrogate approach to mixture evaluation uses a single component as the measure of concentration in relation to the response of the whole mixture. It assumes that the risks associated with each of the components of the complex mixture are proportional to the level of an indicator or index chemical in the mixture. The surrogate approach can be used for a series of compounds that are very closely related chemically (e.g. PAHs) but for which toxicological information on some members is limited. This procedure can be applied with confidence only if a great deal of toxicological information is available on at least one member of the series and if the known toxic properties of the various compounds fall along a well-defined continuum.

JECFA used the surrogate approach for the evaluation of PAHs (FAO/WHO, 2006a). The Committee noted that the TEFs that had previously been proposed for PAHs were derived from studies involving parenteral administration or in vitro approaches and that no data on oral administration were available that were suitable for this purpose. The Committee concluded that a surrogate approach should be used for the evaluation of mixtures of PAHs administered by the oral route, with benzo[*a*]pyrene being used as a marker of exposure to, and carcinogenicity of, the genotoxic and carcinogenic PAHs.

7.4 The formulation of advice on compounds that are both genotoxic and carcinogenic

JECFA has established procedures for determining health-based guidance values, such as the ADI or TI, for chemicals that produce adverse effects that are thought to show a threshold in their dose–response relationships. That is, there is considered to be no appreciable risk at intakes below the health-based guidance value. Some chemicals increase the incidence of cancer in experimental animals by non-genotoxic mechanisms; for these, establishing a health-based guidance value such as a provisional tolerable weekly intake (PTWI) would be appropriate. However, for substances that are both genotoxic and carcinogenic, dose levels that do not show a carcinogenic effect may simply represent the limit of detection in that bioassay, rather than an estimate of a possible threshold. Therefore, JECFA and JMPR do not establish health-based guidance values for compounds that are both genotoxic and carcinogenic using the no-observed-adverse-effect level (NOAEL) approach (see chapter 5). In the absence of evidence on the influence of non-linearity on the incidence of cancer at low levels of exposure, the advice given previously by JECFA on compounds that are both genotoxic and carcinogenic has been that intakes should be as low as reasonably achievable (ALARA). Such advice is of limited value, because it does not take into account either human exposure or carcinogenic potency and has not allowed risk managers to prioritize different contaminants or to target risk management actions. In addition, ever-increasing analytical sensitivity means that the numbers of chemicals with both genotoxic and carcinogenic potential detected in food will increase.

At its sixty-fourth meeting (FAO/WHO, 2006a), JECFA considered a number of contaminants for which genotoxicity and carcinogenicity were important issues and discussed possible approaches to the formulation of advice that would better inform risk managers about the possible magnitude of health concerns at different levels of intake in humans. Hazard identification would normally be based on data from studies on genotoxicity and from cancer bioassays. As described in chapter 5, hazard characterization (dose–response assessment) of substances that are both genotoxic and carcinogenic would be based on the available dose–response data for cancer, which would be derived mostly from studies in rodents given daily doses many orders of magnitude greater than

the estimated intakes in humans. If available, dose–response data from studies of epidemiology may also be used for hazard characterization and would avoid the necessity for interspecies comparisons and extrapolation over many orders of magnitude. An International Programme on Chemical Safety (IPCS) 2004 workshop recommended the use of the lower one-sided confidence limit of the benchmark dose (BMDL) as a starting point for hazard characterization based on data from a bioassay for cancer in experimental animals when the data are suitable for dose–response modelling (IPCS, 2009a).

The dose metric used for modelling could be a biomarker, providing that it was critically related to the process by which cancer arises and had been validated in relation to the external dose or intake. For carcinogenesis, selection of the dose–response data for modelling will need to consider both site-specific incidences of tumours, especially for the site showing the greatest sensitivity, as well as combined data (e.g. numbers of tumour-bearing animals) for compounds that do not show clear organ specificity. Analyses based on the numbers of tumour-bearing animals may also be appropriate under other circumstances—for example, in the assessment of complex mixtures of compounds that are both genotoxic and carcinogenic. Dose–response characterization should aim to define the BMDL for the carcinogenic responses of relevance to human health, at the lowest level of response (the benchmark response [BMR]) that reliably defines the bottom end of the observed experimental dose–response relationship. A BMR of a 10% incidence is likely to be the most appropriate for modelling of data from cancer bioassays, because the values for different mathematical models show wider divergence at incidences below 10%. The consistent use of the same BMR (i.e. 10%) will facilitate comparisons of the risks associated with different compounds that are both genotoxic and carcinogenic.

Exposure (intake) assessment for a compound that is both genotoxic and carcinogenic is no different from that for other types of contaminants. Risk characterization involves comparison of the estimated exposure with the identified BMDL. In principle, this can take different forms (FAO/WHO, 2006a):

- *Calculation of the MOE for substances that are both genotoxic and carcinogenic*. The MOE is the ratio between a point of departure (POD) or reference point (such as the BMDL) on the

dose–response curve from experimental animal or epidemiological studies and the estimated human exposure. The MOE can be used to prioritize different contaminants, providing that a consistent approach has been adopted. The acceptability of an MOE depends on its magnitude and is ultimately a risk management decision (IPCS, 2009a). To aid that decision, the risk assessor should provide information on the nature and magnitude of uncertainties in both the toxicological and exposure data. Although the risk assessor should not provide an assessment of the acceptability of the MOE, guidance should be given on its adequacy, taking into account the inherent uncertainties and variability (Barlow et al., 2006).

- *Dose–response analysis outside the observed dose range.* Quantitative dose–response analysis could be used to calculate the incidence of cancer that is theoretically associated with the estimated exposure for humans or the exposure associated with a predetermined incidence (e.g. 1 in 10^6). In order to provide estimates of the possible carcinogenic effect at the estimated exposure for humans, mathematical modelling would need to take into account the shape of the dose–response curve between the high doses used in the cancer bioassay and much lower intakes by humans. This requires extrapolation outside the observed dose range. In the future, it may be possible to incorporate data on dose–response or concentration–response relationships for the critical biological activities involved in the generation of cancer, such as metabolic bioactivation and detoxification processes, deoxyribonucleic acid (DNA) binding, DNA repair, rates of cell proliferation and apoptosis, into a biologically based dose–response model for cancer that would also incorporate data on species differences in these processes. However, such data are not currently available. At present, any estimate of the possible incidence of cancer for humans has to be based on extrapolation of cancer bioassay data by application of empirical mathematical equations that may not reflect the complexity of the underlying biology. A number of mathematical equations have been proposed for low-dose extrapolation. The resulting risk estimates are dependent on the mathematical model used; the divergence increases as the dose decreases, and the output from different equations can differ by orders of magnitude at very low incidences (see also chapter 5).

- *Linear extrapolation from a POD.* Because the estimated risks at low doses are model dependent, linear extrapolation from the BMDL, which is conservative and simple to apply, has been used as a matter of policy by some scientific bodies or authorities in order to calculate levels of exposure associated with different theoretical incidences of cancer. The incidence used is regarded as an upper-bound estimate for lifetime risk of cancer, and the actual risk may lie anywhere between zero and the calculated upper-bound estimate. Calculation of the intake associated with an incidence of 1 in 10^6 from the BMDL for a 10% incidence using linear extrapolation is simply equivalent to dividing the BMDL by 100 000, and this approach is therefore no more informative than calculation of an MOE.

Of the three options given above, the MOE and linear extrapolation from a POD are the most pragmatic and usable at the present time. Linear extrapolation from a POD offers no advantages over an MOE, and the results are open to misinterpretation, because the numerical estimates may be regarded as quantification of the actual risk. The sixty-fourth JECFA meeting (FAO/WHO, 2006a) therefore decided that advice on compounds that are both genotoxic and carcinogenic should be based on estimated MOEs. The strengths and weaknesses inherent in the data used to calculate the MOE should be given as part of the advice to risk managers, together with advice on its interpretation.

7.5 Subpopulations at risk

It is preferable for risk management and enforcement purposes to set a health-based guidance value, such as an ADI, PTWI, provisional maximum tolerable daily intake (PMTDI) or ARfD, for a substance that will cover the whole population. These values are normally established to protect the most sensitive subpopulation, based on the most sensitive critical health outcome. The use of safety or uncertainty factors has been generally assumed to take into account the differences in sensitivities in human populations, particularly from genotypic and phenotypic variations (Renwick et al., 2003).

However, it is recognized that the most sensitive critical health outcome may not always be relevant to some population subgroups. For example, it is particularly important to ensure that any health-based

guidance value is adequate to protect the embryo or fetus from possible effects in utero. While a health-based guidance value derived from developmental (embryo/fetal) effects would necessarily apply to women of childbearing age, it is recognized that such a value may be unreasonably conservative and not relevant to other population subgroups. Thus, in some situations in which a developmental or other subpopulation-specific end-point determines the health-based guidance value for a substance exhibiting no other toxicity at the developmental or other subpopulation-specific NOAEL, risk managers might request advice regarding a second (higher) value based on another end-point relevant to the rest of the population, as, for example, in the case of methylmercury in fish (FAO/WHO, 2007a).

The critical risk assessment issue that should be considered in recommending different health-based guidance values for different population subgroups is whether the most sensitive critical health outcome is irrelevant for a significant part of the whole population.

The advice provided to risk managers should include the following considerations:

- If a higher health-based guidance value is established based on another end-point, can the exposure be controlled for the sensitive population subgroup?

- Are there potential benefits, such as beneficial food components, for less sensitive populations that would be adversely affected by a health-based guidance value that is based on the most sensitive critical health outcome?

In deciding on the applicability of a health-based guidance value, it should also be considered whether there are particular subpopulations that may be at risk because they are allergic or intolerant to a substance that may be present in food. Examples include the need for individuals with phenylketonuria to avoid sources of phenylalanine, such as the artificial sweetener aspartame, or individuals with hereditary intolerance to fructose, sucrose and sorbitol who should also avoid D-tagatose (FAO/WHO, 2005).

Very young infants are a particularly sensitive subgroup because their metabolic capacities are not yet fully developed. It should be

noted that health-based guidance values are not considered applicable to infants under the age of 12 weeks who might be at risk at lower levels of exposure. Accordingly, risk characterization of exposure of such infants to chemicals (e.g. in infant formula or occurring as contaminants) has to be considered on a case-by-case basis. This is in accordance with similar advice in EHC 70 (IPCS, 1987), where the scientific rationale for this conclusion was originally set out. EHC 237, which provides a systematic analysis of the scientific principles to be considered in assessing health risks in children from exposures to environmental agents during distinct stages of development, is a useful reference in this regard (IPCS, 2006).

7.6 References[1]

Barlow S, Renwick AG, Kleiner J, Bridges JW, Busk L, Dybing E, Edler L, Eisenbrand G, Fink-Gremmels J, Knaap A, Kroes R, Liem D, Müller DJG, Page S, Rolland V, Schlatter J, Tritscher A, Tueting W & Würtzen G (2006) Risk assessment of substances that are both genotoxic and carcinogenic—Report of an international conference organized by EFSA and WHO with support of ILSI Europe. Food Chem Toxicol, **44**: 1636–1650.

Carrington CD, Montwill B & Bolger PM (2004) An intervention analysis for the reduction of exposure to methylmercury from the consumption of seafood by women of child-bearing age. Regul Pharmacol Toxicol, **40**: 272–280.

Cullen AC & Frey HC (1999) Probabilistic techniques in exposure assessment. New York, NY, Plenum Press.

EFSA (2005a) Opinion of the Scientific Committee on a request from EFSA related to a harmonized approach for risk assessment of substances which are both genotoxic and carcinogenic (Request No. EFSA-Q-2004-020). Adopted on 18 October 2005. EFSA J, **282**: 1–31 (http://www.efsa.europa.eu/EFSA/Scientific_Opinion/sc_op_ej282_gentox_en3,0.pdf).

EFSA (2005b) Opinion of the Scientific Committee on a request from EFSA related to exposure assessments (Request No. EFSA-Q-2003–107). Adopted on 22 June 2005. EFSA J, **249**: 1–26 (http://www.efsa.europa.eu/en/efsajournal/doc/sc_op_ej249_exposure_en2.pdf).

FAO/WHO (1999) Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 884; http://whqlibdoc.who.int/trs/WHO_TRS_884.pdf).

[1] Internet links provided in these references were active as of the date of final editing.

FAO/WHO (2000) Evaluation of certain food contaminants. Fifty-third report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 896; http://whqlibdoc.who.int/trs/WHO_TRS_896.pdf).

FAO/WHO (2002) Evaluation of certain food contaminants. Fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 909; http://whqlibdoc.who.int/trs/WHO_TRS_909.pdf).

FAO/WHO (2005) Evaluation of certain food additives. Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 928; http://whqlibdoc.who.int/trs/WHO_TRS_928.pdf).

FAO/WHO (2006a) Evaluation of certain food contaminants. Sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 930; http://whqlibdoc.who.int/trs/WHO_TRS_930_eng.pdf).

FAO/WHO (2006b) Pesticide residues in food—2006. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 187; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/DOWNLOAD/2006_rep/report2006jmpr.pdf).

FAO/WHO (2007a) Evaluation of certain food additives and contaminants. Sixty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 940; http://whqlibdoc.who.int/trs/WHO_TRS_940_eng.pdf).

FAO/WHO (2007b) Evaluation of certain food additives and contaminants. Sixty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 947; http://whqlibdoc.who.int/publications/2007/9789241209472_eng.pdf).

FAO/WHO (2008) Codex Alimentarius Commission procedural manual, 18th ed. Rome, Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission (ftp://ftp.fao.org/codex/Publications/ProcManuals/Manual_18e.pdf).

Feron VJ & Groten JP (2002) Toxicological evaluation of chemical mixtures. Food Chem Toxicol, **40**: 825–839.

Feron VJ, Cassee FR, Groten JP, Van Vliet PW & Van Zorge JA (2002) International issues on human health effects of exposure to chemical mixtures. Environ Health Perspect, **110**(Suppl 6): 893–899.

Frey HC & Patil SR (2002) Identification and review of sensitivity analysis methods. Risk Anal, **22**: 553–578.

FSA (2002) Risk assessment of mixtures of pesticides and similar substances. London, United Kingdom Food Standards Agency, Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (http://www.food.gov.uk/multimedia/pdfs/reportindexed.pdf).

Groten JP, Butler W, Feron VJ, Kozianowski G, Renwick AG & Walker R (2000) An analysis of the possibility for health implications of joint actions and interactions between food additives. Regul Toxicol Pharmacol, **31**: 77–91.

Groten JP, Feron VJ, Sühnel J (2001) Toxicology of simple and complex mixtures. Trends Pharmacol Sci, **22**: 316–322.

Hacking I (1976) The emergence of probability. Cambridge, Cambridge University Press.

Humphreys SH, Carrington CD & Bolger PM (2001) A quantitative risk assessment for fumonisins B1 and B2 in corn. Food Addit Contam, **18**: 211–220.

IPCS (1987) Principles for the safety assessment of food additives and contaminants in food. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 70; http://www.inchem.org/documents/ehc/ehc/ehc70.htm).

IPCS (2006) Principles for evaluating health risks in children associated with exposure to chemicals. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 237; http://www.inchem.org/documents/ehc/ehc/ehc237.pdf).

IPCS (2008) Part 1: Guidance document on characterizing and communicating uncertainty in exposure assessment. In: Uncertainty and data quality in exposure assessment. Geneva, World Health Organization, International Programme on Chemical Safety (Harmonization Project Document, No. 6; http://www.who.int/ipcs/publications/methods/harmonization/exposure_assessment.pdf).

IPCS (2009a) Principles for modelling dose–response for the risk assessment of chemicals. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 239; http://whqlibdoc.who.int/publications/2009/9789241572392_eng.pdf).

IPCS (2009b) Risk assessment of combined exposures to multiple chemicals: a WHO/IPCS framework (Harmonization Project Draft Document for Public and Peer Review). Geneva, World Health Organization, International Programme on Chemical Safety (http://www.who.int/ipcs/methods/harmonization/areas/combinedexposure.pdf).

Ito N, Hasegawa R, Imaida K, Kurata Y, Hagiwara A & Shirai T (1995a) Effect of ingestion of 20 pesticides in combination at acceptable daily intake levels on rat liver carcinogenesis. Food Chem Toxicol, **33**: 159–163.

Ito N, Hagiwara A, Tamano S, Hasegawa R, Imaida K, Hirose M & Shirai T (1995b) Lack of carcinogenicity of pesticide mixtures administered in the

diet at acceptable daily intake (ADI) dose levels in rats. Toxicol Lett, **82–83**: 513–520.

Jonker D, Woutersen RA & Feron VJ (1996) Toxicity of mixtures of nephrotoxicants with similar or dissimilar mode of action. Food Chem Toxicol, **34**: 1075–1082.

Jonker D, Freidig AP, Groten JP, de Hollander AE, Stierum RH, Woutersen RA & Feron VJ (2004) Safety evaluation of chemical mixtures and combinations of chemical and non-chemical stressors. Rev Environ Health, **19**: 83–139.

Krishnan K, Paterson J & Williams DT (1997) Health risk assessment of drinking water contaminants in Canada: the applicability of mixture risk assessment methods. Regul Toxicol Pharmacol, **26**: 179–187.

Mileson B, Faustman E, Olin S, Ryan PB, Ferenc S & Burke T eds (1999) A framework for cumulative risk assessment. Washington, DC, ILSI Press.

O'Brien J, Renwick AG, Constable A, Dybing E, Müller DJG, Schlatter J, Slob W, Tueting W, Van Benthem J, Williams GM & Wolfreys A (2006) Approaches to the risk assessment of genotoxic carcinogens in food: a critical appraisal. Food Chem Toxicol, **44**: 1613–1635.

Pesticide Residues Committee (2007) Pesticide residues monitoring report. Second quarter report 2007. Quarter ended June 2007. York, Pesticide Residues Committee, pp 46–47 (http://www.pesticides.gov.uk/uploadedfiles/Web_Assets/PRC/Report_Q2_2007_13_Dec.pdf).

Renwick AG, Barlow SM, Hertz-Picciotto I, Boobis AR, Dybing E, Edler L, Eisenbrand G, Grieg JB, Kleiner J, Lambe J, Müller DJG, Smith MR, Tritscher A, Tuijtelaars S, Van den Brandt PA, Walker R & Kroes R (2003) Risk characterization of chemicals in food and diet. Food Chem Toxicol, **41**: 1211–1271.

Rescher N (1993) Probability logic: a non-truth-functional system. In: Many-valued logic, 2nd ed. Aldershot, Gregg Revivals, pp 184–187.

Van den Berg M, Birnbaum L, Bosveld AT, Brunström B, Cook P, Feeley M, Giesy JP, Hanberg A, Hasegawa R, Kennedy SW, Kubiak T, Larsen JC, Van Leeuwen FX, Liem AK, Nolt C, Peterson RE, Poellinger L, Safe S, Schrenk D, Tillitt D, Tysklind M, Younes M, Waern F & Zacharewski T (1998) Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ Health Perspect, **106**: 775–792.

Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M, Fiedler H, Hakansson H, Hanberg A, Haws L, Rose M, Safe S, Schrenk D, Tohyama C, Tritscher A, Tuomisto J, Tysklind M, Walker N & Peterson RE (2006) The 2005 World Health Organization re-evaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol Sci, **93**: 223–241.

8. MAXIMUM RESIDUE LIMITS FOR PESTICIDES AND VETERINARY DRUGS

For acronyms and abbreviations used in the text, the reader may refer to the list of acronyms and abbreviations at the front of this monograph. Definitions of select terms may be found in the glossary at the end of the monograph.

8.1 Introduction

Maximum residue limits (MRLs) for pesticide residues and residues of veterinary drugs are the maximum concentrations of residues to be permitted in or on a food by national or regional legislation. MRLs for pesticide residues may also in certain cases be applicable to animal feeds. MRLs are set by the Codex Alimentarius Commission (CAC), acting as the risk manager. Draft MRLs for adoption by CAC are elaborated by the relevant Codex committees, the Codex Committee on Pesticide Residues (CCPR) and the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF), on the basis of scientific expert advice, including recommendations on MRLs, provided by the risk assessors—i.e. the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Meeting on Pesticide Residues (JMPR) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA), respectively.

JMPR evaluates pesticide residue data resulting from pesticide use according to Good Agricultural Practice (GAP) to estimate maximum residue levels[1] in food and feed commodities. Under GAP, a pesticide is used for effective pest control, but leaves a residue that is the

[1] JMPR distinguishes between a "maximum residue level", which is a scientific estimate with its attendant uncertainty, and a "maximum residue limit", or MRL, which is equivalent to a legal limit.

smallest amount practicable. Estimated maximum residue levels are recommended to CCPR (the risk managers) for use as MRLs. If the estimated chronic dietary exposure for a pesticide residue exceeds the acceptable daily intake (ADI) or an estimated short-term exposure exceeds the acute reference dose (ARfD), JMPR flags this situation to CCPR, indicating the type of data that may be useful in refining the dietary intake estimates.

JECFA recommends MRLs for veterinary drugs[1] to CCRVDF. The veterinary drugs proposed for evaluation by JECFA should be registered by national or regional authorities, commercially available and used according to the Good Practice in the Use of Veterinary Drugs (GPVD) approved by the registration authorities. CAC defines GPVD as the "official recommended or authorized usage including withdrawal periods, approved by national authorities, of veterinary drugs under practical conditions" (FAO/WHO, 2008b). If MRLs cannot be proposed such that the estimated chronic dietary exposure to a veterinary drug residue remains below the ADI, JECFA does not recommend MRLs.

In 2005, FAO, the National Institute for Public Health and the Environment of the Netherlands (RIVM) and WHO held a workshop entitled "Updating the Principles and Methods of Risk Assessment: MRLs for Pesticides and Veterinary Drugs" (FAO/WHO, 2006a). This chapter is based on the outcome of that workshop and subsequent considerations by JECFA and JMPR.

8.2 Overview of current principles and practice of JMPR and JECFA for residue evaluation

8.2.1 JMPR assessment processes for pesticide residues

The objective of a JMPR evaluation is to recommend suitable standards for pesticide residues in food commodities. Residue evaluation is complex, and the available information should be used in the context of an understanding of residue behaviour. Residue data requirements

[1] Both JECFA and CCRVDF use the acronym MRL for this limit throughout its stepwise elaboration; however, MRLVD is the acronym of the final standard adopted by CAC on the recommendation of CCRVDF.

and evaluation for JMPR are described in the FAO manual on the submission and evaluation of pesticide residue data for the estimation of maximum residue levels in food and feed (FAO, 2002a).

The FAO Panel on Pesticide Residues in Food and the Environment evaluates pesticide residue data resulting from pesticide use according to GAP to estimate maximum residue levels in food and feed commodities. The use must be safe for the user and the environment, and residues in food must be safe for the consumer.

The substance of interest is identified by systematic and common names, Chemical Abstracts Service (CAS) numbers and chemical formulae. Information on physicochemical properties, such as melting point, water solubility, octanol–water partition coefficient, vapour pressure and hydrolysis, is provided to assist with understanding the stability of the formulated product and the fate and movement of its residues.

The results of animal (livestock) and crop metabolism studies are the prime determinants of the residue definition in food and feed commodities. Substances labelled with radioactive isotopes are used in metabolism studies so that the disposition of the residue can be followed and to help with identification of metabolites. Laboratory animal, usually rat, metabolism studies serve to identify animal metabolites and to suggest times for residue clearance.

The fate of pesticide residues in soil may influence the nature and level of residues in crops, particularly for soil or seed treatments. Rotational crop studies are designed to define the nature and level of pesticide residues that might occur in a crop sowed or planted subsequent to the original crop that received the pesticide treatment.

Analytical methods used in the supervised trials and processing studies must be validated for the substrates and analytes. Analytes will include relevant metabolites that need to be measured in the trials and processing studies as specified in the residue definitions used for monitoring and enforcement and for dietary intake estimates.

Pesticide residue definitions are established for MRL enforcement purposes and for dietary exposure assessment. Residues of parent substance and transformation products are usually expressed as equivalents of the parent substance.

For dietary exposure purposes, it is desirable to include pesticide metabolites and photolysis or other degradation products that have toxicity properties similar to those of the parent substance. For enforcement purposes (testing of food consignments for compliance with MRLs), it is not desirable to include metabolites in the residue definition if they are present as only a minor part of the residue or if they are difficult or expensive to analyse. Metabolites or analytes common to other pesticides are generally avoided in residue definitions if the pesticides are to have separate sets of MRLs; otherwise, anomalies in enforcement work will occur.

JMPR accepts national registered uses of pesticides as GAP. The recommended maximum residue levels depend mainly on the data from supervised residue trials conducted in line with maximum registered uses (highest application rate, minimum preharvest interval, etc.) within GAP. The trials should cover the range of conditions expected to occur in practice, including application methods, seasons, cultural practices and crop varieties.

When the number of trials is sufficient, JMPR estimates a maximum pesticide residue level for the commodity of trade and a supervised trials median residue (STMR) (i.e. median of the valid residue data, one point from each trial) and highest residue (HR) (i.e. highest of the valid residue data, one point from each trial) for the edible portion of the commodity.

The estimated maximum residue level is recommended to CCPR for use as an MRL. The STMR and HR are used in long-term and short-term dietary exposure estimates.

JMPR also requires data from food processing studies on pesticide residues to:

- identify breakdown or reaction products generated by the process;
- find the levels of residue in processed products;
- relate the levels of residue in processed products to levels in the raw agricultural commodity (RAC);
- calculate processing factors from trials that simulate or are equivalent to commercial processes; and
- support dietary exposure calculations.

If residue levels in the processed commodity exceed the residue levels in the RAC by a margin sufficient to require an MRL higher than the RAC MRL, it is necessary for JMPR to estimate a maximum residue level for the processed commodity (FAO/WHO, 2004b).

The aim of livestock feeding studies is to find the levels of pesticide residue likely to occur in animal tissues, milk and eggs from repeated daily dosing of the animals over a few weeks. The nominal feeding levels (equivalent to the doses expressed as concentrations in the feed dry matter) should be close to expected residue level burdens in feed commodities.

The pesticide residue dietary burdens for livestock are derived from HRs and STMRs for feed commodities multiplied by standard animal diets based on Organisation for Economic Co-operation and Development (OECD) livestock feed tables since 2007 (FAO/WHO, 2008a). The dietary burdens are then related to the feeding levels for the pesticide in the livestock feeding studies to estimate animal commodity maximum residue levels. Food residues resulting from the use of external animal pesticide treatments may also need to be taken into account. Trials for these in livestock should employ the recommended formulated product with the dose rate, method of application and timing as required for the registered product. Evaluation of external animal treatments should take into account the disposition and nature of the residues found in a dermal metabolism study.

Estimated maximum residue levels, HRs and STMRs derived from external animal treatments are compared with those derived from exposure through the feed. The recommended maximum residue levels, HRs and STMRs are based on whichever values are higher from this comparison.

For chronic exposure assessment, estimates of likely pesticide residue levels in food are based on the STMRs from the supervised trials and food processing studies and long-term food consumption. Until 2005, JMPR used average daily per capita food consumption estimated for each commodity based on the five regional diets (Middle Eastern, Far Eastern, African, Latin American and European) from the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) derived from FAO food

balance sheets. Since 2006, the five regional diets have been replaced by the 13 GEMS/Food consumption cluster diets. Information on these diets is available on the WHO web site (http://www.who.int/foodsafety/chem/gems/en/index1.html). The chronic intake is calculated as the sum of intakes for each food commodity (residue × food consumption) and compared with the ADI.

For short-term exposure assessment, estimates of high intake of pesticide residue on a single day are based on the HRs from the supervised trials. Large portion sizes and fruit and vegetable unit weights have been provided by a number of countries, but more such data are needed. The short-term intake is calculated for each food separately (large portion size × HR × a variability factor for some cases) and compared with the ARfD (see chapter 6, appendix 6.1).

When an estimate of short-term exposure for a pesticide residue in a food commodity exceeds the ARfD, JMPR examines residue data from supervised trials with alternative GAPs to compare those alternative short-term exposures with the ARfD. If an estimated alternative short-term exposure does not exceed the ARfD, JMPR recommends a maximum residue level based on the alternative GAP.

JMPR, by the use of footnotes to the recommended maximum residue levels, draws attention to those cases where estimates of pesticide residue intake exceed the ADI or ARfD (after examination of alternative GAPs).

The JMPR procedures for recommending MRLs are summarized in Figure 8.1.

8.2.2 JECFA assessment processes for residues of veterinary drugs

JECFA has developed risk assessment principles for residues of veterinary drugs in foods since the first meeting devoted specifically to this topic in 1987 (FAO/WHO, 1988) and has applied conservative approaches and principles to the assessment of residues of veterinary drugs. JECFA develops recommendations for MRLs based on chronic intake estimates calculated from the median residue levels and a theoretical food basket (consisting of 300 g muscle, 100 g liver, 50 g kidney, 50 g fat, 1500 g milk, 100 g eggs and 20 g honey), to estimate a conservative daily intake of residues, known as the estimated daily intake (EDI). The formerly

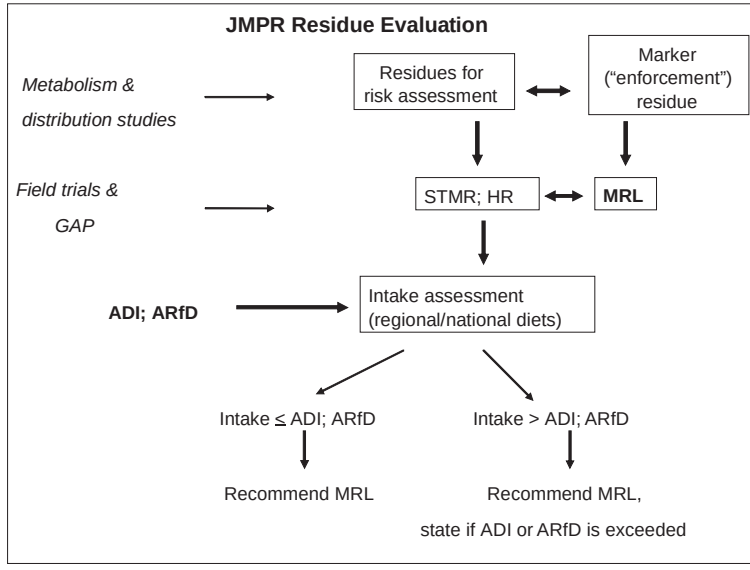

Fig. 8.1. JMPR evaluation of residue data and recommendation of MRLs

used theoretical maximum daily intake (TMDI) utilized the MRL per se as the point estimate for acceptable levels in food, which is a single value representing the upper limit of a high percentile of the distribution of residues, normally the 95th percentile. JECFA concluded at its sixty-sixth meeting (FAO/WHO, 2006b) that this method was not realistic and that all concentrations in the distribution of residues should be considered in the estimation of intake.

In the context of recommending MRLs, JECFA carries out estimates of long-term (chronic) dietary exposures to residues of veterinary drugs in which point estimates of both the amounts of food commodities consumed and the residue concentrations are used (for details, see chapter 6, section 6.3.4.1). The numerical result of this estimation, the EDI, is then compared with the type and amount of residue considered to be without toxicological, pharmacological or microbiological hazard for human health, as expressed by the ADI (for details, see chapter 7). JECFA, at its seventieth meeting (FAO/WHO, 2009), confirmed the utility of the EDI as a tool to ensure that intakes of residues resulting from use of veterinary drugs in accordance with GPVD and the recommended MRLs do not exceed the ADI.

The use of the EDI is currently applicable only to the evaluation of chronic toxicity of, and chronic exposure to, residues as reflected by the ADI. JECFA does not yet use acute dietary exposure estimates for residues of veterinary drugs, but the development of such estimates is under consideration.

JECFA uses residue depletion studies with radiolabelled parent drug as well as additional studies with unlabelled parent drug in intended target animal species for recommending MRLs in raw commodities of animal origin. The first type of study serves to estimate the time course of the concentration of the total residue of concern and to determine a marker residue substance (a substance with a known quantitative relationship between its concentration and the concentration of the total residue of concern; see section 8.3.1.1). The derived MRLs are defined on the basis of the marker residue substance. The second type of study provides information on the time course of the concentration of the marker residue in raw commodities of animal origin under approved practical conditions of use. Information from these studies is used in the derivation of MRLs and for the estimation of dietary exposure using suitable time points on the residue depletion curve. Thus, MRLs are expressed as *concentrations of a marker residue*. However, daily intakes are estimated as *amounts of total residue* of concern ingested by a person. Therefore, the selected point estimate of marker residue concentration has to be converted to equivalents of total residue and multiplied by the point estimate of the amount of the commodity consumed. The details are described in section 6.3.4.1 in chapter 6. The relationships among empirical residue depletion data, MRL, depletion/withdrawal times and EDI are illustrated in Figure 8.2.

MRLs are generally recommended for several edible tissues and products, as appropriate for the intended use—for example, for muscle, liver, kidney and fat of slaughter animals, for fat and skin of poultry (and, where appropriate, of pigs) in natural proportions, for muscle and skin of fish in natural proportions, as well as for milk, eggs and honey. If MRLs cannot be recommended for every commodity of interest, JECFA attempts to include at least appropriate target tissues for regulatory residue analysis of both domestically marketed products and products moving in international trade. Dose treatments in such depletion studies should always include the maximum approved dose, administered in the commercial formulation and under the approved

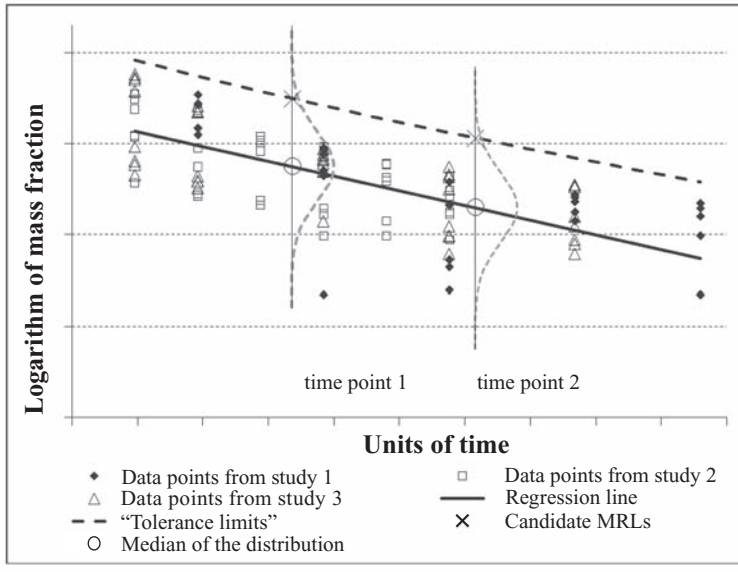

Fig. 8.2. Basic model for the determination of the MRL and of a point estimate of residue concentration used for the dietary exposure estimate

conditions of use. Residues are generally determined in several edible tissues and products, as appropriate for the intended use (e.g. in muscle, liver, kidney and fat of slaughter animals as well as in milk and eggs). These studies also have to provide the necessary information on all types of residues formed, such as free, conjugated and bound residues. For substances with an ADI derived from a toxicological end-point, all residues are considered to have the same toxicological significance as the parent drug unless data are provided to permit JECFA to discard them from consideration or data show that a metabolite has greater toxicity than the parent drug and therefore needs to be addressed separately. Thus, the default assumption is that there may be dose additivity (see chapter 7, section 7.3). Similar considerations apply to substances with a microbiologically defined ADI (see chapter 5).

In addition to specific residue data, JECFA also considers other factors, such as GPVD and the availability of suitable analytical methods for determining residues in food animal tissues. Thus, recommended MRLs may be numerically lower than the theoretical maximum values compatible with the ADI. If, for example, the

concentrations of residues in edible tissues or products estimated from residue depletion studies, when the drug is administered according to GPVD, are *below* those considered toxicologically or microbiologically maximally acceptable, then the levels observed under GPVD will determine the recommended MRLs, provided that practical analytical methods are available for routine compliance monitoring. If the residue exposure estimates found following GPVD *exceed* those compatible with the ADI, then drug use in the food-producing animals may need to be modified to reduce residue concentrations in edible tissues to acceptable concentrations before JECFA can recommend MRLs. Possible modifications include extending the withdrawal period and changing the drug dosage, form or method of delivery (FAO/WHO, 1988).

JECFA requests detailed pharmacological, toxicological, drug metabolism and other related studies to characterize the specific molecules for toxicological evaluation. Generally, identified metabolites that contribute 10% or more of the total residues are candidates for toxicological evaluation. However, in some instances, metabolites consisting of less than 10% of the total residues have been considered.

Microbiological risk has always been addressed by JECFA in its evaluations of substances with antimicrobial activity, and procedures for establishing an ADI on the basis of an antimicrobial no-observed-adverse-effect level (NOAEL) have been developed. The assessment depends on whether or not residues of antimicrobial agents ingested via food of animal origin pose a danger to human health by selective pressure on the intestinal flora, thus favouring the growth of microorganisms with natural or acquired resistance. A decision tree approach for the evaluation of antimicrobial veterinary drugs was introduced by JECFA at its forty-fifth meeting in 1995 (FAO/WHO, 1996) and later adopted at its fifty-second meeting in 1999 (FAO/WHO, 2000) (see chapter 4, section 4.12). In the interest of harmonization of methods, the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) developed a guideline (VICH, 2004) that was a refinement of the JECFA approach, and the Committee agreed at its sixty-sixth meeting (FAO/WHO, 2006b) to incorporate the VICH guideline in future assessments to ensure consistency and transparency in the determination of microbiological ADIs (for details, see chapter 5).

Additional specific data requirements for the consideration of MRLs on the basis of the ADI include authorized mode of administration, dose and formulation, and toxicodynamic, toxicokinetic, metabolism and residue depletion studies. The above data are requested for at least a standard set of edible tissues of the food animal species for which MRLs are to be set, as well as for milk, eggs and honey, if applicable. JECFA also reviews the comparative metabolism between laboratory animals and food animals to determine qualitative or quantitative similarities or differences in metabolites across species.

The data requirements of JECFA flow from the above summarized requirements of the MRL and include information on authorized conditions of use (e.g. mode of administration, dose and formulation of the commercial product, withdrawal times for edible tissues, discard times for eggs and milk), precise identification and properties of the substance under review and used in tests and studies, detailed pharmacological and toxicological studies in laboratory animals, other special studies as necessary on a case-by-case basis (e.g. microbiological studies) and pharmacokinetic and residue depletion studies in the target species of animal. Typically, a dossier of primary data and descriptions of studies conducted with the drug is provided by a sponsor (the manufacturer) or occasionally by a national authority for review by JECFA. In reaching its conclusions on MRLs, JECFA evaluates all data available to it, including those submitted by the sponsor and those identified in a search of the open literature. The Committee's decisions depend largely on consideration of the primary detailed data. Limited reliance is placed on summary or review data alone, if not supported by relevant primary data (FAO/WHO, 2006a).

JECFA may make full recommendations for MRLs of a veterinary drug in appropriate food animal species and tissues on the basis of a permanent ADI and adequate residue data. Where a suitable database is available, the above-described statistical approaches to estimate MRLs may be used. In cases where the basic model is not applicable, JECFA uses other approaches on a case-by-case basis to ensure that, if the recommended MRLs are applied, dietary exposure remains within acceptable limits. These may include using the model diet and the ratio of marker to total residues to perform a check that the MRLs recommended would not exceed the ADI. If the dietary exposure estimate exceeds the ADI, the MRLs are adjusted in an iterative process to

lower concentrations, and the calculation is repeated to ensure that the corresponding dietary exposure estimate is below the ADI.

Temporary MRLs may be recommended either when there is a full ADI but adequate residue or analytical method performance data are lacking or when the ADI is temporary. The Committee may recommend MRLs "not specified" or "unnecessary" when there is a very wide margin of safety between dietary exposure to residues and the ADI, also taking into consideration endogenous levels of the substance, where applicable. Finally, JECFA may determine that MRLs cannot be recommended because of significant deficiencies in either residue data or available analytical methods or when an ADI is not established. JECFA also does not recommend MRLs for uses incompatible with the GPVD established by national authorities.

JECFA has noted on occasions that residues at injection sites may exceed the recommended MRL for the tissue or tissues concerned at practical withdrawal times. To assess the safety implications of residues at the injection site, JECFA requires information on concentrations of residues observed in injection sites sampled under standardized conditions. The Committee has accepted a sampling procedure required by both the European Medicines Agency (EMEA) and the United States Food and Drug Administration (USFDA). It was noted that the EMEA has recently modified its sampling procedure, which now requires, in addition to a core sample of 500 g, a second sample of tissue surrounding the core sample in order to confirm the quality and correctness of the original sampling (EMEA, 2004). JECFA assesses the safety of injection site residues by comparison with an ARfD (e.g. carazolol in injection sites, evaluated at the fifty-second meeting of JECFA; FAO/WHO, 2000), although JECFA has not yet determined consumption figures for estimating acute intakes. Therefore, the consumption figure for muscle normally used for estimates of chronic intake is also used in these cases, and injection site tissue replaces muscle tissue for the estimation of acute intakes. However, JECFA does not include residues that persist at or near the injection site in assessing the contribution of drug residues in edible tissues to the estimated (chronic) daily intake expressed by the EDI.

The JECFA procedures for recommending MRLs are summarized in Figure 8.3.

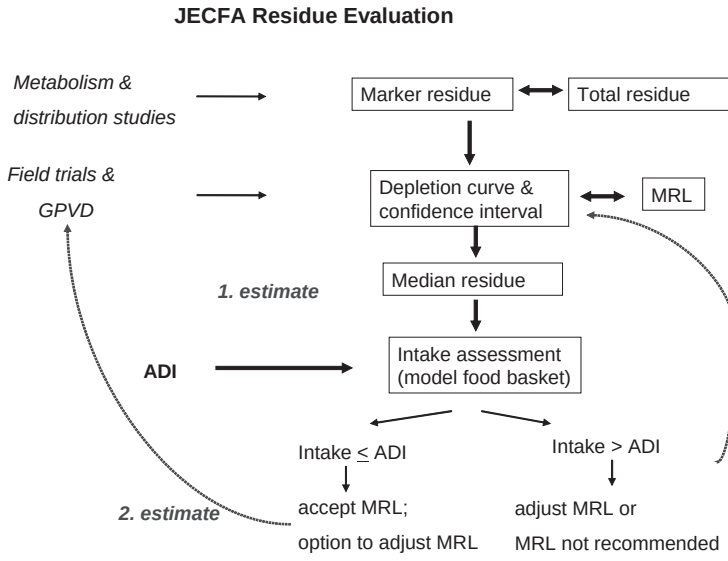

Fig. 8.3. JECFA evaluation of residue data and recommendation of MRLs

8.2.3 Comparison of JMPR and JECFA approaches

The factors considered for the establishment of MRLs include:

- residue definitions;
- animal species or crop;
- commodities (significance in trade and consumption);
- adequacy of the methods used in all studies and tests;
- analytical methods suitable for enforcement purposes; and
- GAP or GPVD.

Table 8.1 compares the options used by JECFA and JMPR in recommending MRLs.

When an ADI has been established but no residues have been detected in a commodity in any of the residue studies using validated methodology, JECFA and JMPR may establish MRLs based on the limit of quantification (LOQ) of the proposed control method. In such cases, it is considered that these MRLs afford the necessary protection for consumers, and adjustment to reflect subsequent developments in analytical methods performance is not required.

Table 8.1. Options used for recommending MRLs: a comparison of JECFA and JMPR evaluations

JECFA	JMPR
• recommended MRL (no request for additional data) - may be based on suitable residue depletion studies, GPVD or requirements of food technological processes, and compatible with toxicological, microbiological or pharmacological ADI	• recommended MRL (no request for additional data) - may be based on a sufficient number of supervised field trial data or adequate livestock feeding studies
• temporary MRL due to: - temporary ADI - deficiencies in residue studies or in analytical methods	• temporary MRL due to: - temporary ADI - deficiencies in residue trials or in analytical methods
• MRLs “unnecessary” or “not specified” (situations with a very wide margin of safety or taking into consideration endogenous levels of the substance)	
• MRLs as guidance limits (in situations where residue concentrations in tissues are below the LOQ of the validated analytical method)	
	• EMRL relating to contamination resulting from former use of the pesticide and based on monitoring data (e.g. DDT)
	• MRL relating to spices based on monitoring data
• no MRL recommended due to: - no ADI established - significant deficiencies in residue or analytical method data	• no MRL recommended due to: - no ADI established - significant deficiencies in residue or analytical method data

DDT, dichlorodiphenyltrichloroethane; EMRL, extraneous maximum residue limit; LOQ, limit of quantification.

The group of spices is a special case where CCPR agreed to consider MRLs estimated from monitoring data (FAO/WHO, 2004a). The 2004 JMPR used spice monitoring data to estimate a 95th-percentile value for the population of samples for which residues were detected at the 95% confidence level, which became the basis for an MRL recommendation (FAO/WHO, 2004c). Such an MRL has no direct relation to a registered or approved use of the pesticide.

JMPR compares the long-term intake assessment, the international estimated daily intake (IEDI), with the ADI, whereas the short-term intake assessment, the international estimated short-term intake (IESTI), is compared with the ARfD (see also chapter 6 on dietary exposure assessment). In cases where the predicted chronic exposure exceeds the ADI or the short-term exposure exceeds the ARfD, even after consideration of alternative GAPs, JMPR will report this fact to CCPR and may, if possible, indicate the data necessary to allow refinement of the risk characterization. In similar cases, JECFA will not generally recommend MRLs to CCRVDF.

To summarize, JMPR recommends MRLs based on evaluation of residue data to estimate likely maximum residue levels in food commodities resulting from pesticide use according to GAP—that is, with pesticide use for effective pest control, but leaving a residue that is the smallest amount practicable. The use must be safe for the user and the environment, and residue levels must be safe for the consumer. JMPR estimates long-term and short-term dietary exposures and compares these with the ADI or the ARfD, respectively.

JECFA recommends MRLs based on evaluation of residues resulting from drug use according to GPVD and estimates dietary exposure to residues. It also takes into account other relevant public health risks, such as allergenicity, as well as food technological aspects. MRLs are recommended only if they are compatible with GPVD and do not cause chronic dietary exposure in excess of the ADI.

8.3 Identification and description of residues and methods

8.3.1 Residue definition, chemical identity and physicochemical properties

A residue, defined in the simplest terms, results when a drug or pesticide is deliberately applied to a food-producing animal or plant. This

differentiates "residues" from "contaminants". The CAC Procedural Manual (FAO/WHO, 2008b) provides the following definitions:

> *Contaminant* means any substance not intentionally added to food, which is present in such food as a result of the production (including operations carried out in crop husbandry, animal husbandry and veterinary medicine), manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food or as a result of environmental contamination. The term does not include insect fragments, rodent hairs and other extraneous matter….
>
> *Pesticide residue* means any specified substance in food, agricultural commodities, or animal feed resulting from the use of a pesticide. The term includes any derivatives of a pesticide, such as conversion products, metabolites, reaction products, and impurities considered to be of toxicological significance….
>
> *Residues of veterinary drugs* include the parent compounds and/or their metabolites in any edible portion of the animal product, and include residues of associated impurities of the veterinary drug concerned.

Thus, the definition of a pesticide residue and a veterinary drug residue are essentially equivalent. The definition for "pesticide residue" differs from the definition for "residues of veterinary drugs" by the addition of the phrase "considered to be of toxicological significance". Neither of these definitions of residues includes reference to other substances that may be present as adjuvants in the formulated products or as carrier or delivery devices.

Both JECFA and JMPR have similar requirements for the identification and characterization of a substance that is under review for the establishment of an ADI and MRLs. A comparison of the data used for these purposes by JECFA and JMPR is given in Table 8.2.

Most of the differences in requirements for physicochemical properties reflect the concern with environmental fate, which is addressed only for pesticides by JMPR. However, there are some additional differences in the respective situations. JMPR considers the properties and relative toxicities of both the pure and the technical forms of the pesticide under review. In certain cases, parameters such as dissociation constant, *n*-octanol–water partition coefficient and photochemical degradation may be relevant for JECFA assessments. In specific cases,

Table 8.2. Identity and physicochemical properties: data used to establish identity of substances by JECFA and JMPR

JECFA	JMPR
Identity	
Chemical name	Chemical name
- IUPAC	- IUPAC
- CAS	- CAS
CAS registry number	CAS registry number
Synonyms (includes common and proprietary names)	Synonyms (includes common and proprietary names)
Structural formula	Structural formula
Molecular formula	Molecular formula
Molecular weight	Molecular weight
Physicochemical properties	
Physical appearance (state, colour)	Physical appearance (state, colour)
	Odour
Solubility in water	Solubility in water (including pH effects)
Solubility in organic solvents	Solubility in organic solvents
Stability of pure material	
Melting point	Melting point
Optical rotation	
Ultraviolet absorbance maximum	
	Vapour pressure
	Volatility (Henry's Law constant)
	Dissociation constant
	n-Octanol–water partition coefficient
	Hydrolysis rate
	Photochemical degradation
	Relative density

IUPAC, International Union of Pure and Applied Chemistry.

a veterinary drug referred to JECFA or a pesticide referred to JMPR for review may be formulated as a salt (or readily hydrolysable ester), which is rapidly dissociated into the pure active substance. It must be clearly stated in the description of the drug or pesticide in the monographs whether the description and properties given refer to the pure active substance or to the salt (or ester).

It is very important also to specify the composition of the active substance, whether it is a pesticide or a veterinary drug, especially when stereoisomers are involved, where the relative proportions of the isomers should be given. In some cases, only one isomer is active, or one may be significantly more biologically active than others.

JMPR requires information on the route of synthesis, composition of the technical-grade material and the representative batches used for the toxicological tests to interpret the results of the studies on toxicity. In general, impurities present at 0.1% or greater in a pesticide are identified, but any presence of highly toxic impurities, such as dioxins or dibenzofurans, is also stated. Mass balance should typically be ≥98%. JECFA generally does not request identification of minor impurities. However, identification of residue components that represent 10% or more of the total residues of the veterinary drug in the edible tissues is generally required. The information on appearance and physical properties may be used to establish purity of analytical standards used in a control laboratory. The information required by JMPR on solubilities, particularly the information on volatility, partition coefficient, hydrolysis and photodegradation, not only helps to establish the stability of standards, but also is critical for predicting the behaviour and fate of pesticides when applied under various typical conditions of field use and during commercial food processing.

8.3.1.1 Marker residue

CAC (FAO/WHO, 2003b) defines a marker residue for veterinary drugs as a "residue whose concentration decreases in a known relationship to the level of total residues in tissues, eggs, milk or other animal tissues", based on a definition used by JECFA. The relationship between the concentrations of the marker residue and total residues is usually established at representative time points during depletion in a study using drug labelled with a radioactive isotope. The concentrations of

total residues (total radioactivity expressed as equivalents of the parent drug) are compared with the concentrations of the marker residue, and the ratio of the concentration of the marker residue to that of total residues can be calculated.

Ideally, the marker residue provides unequivocal evidence of exposure to a specific drug. It may be the parent drug, a major metabolite, a sum of parent drug and metabolites, or a reaction product formed from the drug residues during analysis. In some cases, the marker residue is present as a bound residue and requires chemical or enzymatic treatment to be released for analysis. Not only parent drug, but several metabolites, including releasable bound residues, may possess significant pharmacological, toxicological or antimicrobial properties. However, the marker residue is not necessarily a residue of toxicological or microbiological concern. MRLs recommended by JECFA are expressed as concentrations of the marker residue. The relationship between the marker residue and total residues is used for the conversion of concentrations of the marker residue into concentrations of total residues of concern for the purpose of estimation of dietary exposure.

JMPR and CCPR use an approach similar to that used by JECFA and CCRVDF to designate the residue resulting from application of a pesticide that will be used in the establishment of MRLs, referred to as "the definition of residue for enforcement purposes". A pesticide residue typically may include not only the pesticide, but also its metabolites, degradation products and other transformation products. The situation may vary, from those in which only the parent pesticide is found on treated commodities to situations in which multiple metabolites and degradation or transformation products are present. For each pesticide used on food or feed commodities, JMPR selects the residues to be used for dietary risk assessment and those on which MRLs will be expressed. The term "definition of the residue" or "residue definition" may be used in reference to either of these two purposes.

JMPR selects the residue to be referred to in establishing the MRLs for a pesticide based on the criteria that it is simple (preferably a single substance) and suitable for practical routine monitoring and enforcement of the MRL at a reasonable cost.

There are rare situations for both veterinary drugs and pesticides in which the same metabolite is formed from several closely related parent substances and could be used as marker residue for all of them. In such cases, JECFA or JMPR may establish individual ADIs for the parent drugs or a group ADI for these substances, as appropriate. However, MRLs recommended for the parent substances are then expressed in terms of a common "marker residue".

JECFA uses the same approach for the dietary intake assessment of veterinary drugs with a common marker residue as for individual veterinary drugs (see discussion below). Similar toxicity is not necessarily the case for pesticides with MRLs based on a common "residue for enforcement purposes". For example, JMPR has found it possible, in the case of the dithiocarbamates, to separate the dietary intake assessments, because the dietary intake assessment does not rely on the common MRL, but is based on residue data from supervised trials specific to the individual substances.

8.3.1.2 Definition of residues for dietary intake

In JMPR, residue definitions are established for purposes of enforcement of the MRL and for dietary intake assessment. Residues of parent and transformation products are usually expressed as equivalents of the parent substance. For dietary exposure assessment purposes, it is desirable to include metabolites and photolysis or other degradation products that have toxicity properties similar to those of the parent substance.

The definition of a residue (for estimation of dietary intake) used by JMPR is that combination of the pesticide and its metabolites, impurities and degradation products to which the STMR and HR apply. The residue definition for estimation of dietary intake depends on the results of metabolism and toxicology studies and their general suitability for estimating dietary intake of the residue for comparison with the ADI and ARfD (FAO, 2002a).

In JECFA, data from a study with the radiolabelled drug are assessed to follow the distribution and depletion of the total residues in the edible tissues. The relationships between the total and marker residues are established for each tissue at each time point. Factors are

derived to reflect the ratio between the marker residue and total residues at each time point. These factors are then used to adjust the concentrations of marker residue for each edible tissue to total residues of toxicological concern in the calculation of the EDI.

JECFA recognizes that the use of veterinary drugs in food-producing animals can result in residues that cannot be extracted from tissues using mild procedures. In certain cases, non-extractable residues may be releasable using more specific or vigorous methods, such as the application of procedures for the release of conjugated residue components, without destroying the compounds of interest. The remaining fraction of the bound radioactivity may partly consist of fragments of the drug incorporated into endogenous compounds (endogenous fraction) that would be of no toxicological concern. Bound residues can frequently not be fully characterized. JECFA has developed a procedure to estimate the dietary exposure to residues of a drug that has a bound residue component (FAO/WHO, 1989). It takes into account the toxicological potency and bioavailability of the residues. Using the parenthetical definitions for residues and bound residues (Residues = free residues + bioavailable bound residues; Bound residue = total residue − (extractable fraction + endogenous fraction), the following equation describes the calculation of the total residue of (toxicological) concern for a given tissue:

$$\text{Residue} = P_0 + \sum_{n=n_1}^{n_x} (M_n * A_n) + (\text{Bound residues} * \text{fraction bioavailable} * A_b)$$

where:

- P_0 is the amount of parent drug per kilogram of tissue,
- $n_1 \ldots n_x$ are the different metabolites of the parent drug,
- M_n is the amount of (unbound) drug metabolite n per kilogram of tissue,
- A_n is the toxicological potency of n relative to that of parent drug,
- A_b is the estimated relative toxicological potency of the metabolites in the bound residue (when no information is available, use $A_b = 1$).

Where the endogenous fraction is not known, it should be given a value equal to zero. If the bioavailable fraction of the residues is not known, JECFA considers that a bound residue is of no greater concern than the substance for which the ADI was established, and

therefore this fraction is taken to be equal to 1. In considering the safety of bound residues, JECFA acknowledges that a suitable extractable residue component may be selected as the marker residue used for recommending an MRL if bound residues make up an insignificant portion of the total residue. In these cases, it is not necessary to apply the above calculations. However, where bound residues become a significant portion of the total residues of concern, then the procedure described may be used to assess their safety.

8.3.2 Pharmacokinetic, toxicokinetic and metabolic data used to determine the residue definition

The data requirements for JECFA and JMPR determinations of the residue definition in target species, livestock and food commodities of plant origin are available on WHO and FAO web sites. For JECFA, this information is provided in the call for data for the individual meetings. For JMPR evaluation, detailed guidance is available in chapter 3 of the FAO manual on the submission and evaluation of pesticide residue data for the estimation of maximum residue levels in food and feed (FAO, 2002a).

8.3.2.1 *Pharmacokinetics, toxicokinetics and metabolism*

The residue definition for veterinary drugs and pesticides in edible commodities of animal origin is obtained from metabolism studies conducted in target species and livestock animals (see summary in Table 8.3). The metabolites, degradation products and other transformation products are typically identified and quantified with methods based on the use of substances labelled with radioactive isotopes. Metabolites obtained in these studies are qualitatively compared with metabolites identified in laboratory animals, usually rats, to ensure that substances occurring in significant amounts in edible commodities have been included in the toxicological testing or to determine whether additional testing of individual metabolites is necessary. Metabolism studies in laboratory animals also serve to identify mammalian metabolites and to suggest possible time courses for clearance of residues.

For pesticides, a residue definition in food and feed of plant origin is obtained from plant metabolism, confined rotational crop and soil metabolism studies. Soil metabolites or degradation products might be taken up by plants and occur in edible commodities.

Table 8.3. Information used for residue definition: a comparison of JECFA and JMPR evaluations

JECFA	JMPR
Total residue and metabolism study in livestock	
Kinetic study conducted in the target animal species only	Study conducted typically in lactating goats and laying hens or in related species
Dosing levels sufficient to see total residue depletion and identify metabolites (normally at recommended dosing levels)	Dosing levels sufficient to see total residue (but not necessarily depletion) and identify metabolites
Route of administration as indicated on the label	Mostly oral route of administration; other routes possible depending on the label use
Radiolabelled substances, typically ^{14}C (^{3}H if higher sensitivity is required), to show disposition and distribution of total residues in edible tissues (including milk and eggs as appropriate), body fluids and excreta	Radiolabelled substances, typically ^{14}C, to show disposition and distribution of total residues in edible tissues (including milk and eggs, as appropriate)
Same study or similar studies show metabolic profile of the distributed residues in edible tissues	Same study or similar studies show metabolic profile of the distributed residues in edible tissues and identity of metabolites
Comparative metabolism review to ensure that residues in food animal are adequately tested in toxicology	Comparative metabolism review to ensure that residues in food animal are adequately tested in toxicology
Study intended to provide ratio of marker residue to total residues	Not relevant
Plant metabolism studies	
Not relevant	Radiolabelled substances, typically ^{14}C, to show disposition and distribution of total residues in edible commodities
Not relevant	Same study or similar studies show metabolic profile of the distributed residues in edible tissues and identity of metabolites
Not relevant	Comparative metabolism review to ensure that residues in plants are included in mammalian toxicology testing

Table 8.3. ***(Continued)***

JECFA	JMPR
Pharmacokinetics	
Studies may be conducted in laboratory animal species and target animals; if available, data from studies in humans are also considered	Metabolism studies are conducted in lactating ruminants and laying hens
Studies are conducted to address the pharmacokinetics and relative bioavailability of the veterinary drug by the intended route of administration and to establish oral bioavailability of residues in laboratory animal species	Metabolism studies provide information on the identity and disposition of residues in edible tissues, milk and eggs
Results are informative for assessment of differences in residue profiles depending on formulation, route of administration, dosing regimen and species specificity	For external treatment of animals, studies with formulated products used according to approved label instructions provide information on resulting residue levels
Results may be useful in explaining residue characteristics from sustained release (depot) formulations	Not relevant
May be useful in extrapolation of residue data to other species	Results from feeding studies in cattle and hens are extrapolated to mammalian and poultry livestock, respectively

In summary, livestock metabolism and target animal metabolism studies provide the following information for the residue evaluations by JECFA and JMPR:

- nature of the residue in edible tissues, milk and eggs;
- residue distribution in edible tissues, milk and eggs;
- time course of residue concentrations in edible tissues, milk and eggs; and
- information on fat solubility of residues.

JECFA and JMPR consider the results of the animal metabolism studies to be the prime determinant of residue definition in animal commodities and use the results to suggest which metabolites need to be monitored. For some substances, residues in animal tissues, milk and eggs are not detectable even from the use of relatively high doses.

In these cases, the metabolism studies may justify MRLs on animal commodities being set at the LOQ and may justify a decision that residue levels in edible tissues, milk or eggs are set to zero for dietary intake estimations.

Pesticide residues are described as fat soluble or not on the basis of their distribution between fat and other tissues in animal metabolism and livestock feeding studies with support from the octanol–water partition coefficient. For a fat-soluble substance, it is better to regulate on the basis of the residue in the fat component of the meat, as the residue will be more consistent in fat, compared with meat or muscle, which may contain varying levels of fat. Therefore, the "fat-soluble" status determines the nature of a sample that should be taken for enforcement analysis.

For a fat-soluble substance in meat, JMPR estimates residue levels for both muscle and fat for dietary intake estimation based on dietary consumption of meat and recommends an MRL for the trimmable fat from the meat (i.e. on the fat tissue). JECFA may recommend MRLs for both muscle (without trimmable fat) and fat (for details on definitions, see section 8.4.1.1). These residue definitions for muscle and fat were maintained at the sixty-sixth meeting of JECFA (FAO/WHO, 2006b). The residue control systems should take the differences between the JMPR definition of meat (may contain adhering fat) and the JECFA definition of muscle (which does not contain trimmable fat) into account. However, even if trimmable fat is removed, the residues of fat-soluble substances in muscle are influenced mainly by the intramuscular fat content, which can have considerable variability.

Plant metabolism studies provide the following information for the residue evaluator (JMPR):

- nature of the metabolites and photolysis products;
- plant metabolites not appearing in animals;
- composition of residue at normal harvest;
- surface or absorbed residue;
- foliar absorption;
- root absorption;
- translocation to seeds, fruits or other edible portion;

- absorption of soil metabolites; and
- differences in metabolism in transgenic crops.

Plant metabolism studies provide the background understanding for residue behaviour and support interpretation of the residue trials. For example, if the residue is essentially a surface residue, the edible portions of fruits like bananas and oranges should be relatively free of residues. If residues translocate from treated foliage to seeds, fruits, roots or other edible portion, residue levels might be expected to increase for a time after treatment.

Photolysis products may constitute part of the residue when a pesticide is used on crops in the field. Because photolysis products are generated by a non-biological mechanism, these substances are less likely than plant metabolites to be animal metabolites also.

The fate of the pesticide in soil may influence the residues in crops, particularly for soil or seed treatments. Rotational crop studies are designed to answer questions about the nature and level of pesticide residues that might occur in a crop following treatment.

8.3.2.2 *Purpose of livestock metabolism studies for veterinary drug and pesticide evaluation*

Metabolism studies in livestock are used to qualitatively and quantitatively determine the metabolism and degradation of the active ingredient.

For assessments by JMPR, metabolism studies with oral dosing of dairy livestock or laying hens provide information on the fate of residues resulting from pesticide use in the production of feedstuffs or pesticide treatment of animal housing. For direct animal treatment, dermal application studies are conducted.

For the evaluation of veterinary drugs in food by JECFA, appropriate metabolism and toxicokinetic studies in the food-producing animals that simulate the conditions of use of the drug in animal husbandry are needed. Additionally, toxicokinetic and metabolism studies in the animal species used for toxicological investigation are required.

Livestock metabolism studies fulfil several major purposes:

- to estimate total residues and their major components (and residue depletion for JECFA) in the edible livestock commodities (muscle, fat, offal [= liver and kidney for JECFA], eggs, milk), as well as the excreta;
- to identify the residues to be considered for both dietary exposure calculations and MRL enforcement or residue monitoring;
- to estimate the relative distribution of the parent substance and metabolites in muscle and fat;
- to show the efficiency of extraction procedures for various components of the residue, an element of analytical method validation; and
- to provide the basis for a metabolic profile or degradation pathway.

Toxicokinetic studies with the formulated drug product in healthy animals of each of the target species should be designed to determine the rate and extent of absorption of the active substance and its distribution, metabolism and excretion, including identification and quantification of major metabolites. The proportion of the administered dose eliminated by metabolism (usually by liver) and excretion (in urine and faeces) is also determined. Kinetic parameters, including "flip-flop" kinetics (situations where the rate of excretion exceeds the rate of absorption; Renwick, 2008), when present, are derived from plasma concentration–time data in individual animals or populations based on compartmental or non-compartmental analyses.

Chirality may have a marked impact on both pharmacokinetic behaviour and pharmacodynamic activity. A drug with a single chiral centre exists in two enantiomeric forms, and these enantiomers may have distinct pharmacokinetic and pharmacodynamic properties in vivo. Most registered chiral drugs contain a racemic mixture (50:50) of the two enantiomers. In determining the kinetic properties of such a mixture, it is essential to analyse each enantiomer separately. For both veterinary drugs and pesticides, JECFA and JMPR (respectively) consider it important to consider the possible different properties of enantiomers in the safety assessment and in the process for recommending MRLs.

Injectable sustained-release formulations frequently lead to prolonged persistence of drug at the injection site and "flip-flop" blood kinetics. Injection site residues vary markedly between animals in magnitude of concentration and persistence. They usually comprise a very high proportion of unchanged drug. Hence, the marker residue (if it is not the parent drug molecule) is unlikely to be appropriate for determining residues at the injection site. Risk from exposure to injection site residues is primarily considered short term (acute) in nature (FAO/WHO, 2000), and JECFA has for certain substances established ARfDs based on pharmacological end-points. JMPR has developed specific guidance on the setting of ARfDs, including a proposal for a single-dose study protocol suitable for this purpose (Solecki et al., 2005).

Livestock metabolism studies on pesticides should reflect feeding of individual substances, usually the parent compound. The dosing material for oral studies should not be a mixture of active ingredient and plant metabolites. If the plant metabolites are also found to be animal metabolites, then additional livestock metabolism experiments that involve dosing with plant metabolites need not be considered. If the plant metabolism studies show that a plant metabolite comprises a major portion of the total radioactive residues on a feed item or that it is not also an animal metabolite, a livestock metabolism study involving dosing with that metabolite might be necessary.

8.3.2.3 Purpose of plant metabolism studies

Plant metabolism studies are conducted for pesticides to determine the qualitative metabolic (or degradation) fate of the active ingredient. The composition of the terminal residue must be determined before the residue definition is decided and before analytical methods can be developed for monitoring and for MRL enforcement purposes. Crop metabolism studies are used to elucidate the degradation pathway of the active ingredient—that is, to identify the metabolism and degradation products when a pesticide is applied to a plant directly or indirectly, including the relative quantity of metabolites and degradation products in extracts and non-extractable material.

Crop metabolism studies serve the following major purposes:

- to provide an estimate of total radioactive residues in the various RACs of treated crops;

- to determine the distribution and movement of residues within the plant (e.g. to determine whether the pesticide is absorbed through roots or foliage or whether translocation occurs);
- to identify the components of the terminal residue, which serve as part of the basis for setting the residue definition, thereby defining the components to be quantified by the residue analytical methodology; and
- to demonstrate the efficiency of the extraction procedures for the various components of the residue.

Transgenic and non-transgenic crops may metabolize the pesticide differently. However, the principles for deciding residue definition remain the same. When a commodity produced by a non-transgenic crop cannot be readily distinguished from the transgenic crop commodity, the residue definition should be the same for both, because the residue analyst testing a commodity in trade may not know whether the crop is transgenic or non-transgenic. No single approach is applicable to all situations, and a case-by-case approach is needed at present.

Data on metabolism are used in evaluating both the toxicological and residue profiles of pesticides. JMPR examines the metabolism in experimental animals and compares it with both that in food-producing livestock and that in plant species on which the pesticide is used. This is required to decide upon the relevance of the toxicological studies to humans and to define the residues in plants and livestock products. The ADI estimate, based on toxicological studies in experimental mammalian animals, is relevant for residues in foodstuffs only if the metabolite pattern is qualitatively similar.

Plant metabolites or degradation products (e.g. from photolysis) that have not been identified in laboratory animal metabolism studies are not covered by the initial toxicological database. Separate studies for these substances may be necessary if significant residues occur in food and feed items.

For pesticide evaluation by JMPR, soil metabolism and rotational crop studies provide information on metabolites or degradation products produced in the soil that may be taken up in the target crop or a crop that is planted following the harvest of the target crop. If metabolites occur that had not been previously identified in crops or animals, further information on their toxicological significance is needed.

For paddy rice grown in a water/sediment environment, studies such as photolysis in natural pond water and residue degradation in water/sediment systems are relevant. However, the necessary information on the nature of the residue may be obtained from a paddy rice metabolism study.

8.3.3 Analytical methods and residue stability in stored analytical samples

JECFA and JMPR have similar requirements for analytical method validation (see chapter 3). For methods used in pharmacokinetic or toxicokinetic studies, residue depletion studies, supervised field trials and processing studies, the emphasis is on demonstrating that the method performed reliably in the hands of the analysts involved in that specific study. Most contemporary studies are conducted according to Good Laboratory Practice (GLP) and provide detailed records of the data provided for assessment. JECFA and JMPR always perform an independent review of the validation data for the methods used in the studies. When a method is assessed for its suitability to support MRL enforcement and monitoring of residues, the practicability of use of the method in a routine setting is additionally an important consideration.

Although the requirements for analytical methods and analyte stability determinations are very similar for both JECFA and JMPR, there are some differences in how they evaluate the submitted data. The comparison is summarized in Table 8.4. More details are provided in the following sections.

8.3.3.1 *Method performance requirements*

JECFA and JMPR have devoted significant efforts to evaluating the performance of analytical methods because of the influence it has in recommending MRLs. Both have adopted performance criteria that are used when evaluating methods proposed for monitoring of compliance of commodities with a recommended MRL. Major considerations include accuracy (frequently estimated from analyte recoveries), precision (repeatability and reproducibility), sensitivity (slope of the calibration curve) and selectivity. Use of commonly—usually commercially—available laboratory instruments and use of solvents that do not pose potential environmental or human health risks are

Table 8.4. Information on analytical methods and stability of residues in frozen storage prior to analysis: a comparison of JECFA and JMPR evaluations

JECFA	JMPR
Validation and verification of marker residue methods	Validation and verification of enforcement residue methods
Usually single (marker) residue	Emphasis on multiresidue method for enforcement, single-residue methods for field trials
Recovery correction used	No recovery correction used, but monitored (also no correction for loss of analyte during frozen storage of samples)
Stability of marker residue in matrices	Stability of parent and relevant metabolites in representative matrices
Raw commodities only	Includes assay validation for processed food studies

also important factors to consider. In addition, adequate method performance testing for specific techniques (e.g. microbiological detection) is required. Guidance for analytical method performance factors has been described in individual reports. Based on JECFA and JMPR advice, CCRVDF and CCPR have established performance criteria for analytical methods for controlling compliance with MRLs (FAO, 2002b; FAO/WHO, 2003a). Target values for method precision and recovery have been established for the residue concentrations typically required to support MRLs.

Evaluations of analytical assays for veterinary drugs and pesticides are arrived at using similar procedures, but the interpretation of the results is different. For veterinary drugs, the analyte is the marker residue, and all validation and stability requirements are directed towards that molecule. Results are corrected for recovery. Decisions for rejection of assay validation results due to low recovery are made on a case-by-case basis. Low recoveries may occasionally be acceptable if the concentration of an internal standard is used as a reference point for quantification of the analyte.

For pesticide field trials, the analytes include parent substance and all relevant metabolites. Analytical methods are required to determine all residue components needed for the residue definitions for compliance with the MRL and for estimation of dietary intake. The major residue components are determined individually as far as technically possible. The LOQ of the analytical method is taken as the lowest residue level where analytical recoveries were tested and shown to be acceptable. Decisions for rejection of assay validation results due to low recovery are made on a case-by-case basis; in general, analytical recoveries are acceptable in the range 70–130%. Extractability of the residue should be tested by analysis of samples from the metabolism studies, where concentrations of parent and metabolites are already known from radiolabel (usually ^{14}C) measurement.

For pesticides, the preferred regulatory method is a multiresidue procedure, even if its recoveries are not as good as those of a substance-specific individual method. Where the residue definition for dietary exposure assessment is different from that for regulatory purposes, analytical methods specially developed for determination of specified metabolites are also required.

In summary, the main difference in the procedures is that JMPR uses analytical recovery to assess the acceptability of data, whereas JECFA accepts adjustments of analytical results for analytical recovery. This is consistent with analytical practices in the respective areas of veterinary drugs and pesticides and with International Union of Pure and Applied Chemistry (IUPAC) guidance on recovery correction (Thompson et al., 1999).

8.3.3.2 Analyte stability

The purpose of the stability studies is to show that the analyte is stable under conditions of analysis and storage. Similar analyte stability information is evaluated by JECFA and JMPR, including the stability of pure standards as normally constituted and in solution and during sample processing.

Stability studies are conducted to determine if pesticide levels in stored analytical samples remain stable during the period of storage under controlled freezer conditions. The results of storage stability

tests conducted on residue samples held in storage from representative substrates should be provided. For plant materials, the number of crops depends on the uses of the pesticide. Typical matrices are selected to include materials containing predominantly water, oil, protein or starch. Animal tissues, milk and eggs are tested for residue storage stability when animal commodity MRLs are needed. The study conditions reflect those to which the samples from the residue trials have been subjected (often with storage for a year or more). Where sample extracts have been stored for more than 24 h prior to analysis, the stability of residues is demonstrated with recovery studies performed under similar conditions.

Freezer storage stability studies are needed to provide assurance that the residues in the stored sample are essentially the same as those in the fresh sample (FAO, 2002a). When the analytical method determines the "total residues", storage stability studies include not only the total residues, but also separate analyses of all substances that may be included in the residue definitions.

JMPR considers that residue data from supervised trials and other studies would generally not be valid when the samples have been stored in conditions and for a time shown by the frozen storage stability studies to result in more than 30% reduction of residue concentration. JMPR does not adjust residue data for possible losses during frozen storage.

For veterinary drugs, the stability of the analyte under normal conditions of storage is investigated to demonstrate the period for which the marker residue remains stable in target tissues, to ensure the accuracy of the analytical result obtained in the residue depletion studies and for validation of the regulatory assays. For example, in a veterinary drug, stability is demonstrated during frozen storage at −20 °C over a period of at least 6 weeks to reflect the typical period of time for which a survey sample may be stored awaiting regulatory analysis. Decisions on acceptable stability criteria (usually ≥70%) are made on a case-by-case basis. If the analyte is not stable in tissues under these conditions of storage, other conditions, such as storage at −70 °C, may be required. As a positive result may lead to reanalysis, possibly by a second laboratory, it is preferable that stability is investigated over a prolonged period of 3–6 months to represent the potential time that may elapse between an initial analysis and a subsequent reanalysis

of a regulatory sample. Preferably, such studies are conducted with both fortified blank matrix and incurred materials, as the behaviour of residues in fortified matrix may not be the same as observed when incurred residues are investigated.

8.3.3.3 Fate of residues during commercial food processing

The aim of food processing studies on pesticide residues is to identify breakdown or reaction products generated by the process, to find the levels of residues in processed products and to support dietary exposure calculations. JECFA does not consider processing and evaluates residues of veterinary drugs only in the raw product. Also, JMPR does not require any processing data for meat or dairy commodities.

JECFA also considers other factors when setting MRLs. For example, the antimicrobial activity of substances may interfere with fermentation processes in food production in foods of animal origin, and therefore the MRLs may be set at levels to avoid such interference. Such cases are described explicitly and transparently in JECFA evaluation reports. It should be noted that MRLs accommodating food technological requirements are set by JECFA following a specific request from CCRVDF.

JMPR evaluates changes in the nature of the residues during commercial food processing and levels occurring in processed plant commodities. It evaluates food processing data on residue behaviour where significant residues occur in plants or plant products that are processed into food. For example, information on the fate of pesticide residues in wheat during milling is needed, because residue levels in bran and flour are likely to be higher and lower, respectively, than those in the wheat, necessitating the recommendation of an MRL for bran. "Significant residues" are generally defined as >0.1 mg/kg, unless the substance has a high acute or chronic toxicity. Special attention should be given to residue concentrations below 0.1 mg/kg in case residues concentrate in further processing steps (see chapter 3 of FAO, 2002a). The FAO manual (FAO, 2002a) gives general advice on planning and conducting food processing studies.

Effects on the nature of the residues during processing and the identification of breakdown products are commonly determined

by in vitro hydrolysis procedures. Therefore, a concept is adopted of selecting three different hydrolytic conditions to represent the processing effects of pasteurization, boiling (also baking and brewing) and sterilization. The hydrolysis studies are the basis for the subsequent studies on the levels of residues in processed products. They make it possible to confirm the definition of the marker residue for processed products or to define extra breakdown products to be analysed in further studies.

Based on the effect on residue levels and the disposition of the residues in the various processed products, processing factors are calculated and considered by JMPR as follows:

$$\text{Processing factor} = \frac{\text{Residue level in processed commodity}}{\text{Residue level in raw commodity}}$$

Processing factors assist in the dietary intake assessment of processed commodities. They are also used in recommending MRLs for processed products with an existing Codex commodity code, but only if the processing leads to an increase of the residue level.

Residues in processed dairy commodities with higher fat content than milk will have a higher residue level in the processed commodity than in the raw product for fat-soluble substances. Partitioning of residues into the fat in milk is influenced by the molecular structure of the substance. Furthermore, the fat content of milk is variable. JMPR decided to recommend two MRLs for fat-soluble substances, one on whole milk and one on milk fat (FAO/WHO, 2004c). This is necessary to estimate residues in processed dairy commodities. Until its sixty-sixth meeting (FAO/WHO, 2006b), JECFA had recommended MRLs only on a whole milk basis, but at that meeting it adopted the JMPR approach. For this purpose, residue depletion studies involving milk should include analysis of the marker residue in both whole milk and the fat portion of the milk.

8.3.4 Field study data used to identify the MRL: livestock feeding studies and animal treatments

The aim of livestock feeding studies for pesticides is to find the levels of residue likely to occur in animal tissues, milk and eggs

from repeated daily dosing of the animals over a few weeks. This is comparable to the residue depletion studies conducted for veterinary drugs chronically administered in feed or in drinking-water. The JMPR and JECFA approaches to these study types are presented in Table 8.5.

The nominal lowest feeding level for pesticides (equivalent to the doses expressed as concentrations in the feed dry matter) should be close to the expected residue level burdens in feed commodities. Additionally, animals are fed levels of 3 and 10 times this dose. For pesticides, milk from dairy cows and eggs from poultry are collected daily during treatment and recovery. Collection of residue depletion data in fat is particularly useful for persistent pesticides with slow depletion rates.

Veterinary drugs are administered at the maximum label dose and duration. Sampling of edible tissues, milk and eggs may be appropriate during treatment, depending on the type of product and treatment, but is typically performed less frequently than sampling after the cessation of treatment for veterinary drugs.

Although JECFA (for direct drug treatment) requires only that a veterinary drug is administered according to the approved label instructions, both JECFA (for chronic feed and water treatments) and JMPR consider it important for studies to continue at least until residue levels reach a plateau in relevant tissues and products, such as milk and eggs.

Both pesticides and veterinary drugs may result in residues in the food animal as a result of direct treatments. A comparison of the JECFA and JMPR approaches to these types of studies is presented in Table 8.6.

Residue depletion studies with external animal treatments of pesticides and veterinary drugs should employ the recommended formulated product with the maximum dose rate, method of application and timing as required for the registered product. Evaluation of external animal treatments takes into account the disposition and nature of the residues found in metabolism studies based on the same route of exposure.

Table 8.5. Information on livestock feeding studies and animal treatments: a comparison of JECFA and JMPR evaluations

JECFA	JMPR
Use of veterinary drug in line with label instructions (use of veterinary drug in medicated feed or drinking-water products)	
Trials in typical breeds in commercial production and conditions	
Study conducted in target animal species	Lactating dairy cows to represent mammals, laying hens to represent poultry
Use of approved formulation at maximum label dose and duration under typical field conditions	Dosing daily via capsule at approximately 1×, 3× and 10× expected dietary burden
For chronic feed and water treatment, duration sufficient to reach plateau concentrations of residue in edible tissues and in milk and eggs	Duration typically 28 days with 5- to 7-day recovery period; target is to reach plateau concentrations of residue in milk and eggs
Slaughter intervals and number of animals slaughtered for tissue collection sufficient to estimate maximum concentrations of residues and time of occurrence of maximum residue concentrations and kinetic parameters of subsequent depletion	
Measure residue levels in muscle, fat, liver and kidney (whole milk and eggs, if applicable)	Measure residue levels in the four edible tissues at end of treatment and recovery
Measure residue levels in milk and eggs regularly during and after cessation of treatment	Measure residue levels in milk and eggs collected daily during treatment and recovery period
Residues to be measured are the marker residues, used to derive the MRLs, to estimate the exposure to residues and for the risk assessment	Residues to be measured include the components of the residue definitions for MRL enforcement and risk assessment
Residue depletion study	
Conduct under GLP	Conduct under GLP

Table 8.6. Information on direct treatment of livestock: a comparison of JECFA and JMPR evaluations

JECFA	JMPR
Use of veterinary drug in line with label instructions (all treatments)	Use of pesticide in line with label instructions (external treatment only)
Trials in typical commercial animals and conditions	Trials in animals expected to generate highest residue (preferred)
Study conducted in target animal species using approved formulation and method of application at the maximum label dose and duration under typical field conditions	Study conducted in target animal species using approved formulation at maximum label dose and duration under typical field conditions
Slaughter intervals to demonstrate time course to the maximum concentration of residues and subsequent depletion	Slaughter intervals to demonstrate time to and duration of maximum residue concentrations and subsequent depletion
Trials to cover typical breeds in commercial production	Trials to cover typical breeds in commercial production
Measure residues in muscle, fat, liver and kidney (whole milk, eggs and honey, if applicable)	Measure residues in muscle, fat, liver and kidney (whole milk, milk fat for fat-soluble substances and eggs)
Sample muscle and, where applicable, fat from the treatment site	Sample fat from the treatment site
Residues to be measured are the marker residues, used to establish the MRL and for risk assessment	Residues to be measured to cover enforcement and risk assessment residue definitions
Depletion study	Depletion study
Conduct under GLP	Conduct under GLP not stressed

8.4 Criteria for selecting data, species and commodities

8.4.1 Comparability of definitions for species, tissues and commodities of foods of animal origin

The evaluation of pesticide and veterinary drug residues is similar conceptually in a number of areas, but some details and assumptions are at variance, as can be seen from a comparison of the Codex Classification of Foods and Animal Feeds (FAO/WHO, 2006c) with

the Codex Glossary of Terms and Definitions (Residues of Veterinary Drugs in Foods) (FAO/WHO, 2003b). The relevant points of discussion on definitions are noted below.

8.4.1.1 Meat and muscle

JMPR (FAO/WHO, 2006c) refers to *meats* (from mammals other than marine mammals) as

> muscular tissues, including adhering fatty tissues such as intramuscular, intermuscular and subcutaneous fat from animal carcases or cuts of these as prepared for wholesale or retail distribution in a fresh state.

JECFA (FAO/WHO, 2003b) refers to *muscle* as "skeletal tissue of an animal carcass or cuts of these tissues from an animal carcass that contains interstitial and intramuscular fat". This includes "bone, connective tissue, tendons as well as nerves and lymph nodes in natural portions", but does not include edible offal or trimmable fat. *Meat* is considered the edible part of any mammal.

JMPR (FAO/WHO, 2006c) refers to *poultry meats* as "the muscular tissues including adhering fat and skin from poultry carcasses as prepared for wholesale or retail distribution" and specifies that "for fat-soluble pesticides a portion of adhering fat is analysed and MRLs apply to the poultry fat".

JECFA (FAO/WHO, 2003b) refers to *poultry* as "domesticated birds including chickens, turkeys, ducks, geese, guinea-fowls or pigeons".

8.4.1.2 Milk

The definitions for *milk* used by JMPR and JECFA are substantially the same (FAO/WHO, 2003b, 2006c).

8.4.1.3 Eggs

The definitions used by JMPR and JECFA for *eggs* are the same. The classification used by JMPR and JECFA allows for specific commodities (e.g. duck eggs, goose eggs); JECFA may use a wider species grouping for commodities, depending on the available data (e.g. poultry eggs) (FAO/WHO, 2003b, 2006c).

8.4.1.4 Aquatic species

JMPR uses definitions for *fish* that range from general category to specific species (e.g. trout). JECFA uses a definition that allows for inclusion of several *aquatic species*, and the term may also apply in certain cases to invertebrates. Some differences may be in relation to the portion of the commodity to which the MRL applies. For JMPR, the portion of fish is the whole commodity in general after removal of the digestive tract; for JECFA, the portion of aquatic species refers to muscle tissue or muscle and skin in natural proportions (FAO/WHO, 2003b, 2006c).

8.4.1.5 Edible offal

The definition used by JMPR for edible offal includes a much broader list of organs (e.g. liver, kidney, tongue, heart, stomach, thymus gland, brain) than the definition of edible offal considered by JECFA (i.e. liver and kidney). Specific species/food categories for liver and kidney that correspond with the JECFA species/tissue combination also exist in the Codex Classification of Foods and Animal Feeds used by JMPR (FAO/WHO, 2003b, 2006c).

8.4.2 Data evaluation based on the application of GLP, GAP and GPVD

JECFA and JMPR consider all the relevant information on the uses of the substance as it is authorized in commercial products by national authorities. Many national governments have established data quality requirements for substances intended for new uses and new registrations. This is generally referred to as consideration of data from studies conducted according to GLP. The principles of GLP define a set of rules and criteria for a quality system applied to the processes and conditions under which non-clinical health and safety studies are planned, performed, monitored, recorded, archived and reported.

GAP and GPVD refer to those uses that are authorized by national registration authorities and issued as directions for use and printed on pesticide product and veterinary drug preparation labels. The GAP and GPVD authorizations may vary among national governments to satisfy the practical needs of plant production and animal husbandry and relevant national legislation.

MRLs for residues of pesticides and veterinary drugs are recommended based on the results of analysis of residue trials reflecting the registered or authorized uses of the substance and available analytical methods. In order to identify whether a specific study and its data are suitable for recommending an MRL, JMPR considers the approved product label that describes the registered or authorized uses reflecting GAP. Similarly, JECFA reviews information from residue and metabolism studies from the approved uses of commercial products as guidance to determine whether data from studies were conducted according to GPVD. In practice, this translates into the consideration of the types of study data given in the following sections to recommend MRLs for appropriate commodities and species and uses. It should be noted that evaluations and recommended MRLs do not consider off-label use or potential misuses of the substance.

8.4.2.1 JMPR

Information requested and considered by JMPR is specified in the FAO manual on the submission and evaluation of pesticide residue data for the estimation of maximum residue levels in food and feed (FAO, 2002a) and comprises the following:

- identity and physical and chemical properties;
- metabolism and environmental fate;
- residue analysis and stability of pesticide residues in stored samples;
- use pattern, including major pests or diseases to be controlled, crops and situations, and formulations and type of treatment (route of application: e.g. foliar, dip, pour-on);
- results from supervised trials on crops;
- results from farm animal feeding studies;
- fates of residues in storage and processing;
- residues in food in commerce and at consumption;
- direct treatment of animals, if applicable (not covered by animal feeding studies; this refers to a dermal treatment);
- labels of the commercial products authorized, confirming the above use patterns; and
- national residue definitions.

8.4.2.2 JECFA

JECFA considers the conditions of use of commercial products authorized. In its call for data, the JECFA Secretariat requests:

- chemical identity and properties;
- use and dosage forms;
- pharmacokinetic/toxicokinetic and metabolism studies in experimental and target animals;
- residue depletion studies in target animals using substances labelled with radioactive isotopes (to provide information on total residues and major residue components);
- residue depletion studies with unlabelled drug for analysis of marker residue in target animals, eggs, milk and honey, as appropriate;
- a description of the analytical procedures for detection and determination of residues;
- labels of the commercial products authorized, confirming the above use patterns; and
- a review of the routine analytical procedures for determination of residues, including quality assurance systems.

Registered and approved veterinary uses may vary from country to country, because, among other reasons, the efficacious use patterns may be different, especially in regions with great differences in disease distribution, predominant parasites, production methods (e.g. extensive or intensive), predominant animal breeds, climate and water temperature (e.g. aquaculture).

8.4.3 Direct external animal treatment—dossier submissions to JMPR and JECFA

Residue studies relating to substances with ectoparasiticidal uses may be submitted to JMPR or JECFA for evaluation and MRL recommendations. The majority of such submissions regarding direct external animal treatment are provided to JECFA.

Where the substance primarily has pesticidal uses on food crops, the data submission for direct external animal treatments is likely to be included as part of the pesticide dossier submission to JMPR.

If the substance has been developed by a company whose business is primarily animal health, it is likely that the dossier will be sent to JECFA.

8.5 Extrapolation issues

8.5.1 Proposal for expanding the scope of MRLs

Both JECFA and JMPR have no fixed rules on extrapolation of MRLs to other crops and species or between regions, but have extrapolated data on a case-by-case basis.

8.5.1.1 Pesticide residues

JMPR relies on the registrations of national authorities. Consequently, JMPR does not recommend separate MRLs unless there are nationally registered or approved uses. In order to make recommendations for any MRL, JMPR would expect to receive information on the national registered uses and data from appropriate residue trials.

Where residue data are unavailable or are very limited, JMPR will consider extrapolating from one crop with relevant data to another crop where relevant data are incomplete. The 1997 JMPR listed the information needed for extrapolation to additional crops, including "minor crops" (FAO/WHO, 1997). No definition of "minor crop" is widely accepted, although attempts to produce an acceptable definition have been made based on consumption and trade data (Harris & Gaston, 2004). In particular, the information requested includes the description of the cultural practices for the production, the approved or registered uses of the pesticide and the reasons for expecting residue levels on the "minor crop" to be similar to those on the major crop. Information on the potential problems in international trade is also useful.

The current JMPR approach to the estimation of group maximum residue levels is explained in the FAO manual on the submission and evaluation of pesticide residue data for the estimation of maximum residue levels in food and feed (FAO, 2002a). Group tolerances may be proposed where data are available on a number of crops within that crop group or at least two species are included in products of animal origin.

Commodity groupings described in the Codex Classification of Foods and Animal Feeds (FAO/WHO, 2006c) are the basis for group maximum residue levels.

The approach was amended by JMPR in 2006 (FAO/WHO, 2007) in responding to recommendations from a workshop (FAO/WHO, 2006a). Commodity group MRLs may be proposed on the basis of the following minimum conditions: the pesticide is registered or authorized on the crop group, and relevant and adequate residue data are available for at least one major commodity of the group. However, all relevant data for the commodities of the group should be taken into account.

In some cases, where the residues on one or a few commodities in the group are quite different from the rest, it may be possible to recommend a limit for, for example, group X, except for commodities Y and Z.

A general principle on recommending group MRLs in wider circumstances should be considered in an attempt to cover more uses where national authorizations exist. Overall, to facilitate international trade and protect consumer health, it may be better to recommend these MRLs rather than to have no standards at all.

In an FAO-sponsored project on minimum data requirements, Harris & Gaston (2004) recommended a number of possibilities for plant commodity group tolerances and extrapolations that were based on a comparison of the national rules from Australia, the United States of America (USA) and the European Union (Table 8.7). It was proposed that these extrapolations were most likely to be acceptable from a risk management perspective, as these minimum data requirements were already routinely applied in these countries.

8.5.1.2 *Residues of veterinary drugs*

JECFA has routinely recommended MRLs in animal species such as cattle, pigs, sheep, chickens and turkeys. JECFA has recommended MRLs for at least 15 substances in some species, including horses, goats, deer and rabbits, on the basis of data from related species (FAO, 2004). This extension of MRLs from one species with a comprehensive data set to another species without such a data set has been based

Table 8.7. Extrapolations that can be used in situations of comparable GAP[a]

Crop	Recommended extrapolations
Citrus fruit	Oranges and a small citrus to whole group
Tree nuts	Almonds plus one other nut (except coconuts) to whole group
Pome fruit	Apples and pears to whole group
Stone fruit	Peaches, nectarine and cherry or peaches, plum and cherry to whole group
Berries and other small fruit	Any berry and currant to whole group (excluding grapes)
Root and tuber vegetables	Potato, carrot and one other root crop to whole group
	Potato to tuber and corm subgroup
	Sweet potato or yam to tuber and corm excluding potato subgroup
Bulb vegetables	Onions green and dry to whole group
Fruiting vegetables (non-cucurbits)	Tomato and peppers to whole group
Fruiting vegetables (cucurbits)	Cucumber, melon and other cucurbits to whole group
Brassicas	Cauliflower or broccoli and cabbage and one other *Brassica* to whole group
Leafy vegetables (also see stem vegetables)	Head and leafy lettuce and spinach to leafy vegetables
	Cos lettuce to leafy Asian vegetables
Herbs	Two leafy herbs to whole group
Legume vegetables (fresh)	Beans green and peas green to whole group
Stem vegetables	Celery to leafy petioles subgroup
Pulses	Any dried bean and dried pea to whole group
Oilseeds	Any three oilseeds to whole group
Cereals	Rice plus any two other cereals to whole group including rice

[a] From Harris & Gaston (2004).

on considerations such as the choice of a marker residue and how similar the MRLs are for the species for which recommendations on MRLs have already been made based on data.

For the majority of substances with MRLs for more than one species, the same marker residue has been identified. For products such as eggs and milk, the marker residue is not different from those defined for edible tissues, including liver and kidney. The parent drug has been chosen as the marker residue in almost all cases.

The range of variation of the MRLs between species has routinely been a factor of 3 or less (e.g. cattle and pig muscle 300 µg/kg, poultry muscle 800 µg/kg). From the examination of the variations of MRLs between species, most of the differences can be explained by variations in ratios of the marker residue to total residues. When these differences in the ratios exist, harmonization of the MRLs across species could result in the EDI exceeding the exposure to residues permitted by the ADI for those species.

JECFA has based its recommendations on two situations:

- substances with a residue depletion study using unlabelled drug in the specific species in conjunction with data on comparative metabolism or relevant data on metabolism in another species; and
- substances where MRLs were recommended only by extrapolation of information available for another relevant species.

8.5.1.3 *Possible extension of MRLs to other animal species*

For substances that have no MRLs recommended in any species, a full set of residue data in all relevant species and tissues should be provided so that the most complete set of MRLs can be recommended.

For substances that have MRLs recommended in one or more species, MRLs could be extended to a related species provided that the metabolic profile is comparable, the marker residue is present in the species for which the extension is considered at sufficient levels for monitoring by validated analytical methods and there is an approved use. Extension of MRLs from one species to another may be reviewed on a case-by-case basis; however, possible examples are shown in Table 8.8.

Table 8.8. Possible extrapolations between animal species

Species with a full set of available data	Recommended extrapolations
Ruminant (muscle, liver, kidney, fat)	All ruminants
Non-ruminant mammals (muscle, liver, kidney, fat)	All non-ruminant mammals
Chicken and eggs	Poultry and poultry eggs

8.5.1.4 Honey

It is not appropriate to consider honey as a candidate for extension of MRLs from one species to another because of the difficulty in extrapolating from mammals, birds or fish to bees, as the treatment modalities are not comparable. The factors likely to influence the extent of formation and the kinetic behaviour of residues in honey are more numerous than those for the foods derived from other animal species. The main groups of substances that typically leave residues in edible bee products are antibiotics (residues mainly in honey and royal jelly) and persistent lipophilic acaricides (residues mainly in wax and propolis). The stability of some of these substances in honey may be limited; however, a decrease in concentration over time will be a factor mainly of dilution as more honey is produced. Furthermore, the marker residue concept is not normally or easily applicable.

8.5.2 Geographic extrapolation

8.5.2.1 Pesticide residues

Residue data from countries are compared with national registered uses in the country of the trials or in a neighbouring country with similar climate and cultural practices.

The 2004 JMPR (FAO/WHO, 2004d) assessed the results of work carried out by an OECD/FAO project (OECD, 2003), which reviewed supervised residue trials on a given crop conducted under the same GAP with the commodity harvested on day zero after the final pesticide application and showed that residue levels were at least as variable within geographic zones as between geographic zones. It was suggested that application method, crop type and local agricultural

practices were major contributors to differences in residue levels among trials conducted under the same GAP. Climate had only a minor direct effect. JMPR suggested, therefore, that hypothetical zones (not geographical zones) could be developed on the basis of crop type and variations in agricultural practice. For example, wheat is grown in a relatively uniform manner worldwide (one zone), whereas grapes are grown under a variety of conditions, such as crop height, leaf number and plant density (multiple zones). JMPR concluded that some of the recommendations of a workshop examining these issues (Harris & Pim, 1999) and the project steering group (OECD, 2003) would continue to be considered as auxiliary advice, but that substantial additional work would be required to make the recommendations generally applicable as guidance.

8.5.2.2 Veterinary drug residues

There are very few examples in JECFA where climate may have had an effect on residue levels of veterinary drugs, and therefore additional data to address geographic extrapolation are not justified. JECFA is aware, however, that climate (e.g. tropical versus temperate) may require different animal breeds to adequately adapt to different climates, and these animal breeds may have different metabolic profiles. In addition, different climates may result in different insect infestations in food animals, such that approved uses in temperate climates may not be effective in tropical climates. More data are necessary to clarify these types of situations.

8.6 References[1]

EMEA (2004) Guideline on injection site residues. London, European Medicines Agency, Committee for Veterinary Medicinal Products (EMEA/CVMP/542/03-FINAL; http://www.emea.europa.eu/pdfs/vet/swp/054203en.pdf).

FAO (2002a) Submission and evaluation of pesticide residues data for the estimation of maximum residue levels in food and feed, 1st ed. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 170; http://www.fao.org/ag/agp/agpp/pesticid/JMPR/Download/FAOM2002.pdf).

[1] Internet links provided in these references were active as of the date of final editing.

FAO (2002b) Residues of some veterinary drugs in animals and foods. Rome, Food and Agriculture Organization of the United Nations (FAO Food and Nutrition Paper, No. 41/14).

FAO (2004) Summary of JECFA evaluations of veterinary drug residues from the 32nd meeting to the present. In: Residues of some veterinary drugs in animals and foods. Rome, Food and Agriculture Organization of the United Nations (FAO Food and Nutrition Paper, No. 41/16; http://www.fao.org/docrep/007/y5612e/y5612e00.htm).

FAO/WHO (1988) Evaluation of certain veterinary drug residues in food. Thirty-second report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 763; http://whqlibdoc.who.int/trs/WHO_TRS_763.pdf).

FAO/WHO (1989) Evaluation of certain veterinary drug residues in food. Thirty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 788; http://whqlibdoc.who.int/trs/WHO_TRS_788.pdf).

FAO/WHO (1996) Evaluation of certain veterinary drug residues in food. Forty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives (WHO Technical Report Series, No. 864; http://whqlibdoc.who.int/trs/WHO_TRS_864.pdf).

FAO/WHO (1997) Extrapolation of residue data to minor crops. In: Pesticide residues in food—1997. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations, pp 9–10 (FAO Plant Production and Protection Paper, No. 145; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/97_rep/Report97.pdf).

FAO/WHO (2000) Evaluation of certain veterinary drug residues in food. Fifty-second report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 893; http://whqlibdoc.who.int/trs/WHO_TRS_893.pdf).

FAO/WHO (2003a) Report of the thirty-fifth session of the Codex Committee on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission (ftp://ftp.fao.org/codex/alinorm03/Al0324Ae.pdf).

FAO/WHO (2003b) Codex glossary of terms and definitions (residues of veterinary drugs in food). Rome, Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission (CAC/MISC 5; http://www.codexalimentarius.net/web/standard_list.do?lang=en).

FAO/WHO (2004a) Report of the thirty-sixth session of the Codex Committee on Pesticide Residues. Rome, Food and Agriculture Organization of the

United Nations, Codex Alimentarius Commission (ALINORM 04/27/24; http://www.fao.org/docrep/meeting/008/j2393e/j2393e00.htm).

FAO/WHO (2004b) Refinement of the estimations of maximum residue levels for processed commodities. In: Pesticide residues in food—2003. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations, pp 8–9 (FAO Plant Production and Protection Paper, No. 176; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/2003.pdf).

FAO/WHO (2004c) Estimation of maximum residue levels of pesticides in or on spices on the basis of monitoring results. In: Pesticide residues in food—2004. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations, pp 19–24 (FAO Plant Production and Protection Paper, No. 178; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/DOWNLOAD/2004_rep/report2004jmpr.pdf).

FAO/WHO (2004d) Application of the recommendations of the OECD project on minimum data requirements to the work of the JMPR. In: Pesticide residues in food—2004. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations, pp 30–33 (FAO Plant Production and Protection Paper, No. 178; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/DOWNLOAD/2004_rep/report2004jmpr.pdf).

FAO/WHO (2006a) Updating the principles and methods of risk assessment: MRLs for pesticides and veterinary drugs. Rome, Food and Agriculture Organization of the United Nations (ftp://ftp.fao.org/ag/agn/jecfa/bilthoven_2005.pdf).

FAO/WHO (2006b) Evaluation of certain veterinary drug residues in food. Sixty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 939; http://whqlibdoc.who.int/publications/2006/9241209399_eng.pdf).

FAO/WHO (2006c) Codex classification of foods and animal feeds. Rome, Food and Agriculture Organization of the United Nations and World Health Organization, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (Draft Revision-1 (2006); ftp://ftp.fao.org/codex/ccpr38/pr38CxCl.pdf).

FAO/WHO (2007) Updating the principles and methods of risk assessment: MRLs for pesticides and veterinary drugs. In: Pesticide residues in food—2006. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group

on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations, pp 17–20 (FAO Plant Production and Protection Paper, No. 187; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/DOWNLOAD/2006_rep/report2006jmpr.pdf).

FAO/WHO (2008a) OECD livestock feed tables—JMPR calculation of livestock dietary burden. In: Pesticide residues in food—2007. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations, pp 21–24 (FAO Plant Production and Protection Paper, No. 191; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/DOWNLOAD/2007_rep/report2007jmpr.pdf).

FAO/WHO (2008b) Definitions for the purposes of the Codex Alimentarius. In: Procedural manual, 18th ed. Rome, Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission, pp 17–19 (ftp://ftp.fao.org/codex/Publications/ProcManuals/Manual_18e.pdf).

FAO/WHO (2009) Evaluation of certain veterinary drug residues in food. Seventieth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 954; http://who.int/ipcs/publications/jecfa/trs_954.pdf).

Harris CA & Gaston CP (2004) A survey report to follow-up the development of the concept of minimum data requirements for establishing maximum residue limits (MRLs) including import tolerances for pesticides. Rome, Food and Agriculture Organization of the United Nations (http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/DOWNLOAD/survey_min_data_req_mrls.pdf).

Harris C & Pim J (1999) Minimum data requirements for establishing maximum residue limits (MRLs) including import tolerances. Recommendations from the scientific workshop held at the Pesticides Safety Directorate, York, UK on 6–8 September 1999. York, Pesticides Safety Directorate (Document 2734/SANCO/99; http://ec.europa.eu/food/plant/protection/resources/min_data_en.pdf).

OECD (2003) Report of the OECD/FAO zoning project. Paris, Organisation for Economic Co-operation and Development, Environment Directorate, Working Group on Pesticides (OECD Series on Pesticides, No. 19; ENV/JM/MONO(2003)4; http://www.oecd.org/dataoecd/27/0/2955870.pdf).

Renwick AG (2008) Toxicokinetics. In: Hayes AW ed. Principles and methods of toxicology, 5th ed. Boca Raton, FL, CRC Press, pp 179–230.

Solecki R, Davies L, Dellarco V, Dewhurst I, Van Raaij M & Tritscher A (2005) Guidance on setting of acute reference dose (ARfD) for pesticides. Food Chem Toxicol, **43**: 1569–1593.

Thompson M, Ellison SLR, Fajgelj A, Willets P & Wood R (1999) Harmonised guidelines for the use of recovery information in analytical measurement. Pure Appl Chem, **71**(2): 337–348.

VICH (2004) Studies to evaluate the safety of residues of veterinary drugs in human food: general approach to establish a microbiological ADI. Brussels, International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH GL36; http://www.vichsec.org/pdf/05_2004/Gl36_st7_F_rev.pdf).

9. PRINCIPLES RELATED TO SPECIFIC GROUPS OF SUBSTANCES

For acronyms and abbreviations used in the text, the reader may refer to the list of acronyms and abbreviations at the front of this monograph. Definitions of select terms may be found in the glossary at the end of the monograph.

9.1 Special considerations for substances consumed in small amounts

Many of the substances evaluated by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) are present in food at low concentrations. Examples include flavouring substances, which are added to food to enhance organoleptic appeal, processing aids, extraction solvents and enzymes used in food production. Also included are residues migrating into food from packaging materials, environmental contaminants, such as lead, cadmium, mercury and chlorinated organic chemicals, and residual amounts of pesticides and veterinary drugs used in livestock production. Residues in food from pesticides and veterinary drug use are not considered further here, as they have been discussed in detail in chapter 8.

9.1.1 Threshold of toxicological concern (TTC)

The establishment of safe exposure levels for food chemicals typically involves the first two steps of the risk assessment process, in which no-observed-adverse-effect levels (NOAELs) are determined, either from laboratory animal studies or from human observations, and translated into acceptable exposure levels or health-based guidance values, such as an acceptable daily intake (ADI) (see chapter 5). This traditional approach, which has been in constant use for over 50 years, generally requires that toxicological data on each chemical substance are available in order to perform a safety assessment.

The toxicological potency of the chemicals to which humans are exposed via the diet varies up to 6 or more orders of magnitude. This means that the exposure at which adverse effects are triggered, in terms of the amount of substance ingested per unit body weight, varies considerably between substances. Many factors influence the in vivo toxicity of chemicals, including chemical reactivity, metabolism and toxicokinetics, and the nature and magnitude of their interaction with molecular targets (toxicodynamics). Among organic chemicals, the principal determinant of toxicity is chemical structure; information accumulated over time indicates that the presence of functional groups on a molecule is a primary determinant of inherent toxicity. For example, for most chemical carcinogens, the structural features

leading to deoxyribonucleic acid (DNA) reactivity and subsequent carcinogenesis have been elaborated (Ashby & Tennant, 1991).

The knowledge that toxicity is a function of both chemical structure and the extent of exposure is the basis of the concept of the threshold of toxicological concern (TTC). The TTC approach can be used to facilitate risk assessment of substances present at low levels in the diet for which there are few or no toxicity data. The approach is based on the concept that a human exposure threshold value can be determined for substances, below which there is a very low probability of any appreciable risk to human health (Munro et al., 1996). The TTC concept has been developed and refined (Kroes et al., 2000, 2004).

Regulatory agencies have long had an interest in this concept, because humans may be exposed to very small amounts of an enormous number of naturally occurring and human-made chemicals from a wide variety of sources. The TTC concept was initially proposed by Rulis (1986, 1989, 1992) as a way for the United States Food and Drug Administration (USFDA) to remove unnecessary requirements for testing of components of packaging materials that could migrate in extremely low amounts into foods.

Based on the assumption that carcinogenicity would be the most critical effect at low exposures, Rulis (1986, 1989, 1992) applied a mathematical approach to the development of a threshold of concern for food contact materials. Rulis (1986) transformed the potencies (expressed as tumorigenic dose for 50% of test species, or TD_{50} values) of 343 orally administered carcinogens, compiled by Gold et al. (1984), into a distribution of exposures calculated to present a theoretical lifetime cancer risk of 1 in 1 million by simple linear extrapolation. His analysis indicated that it was highly probable that dietary exposures to organic chemicals at levels of 0.05 µg/kg of diet or less would not present a carcinogenic risk to humans, regardless of chemical structure, and therefore it was not necessary to obtain laboratory animal toxicity data to evaluate such exposures.

Munro (1990) reanalysed the data assessed by Rulis (1986) using the same methodology and also applied a probabilistic approach to three alternative data sets, consisting of 1) carcinogens from the updated database of Gold et al. (1989), 2) the United States National

Toxicology Program (NTP) carcinogens as defined by Ashby & Tennant (1988) and Ashby et al. (1989) and 3) carcinogens selected using conservative biological criteria. Overall, the results of the reanalysis indicated that there was low probability that exposure to a substance of unknown toxicity at a level of 1 μg/kg of diet would present a greater than 1 in 1 million risk of cancer.

On the basis of this work, the USFDA established a "threshold of regulation" for indirect food additives (the term used by the USFDA for migrants from food contact materials) of 0.5 μg/kg total diet (USFDA, 1995). This is equivalent to a daily dietary exposure of 1.5 μg, assuming consumption of 3 kg of food and liquid per day. The USFDA stated that this threshold of regulation would be applied to indirect food additives that are not known to be carcinogens and that do not contain structural alerts indicative of carcinogenicity. Substances meeting these criteria and with intakes less than the TTC would not require toxicological testing.

It should be noted that the threshold of regulation adopted by the USFDA was based on a presumption that migrating packaging material components might be carcinogenic. Assuming that 1 in 10 compounds assessed might be a carcinogen, a TTC value of 1.5 μg/person per day was derived from the distribution of TD_{50} values in the Gold et al. (1989) carcinogen database: at this intake, there is a 96% probability that the risk of cancer would be 1 in 1 million or less. If carcinogenic potential could be ruled out, presumably higher threshold values could be generated for non-carcinogenic components. To this end, the analyses conducted by the USFDA (1995), Rulis (1986, 1989, 1992) and Munro (1990) were further developed by Munro et al. (1996) through compilation of a database consisting of over 600 reference substances from which distributions of no-observed-effect levels (NOELs) were derived. The reference database presented the toxicity in terms of NOELs for a wide variety of organic chemicals of diverse structure, similar to the efforts of the previous workers but, in this case, grouped into three general classes based on chemical structure using the decision tree of Cramer et al. (1978). The use of a structural classification is based on the well-accepted tenet that inherent toxicity is related to chemical structure. This reference database was used to derive a threshold of human exposure that would be without safety concern for each

of the three structural classes and that can be applied to substances lacking toxicity data.

Munro et al. (1996) plotted the distribution of NOELs for 600 chemical substances, which included food additives, drugs, industrial chemicals and pesticides, arranged according to the three structural classes of Cramer et al. (1978). The 5th percentile of the distribution of NOEL values was calculated for each of the three structural classes. These 5th-percentile NOELs were then transformed into human exposure threshold values, referred to as TTCs, by dividing the 5th-percentile NOEL for each structural class by a 100-fold uncertainty factor. The TTC values for Cramer et al. (1978) structural classes I, II and III were 1800, 540 and 90 µg/person per day, respectively. As the TTC approach compares human exposure threshold values with exposure data, it requires sound estimates of human exposure.

Subsequent work conducted by Kroes et al. (2000, 2004) attempted to further evaluate the appropriateness of the thresholds proposed by Munro et al. (1996) to the distributions of NOELs for various specific forms of toxicity, such as developmental toxicity, neurotoxicity and immunotoxicity. With the exception of neurotoxicity induced by organophosphorus compounds, none of the end-points examined produced TTC values less than the TTC for Cramer et al. (1978) structural class III of 90 µg/person per day, and all classes of substances examined (including endocrine disrupting chemicals) would be accommodated within the TTC based on the carcinogen database of 1.5 µg/person per day.

Kroes et al. (2004) developed a decision tree for the application of the TTC concept for substances in structural classes I, II and III. The decision tree also includes a TTC for potential genotoxic carcinogens, based on the carcinogenic potencies associated with 730 compounds, mostly drawn from the Gold et al. (1989) carcinogen database (Gold & Zeiger, 1997). Analyses by Cheeseman et al. (1999) had indicated that the TD_{50} values for different structural alerts could be used to identify the most potent genotoxic carcinogens. Kroes et al. (2004) incorporated into their decision tree (Figure 9.1) a TTC value of 0.15 µg/person per day for those compounds that contained certain structural alerts for genotoxicity. They excluded substances with aflatoxin-like, azoxy- and nitrosamine groups, because such substances would

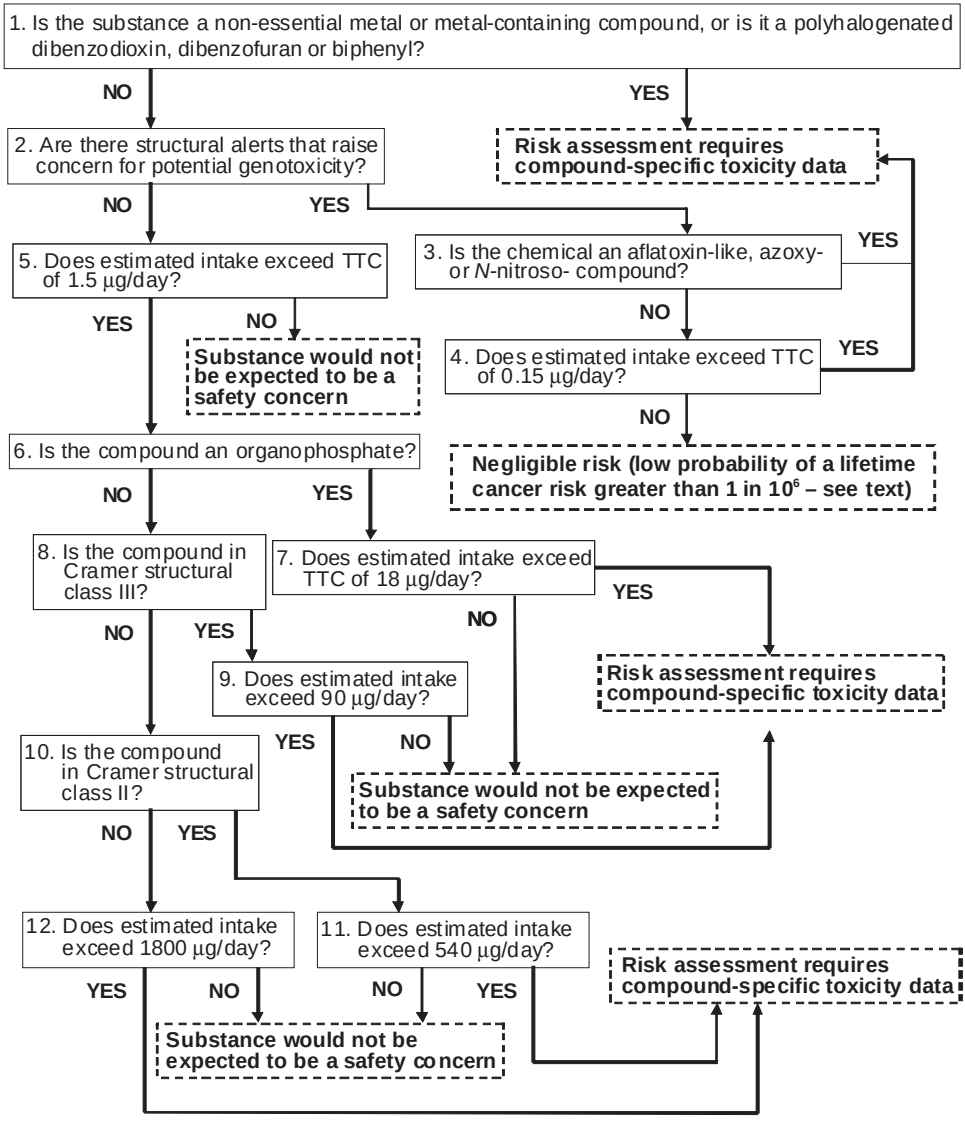

Fig. 9.1. Decision tree of Kroes et al. (2004) for application of the TTC approach

give a high probability of a theoretical lifetime cancer risk greater than 1 in 1 million at such an intake, whereas other substances with structural alerts for genotoxicity would present a 95% probability of less than 1 in 1 million risk. They also excluded metals and metal-containing compounds and proteins, because the database from which the TTC values were derived did not include these types of substances. Polyhalogenated dibenzodioxins, dibenzofurans or biphenyls were also excluded because of their long half-lives and wide species differences in toxicokinetics; in addition, such substances would be evaluated by the toxic equivalency factor (TEF) approach, so the TTC concept would not be appropriate. The rationale for the TTC value of 0.15 µg/person per day applicable to compounds with certain structural alerts for genotoxicity is similar to that for the TTC value of 1.5 µg/person per day (discussed previously), except that it was assumed that all compounds with such structures could be potential DNA-reactive carcinogens, rather than 1 in 10, as used in the derivation of the higher value. The TTC value of 0.15 µg/person per day is designed to allow the formulation of timely advice to risk managers about the possible risk due to very low levels of a compound with a structural alert for genotoxicity or with positive evidence of genotoxicity and is not intended to provide a rationale for the deliberate addition of such a compound to the food supply.

A major advantage of the TTC concept is that it presents a method for focusing resources on public health problems of greatest significance. Substances having exposures below the relevant TTC have low potential for human harm and low priority for testing. The procedure provides confidence that substances consumed in very small amounts present only a minimal potential for risk. Moreover, the TTC provides a reasonable and science-based alternative to laboratory animal testing of substances with innocuous structures and minimal exposure.

At its sixty-fifth meeting in 2006 (FAO/WHO, 2006a), JECFA considered the application of approaches involving the TTC, not only for the risk characterization of flavourings, for which the TTC concept had been used by JECFA for a decade (see section 9.1.2), but also for other substances present in the diet in small amounts. The Committee noted that the following considerations should be taken into account for further application of TTC approaches:

- The approaches should be used in conjunction with conservative estimates of dietary exposure.

- Additional data on the toxicity of structurally related substances might be required.

It further recommended that guidance be drawn up on application of the approach with regard to substances present in the diet in small amounts, such as certain residues of processing aids, packaging materials and contaminants, to provide advice on the risk assessment of substances for which full toxicological data sets are not available or are unnecessary.

The TTC concept was introduced to allow risk assessors to provide science-based advice when there is a high probability of negligible harm based on dietary exposure and chemical structure alone. It is not intended to replace established risk assessment procedures used by JECFA and JMPR for substances such as food additives and pesticide residues, which undergo prior approval based on the generation of a comprehensive database. Also, the TTC approach would not replace the established procedures for dioxin-like compounds or certain heavy metals or where there are sufficient data to allow the establishment of a health-based guidance value.

9.1.2 Flavouring agents

9.1.2.1 The JECFA procedure for safety evaluation

For flavouring agents, JECFA has noted that in most cases dietary exposure to these substances is low and self-limiting, and the majority of flavours are metabolized rapidly to innocuous end-products (FAO/WHO, 1995). This fact limits the need for toxicological testing of many flavouring agents, and therefore metabolic data (e.g. hydrolysis of esters) and structure–activity relationships can play a key role in their safety evaluation.

Flavouring agents are composed of divergent groups of materials, including:

- artificial substances unlikely to occur naturally in food;
- natural materials not normally consumed as food, their derived products and the equivalent nature-identical flavourings;

- herbs and spices, their derived products, and the equivalent nature-identical flavourings; and
- natural flavouring substances obtained from vegetable and animal products and normally consumed as food, whether processed or not, and their synthetic equivalents.

The safety evaluation of flavouring agents presents a special challenge. Flavouring substances are generally consumed in low amounts, and there are several thousand individual flavouring substances in commercial use worldwide. All of the existing individual flavouring substances can be arranged into about 40 groups comprising substances with related chemical structures and similar known or predicted metabolic fates. Testing all these substances for toxicity using classical toxicological approaches would present a formidable challenge and require a massive use of resources. The safety evaluation of flavours presents an opportunity to combine data on intake, metabolic fate and toxicity, including the application of the TTC concept (see section 9.1.1), to perform assessments of flavourings in related structural groups.[1]

The current JECFA Procedure for the Safety Evaluation of Flavouring Agents (the "Procedure") was first considered in 1995 (FAO/WHO, 1995), based on work subsequently published by Munro et al. (1999). The Procedure was adopted by JECFA for the evaluation of flavouring agents at its forty-sixth meeting in 1997 (FAO/WHO, 1997) and has since been modified several times (FAO/WHO, 1999, 2006a, 2009), as outlined in chapter 1. At the sixty-fifth JECFA meeting in 2005 (FAO/WHO, 2006a), the Committee reaffirmed the use of the TTC approach in the evaluation procedure for flavouring agents. The Procedure is outlined in Figure 9.2.

The approach incorporates a series of criteria designed to provide a method to evaluate flavouring substances in a consistent and timely manner. The criteria take account of available information on dietary exposure from current uses, structure–activity relationships and known or predicted metabolism, plus any available toxicity data on

[1] A JECFA number is assigned consecutively to every flavouring substance specified and evaluated by JECFA.

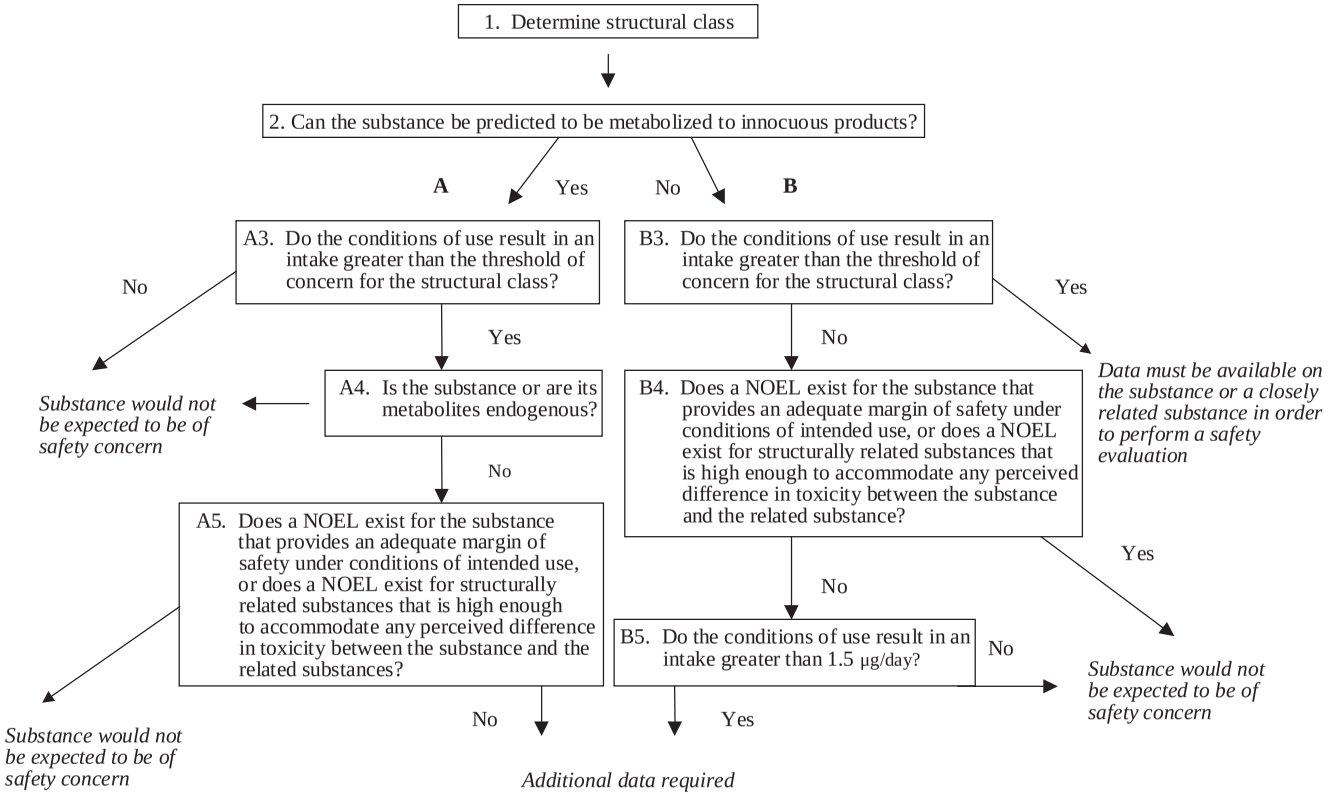

Fig. 9.2. Procedure for the Safety Evaluation of Flavouring Agents adopted by the Committee at its sixty-fifth meeting (FAO/WHO, 2006a)

the compound or structurally related compounds. The use of these criteria provides a means of sorting flavouring substances in terms of the presence or absence of safety concerns and provides guidance on the nature and extent of the data required to perform a safety evaluation.

The criteria take advantage of the fact that some flavouring agents occur as normal constituents of mammalian tissues or are metabolized to form such constituents and are then completely metabolized to innocuous end-products, such as carbon dioxide and water. Flavouring agents with these characteristics are considered to be safe for consumption if dietary exposure is below the threshold of concern for the structural class, but are evaluated on the basis of toxicity data if dietary exposure is above the threshold of concern for the structural class. This safety evaluation may involve the use of toxicity data on the individual substance concerned or may rely, at least in part, on toxicity data on substances of closely related structure.

For flavouring agents that are not known or predicted to be metabolized to innocuous end-products, the safety evaluation must be based on toxicity data, even if estimated dietary exposure is low. In such cases, there must be an adequate margin of safety between dietary exposure to the flavouring agent and the NOEL/NOAEL for the substance or the NOEL/NOAEL for a substance of closely related structure on which the safety evaluation relies. Flavouring agents currently in use for which no toxicity or metabolic data exist, and for which estimated dietary exposure is extremely low, less than 1.5 μg/day, could be considered not to present a safety concern provided they do not contain structural alerts for genotoxicity.

It has been noted that the safety evaluation procedure is not intended to be applied to flavouring agents with existing unresolved problems of toxicity. As with any scheme, its application calls for judgement, and it should not replace expert opinion; JECFA therefore reserved the right to use alternative approaches when data on specific flavouring agents warranted such action.

It was noted that a key element of the Procedure involves determining whether a flavouring agent and the products of its metabolism are innocuous or endogenous substances. The Committee considered that these terms require definition. It recommended that *innocuous*

metabolic products should be defined as products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent, whereas *endogenous substances* are intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included. The estimated dietary exposure to a flavouring agent that is, or is metabolized to, an endogenous substance should be judged not to give rise to perturbations outside the physiological range.

JECFA has noted that ADIs had previously been established for some flavouring agents or groups of flavouring agents and recommended that these should be retained, as the information on which they are based is relevant to an evaluation of their safety and, in addition, they may have uses other than as flavouring agents (e.g. as food additives).

9.1.2.2 *Consideration of dietary exposure estimates*

When the Procedure for the Safety Evaluation of Flavouring Agents was first adopted at its forty-sixth meeting in 1996 (FAO/WHO, 1997), JECFA decided that a practical and realistic approach to derive estimated dietary exposures for consumers of flavouring agents was to use annual production volume data for different regions. This estimate, termed the maximum survey-derived intake (MSDI), was derived from figures for the total annual production of flavouring agents, adjusting for the fact that not all the chemical produced would be reported (60–80% reported) and assuming that the flavouring agent would be consumed by only 10% of each population considered. MSDI estimates were originally based on production and population data for the United States of America (USA) and Europe, but now include data from Japan, with a requirement for recent production data to be submitted by the industry to each meeting. At the sixty-eighth meeting (FAO/WHO, 2007b), a correction factor of 0.8 was applied to the annual production volumes reported in the surveys from Europe, Japan and the USA.

Although JECFA re-endorsed the MSDI approach at meetings subsequent to the forty-sixth meeting, it also discussed limitations to the use of the MSDI for estimating dietary exposure to flavouring agents

(FAO/WHO, 2001, 2005, 2006a, 2007b, 2009). Specific concerns were that low production volume flavouring agents may be added at high levels to certain foods and that high production volume flavouring agents could be present in a large number of foods at different added use levels. The uneven distribution of added use levels for some flavouring agents across different food categories and within food categories and the consequent uneven distribution of dietary exposures to a flavouring agent could not be taken into account in the MSDI estimate. JECFA noted that use of the MSDI might result in an underestimation of dietary exposure to a flavouring agent for regular consumers of certain foods containing that flavouring agent.

At its sixty-fifth meeting (FAO/WHO, 2006a), JECFA reviewed existing model diets for estimating potential dietary exposure to flavouring agents based on generally recognized as safe (GRAS)[1] levels published in the USA or added use level data. These models for dietary exposure estimation assume daily consumption of large portions of several food categories containing the same flavouring agent (possible average daily intake [PADI], theoretical added maximum daily intake [TAMDI]) (see chapter 6, section 6.3.4.1). However, the dietary exposure estimates from these model diets were not considered to be realistic estimates of dietary exposure to flavouring agents as a result of the conservative assumptions made and therefore were not suitable for use in the Procedure. JECFA therefore recommended that there should be further consideration of the most appropriate approach for evaluating the safety of flavouring agents.

JECFA considered further information on recommended use levels supplied by industry on flavouring agents evaluated at subsequent meetings (FAO/WHO, 2007a,b, 2009). An additional new method of estimating dietary exposure for flavouring agents, using the single

[1] GRAS, or generally recognized as safe, is a regulatory concept specific to the United States Federal Food, Drug, and Cosmetic Act. Any substance added to food requires a food additive regulation for its use, unless its intended use is GRAS. Food ingredients whose use is GRAS are not required by law to receive USFDA approval before marketing. The Flavour and Extract Manufacturers Association (FEMA) has been publishing lists of flavouring substances and associated use levels at or below which they have deemed their use to be GRAS for over 30 years.

portion exposure technique (SPET), was agreed upon in 2008 (FAO/WHO, 2009).

The SPET estimate assumes a daily consumption of only a single portion of food containing the flavouring agent, based on added use levels provided by the industry, rather than FEMA GRAS levels.[1] It aims to represent the chronic dietary exposure for a regular consumer who consumes a specific food product containing the flavouring agent of interest daily and not a high consumer of these foods.

The SPET identifies all food categories likely to contain the flavouring agent, assigns an added use level to a single "standard" portion of each of these categories and then identifies the single food category that is likely to contribute the highest dietary exposure. The standard portion is taken to represent the mean food consumption amount for consumers of that food category, assuming daily consumption over a long period of time. The standard portion does not reflect high food consumption amounts reported in national dietary surveys for the food category and is therefore a more realistic prediction of long-term consumption patterns.

A summary of an analysis of MSDI and SPET estimates for 225 flavours for which added use level and production data for one of the three geographic regions (Europe, Japan and the USA) were available was reported at the sixty-ninth meeting of JECFA (FAO/WHO, 2009). In nearly all cases (>90%), the SPET estimate was above the MSDI, and the SPET estimate was more likely than the corresponding MSDI to be above the TTC of the relevant structural class. The SPET estimate was most frequently above the TTC in class III, but this also occurred in classes I and II.

JECFA concluded that the MSDI and SPET dietary exposure estimates provide different and complementary information (FAO/WHO, 2009). Inclusion of the SPET estimate in the Procedure addressed previous concerns about the MSDI estimate of dietary exposure, because the SPET estimate takes account of the possible

[1] Lists of flavouring substances and associated use levels at or below which they have deemed their use to be GRAS are published regularly by FEMA.

uneven distribution of dietary exposures to a flavouring agent for consumers of foods containing that substance. The higher value of the two dietary exposure estimates (MSDI or SPET) will be used within the Procedure.

As it was not possible to elaborate criteria, based on structure, production level or group of flavouring agents, to identify the flavouring agents for which the MSDI underestimated dietary exposure and SPET estimates should be used, JECFA concluded that it was necessary to incorporate SPET estimates into the Procedure for all flavouring agents considered at future meetings. JECFA also noted that the addition of the SPET dietary exposure estimate, where it was higher than the MSDI, to the relevant steps A3 and B3 of the decision tree in the Procedure (see Figure 9.2) would be likely to lead to a more extended evaluation in only a limited number of cases. It was not considered necessary to re-evaluate flavouring agents already assessed using the Procedure.

9.1.3 Food contact materials/packaging migrants

Many food contact materials are made from polymers that are usually inert biologically as a result of their high molecular weight. However, constituents of these polymers, such as monomers, additives, catalysts and other substances used in their manufacture, are low molecular weight substances, which theoretically could migrate from the food contact material into foods. The same can be said for other constituents of the food contact materials, such as inks used in labelling. Migration may occur during storage and be enhanced during food preparation, such as heating, microwave cooking or processing with ionizing radiation. Also, the food matrix may affect the degree of migration, such that fat-soluble substances will migrate more readily into fatty foods, whereas water-soluble substances will migrate more readily into aqueous foods.

The safety evaluation of food packaging materials presents special problems because of the very large number of them in use and the anticipated low level of migration of substances from food contact materials and consequent low dietary exposure. JECFA (IPCS, 1987) has previously set out criteria for the evaluation of these substances, noting that the following information is required:

- the chemical identity and toxicological status of the substances that enter food;
- the possible exposure, details of which can be derived from migration studies using suitable extraction procedures and/or the analysis of food samples; and
- the nature and amount of food contact with the packaging materials, and the intake of such food.

These criteria define the fundamental data required to identify those substances that migrate, the amounts that may be present in food and consequent exposures.

In principle, two alternatives exist for performing safety evaluations on food contact materials. One is to require toxicological data regardless of the level of potential dietary exposure so that a safety evaluation can be performed. A second option is to apply a tiered approach in which the number of toxicological data required are related to the extent of anticipated exposure as measured by migration studies. As discussed previously (see section 9.1.1), in 1995, the USFDA adopted a "threshold of regulation" for food packaging migrants such that a substance would be exempt from USFDA regulation if exposures were less than 1.5 µg/person per day, provided the migrant was not carcinogenic or did not contain structural alerts for carcinogenicity (USFDA, 1995). Given the large number of food contact materials in commerce, such an approach provides a reasonable alternative to requiring that all such migrating substances be tested for toxicity.

Models for estimating potential dietary exposures to packaging materials are discussed in chapter 6 (section 6.3.4.1).

9.1.4 Processing aids

Processing aids are composed of diverse substances, including, but not limited to, carrier or extraction solvents and enzymes used in food processing.

9.1.4.1 Solvents

Extraction solvents are used in, for example, the extraction of fats and oils, defatting fish and other meals, and decaffeinating coffee and tea. They are chosen mainly for their ability to dissolve the desired

food constituents selectively and for their volatility, which enables them to be separated easily from the extracted material with minimum damage. The points raised by their use relate to:

- the toxicity of their residues;
- the toxicity of any impurities in them;
- the toxicity of substances such as solvent stabilizers and additives that may be left behind after the solvent is removed; and
- the toxicity of any substances produced as a result of a reaction between the solvent and food ingredients.

Before any extraction solvent can be evaluated, information is required on:

- the identity and amount of impurities in the solvent (including those that are formed, acquired or concentrated owing to continuous reuse of the solvent);
- the identity and amount of stabilizers and other additives; and
- the toxicity of residues of solvents, additives and impurities.

Impurities are particularly important, because there are wide differences in the purities of food-grade and industrial-grade solvents. The food use of extraction solvents is frequently much less than the industrial use, and considerable problems may arise in their evaluation if toxicological data exist only on the industrial grade of the solvent, which contains potentially toxic impurities that may not be present in the food-grade material. For example, when evaluating the solvents 1,1,1-trichloroethane, trichloroethene and tetrachloroethene, it was noted that the toxicological data indicated the presence of certain known toxic and carcinogenic substances. The interpretation of these data became extremely difficult because industrial-grade material had been used in the studies. Only food-grade material should be used in toxicological studies, and the impurities in the material should be fully identified.

Carrier solvents raise somewhat different issues. They are used for dissolving and dispersing nutrients, flavours, antioxidants, emulsifiers and a wide variety of other food ingredients and additives. With the exception of carrier solvents for flavours, they tend to occur in food at levels higher than those of extraction solvents, mainly because some

of them are relatively non-volatile. As carrier solvents are intentional additives and are often not removed from the processed food, it is important to evaluate their safety together with the safety of any additives or stabilizers in them.

9.1.4.2 Enzymes

Enzymes used in food processing are derived from animal tissues, plants and microorganisms. Enzymes isolated from these sources are blended with formulation ingredients, such as diluents, stabilizing agents and preserving agents. The formulation ingredients may include water, salt, sucrose, sorbitol, dextrin, cellulose or other suitable compounds. The formulated enzymes are referred to as enzyme preparations. Depending on the application, an enzyme preparation may be formulated as a liquid, semiliquid or dried product. Enzyme preparations contain either one major active enzyme that catalyses a specific reaction during food processing or two or more active enzymes that catalyse different reactions. Enzyme preparations often contain constituents of the source organism and compounds derived from the manufacturing process—for example, the residues of the fermentation broth.

JECFA has elaborated and periodically updated principles and procedures for the safety assessment of enzyme preparations. An enzyme preparation evaluated by JECFA must comply with the General Specifications and Considerations for Enzyme Preparations Used in Food Processing (FAO, 2006a), which were last updated at the sixty-seventh meeting of JECFA in 2006 (FAO/WHO, 2007a). The document addresses certain aspects of safety evaluation that apply to all enzyme preparations, including safety evaluation of the production organism, the enzyme component, side activities, the manufacturing process and the consideration of dietary exposure. It states that evaluation of the enzyme component should include considerations of its potential to cause an allergic reaction. The document also addresses certain safety concerns that pertain to enzyme preparations derived from genetically modified microorganisms. It includes recommendations for safety assessment of the genetic material inserted into the genome of the production microorganism and for providing evidence that the enzyme preparation contains neither antibiotic inactivating proteins at concentrations that would interfere with antibiotic

treatment nor transformable DNA that could potentially contribute to the spread of antibiotic resistance. For further details, the online document should be consulted (FAO, 2006a).

An enzyme preparation must also comply with the identity and purity specifications, which are established for each enzyme preparation on a case-by-case basis (FAO, 2006b). Dietary exposure is calculated on the basis of the total organic solids (TOS) content in the final (commercial) enzyme preparation and is usually expressed in milligrams or micrograms TOS per kilogram body weight per day. TOS encompasses the enzyme component and other organic material derived from the enzyme source and manufacturing process while excluding intentionally added formulation ingredients. Toxicological studies are usually performed using the concentrated enzyme prior to the addition of the formulation ingredients. The TOS content of the toxicology batch is provided to enable the derivation of the NOAEL expressed in milligrams or micrograms TOS per kilogram body weight per day, on which JECFA bases the ADI. JECFA then considers dietary exposure to an enzyme preparation in relation to the ADI.

For the purpose of toxicological evaluation, enzyme preparations used in food processing can be grouped into five major classes:

1) Enzymes obtained from edible tissues of animals commonly used as foods. These are regarded as foods and, consequently, considered acceptable, provided that satisfactory chemical and microbiological specifications can be established.

2) Enzymes obtained from edible portions of plants. These are regarded as foods and, consequently, considered acceptable, provided that satisfactory chemical and microbiological specifications can be established.

3) Enzymes derived from microorganisms that are traditionally accepted as constituents of foods or are normally used in preparation of foods. These products are regarded as foods and, consequently, considered acceptable, provided that satisfactory chemical and microbiological specifications can be established.

4) Enzymes derived from non-pathogenic microorganisms commonly found as contaminants of foods. These materials are not

considered as foods. It is necessary to establish chemical and microbiological specifications and to conduct short-term toxicity studies to ensure the absence of toxicity. Each preparation must be evaluated individually, and an ADI must be established.

5) Enzymes derived from microorganisms that are less well known. These materials also require chemical and microbiological specifications and more extensive toxicological studies, including a long-term study in a rodent species.

Safety assessments for enzymes belonging to classes 1–3 will be the same regardless of whether the enzyme is added directly to food or is used in an immobilized form. Separate situations should be considered with respect to the enzymes described in classes 4–5, dependent on whether they are:

a) enzyme preparations added directly to food but not removed;
b) enzyme preparations added to food but removed from the final product according to Good Manufacturing Practice (GMP); or
c) immobilized enzyme preparations that are in contact with food only during processing.

For a) above, an ADI should be established to ensure that levels of the enzyme product present in food are safe. The studies indicated in these guidelines are appropriate for establishing ADIs (the guidelines were originally drafted for this situation). For b), an ADI "not specified" may be established, provided that a large margin of safety exists between possible residues and their acceptable intake. For c), it may not be necessary to set an ADI for residues that could occur in food as a result of using the immobilized form of the enzyme. It is acceptable to perform the toxicity studies relating to the safety of the enzyme on the immobilized enzyme preparation, provided that information is given on the enzyme content in the preparation.

9.1.4.3 Immobilizing agents

A number of procedures involving different chemical substances are used for immobilizing enzymes. These processes include microencapsulation (e.g. entrapment in gelatine to form an immobilized complex), immobilization by direct addition of glutaraldehyde, immobilization by entrapment in porous ceramic carrier and complexation

with agents such as diethylaminoethyl cellulose or polyethylenimine. Several agents may be used in the immobilizing process. Substances derived from the immobilizing material may be in the final product due to either the physical breakdown of the immobilizing system or impurities contained in the system.

The number of data necessary to establish the safety of the immobilizing agent depend on its chemical nature. The levels of residues in the final product are expected to be extremely low.

Some of the substances used in the preparation of immobilizing systems are extremely toxic. The levels of these substances or their contaminants permitted in the final product should be at the lowest levels that are technologically feasible, provided that these levels are below those of any toxicological concern. An ADI is not established, but there must be adequate safety for their approved uses.

9.2 Special considerations for nutrients and substances consumed in large amounts

9.2.1 Introduction

The safety assessment of substances that are consumed in relatively large amounts presents a number of special problems. Such materials include defined chemical substances such as the bulk sweeteners sorbitol and xylitol, modified food ingredients such as modified starches, nutrients and related substances, and non-traditional whole foods.

The safety assessment of such substances should differ from that of other food additives, such as colouring and flavouring agents and antioxidants, for the following reasons:

- Many will have a high daily intake; thus, minor constituents and processing impurities assume greater than usual significance.
- Even though they are often structurally similar or even identical to natural products used as food and thus may appear to be of low toxicity, they may require extensive toxicity testing because of their high daily intake.
- Some may be metabolized into normal body constituents.

- Some substances, particularly foods from novel sources, may replace traditional foods of nutritional importance in the diet.

- Many are complex mixtures rather than defined chemical substances.

- The difference between the maximum quantity that can be fed to laboratory animals in feeding tests without impairing the nutritional quality of the diet and the amount consumed by human beings is often relatively small on a body weight basis.

9.2.1.1 *Chemical composition, specifications and impurities*

Thorough chemical analysis should be performed on high-consumption substances to measure potential impurities and to provide information on nutritional adequacy, especially when such substances replace traditional food. It is not possible to provide a checklist of necessary chemical studies to cover all high-consumption compounds. However, the substance should be subjected to a full proximate analysis, and particular attention should be paid to the points discussed in the following paragraphs.

Because the intake of undesirable impurities concomitant with the intake of bulk ingredients is potentially high, special effort should be made to identify the impurities. Information on the production process, including the materials and procedures involved, will point to the types of contaminants for which limits may need to be specified. The specifications should be accompanied by details of product variability and of the analytical methods used to check the specifications and details of the sampling protocols. If the substance is so complex that comprehensive product specifications on chemical composition are impractical (as they might be, for example, for a microbial protein), the description of the substance in the specifications may include relevant aspects of its manufacturing process. If manufacturing data are based on production on a pilot scale, the manufacturer should demonstrate that, when produced in a large-scale plant, the substance will meet the specifications established on the basis of pilot data.

The permissible limits for impurities may in some cases correspond to the levels accepted for natural foods that have similar structure or function or that are intended to be replaced by the new

material. If the substance is prepared by a biological process, special attention should be paid to the possible occurrence of natural toxins (e.g. mycotoxins).

If the nature of the substance or manufacturing process indicates the possible presence of naturally occurring or adventitious antinutritional factors (e.g. phytate, trypsin inhibitors) or toxins (e.g. haemagglutinins, mycotoxins, nicotine), the product should be analysed for them specifically. Biological tests, either as part of the nutritional evaluation in the case of enzyme inhibitors or more specifically as part of a mycotoxin screening programme, will provide useful backup evidence concerning the presence or absence of these contaminants.

Finally, if, under the intended conditions of use, the substance may be unstable or is likely to interact chemically with other food components (e.g. degradation or rearrangement of the substance during heat processing), data should be provided on its stability and reactivity. The various tests should be conducted under conditions relevant to the use of the substance (e.g. at the acidity and temperature of the environment and in the presence of other compounds that may react).

9.2.1.2 *Nutritional studies*

With some substances, particularly novel foods, nutritional studies may be necessary to predict the likely impact of their introduction on the nutritional status of consumers. In addition to affecting the nutritional content of the diet, such substances may influence the biological availability of nutrients in the diet. The nutritional consequences of the introduction of such a substance in the diet can be judged only in the light of information about its intended use. Therefore, as much information as possible should be obtained about potential markets and uses, and the likely maximum consumption by particular subpopulations should be estimated. It is also possible to check the accuracy of premarketing predictions by use of post-marketing monitoring studies (see, for example, Allgood et al., 2001; Hlywka et al., 2003; Amanor-Boadu, 2004; Lea & Hepburn, 2006; Hepburn et al., 2008; and chapter 4, section 4.11.3).

9.2.1.3 Toxicity studies

When testing high-consumption additives, laboratory animals should generally be fed the highest levels that are consistent with palatability and nutritional status. Therefore, before beginning such studies, it is desirable to investigate the palatability of the test diet in the test animals. If a palatability problem is encountered, it may be necessary to increase the amount of the test substance to the required level gradually. Paired-feeding techniques should be used if the problem cannot be overcome. It should always be borne in mind that there are practical limits to the amounts of certain foods that can be added to animal diets without adversely affecting the animals' nutrition and health.

To ensure that the nutritional status of the test animal is not distorted, the test and control diets should have the same nutritive value in terms of both macronutrients (e.g. protein, fat, carbohydrate and total calories) and micronutrients (e.g. vitamins and minerals). When feeding substances at high levels, it is usually advisable to formulate diets from individual ingredients (rather than adding the test material to a standard laboratory diet) to provide the same nutrient levels in the control and test diets. Comprehensive nutrient analyses of the test and control diets should be performed to ensure that they are comparable. Sometimes nutritional studies are advisable before toxicological studies are performed to ensure that test diets are correctly balanced. Without due regard to nutritional balance, excessive exposure may mean that a study investigates the adverse effects of long-term dietary imbalance rather than the toxic effects of the substance.

Metabolic studies are useful and necessary for assessing the safety of high-consumption additives. With complex mixtures, studies on the metabolic fate of every constituent would be impractical. However, if contaminants or minor components are suspected as the cause of toxicity, their metabolism should be investigated. If the material, or a major component of it, consists of a new chemical compound that does not normally occur in the diet (e.g. a novel carbohydrate), studies of the metabolic fate of the new compound would be appropriate.

If biochemical and metabolic studies show that the test material is completely broken down in the food or in the gastrointestinal tract to substances that are common dietary or body constituents, then other

toxicity studies may not be necessary. The results of metabolic studies can stand on their own if it is shown that breakdown into these common constituents occurs under the conditions of normal consumption of the material, that the material contributes only a small proportion of these common constituents in the daily diet and that side reactions giving rise to toxic products do not occur.

Analysis of urine and faeces may provide important information relating to changes in normal excretory functions caused by the test substance. For example, the gut flora may be altered or preferential loss of a mineral or vitamin may occur, resulting in detrimental effects on the health of the test animals. If the substance is incompletely degraded or not degraded by the digestive enzymes of the stomach or the small intestine, appreciable concentrations may be found in the faeces or in the distal gut compartments. Such substances may also induce laxation. As a result, changes in the absorption of dietary constituents or changes in the composition and metabolic activity of the intestinal flora may be observed. Because of anatomical differences in the digestive tract and because of considerable differences in the composition of the basal diet, such effects may occur only in humans but not in rodents, or vice versa. Therefore, short-term studies should be performed in laboratory animals and humans (if possible; see chapter 4, section 4.11), in which variables likely to be affected by the test compound are examined in detail. It is especially important to investigate questions relating to whether the eventual effects are progressive or transient and whether they occur in subjects exposed to the compound for the first time or in subjects adapted to a daily intake of the substance. Clearly, no standard design for such studies can be devised. Only a thorough knowledge of the nutritional and biochemical literature can serve as a guideline.

Separate toxicological tests should be performed on toxicologically suspect impurities or minor components present in the test material. If any observed toxicity can be attributed to one of the impurities or minor components, its maximum level should be established in the specification.

Because of the relative non-toxicity of high-consumption additives, toxicity tests in animals may not show any adverse effects even at the highest dose tested. When establishing an ADI, the traditional

concept of utilizing a 100-fold safety factor is often not possible if the human consumption level is high and feeding studies do not produce adverse effects. In such cases, new approaches are indicated. It may be possible, for example, to establish a large safety margin between the highest dose tested and the expected consumption of such substances by humans. Or the ADI may be set on the basis of a smaller safety factor, which may be permissible when aspects such as similarity to traditional foods, metabolism into normal body constituents and lack of overt toxicity are considered. For a compound, such as a bulking agent, that may influence the nutritional balance or the digestive physiology by its mere bulk and that may be absorbed from the gut only incompletely or not at all, it may be more appropriate to consider the dose level in terms of the percentage inclusion in the diet. If several similar types of compounds are likely to be consumed, a group ADI (limiting the cumulative intake) should be allocated.

The results of human studies, which are discussed in relation to novel foods in section 9.2.3, may allow the use of a lower safety factor than that obtained from laboratory animal studies.

9.2.2 Nutrients and related substances

The increased use of fortified foods, dietary or food supplements, specially formulated foods and so-called "functional foods" has increased the intake of nutrient substances around the world. In turn, there has been growing interest in an international basis for determining the levels of intake that may pose a risk. JECFA has evaluated the safety of several substances that were claimed to have nutritional or health benefits. The sixty-third JECFA noted that whether such products meet appropriate definitions as nutrients or are worthy of health, nutrient or other claims was outside its remit (FAO/WHO, 2005). Therefore, JECFA reiterated that it would evaluate only the safety of these ingredients and expressed the view that its evaluation of the safety of these ingredients should not be interpreted to mean that the Committee endorses the use of these substances for their claimed nutritional or health benefits.

JECFA has assigned ADIs for several nutrients or determined "no safety concern" under the proposed conditions of use (e.g. L-5-methyltetrahydrofolic acid; FAO/WHO, 2006a).

In the risk assessment for non-nutrients, it is assumed that:

- the substance has no desirable or essential physiological roles;
- homeostatic mechanisms for the specific substance do not exist and/or detoxification pathways are not likely to be chemical specific; and
- there are no health risks if the intake is zero.

Unlike non-nutrients, nutrient substances are biologically essential or have a demonstrated favourable impact on health at specified levels of intake. This consideration influences approaches used to adjust for uncertainty associated with the data used to estimate a health-based guidance value, such as an upper level of intake (UL), and also necessitates that the homeostatic mechanisms specific to essential nutrient substances be taken into account. Therefore, modifications to the classic non-nutrient risk assessment approach are needed.

The relationship between intake and risk for nutrient substances is illustrated in Figure 9.3. For most essential nutrients, homeostatic mechanisms that maintain the amount of nutrient substance in the body within a physiological range are associated with both low and high levels of intake. Should intakes increase or decrease, it is assumed that homeostatic responses of some type occur and that the responses may vary by age, sex or life stage. However, homeostatic adaptations have a limited capacity and can be overwhelmed by excessive intake. At the extremes, as the capacity of a homeostatic mechanism is exceeded, the incidence or impact of specific adverse health effects is likely to increase. Nutrient substances that are not established as essential may also show dual curves, with the left-hand curve reflecting the failure to optimize health. The distinctions between essentiality and a demonstrated favourable health impact require further elucidation and clarification as data evolve.

Several international working groups have provided guidance for the risk assessment of nutrients and related substances (IPCS, 2002; Renwick et al., 2003, 2004; FAO/WHO, 2006b). For the safety evaluation of nutrients and related substances, these groups recommended the use of the UL, which is defined as the maximum level of habitual intake from all sources of a nutrient or related substance judged to be unlikely to lead to adverse health effects in humans.

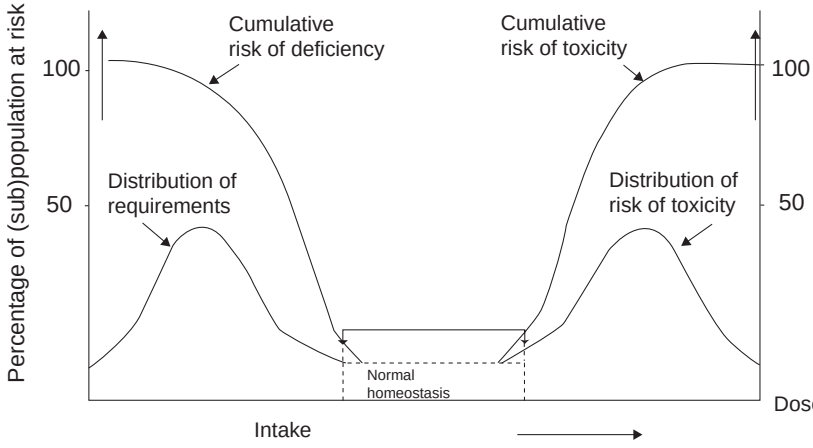

Fig. 9.3. Dual curves for risk relationship of nutrients: percentage of (sub)-population at risk of "deficiency" and then "adverse health effects" as intakes move from low to high (modified from IPCS, 2002)

The UL is not a recommended level of intake but an estimate of the highest level of regular intake that carries no appreciable risk of adverse health effects (criteria for setting a UL are discussed in section 9.2.2.2). As with all health-based guidance values, exceeding the UL is not in itself an indication of risk, but the UL does not give any indication of the magnitude of risk that may be associated with intakes in excess of the UL.

Where possible, ULs that apply to all groups of the general population, including all life stages, should be established. A generally applicable UL can be used with data from intake assessments to identify those individuals or population groups potentially at risk and the circumstances in which harm is likely to occur. However, ULs for nutrients may vary with age or for specific groups (e.g. sex and life stage, including pregnancy) because of different balances between requirements and sensitivities to adverse effects. The WHO review of the principles and methods for the assessment of risk from essential trace elements pointed out age-related factors associated with variable responses to levels of intake (IPCS, 2002). The FAO/WHO Technical Workshop on Nutrient Risk Assessment (FAO/WHO, 2006b) concluded that the most appropriate approach is to develop separate ULs for age, sex and life stage subpopulations. As the data allow, the ULs

can be based on different end-points as applicable to the sensitivity of the subpopulation.

The appropriateness of a UL established for adequately nourished (sub)populations cannot be assumed to transfer to inadequately nourished (sub)populations. For example, an intake well above the UL may be recommended clinically to correct a deficiency. Although the basic process of nutrient risk assessment decision-making would remain the same regardless of the nutritional status of the (sub)population of interest, it is likely that inadequately nourished (sub)populations would need a different set of ULs because of important differences in metabolism and the vulnerability that can result from these differences. However, it should be noted that too little is known about the effects of inadequate nutrition on the absorption, distribution, metabolism and elimination of nutrient substances to allow specification of considerations relevant to adjusting ULs to make them appropriate for inadequately nourished (sub)populations.

The UL is not meant to apply to individuals receiving the nutrient under medical supervision or to individuals with predisposing conditions that render them especially sensitive to one or more adverse effects of the nutrient (e.g. those with genetic predisposition or certain metabolic disorders or disease states).

For some nutrient substances, no credible evidence has demonstrated adverse health effects even at the highest intake used or observed. Vitamin B12 is an example of such a nutrient substance (IOM, 1998). In such cases, the biological threshold for an adverse health effect, if it exists, may be many times higher than the highest intake studied. Lacking data, however, this amount is not known. If no studies have revealed adverse health effects for a nutrient substance but the risk manager needs scientific advice concerning an upper intake, the FAO/WHO Technical Workshop on Nutrient Risk Assessment (FAO/WHO, 2006b) recommended that the highest observed intake (HOI) be used to give guidance. The HOI is defined as the highest level of intake observed or administered as reported within a study of acceptable quality. It is derived only when no adverse health effects have been identified.

There are some special considerations for the risk characterization of micronutrients and macronutrients (Renwick et al., 2003).

Micronutrients are vitamins and minerals that are essential for normal growth and physiological and biochemical functioning. It should be noted that micronutrients used in dietary or food supplements and fortified foods may be in different physical or chemical forms from those present naturally in the food or endogenously in the body. Macronutrients include dietary fats, proteins and carbohydrates, as well as their subcomponents and substitutes. In addition to those substances currently considered as macronutrients, these considerations can also be appropriate for the risk characterization of new substances, including dietary supplements and functional foods. Decision trees that could be considered for the risk characterization of micronutrients and macronutrients are given in Figures 9.4 and 9.5, respectively (Renwick et al., 2003). These are not intended to cover all eventualities, but indicate some matters of particular concern.

9.2.2.1 *Adverse health effects of nutrients and related substances—general concepts*

The general concepts concerning adverse health effects of nutrients have been described by Renwick et al. (2004). An adverse health effect has been defined as any impairment of a physiologically important function that could lead to an adverse health effect in humans (IOM, 1998) and as any change in morphology, physiology, growth, development or lifespan of an organism that results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase in susceptibility to the harmful effects of other environmental influences (IPCS, 2004). Indicators of adverse health effects, which may be used for the derivation of the UL, range from biochemical changes without adverse health effects through to irreversible pathological changes in the functioning of the organism (Figure 9.6). In practice, because of limited availability of data on adverse effects in humans, and as biochemical indicators of adverse effects are often not available, adverse effects selected for establishing ULs may cover the full range indicated in Figure 9.6, including clinical outcomes.

There is an established paradigm for determining safe intakes of foreign compounds, such as food additives, based on the dose–response relationship for adverse effects in laboratory animals or humans (see Edler et al., 2002 and chapter 5). For most types of toxicity from either

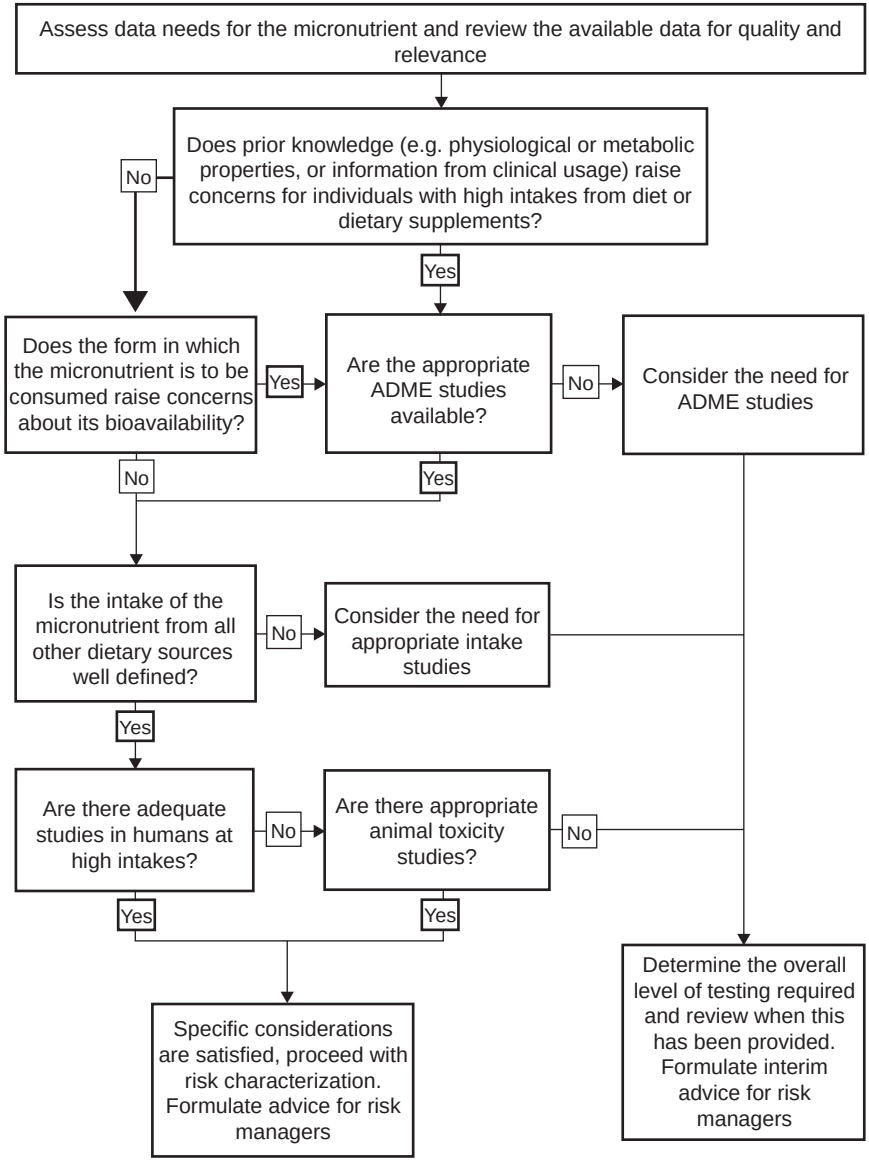

Fig. 9.4. Decision tree outlining the special considerations for the risk characterization of micronutrients (adapted from Renwick et al., 2003) [ADME, absorption, distribution, metabolism, excretion]

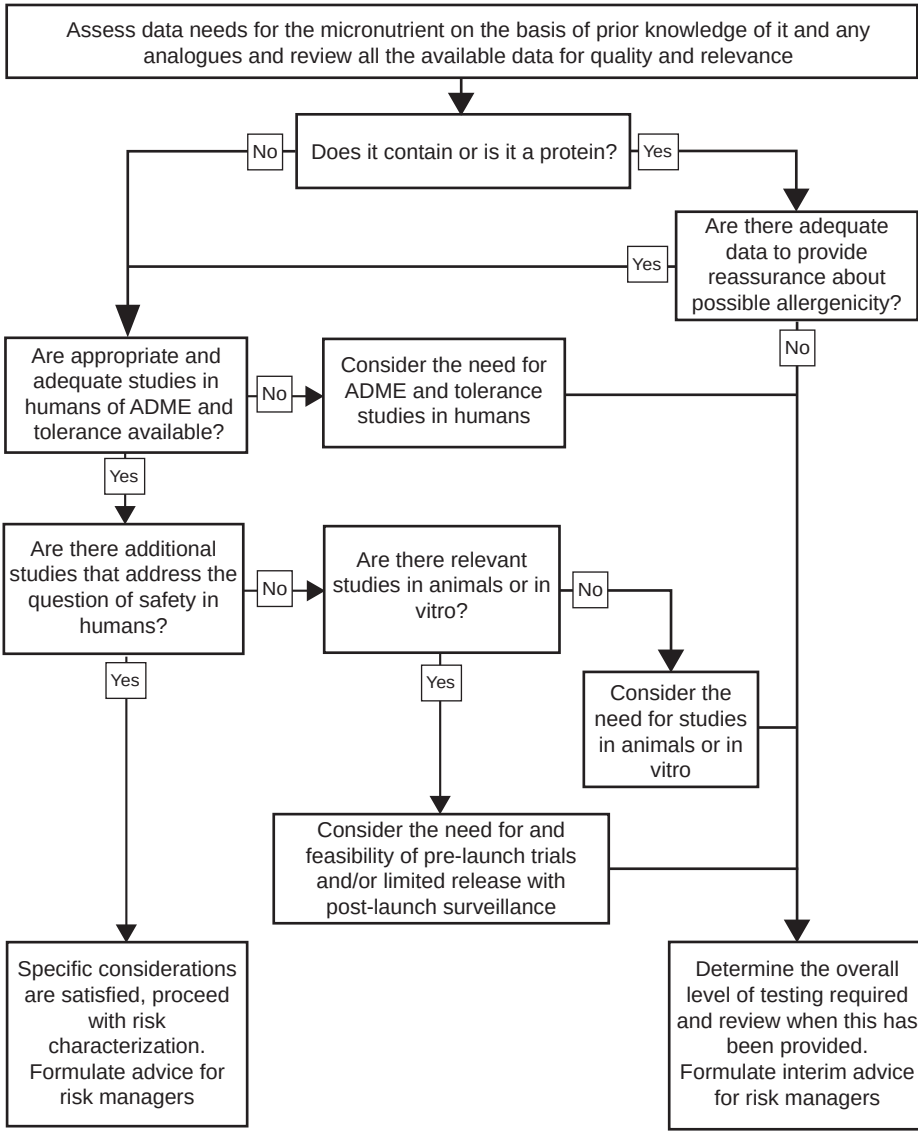

Fig. 9.5. Decision tree outlining the special considerations for the risk characterization of macronutrients (adapted from Renwick et al., 2003)

1. Biochemical changes within the homeostatic range and without indication of adverse sequelae

↓

2. Biochemical changes outside the homeostatic range without known sequelae

↓

3. Biochemical changes outside the homeostatic range that represent a biomarker of potential adverse effects due to excess

↓

4. Clinical features indicative of a minor but reversible change

↓

5. Clinical features of significant but reversible effects

↓

6. Clinical features indicative of significant but reversible organ damage

↓

7. Clinical features indicative of irreversible organ damage

Fig. 9.6. Identifying adverse health effects: sequence of "effects" in increasing order of severity (adapted from Renwick et al., 2004; "features" includes signs and symptoms)

foreign compounds or nutrients, there is believed to be a threshold dose (or intake) below which adverse health effects are not produced. Thresholds for any given adverse effect vary among members of the population. In general, there are insufficient data to establish the distribution of thresholds within the population for individual adverse effects, and uncertainty factors are used to allow for human variability (and for species differences, when necessary) (Edler et al., 2002).

Steps 4 through 7 in Figure 9.6 represent adverse health effects manifesting specific clinical features such as signs and symptoms, and for this reason they can be used readily for risk assessment in the usual manner. However, some of the effects that occur prior to step 4 could constitute appropriate "biomarkers". Because such effects can reflect "critical events", they could serve as surrogates or biomarkers for adverse health effects. However, it should be noted that biochemical effects without functional significance should not be regarded as adverse health effects (IPCS, 2002).

The following criteria have been proposed for the use of these indicators of adverse health effects (FAO/WHO, 2006b):

- The optimal end-point for use in setting a UL would be an effect at step 3 and possibly step 2, with steps 4–7 reflective of clinical features such as signs or symptoms. Step 2 may be applicable in some cases in which sufficient information is available to suggest that changes outside a homeostatic range that occur without known sequelae would be relevant as a surrogate for an adverse health effect.

- The increased use of valid, causally associated biomarkers as surrogates for adverse health effects is desirable for the purposes of nutrient risk assessment. After identifying the sequence of observable effects in the causal pathway for adverse health effects—from initial nonspecific biochemical changes to clear clinical outcomes—if the biomarker meets other relevant criteria, including causal association, biochemical changes outside the homeostatic range can be relevant surrogates for adverse health effects associated with nutrient substances.

9.2.2.2 *Deriving the UL*

The UL can be derived for nutrients using the principles of risk assessment similar to those that have been developed for biological and chemical agents. A pivotal point in the assessment process is the selection of the critical adverse health effect. This is the effect upon which the UL is based—or, more specifically, the effect upon which a set of ULs for the various age, sex and life stage subpopulations is based. The critical adverse health effect is usually the effect that occurs at the lowest level of excessive intake within the (sub)population of interest or at the lowest experimental dose if only laboratory animal data are available. For a given nutrient substance, different critical adverse health effects may be selected for the different age, sex and life stage subpopulations, because metabolic and physiological differences among these subpopulations mean that adverse health effects may manifest differently. Issues related to the physiological severity of the adverse health effect are considered separately rather than as a component of selecting the critical adverse health effect (FAO/WHO, 2006b).

Once the critical adverse health effect is identified, the process moves to deriving the UL. Again, iterations may occur between this activity and those conducted under hazard identification. The first step is to analyse and describe clearly the relationship between the intake of the nutrient substance and the onset of the adverse health effect for those age, sex and life stage subpopulations for which data are available. The analysis (see also chapter 5) is called the intake–response assessment, and its outcome is the determination of one or more of the following three values, depending upon the nature of the existing evidence:

1) a benchmark dose (BMD) (or benchmark intake [BI]): the intake of a substance that is expected to result in a prespecified level of effect (the benchmark response [BMR]; see chapter 5);
2) a NOAEL: the greatest concentration or amount of a substance, found by experiment or observation, that causes no detectable adverse alteration of morphology, functional capacity, growth, development or lifespan of the target organism under defined conditions of exposure (IPCS, 1994); or
3) a lowest-observed-adverse-effect level (LOAEL): the lowest concentration or amount of a substance, found by experiment or observation, that causes a detectable adverse alteration of morphology, functional capacity, growth, development or lifespan of the target organism under defined conditions of exposure (IPCS, 1994).

The NOAEL and LOAEL are based on observed intake levels that are set as part of the study design. Neither takes into account the shape of the intake–response curve that would be seen at other levels of intake. If data allow, the specification of a BMD (BI) permits the derivation of the ULs to be carried out with greater certainty. In any case, any of the three values can serve as the starting point for deriving the UL. The BMD (BI) approach can be particularly useful when the adverse health effect is seen within the range of the current levels of human intake and a NOAEL cannot be identified. This would apply to sodium, for example. Under such circumstances, the BMD (BI or lower confidence limit of the BI, the BIL) is useful, because it defines a point on the intake–response curve that is reliable and relevant to the minimization of the risk of adverse health effects that result from high intake.

Overall, the data sets available for nutrient substances usually are not designed to assess intake–response for adverse health effects. Therefore, not only is the estimation of a BMD (BI) problematic, there are challenges associated with establishing the NOAEL or LOAEL. In addition, the uncertainties and limitations of the usual data sets could, in most cases, result in a value for the lower confidence limit of the BMD (BMDL) (see chapter 5) that was so low that it might lead to nutritional inadequacy. Study quality and design for both human and laboratory animal data are notable issues for the NOAEL (or LOAEL), and they should be considered carefully. Several "study-dependent" factors that influence the magnitude of the value observed include the group size, the sensitivity of the methods used to measure the response, the duration of intake and the selection of intake levels. For laboratory animal studies, important factors include species, strain, sex, age and developmental status.

The NOAEL or LOAEL cannot be used as the final value for the UL—except in the unlikely situation that the value was derived from a large study that is truly representative of the exposed population and contains no uncertainties and negligible errors. Given that available data will usually contain uncertainties, risk assessment principles stipulate that the risk assessor must take these into account. Therefore, an allowance is made for these uncertainties by establishing a UL at some value less than the NOAEL or LOAEL. A similar allowance would need to be made if a BMD (BI) were to be used, but only the NOAEL and LOAEL were discussed at the FAO/WHO Technical Workshop on Nutrient Risk Assessment (FAO/WHO, 2006b).

Following the identification of a NOAEL, LOAEL or BMD (BI), allowances for uncertainty must be made in order to establish a UL. If needed, this is followed by scaling or extrapolating the data to derive ULs for those age, sex and life stage subpopulations for which no data are available. If available data allow, a quantitative allowance for uncertainties may be applied to the NOAEL, LOAEL or BMD (BI) value derived from the intake–response assessment. The first consideration is whether there are sufficient data to make a quantitative allowance for uncertainty: that is, do the data allow the magnitude of uncertainty or variability to be defined? This consideration is equivalent to the determination of a chemical-specific adjustment factor (CSAF) for a non-nutrient substance (see chapter 5, section 5.2.3).

Quantitative allowances are data-derived factors that can be applied to the NOAEL or LOAEL to derive a lower (or sometimes higher) health-based guidance value (a UL), based on information relevant to the target population but not addressed in the data used to derive the values. These adjustments are objective and based on specific data, and they can relate to either kinetic or dynamic aspects of the nutrient substance in different species (IPCS, 1994). While quantitative allowances are theoretically possible for all uncertainties, in practice available data usually allow relatively few quantitative allowances to be made when setting the ULs for nutrient substances. One example of the use of quantitative allowances is the process used to address differences in body size between test animals and humans. Bioavailability is another uncertainty for which quantitative allowances may be used, particularly when data are available for different forms of the same nutrient substance. This allowance could, in principle, lead to setting different ULs for different forms of the nutrient substance—for example, the nicotinic acid and nicotinamide forms of niacin.

Generally, however, allowances for uncertainty must make use of uncertainty factors. Application of the default uncertainty factors that are used for non-nutrient substances poses a potential problem for nutrient substances: the resulting UL could be a value that is below the intake required to ensure nutritional adequacy. This issue arises primarily for those nutrient substances that have recommended intakes that are relatively close to intake levels that may pose a risk; examples commonly quoted include iron, zinc, copper and sometimes calcium. It is now widely recognized that the use of large generic default factors is not usually applicable to nutrient risk assessment. Instead, uncertainty factors used in nutrient risk assessment require consideration on a case-by-case basis and must be placed within the context of established intake requirements.

The FAO/WHO Technical Workshop on Nutrient Risk Assessment (FAO/WHO, 2006b) concluded that it is preferable to develop a composite uncertainty factor case by case rather than apply separate uncertainty factors for different issues. The substance-specific composite factor for uncertainty is applied to the NOAEL or LOAEL after any available quantitative allowances have been made. Because the risk assessment of nutrient substances has to consider both toxicity and essentiality, the use of a composite factor increases the likelihood that

the final value will not be so large as to result in a UL that is lower than the required intake of the nutrient substance. The impact of uncertainty considerations related to the toxicity data must be checked against the level of recommended intake for biological essentiality or for normal health. After uncertainties are taken into account, the resulting value is the UL for the specified subpopulation. When data are insufficient for setting a UL for one or more age, sex and life stage subpopulations (as often is the case), the gap is filled by adjusting a UL that has been established for another subpopulation. Therefore, although it is desirable to establish ULs based on data and end-points, such as differences in the metabolism, homeostatic mechanisms and toxicokinetics between children and adults, in the absence of such data, appropriate scaling is needed. Adjusting or scaling an adult UL into a UL relevant to children may be undertaken by correction using:

- the quantified reference body weight established for the age group;
- body surface area, which is calculated using the reference body weight taken to the power of 0.66 (i.e. $BW^{0.66}$); or
- energy requirement, which is sometimes referred to as metabolic body weight and is calculated using the reference body weight taken to the power of 0.75 (i.e. $BW^{0.75}$).

Because nutrient substances usually are components of normal intermediary metabolism, scaling on the basis of either surface area (i.e. $BW^{0.66}$) or energy requirement (i.e. $BW^{0.75}$) is likely to be more appropriate.

Quantitative data on the dietary intake of a nutrient substance by the (sub)population of interest are required to estimate the proportion of the (sub)population that is likely to exceed the UL. Data on the basis for derivation of the UL and other information gleaned from hazard identification and characterization are essential for describing the risk associated with intake above the UL.

There are several special considerations for the intake assessment for nutrients and related substances. The exposure or intake assessment is population relevant rather than globally relevant. That is, it is dependent on the types of foods and supplements consumed and on dietary patterns within a region or nation-state. This means that risk characterizations can be inherently different depending upon the

target population. This difference holds true even when the derivation of the UL is conducted in a consistent manner using internationally applicable guiding principles. There are wide variations in data types used for dietary intake assessment and in the methods of analysis and presentation of the findings. The FAO/WHO Technical Workshop on Nutrient Risk Assessment reviewed in detail the approaches to nutrient intake assessment and proposed harmonized protocols to improve these data (FAO/WHO, 2006b).

9.2.3 Foods from novel sources

Developments have made possible the production of foods from unconventional sources (e.g. fungal mycelia and yeast cells). In addition, so-called "exotic" fruits and vegetables are being introduced from their region of origin to other regions. Foods that are well known and traditional in one country or region may be unknown and thereby novel in another country or region.

These foods are intended for consumption, either directly or after simple physical modification to provide a more acceptable product. They may be consumed in large amounts, even by infants and children, particularly if they are permitted for use as protein supplements in otherwise protein-deficient diets.

Although the definition of what constitutes a novel food is basically a risk management decision, the following working definitions have been proposed (adapted in part from IPCS, 1987 and Knudsen et al., 2005):

- *History of safe use for a food*: Term used for the qualified presumption of safety. There is evidence for the safety of the food from compositional data and from experience since the food has been an ongoing part of the diet for a number of generations in a large, genetically diverse population. This presumption is for a certain context of use (conditions of use, defined part of the plant used and required processing) and allows for minor population predispositions, such as intolerance and allergenicity.

- *Traditional foods*: Foods that have a history of significant human consumption by the broad community for several generations as

part of the ordinary diet at the global, regional or local level or as a part of an ethnic diet.

- *Non-traditional foods*: Foods that do not have a history of significant human consumption by the broad community for several generations as part of the ordinary diet.

- *Novel foods*: Non-traditional foods for which there is insufficient knowledge in the broad community to ensure safe use or that have characteristics that raise safety concerns due to composition, levels of undesirable substances, potential for adverse effects, traditional preparation and cooking, and patterns and levels of consumption. These include food or food ingredients produced from raw materials not normally used for human consumption or food that is severely modified by the introduction of new processes not previously used in the production of food.

- *Foods for special dietary uses*: Those foods that are specially processed or formulated to satisfy particular dietary requirements that exist because of a particular physical or physiological condition or specific diseases and disorders and that are presented as such. These include foods for infants and young children. The composition of these foodstuffs must differ significantly from the composition of ordinary foods of comparable nature, if such ordinary foods exist.

A decision tree for points that could be considered in the evaluation of whole foods has been proposed by Renwick et al. (2003) and is shown in Figure 9.7.

9.2.3.1 *Chemical composition*

Complete chemical identification of whole foods may not be feasible, but specifications are necessary to ensure that levels of potentially hazardous contaminants, such as mycotoxins and heavy metals or other substances of concern, are kept to a minimum. Toxicological evaluations must be closely related to well-defined materials, and evaluations may not be valid for all preparations from the same source material, if different processing methods are used.

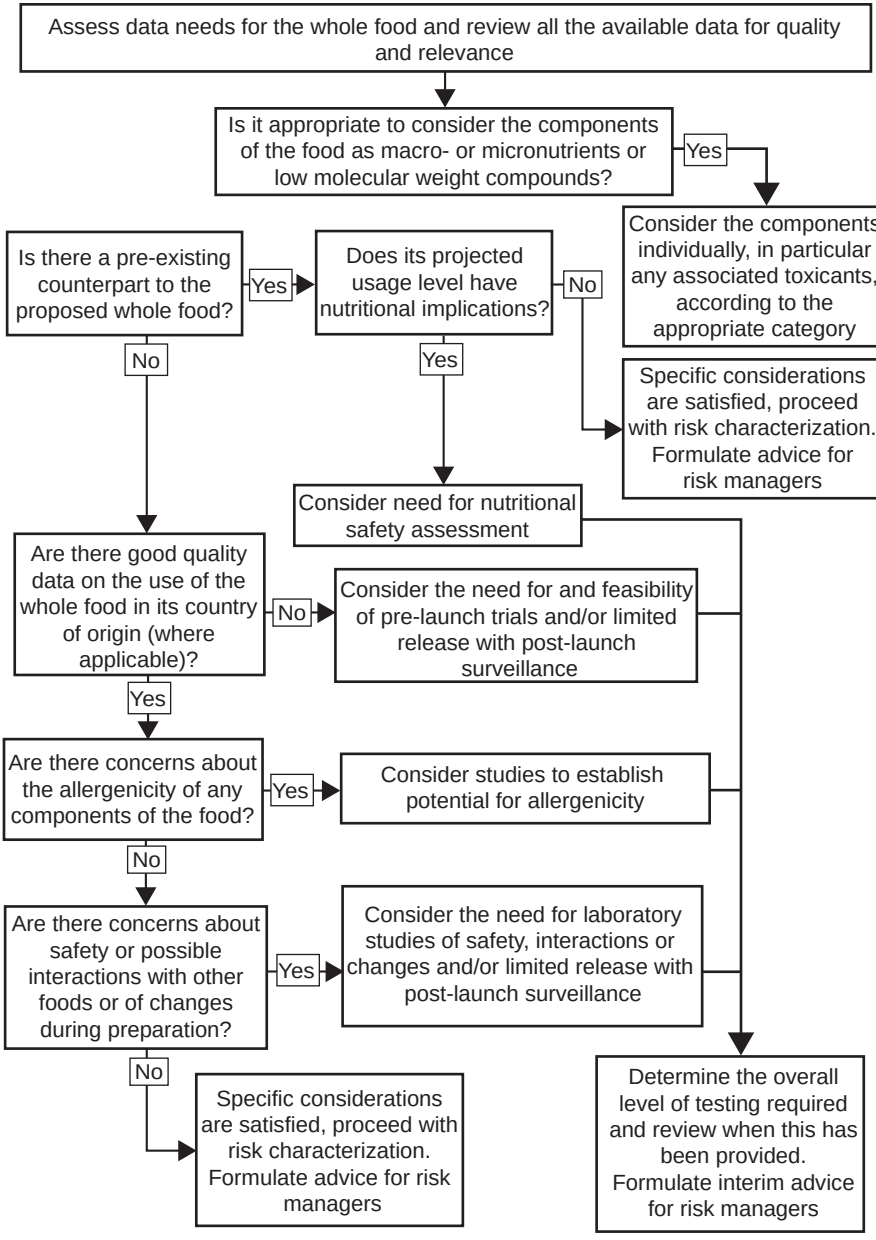

Fig. 9.7. Decision tree outlining the special considerations for the risk characterization of whole foods (adapted from Renwick et al., 2003)

9.2.3.2 Nutritional considerations

When a novel food is intended to replace a significant portion of traditional food in the diet, its likely impact on the nutritional status of consumers requires special consideration.

The influence of the introduction of the new substance on the nutrient composition of the diet as a whole should be identified, particularly with respect to groups such as children, the elderly and "captive populations" (e.g. hospital patients and schoolchildren). In order not to adversely affect the nutritional quality of the diet, it may be necessary to fortify the substance with vitamins, minerals or other nutrients.

The nutritional value of the novel food should be assessed initially from its chemical composition with respect to both macronutrients and micronutrients, taking into account the effects of any further processing and storage. The possible influence of components of the novel food, such as antinutritional factors (e.g. inhibitors of enzyme activity or mineral metabolism), on the nutritional value or keeping quality of the remainder of the diet should also be established.

9.2.3.3 Toxicological evaluations

Depending on the nature and intended uses of the novel food, studies in laboratory animals may be needed to supplement the chemical studies. If the novel food is intended to be an alternative significant supply of protein, tests on its protein quality will be necessary. In vivo studies will also be needed when it is appropriate to determine 1) the availability of vitamins and minerals in the novel food in comparison with the food it would replace and 2) any interaction the novel food might have with other items of the diet that would reduce the whole diet's nutritional value. If the novel food is expected to play an important role in the diet, it may be necessary to verify that the results of laboratory animal studies can be extrapolated to humans by measuring the availability of nutrients to human subjects.

In most cases, novel foods constitute a large percentage of the daily diet in laboratory animal studies because they are of a non-toxic nature. Therefore, the considerations discussed in section 9.2.1.3 apply to the toxicological testing and evaluation of foods from novel sources.

9.2.3.4 Human data

The general principles of studies in humans have been set out in section 4.11 of chapter 4. Human studies on novel foods need to be designed on a case-by-case basis. Human studies should not be embarked upon until there has been a full appraisal of the safety of the novel food using all available data (e.g. history of safe use, data on chemical and microbiological impurities, composition and toxicology). After the launch of a novel food on the market, post-marketing surveillance studies may also be helpful in providing confirmation of anticipated usage patterns and exposure levels. It may be necessary to conduct allergenicity studies on the novel food because of its composition (e.g. if it is highly proteinaceous) or because the results of laboratory animal or human feeding studies suggest that the food might produce hypersensitivity in some people. Important information can be gained by monitoring the health of workers, such as laboratory staff and employees in the manufacturing plant, coming into contact with the novel food. It is not realistic to strive for absolute absence of risk for allergenicity, and the aim of any study should be to ensure that a novel food is at least as safe as its traditional counterpart (i.e. the food that it will replace in the diet).

9.2.3.5 History of use

Human experience, but normally not formal human scientific studies, is an essential part of the data collection in the history of use. The human experience with respect to the consumption of a certain food in a region different from the one that has deemed the food to be novel is normally just an empirical observation that the food in question has been eaten for generations in that region. It will normally be coupled with information on how it is prepared, how it is eaten, how much is eaten and whether the food in question has had any special claims linked to it. This kind of information is often anecdotal and not scientifically well documented and is a history of "use"; however, owing to the absence of health measurements, it is not a history of "safe use".

The following information can be considered for the evaluation of a history of use (adapted from Health Canada, 2006):

- Historical evidence indicating ongoing, frequent consumption by a cross-section of the population where it has been used over several generations. This evidence may be derived from various

sources, including, but not limited to, scientific publications and patents, non-scientific publications and books, cookbooks, books on the history of food culture or affidavits from two or more independent, reputable authorities that include well-documented accounts of the way in which the food is used and how they know it has the history it does. Limited usage or short-term exposure would not be adequate to demonstrate a history of safe use.

- A declaration of any possible adverse effects linked to the food documented in its country of origin or a country where there is a high degree of consumption.

- A description of the standard methods of commercial or domestic processing and preparation for consumption.

- A description of how the food is cultivated or (if from wild sources) harvested.

- Amounts of the food that people are likely to consume, including typical serving sizes and expected frequency of consumption, at both average and high consumption levels.

- Analysis of the composition of the food based on randomly selected, statistically valid samples. This analysis should include proximate data as well as amino acid profile, fatty acid profile, mineral and trace mineral composition and vitamin composition, as well as any nutrients, antinutrients or bioactive phytochemicals in the product that are known to be of particular interest. The analysis should pay special attention to the presence of compounds in the food that may have implications for the health of any subgroups of the population (e.g. possible toxicants or allergens or unusually high levels of nutrients in the food source or final food product).

- Metabolism or gastrointestinal effects in humans.

9.2.3.6 *Exposure assessment*

For novel foods, exposure will need to be estimated from proposed uses. For many novel foods, accurate prediction of the likely commercial success, and therefore intakes, is particularly difficult. Therefore, post-launch monitoring can be essential to verify that the

risk characterization was appropriate to the exposure. Information on the intended or anticipated uses of the novel food is essential for the assessment of whether the uses will be safe or will constitute a risk. For exotic fruits and vegetables, experience from the region from which they originate can provide helpful information; consumption patterns must be considered in the local context of the novel use proposed. A food traditionally consumed only occasionally or exclusively in combination with another material may cause problems when consumed in larger quantities or in a different combination.

The exposure assessment should also consider the appropriate ways of preparing and cooking the novel plant food. Some are to be eaten raw; some are to be milled to flour and go through baking processes; some are to be peeled and cooked; some are to be extracted, treated with acids or bases, dried and fried. All these processes greatly influence the contents and digestive availability of inherent toxicants, macronutrients and micronutrients of the individual novel food as assessed in the hazard characterization.

9.2.3.7 Risk characterization

For the risk characterization of novel foods, the margin of exposure (MOE) approach may be suitable. The MOE is calculated from the estimated daily safe intake divided by the likely human daily exposure. This value can then be used by the risk managers to guide further decisions on the use of the novel plant food in the general food supply and—if properly indicated on the food—by the individual consumer to guide his or her choice for proper food that meets individual expectations and needs.

9.3 References[1]

Allgood GS, Kuter DJ, Roll KT, Taylor SL & Zorich NL (2001) Postmarketing surveillance of new food ingredients: results from the program with the fat replacer olestra. Regul Toxicol Pharmacol, **33**: 224–233.

Amanor-Boadu V (2004) Post-market surveillance model for potential human health effects of novel foods. Food Policy, **29**: 609–620.

[1] Internet links provided in these references were active as of the date of final editing.

Ashby J & Tennant RW (1988) Chemical structure, *Salmonella* mutagenicity and extent of carcinogenicity as indicators of genotoxic carcinogenesis among 222 chemicals tested in rodents by the U.S. NCI/NTP. Mutat Res, **204**(1): 17–115.

Ashby J & Tennant RW (1991) Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the U.S. NTP (National Toxicology Program). Mutat Res, **257**(3): 229–306 [Corrigendum, 1994, **317**(2): 175].

Ashby J, Tennant RW, Zeiger E & Stasiewicz S (1989) Classification according to chemical structure, mutagenicity to *Salmonella* and level of carcinogenicity of a further 42 chemicals tested for carcinogenicity by the U.S. National Toxicology Program. Mutat Res, **223**(2): 73–103.

Cheeseman MA, Machuga EJ & Bailey AB (1999) A tiered approach to threshold of regulation. Food Chem Toxicol, **37**(4): 387–412.

Cramer GM, Ford RA & Hall RL (1978) Estimation of toxic hazard—A decision tree approach. Food Cosmet Toxicol, **16**: 255–276 [& Erratum].

Edler L, Poirier K, Dourson M, Kleiner J, Mileson B, Nordmann H, Renwick A, Slob W, Walton K & Wurtzen G (2002) Mathematical modeling and quantitative methods. Food Chem Toxicol, **40**: 283–326.

FAO (2006a) General specifications and considerations for enzyme preparations used in food processing. Rome, Food and Agriculture Organization of the United Nations (http://www.fao.org/ag/agn/jecfa-additives/docs/enzymes_en.htm).

FAO (2006b) Combined compendium on food additive specifications. Vol. 4. Analytical methods, test procedures and laboratory solutions used by and referenced in the food additive specifications. Rome, Food and Agriculture Organization of the United Nations (FAO JECFA Monographs, No. 1; ftp://ftp.fao.org/docrep/fao/009/a0691e/a0691e00a.pdf).

FAO/WHO (1995) Evaluation of certain food additives and contaminants. Forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 859; http://whqlibdoc.who.int/trs/WHO_TRS_859.pdf).

FAO/WHO (1997) Evaluation of certain food additives and contaminants. Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 868; http://whqlibdoc.who.int/trs/WHO_TRS_868.pdf).

FAO/WHO (1999) Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 884; http://whqlibdoc.who.int/trs/WHO_TRS_884.pdf).

FAO/WHO (2001) Evaluation of certain food additives and contaminants. Fifty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 901; http://whqlibdoc.who.int/trs/WHO_TRS_901.pdf).

FAO/WHO (2005) Evaluation of certain food additives. Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 928; http://whqlibdoc.who.int/trs/WHO_TRS_928.pdf).

FAO/WHO (2006a) Evaluation of certain food additives and contaminants. Sixty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 934; http://whqlibdoc.who.int/trs/WHO_TRS_934_eng.pdf).

FAO/WHO (2006b) A model for establishing upper levels of intake for nutrients and related substances: report of a Joint FAO/WHO technical workshop on nutrient risk assessment, 2–6 May 2005. Geneva, World Health Organization (http://www.who.int/ipcs/highlights/full_report.pdf).

FAO/WHO (2007a) Evaluation of certain food additives and contaminants. Sixty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 940; http://whqlibdoc.who.int/trs/WHO_TRS_940_eng.pdf).

FAO/WHO (2007b) Evaluation of certain food additives and contaminants. Sixty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 947; http://whqlibdoc.who.int/publications/2007/9789241209472_eng.pdf).

FAO/WHO (2009) Evaluation of certain food additives and contaminants. Sixty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 952; http://whqlibdoc.who.int/trs/WHO_TRS_952_eng.pdf).

Gold LS & Zeiger E eds (1997) Handbook of carcinogenic potency and genotoxicity databases. Boca Raton, FL, CRC Press.

Gold LS, Sawyer CB, Magaw R, Backman GM, de Veciana M, Levinson R, Hooper NK, Havender WR, Bernstein L, Peto R, Pike MC & Ames BN (1984) A carcinogenic potency database of the standardized results of animal bioassays. Environ Health Perspect, **58**: 9–319.

Gold LS, Slone TH & Bernstein L (1989) Summary of carcinogenic potency and positivity for 492 rodent carcinogens in the carcinogenic potency database. Environ Health Perspect, **79**: 259–272.

Health Canada (2006) Guidelines for the safety assessment of novel foods. Ottawa, Ontario, Health Canada, Health Protection Branch, Food Directorate (http://www.hc-sc.gc.ca/fn-an/legislation/guide-ld/nf-an/guidelines-lignesdirectrices_e.html).

Hepburn P, Howlett J, Boeing H, Cockburn A, Constable A, Davi A, de Jong N, Moseley B, Oberdörfer R, Robertson C, Wal JM & Samuels F (2008) The application of post-market monitoring to novel foods. Food Chem Toxicol, **46**(1): 9–33.

Hlywka JJ, Reid JE & Munro IC (2003) The use of consumption data to assess exposure to biotechnology-derived foods and the feasibility of identifying effects on human health through post-market monitoring. Food Chem Toxicol, **41**: 1273–1282.

IOM (1998) Dietary reference intakes: a risk assessment model for establishing upper intake levels for nutrients. Prepared by the Food and Nutrition Board, Institute of Medicine. Washington, DC, National Academy Press (http://books.nap.edu/openbook.php?record_id=6432).

IPCS (1987) Principles for the safety assessment of food additives and contaminants in food. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 70; http://www.inchem.org/documents/ehc/ehc/ehc70.htm).

IPCS (1994) Assessing human health risks of chemicals: derivation of guidance values for health-based exposure limits. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 170; http://www.inchem.org/documents/ehc/ehc/ehc170.htm).

IPCS (2002) Principles and methods for the assessment of risk from essential trace elements. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 228; http://www.inchem.org/documents/ehc/ehc/ehc228.htm).

IPCS (2004) IPCS risk assessment terminology. Geneva, World Health Organization, International Programme on Chemical Safety (Harmonization Project Document, No. 1; http://www.who.int/ipcs/methods/harmonization/areas/ipcsterminologyparts1and2.pdf).

Knudsen I, Søborg I, Eriksen F, Pilegaard K & Pedersen P (2005) Risk assessment and risk management of novel plant foods. Concepts and principles. Copenhagen, Nordic Council of Ministers (Report TemaNord 2005:588; http://www.norden.org/en/publications/publications/2005-588/at_download/publicationfile).

Kroes R, Galli C, Munro I, Schilter B, Tran L, Walker R & Würtzen G (2000) Threshold of toxicological concern for chemical substances present in the diet: a practical tool for assessing the need for toxicity testing. Food Chem Toxicol, **38**: 255–312.

Kroes R, Renwick AG, Cheeseman M, Kleiner J, Mangelsdorf I, Piersma A, Schilter B, Schlatter J, Van Schothorst F, Vos JG & Würtzen G (2004) Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. Food Chem Toxicol, **42**: 65–83.

Lea LJ & Hepburn PA (2006) Safety evaluation of phytosterol-esters. Part 9: Results of a European post-launch monitoring programme. Food Chem Toxicol, **44**: 1213–1222.

Munro IC (1990) Safety assessment procedures for indirect food additives: an overview. Report of a workshop. Regul Toxicol Pharmacol, **12**: 2–12.

Munro IC, Ford RA, Kennepohl E & Sprenger JG (1996) Correlation of structural class with no-observed-effect levels: a proposal for establishing a threshold of concern. Food Chem Toxicol, **34**: 829–867.

Munro IC, Kennepohl E & Kroes R (1999) A procedure for the safety evaluation of flavouring substances. Food Chem Toxicol, **37**: 207–232.

Renwick AG, Barlow SM, Hertz-Picciotto I, Boobis AR, Dybing E, Edler L, Eisenbrand G, Grieg JB, Kleiner J, Lambe J, Müller DJG, Smith MR, Tritscher A, Tuijtelaars S, Van den Brandt PA, Walker R & Kroes R (2003) Risk chacterization of chemicals in food and diet. Food Chem Toxicol, **41**: 1211–1271.

Renwick AG, Flynn A, Fletcher RJ, Müller DJ, Tuijtelaars S & Verhagen H (2004) Risk–benefit analysis of micronutrients. Food Chem Toxicol, **42**: 1903–1922.

Rulis AM (1986) De minimis and the threshold of regulation. In: Felix CW ed. Food protection technology. Chelsea, MI, Lewis Publishers, pp 29–37.

Rulis AM (1989) Establishing a threshold of concern. In: Bonin JJ & Stevenson DE eds. Risk assessment in setting national priorities. Vol. 7. New York, NY, Plenum Press, pp 271–278.

Rulis AM (1992) Threshold of regulation: options for handling minimal risk situations. In: Finley JW, Robinson SF & Armstrong DJ eds. Food safety assessment. Washington, DC, American Chemical Society, pp 132–139 (ACS Symposium Series, No. 484).

USFDA (1995) Food additives; threshold of regulation for substances used in food-contact articles (final rule). United States Food and Drug Administration. Fed Regist, **60**(136): 36582–36596.

ANNEX 1: GLOSSARY OF TERMS

Note: The primary sources of the definitions found in this glossary of terms are listed at the end of the glossary. Some definitions have been taken directly from the original source, whereas others have been modified for the purposes of this document. Still others derive from the text of this monograph. Not all terms provided in the glossary are used in this monograph, but they are included here to help the reader understand previous evaluations of the Joint FAO/WHO Expert Committee on Food Additives and the Joint FAO/WHO Meeting on Pesticide Residues.

Absorption
The process by which a substance is transferred from the site of administration into the circulation. For chemicals in food, absorption usually refers to passage across the gut wall into the circulation, although for some chemicals, uptake may be only as far as the epithelium of the gastrointestinal tract.

Acceptable
A term previously used as the outcome of the safety assessment of food additives. Now replaced mainly by the term "not specified" or "no safety concern at current estimated levels of intake".

Enzyme preparations: Used to describe enzymes that are obtained from edible tissues of animals or plants commonly used as foods or are derived from microorganisms that are traditionally accepted as constituents of foods or are normally used in the preparation of foods. Such enzyme preparations are considered to be acceptable provided that satisfactory chemical and microbiological specifications can be established.

Flavouring agents: Used to describe flavouring agents that are of no safety concern at current levels of intake. If an acceptable daily intake has been allocated to the agent, it is maintained unless otherwise indicated.

Food additives: Used on some occasions when present uses are not of toxicological concern or when intake is self-limiting for technological or organoleptic reasons.

Acceptable daily intake (ADI)
The estimate of the amount of a chemical in food or drinking-water, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risk to the consumer. It is derived on the basis of all the known facts at the time of the evaluation. The ADI is expressed in milligrams of the chemical per kilogram of body weight (a standard adult person weighs 60 kg). It is applied to food additives, residues of pesticides and residues of veterinary drugs in food.

Acceptable daily intake (ADI) "not limited"
A term no longer used by the Joint FAO/WHO Expert Committee on Food Additives that has the same meaning as ADI "not specified".

Acceptable daily intake (ADI) "not specified"
Food additives: A term applicable to a food substance of very low toxicity that, on the basis of the available chemical, biochemical and toxicological data as well as the total dietary intake of the substance (from its use at the levels necessary to achieve the desired effect and from its acceptable background in food), does not, in the opinion of the Joint FAO/WHO Expert Committee on Food Additives, represent a hazard to health. For that reason, and for reasons stated in individual evaluations, the establishment of an ADI expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of Good Manufacturing Practice: that is, it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal inferior food quality or adulteration, and it should not create a nutritional imbalance.

Veterinary drugs: A term applicable to a veterinary drug for which available data on its toxicity and intake indicate a large margin of safety for consumption of residues in food when the drug is used according to Good Practice in the Use of Veterinary Drugs. For that reason, and for the reasons stated in the individual evaluation, the Committee has concluded that use of the veterinary drug does not represent a dietary hazard to human health and that there is no need to specify a numerical ADI.

Acceptable level of treatment
Acceptable daily intakes (ADIs) are expressed in terms of milligrams per kilogram of body weight. In certain cases, however, food additives

are more appropriately limited by their levels of treatment. This situation occurs most frequently with flour treatment agents. It should be noted that the acceptable level of treatment is expressed as milligrams per kilogram of the commodity. This should not be confused with an ADI.

Acceptable risk
A risk management term. The acceptability of the risk depends on scientific data, social, economic and political factors, and the perceived benefits arising from exposure to an agent.

Accuracy
Degree of agreement between average predictions of a model or the average of measurements and the true value of the quantity being predicted or measured.

Acute exposure
A short-term exposure to a chemical, usually consisting of a single exposure or dose administered for a period of 24 h or less.

Acute reference dose (ARfD)
The estimate of the amount of a substance in food or drinking-water, expressed on a body weight basis, that can be ingested in a period of 24 h or less without appreciable health risk to the consumer. It is derived on the basis of all the known facts at the time of evaluation. The ARfD is expressed in milligrams of the chemical per kilogram of body weight.

Adverse effect
Change in the morphology, physiology, growth, development, reproduction or lifespan of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences.

Aggregate exposure
The combined exposures to a single chemical across multiple routes (oral, dermal, inhalation) and across multiple pathways (food, drinking-water, residential). *Related term*: Cumulative exposure.

Allergy
See Food allergy.

Assessment factor
Numerical adjustment used to extrapolate from experimentally determined (dose–response) relationships to estimate the exposure to an agent below which an adverse effect is not likely to occur. *Related terms*: Safety factor, Uncertainty factor.

Benchmark dose (BMD)
A dose of a substance associated with a specified low incidence of risk, generally in the range of 1–10%, of a health effect; the dose associated with a specified measure or change of a biological effect.

Benchmark dose lower confidence limit (BMDL)
The lower boundary of the confidence interval on the benchmark dose. The BMDL accounts for the uncertainty in the estimate of the dose–response that is due to characteristics of the experimental design, such as sample size. The BMDL can be used as the point of departure for derivation of a health-based guidance value or a margin of exposure.

Benchmark intake (BI)
The intake of a substance that is expected to result in a prespecified level of effect. *Related term*: Benchmark dose.

Benchmark intake lower confidence limit (BIL)
The lower boundary of the confidence interval on the benchmark intake. *Related term*: Benchmark dose lower confidence limit.

Benchmark response (BMR)
The response for which the benchmark dose is to be calculated.

Bias
The sum of all the systematic errors in an experiment. *Related term*: Error.

Bioavailability
For food additives, contaminants and pesticide residues, a term referring to the proportion of a substance that reaches the systemic circulation unchanged after a particular route of administration. For veterinary drug residues in food, it is used to reflect the fraction that can be released from the food matrix and is available for absorption.

Biomarkers
Indicators of changes or events in human biological systems. *Biomarkers of exposure* refer to cellular, biochemical or molecular measures that are obtained from biological media such as human tissues, cells or fluids and are indicative of exposure to a substance. *Biomarkers of effect* refer to biological changes that represent an alteration in endogenous body constituents (e.g. depression of cholinesterase levels as an indicator of exposure to pesticides).

Budget method
A screening method used for estimating dietary exposure to a food additive that is based on default maximum consumption amounts of solid food and liquids derived from physiological consumption limits and the maximum use levels of the additive.

Central tendency
The central tendency of a probability distribution typically refers to the mean (arithmetic average) or median (50th percentile) value estimated from the distribution. For some very highly skewed distributions, the mean might not represent central tendency, and some analysts prefer to use the median as a central tendency estimate.

Chemical-specific adjustment factor (CSAF)
A modified default 10-fold uncertainty factor that incorporates appropriate data on species differences or human variability in either toxicokinetics (fate of the chemical in the body) or toxicodynamics (actions of the chemical on the body).

Chronic exposure
A continuous or intermittent long-term contact between an agent and a target.

Clastogenicity
The condition of causing structural chromosomal aberrations in populations of cells or organisms.

Codex Alimentarius Commission (CAC)
CAC was formed in 1962 to implement the Joint FAO/WHO Food Standards Programme. It is an intergovernmental body made up of more than 170 member nations, the delegates of which represent their

own countries. CAC's work of harmonizing food standards is carried out through various committees, such as the Codex Committee on Food Additives (CCFA), the Codex Committee on Contaminants in Food (CCCF), the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) and the Codex Committee on Pesticide Residues (CCPR). The Joint FAO/WHO Expert Committee on Food Additives serves as the advisory body to CAC on all scientific matters concerning food additives, food contaminants, naturally occurring toxicants and residues of veterinary drugs in food. The Joint FAO/WHO Meeting on Pesticide Residues serves as the advisory body to CAC on all scientific matters concerning pesticide residues.

Composite sample
Often prepared as a representative mixture of several different (usually bulk) samples, from which the laboratory sample is taken.

Concentration
The amount of one substance (e.g. milligrams of pesticide residue) contained in a given amount of another substance (e.g. kilograms of food).

Concentration–effect relationship
Relationship between the exposure, expressed in concentration, of a given organism, system or (sub)population to an agent in a specific pattern during a given time and the magnitude of a continuously graded effect to that organism, system or (sub)population. *Related terms*: Dose–effect relationship, Dose–response relationship.

Conditional acceptable daily intake (ADI)
A term no longer used by JECFA to signify a range above the "unconditional ADI", which may signify an acceptable intake when special problems, different patterns of dietary intake, and special groups of the population that may require consideration are taken into account.

Confidence interval
An estimated two-sided interval from the lower to upper confidence limit of a statistical parameter. This interval is expected to enclose the true value of the parameter with a specified confidence. For example, 95% confidence intervals are expected to enclose the true values of estimated parameters with a frequency of 95%.

Conservative estimate
An estimate that tends to err on the side of caution. A conservative estimate of dietary exposure, for example, assigns the "worst case" food chemical concentrations and/or food consumption levels to maximize (or minimize, in the case of nutrients, when assessing nutrient deficiency) the estimated food chemical exposure.

Consumer days
From the total number of records in a food consumption survey (i.e. total survey days), those days on which individuals reported consuming the food or foods of interest.

Consumer loyalty
The tendency of consumers to repeatedly purchase and consume the same processed food products.

Consumption cluster diets
See GEMS/Food consumption cluster diets.

Contaminant
Any substance not intentionally added to food that is present in such food as a result of the production (including operations carried out in crop husbandry, animal husbandry and veterinary medicine), manufacture, processing, preparation, treatment, packaging, transport or holding of such food or as a result of environmental contamination. The term does not include insect fragments, rodent hairs and other extraneous matter.

Cumulative exposure
The sum of exposures to two or more food chemicals that have a common mechanism of toxicity. *Related term*: Aggregate exposure.

Deterministic estimate
In exposure assessment, an estimate that is based on a single value for each model input and a corresponding individual value for a model output, without quantification of the cumulative probability or, in some cases, plausibility of the estimate with respect to the real-world system being modelled. This term is also used to refer to a model for which the output is uniquely specified based on selected single values for each of its inputs.

Developmental toxicity
Any adverse effects induced prior to attainment of adult life, including effects induced or manifested in the embryonic or fetal period and those induced or manifested postnatally (before sexual maturity). These may include prenatal or early postnatal death, structural abnormalities, altered growth and functional deficits. *Related terms*: Reproductive toxicity, Teratogenicity.

Dietary exposure
See Intake.

Dietary exposure assessment
The qualitative and/or quantitative evaluation of the likely intake of chemicals (including nutrients) via food, beverages, drinking-water and food supplements. *Synonymous with*: Intake assessment.

Dietary recall (24 h dietary recall)
A retrospective assessment method in which an interviewer prompts a respondent to recall and describe all foods and beverages consumed in the preceding 24 h or the preceding day. The interview may be conducted in person or by telephone and may be recorded by paper and pencil or computer assisted. Portion size estimating aids assist the respondent to recall amounts consumed.

Dietary record
See Food record.

Dietary supplement
See Food supplement.

Diet history questionnaire
A retrospective assessment method ascertaining a respondent's "usual" food intake by collecting descriptive detail and amount information about each food. Questionnaires may include questions on meal patterns, lists of common foods and groups of generic food. They are typically administered by a trained interviewer either in person or by telephone, but they can also be self-reported.

Distribution
See Probability distribution.

Dose
Total amount of an agent administered to, taken up by or absorbed by an organism, system or (sub)population.

Dose–effect relationship
Relationship between the total amount of an agent administered to, taken up by or absorbed by an organism, system or (sub)population and the magnitude of a continuously graded effect to that organism, system or (sub)population. *Related terms*: Concentration–effect relationship, Dose–response relationship.

Dose-related effect
Any effect to an organism, system or (sub)population as a result of the quantity of an agent administered to, taken up by or absorbed by that organism, system or (sub)population.

Dose–response
Relationship between the amount of an agent administered to, taken up by or absorbed by an organism, system or (sub)population and the change developed in that organism, system or (sub)population in reaction to the agent.

Dose–response assessment
Analysis of the relationship between the total amount of an agent administered to, taken up by or absorbed by an organism, system or (sub)population and the changes developed in that organism, system or (sub)population in reaction to that agent, and inferences derived from such an analysis with respect to the entire population. Dose–response assessment is the second of four steps in risk assessment.

Dose–response curve
Graphical presentation of a dose–response relationship.

Dose–response relationship
Relationship between the amount of an agent administered to, taken up by or absorbed by an organism, system or (sub)population and the change developed in that organism, system or (sub)population in reaction to the agent. *Related terms*: Concentration–effect relationship, Dose–effect relationship.

Double-blind placebo-controlled food challenge
A study in which neither the patient nor the test administrator is aware of the food or placebo being tested. In the test, the patient ingests a food that has been disguised so that neither the patient nor the observer is aware of the contents of the challenge. This type of challenge is designed to reduce the subjective attitudes of both participants during the challenge. *Related term*: Single-blind placebo-controlled food challenge.

Duplicate diets/Duplicate portion study
A method for estimating dietary intakes that involves collection and analysis of identical portions of foods and beverages consumed by an individual.

Effect
A change in the state or dynamics of an organism, system or (sub)-population caused by the exposure to an agent.

Effect assessment
Combination of analysis and inference of possible consequences of the exposure to a particular agent based on knowledge of the dose–effect relationship associated with that agent in a specific target organism, system or (sub)population.

Elimination
The expelling of a substance or other material from the body (or a defined part thereof), usually by a process of extrusion or exclusion, but sometimes through metabolic transformation.

Embryo/fetotoxicity
Any toxic effect on the embryo or fetus resulting from prenatal exposure, including structural or functional abnormalities or postnatal manifestation of such effects.

Endogenous substances
Intermediary metabolites normally present in human tissues and fluids, whether free or conjugated. Hormones and other substances with biochemical or physiological regulatory functions are not included.

End-point
Qualitative or quantitative expression of a specific factor with which a risk may be associated as determined through an appropriate risk assessment.

Enterohepatic circulation
Intestinal reabsorption of material that has been excreted through the bile followed by transfer back to the liver, making it available for biliary excretion again.

Epigenetic event
Any heritable influence in the progeny of cells or of individuals on chromosome or gene function that is not accompanied by a change in deoxyribonucleic acid nucleotide sequence.

Error (gross, random, systematic)
Any discrepancy between a computed, observed or measured quantity and the true, specified or theoretically correct value of that quantity. *Gross errors* refer to unintentional or unpredictable errors while generating the analytical result. Errors of this type invalidate the measurement. It is not possible or desirable to statistically evaluate and include the gross errors in the estimation of uncertainty. *Random errors* are present in all measurements and cause replicate results to fall on either side of the mean value. The random error of a measurement cannot be compensated for, but increasing the number of observations and training of the analyst may reduce the effects. *Systematic errors* are those resulting from some bias in the measurement process and are not due to chance. Systematic errors occur in most experiments, but their effects are quite different. The sum of all the systematic errors in an experiment is referred to as the *bias*.

Expert judgement
Opinion of an authoritative person on a particular subject.

Exposure
Concentration or amount of a particular agent that reaches a target organism, system or (sub)population in a specific frequency for a defined duration.

Exposure assessment
Evaluation of the exposure of an organism, system or (sub)population to an agent (and its derivatives). Exposure assessment is one of the steps in the process of risk assessment.

Exposure route
The way in which an agent enters a target after contact (e.g. by ingestion, inhalation or dermal absorption).

Exposure scenario
A set of conditions or assumptions about sources, exposure pathways, amounts or concentrations of agents involved and exposed organisms, systems or (sub)populations (i.e. numbers, characteristics, habits) used to aid in the evaluation and quantification of exposures in a given situation.

Extraneous maximum residue limit (EMRL)
Refers to a pesticide residue or a contaminant arising from environmental sources (including former agricultural uses) other than the use of the pesticide or contaminant directly or indirectly on the commodity. It is the maximum concentration of a pesticide residue that is recommended by the Codex Alimentarius Commission to be legally permitted or recognized as acceptable in or on a food, agricultural commodity or animal feed. The concentration is expressed in milligrams of pesticide residue or contaminant per kilogram of the commodity.

Fate
Pattern of distribution of an agent, its derivatives or metabolites in an organism, system, compartment or (sub)population of concern as a result of transport, partitioning, transformation or degradation.

First-pass metabolism
A phenomenon of metabolism (especially in the liver) whereby the concentration of a substance is greatly reduced before it reaches the systemic circulation.

Food
In the Codex Alimentarius Commission context, any substance, whether processed, semiprocessed or raw, that is intended for human

consumption. It includes drink, chewing gum and any substance that has been used in the manufacture, preparation or treatment of food, but it does not include cosmetics or tobacco or substances used only as drugs.

Food additive
In the Codex Alimentarius Commission context, any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food results, or may be reasonably expected to result, (directly or indirectly) in it or its by-products becoming a component of or otherwise affecting the characteristics of such foods. The term does not include contaminants or substances added to food for maintaining or improving nutritional qualities.

Food allergy
A form of food intolerance in which there is evidence of an abnormal immunological reaction to the food. "Immediate allergic reactions" are those that occur within minutes to hours after ingestion of the offending food, whereas reactions beginning several hours to days after food exposure are characterized as "delayed allergic reactions".

Food balance sheet
Gross estimates of national per capita availability of food commodities derived from a country's annual food production plus imports minus exports. Food waste, refuse, losses from spoilage and other sources of waste are not taken into account.

Food composition data
Data on the composition of foods, mainly on nutrients but also on non-nutrients (e.g. phytochemicals) and contaminants (e.g. acrylamides).

Food consumption
For assessing dietary chemical hazards, an estimate of the quantity of a food or group of foods (including beverages and drinking-water) consumed by a specified population or individual. Food consumption is expressed in grams of food per person per day.

Food diary
See Food record.

Foods for special dietary uses
Foods that are specially processed or formulated to satisfy particular dietary requirements that exist because of a particular physical or physiological condition or specific diseases and disorders and that are presented as such. The composition of these foodstuffs must differ significantly from the composition of ordinary foods of comparable nature, if such ordinary foods exist.

Food frequency questionnaire (FFQ)
A retrospective method asking respondents to report their usual frequency of consumption of each food from a list of foods for a specific period (several months or a year). Food lists vary by the purpose of the study and study population. Frequency of consumption categories also vary by questionnaire, but usually include per day, week or month. In a *semiquantitative FFQ*, portion size information is collected; portion sizes are specified as standardized portions or choice (range of portions). In a *non-quantitative FFQ*, portion size information is not collected.

Food habit questionnaire
A method for collecting information about an individual's beliefs or practices related to food and beverage consumption (e.g. perceptions about foods, food likes and dislikes, methods of preparation).

Food intolerance
A reproducible, unpleasant reaction to a food or food ingredient, including reactions due to immunological effects, biochemical factors such as enzyme deficiencies and anaphylactic reactions that often include histamine release.

Food record (food diary)
Food records are used to record food intake at the time of consumption over a number of days that are not necessarily sequential. Most studies ask respondents to enter the information in hard copy form, although tape recording, bar coding and electronic weighing have also been used to collect descriptive and quantity information. In a *weighed food record*, the respondent weighs all food and beverages consumed on a

small scale. In an *estimated food record*, the respondent estimates all food consumed using household measures or portion size estimating aids.

Food supplement
A product taken by mouth that contains a "dietary ingredient" (e.g. mineral, vitamin, herb, enzyme) and is intended to supplement the intake of that ingredient from the normal diet.

Fortified food
A food to which vitamins, minerals or other components have been added in addition to the levels that were originally found before the food was refined.

Functional food
Any food claiming to have a health-promoting or disease-preventing property beyond the basic function of supplying nutrients.

GEMS/Food
The World Health Organization's Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme, which maintains databases on contaminant levels in foods and estimates of dietary exposure to food chemicals.

GEMS/Food consumption cluster diets
Per capita consumption of raw and semiprocessed agricultural commodities expressed in grams per person per day for distinct groups of the world's population that share similar dietary patterns. Based on Food and Agriculture Organization of the United Nations food balance sheet data, the diets were generated using a cluster analysis, which assigned countries to one of the 13 cluster diets. *Related term*: GEMS/Food regional diets.

GEMS/Food regional diets
Per capita consumption of raw and semiprocessed agricultural commodities expressed in grams per person per day for regional and cultural groups of the world. The diets were generated using Food and Agriculture Organization of the United Nations food balance sheet data from selected representative countries for each of the five regions (Middle Eastern, Far Eastern, African, Latin American and European).

The GEMS/Food regional diets have now been replaced by the GEMS/Food consumption cluster diets. *Related term*: GEMS/Food consumption cluster diets.

Genotoxic carcinogen
Carcinogen whose primary mode of action involves deoxyribonucleic acid or chromosomal alterations.

Genotoxicity
Refers to potentially harmful effects on genetic material that may be mediated directly or indirectly and are not necessarily associated with mutagenicity. Tests of genotoxicity include measures that provide an indication of induced damage to deoxyribonucleic acid (DNA) (but not direct evidence of mutation) via effects such as DNA adduct formation, unscheduled DNA synthesis, sister chromatid exchange or mitotic recombination, as well as tests for mutagenicity. *Related term*: Mutagenicity.

Good Agricultural Practice (GAP)
For pesticide use, includes the nationally authorized safe uses of pesticides under actual conditions necessary for effective and reliable pest control. It encompasses a range of levels of pesticide applications up to the highest authorized use, applied in a manner that leaves a residue that is the smallest amount practicable. Authorized safe uses are determined at the national level and include nationally registered or recommended uses, which take into account public and occupational health and environmental safety considerations. Actual conditions include any stage in the production, storage, transport, distribution and processing of food commodities and animal feed.

Good Clinical Practice (GCP)
A standard for the design, conduct, performance, monitoring, auditing, recording, analyses and reporting of clinical trials that provides assurance that the data and reported results are credible and accurate and that the rights, integrity and confidentiality of trial subjects are protected.

Good Laboratory Practice (GLP)
The formalized process and conditions under which laboratory studies are planned, performed, monitored, recorded, reported and audited. Studies performed under GLP are based on the national regulations of

a country and are designed to assure the reliability and integrity of the studies and associated data.

Good Manufacturing Practice (GMP)
For food additives, includes the following: the quantity of the additive added to food does not exceed the amount reasonably required to accomplish its intended physical, nutritional or other technical effect in food; the quantity of the additive that becomes a component of food as a result of its use in the manufacturing, processing or packaging of a food and that is not intended to accomplish any physical or other technological effect in the food itself is reduced to the extent reasonably possible; the additive is of appropriate food-grade quality and is prepared and handled in the same way as a food ingredient.

Good Practice in the Use of Veterinary Drugs (GPVD)
The official recommended or authorized usage including withdrawal periods, approved by national authorities, of veterinary drugs under practical conditions.

Gross error
See Error.

Group acceptable daily intake (ADI)
An ADI established for a group of compounds that display similar toxic effects or share a common toxic metabolite, thus limiting their cumulative intake.

Guidance value
Value, such as concentration in air or water, that is derived after allocation of the health-based guidance value (e.g. acceptable daily intake) among the different possible media (routes) of exposure.

Hazard
Inherent property of an agent or situation having the potential to cause adverse effects when an organism, system or (sub)population is exposed to that agent.

Hazard assessment
A process designed to determine the possible adverse effects of an agent or situation to which an organism, system or (sub)population could be

exposed. The process includes hazard identification and hazard characterization. The process focuses on the hazard, in contrast to risk assessment, where exposure assessment is a distinct additional step.

Hazard characterization
The qualitative and, wherever possible, quantitative description of the inherent properties of an agent or situation having the potential to cause adverse effects. This should, where possible, include a dose–response assessment and its attendant uncertainties. Hazard characterization is the second stage in the process of hazard assessment and the second step in risk assessment.

Hazard identification
The identification of the type and nature of adverse effects that an agent has an inherent capacity to cause in an organism, system or (sub)-population. Hazard identification is the first stage in hazard assessment and the first step in the process of risk assessment.

Health-based guidance value
A numerical value derived by dividing a point of departure (a no-observed-adverse-effect level, benchmark dose or benchmark dose lower confidence limit) by a composite uncertainty factor to determine a level that can be ingested over a defined time period (e.g. lifetime or 24 h) without appreciable health risk. *Related terms*: Acceptable daily intake, Provisional maximum tolerable daily intake, Provisional tolerable monthly intake, Provisional tolerable weekly intake, Tolerable daily intake.

Highest residue (HR)
The highest residue level (expressed as milligrams per kilogram) in a composite sample of the edible portion of a food commodity when a pesticide has been used according to maximum Good Agricultural Practice (GAP) conditions. The HR is estimated as the highest of the residue values (one from each trial) from supervised trials conducted according to maximum GAP conditions and includes residue components defined by the Joint FAO/WHO Meeting on Pesticide Residues for estimation of dietary intake.

Highest residue – processing (HR-P)
Highest residue in a processed commodity calculated by multiplying the highest residue in the raw commodity by the processing factor.

Incurred residue
Residue present in food or feed as a result of treatment with pesticides or veterinary drugs, for example, in the field (as opposed to residue resulting from spiking samples in the laboratory).

Innocuous metabolic products
Products that are known or readily predicted to be harmless to humans at the estimated intakes of the parent compound.

Intake
For the purposes of food and feed risk assessment, the amount of a substance (including nutrients) ingested by a person or an animal as part of its diet (via food, beverages, drinking-water and food supplements). This term does not refer to whole foods. The "intake" of whole foods is termed "food consumption".

Intake assessment
The qualitative and/or quantitative evaluation of the likely intake of chemicals (including nutrients) via food, beverages, drinking-water and food supplements. *Synonymous with*: Dietary exposure assessment.

International estimated daily intake (IEDI)
A prediction of the long-term daily intake of a pesticide residue on the basis of the assumptions of average daily food consumption per person and median residues from supervised trials, allowing for residues in the edible portion of a commodity and including residue components defined by the Joint FAO/WHO Meeting on Pesticide Residues for estimation of dietary intake. Changes in residue levels resulting from preparation, cooking or commercial processing are included. When information is available, dietary intake of residues resulting from other sources should be included. The IEDI is expressed in milligrams of residue per person.

International estimated short-term intake (IESTI)
A prediction of the short-term intake of a pesticide residue on the basis of the assumptions of high daily food consumption per person and highest residues from supervised trials, allowing for residues in the edible portion of a commodity and including residue components defined by the Joint FAO/WHO Meeting on Pesticide Residues for estimation of dietary intake. The IESTI is expressed in milligrams of residue per kilogram of body weight.

JECFA numbers for flavouring agents
Flavouring agents evaluated by the Joint FAO/WHO Expert Committee on Food Additives have been numbered consecutively for administrative purposes since the forty-ninth meeting by the FAO Joint Secretariat. The flavouring agents evaluated at the forty-sixth meeting have been numbered retroactively.

Joint FAO/WHO Expert Committee on Food Additives (JECFA)
An expert committee that has been meeting since 1956. JECFA has been engaged in collecting and evaluating scientific data on food additives and making recommendations on safe levels of use. This has been accomplished 1) by elaborating specifications for the identity and purity of individual food additives that have been toxicologically tested and are in commerce and 2) by evaluating toxicological data on these food additives and estimating acceptable intakes by humans. In 1972, the scope of the evaluations was extended to include contaminants in food, whereas in 1987, the scope was extended even further to include residues of veterinary drugs in food. When evaluating the latter compounds, maximum residue limits are recommended based upon acceptable intakes estimated by the Committee and data relating to Good Practice in the Use of Veterinary Drugs.

JECFA is a technical committee of specialists acting in their individual capacities. Each JECFA is a separately constituted committee. When the term "JECFA" or "the Committee" is used without reference to a specific meeting, it is meant to imply the common policy or combined output of the separate meetings over the years.

Joint FAO/WHO Meeting on Pesticide Residues (JMPR)
The abbreviated title for the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues, which has been meeting since 1963. The meetings are normally convened annually. The FAO Panel of Experts is responsible for reviewing residue and analytical aspects of the pesticides considered, including data on their metabolism, fate in the environment and use patterns, and for estimating the maximum residue levels and supervised trials median residue levels that might occur as a result of the use of the pesticide according to Good Agricultural Practice. The WHO Core Assessment Group on Pesticide Residues is responsible for reviewing toxicological and

related data on the pesticides and, when possible, for estimating acceptable daily intakes and long-term dietary intakes of residues. As necessary, acute reference doses for pesticides are estimated along with appropriate estimates of short-term dietary intake.

JMPR is a technical committee of specialists acting in their individual capacities. Each is a separately constituted committee. When the term "JMPR" or "the Meeting" is used without reference to a specific meeting, it is meant to imply the common policy or combined output of the separate meetings over the years.

Large portion size
A food consumption amount that represents the 97.5th-percentile consumption (eaters only) of a food that is derived from individual consumer days in a food consumption survey. This is useful in calculating acute dietary exposures.

Limit of detection (LOD)
The minimum concentration of a component in a dietary sample that can be qualitatively detected, but cannot be quantitatively determined, under a pre-established set of analytical conditions.

Limit of quantification (LOQ)
The minimum concentration of a component that can be determined quantitatively with acceptable accuracy and consistency. It often approximates to a value of 3 times the limit of detection.

Long-term exposure
See Chronic exposure.

Long-term toxicity study
A study in which animals are observed during their whole lifespan (or the major part of their lifespan) and in which exposure to the test material takes place over the whole observation time or a substantial part thereof. The term *chronic toxicity study* is used sometimes as a synonym for long-term toxicity study.

Lowest-observed-adverse-effect level (LOAEL)
Lowest concentration or amount of a substance, found by experiment or observation, that causes an adverse alteration of morphology, functional capacity, growth, development or lifespan of the target organism

distinguishable from normal (control) organisms of the same species and strain under the same defined conditions of exposure.

Lowest-observed-effect level (LOEL)
Lowest concentration or amount of a substance, found by experiment or observation, that causes any alteration of morphology, functional capacity, growth, development or lifespan of the target organism distinguishable from normal (control) organisms of the same species and strain under the same defined conditions of exposure.

Margin of exposure (MOE)
Ratio of the no-observed-adverse-effect level or benchmark dose lower confidence limit for the critical effect to the theoretical, predicted or estimated exposure dose or concentration. *Related term*: Margin of safety.

Margin of safety
The margin between the health-based guidance value (reference dose) and the actual or estimated exposure dose or concentration. For some experts, the margin of safety has the same meaning as the margin of exposure. *Related term*: Margin of exposure.

Marker residue (veterinary drugs)
The parent drug, or any of its metabolites, or a combination of any of these, with a known relationship to the concentration of the total residue in each of the various edible tissues at any time between administration of the drug and the depletion of residues to safe levels.

Maximum level (ML)
For contaminants, naturally occurring toxicants and nutrients, the maximum concentration of a substance recommended by the Codex Alimentarius Commission to be legally permitted in a given commodity. For food additives, the level of permission of use given in food standards for the additive in that food or food category.

Maximum residue level for pesticides[1]
Estimated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) as the maximum concentration of residues (expressed as milligrams per

[1] It should be noted that "maximum residue limit" and "maximum residue level" are frequently abbreviated using the same acronym MRL, irrespective of the different meaning and context in which they are used.

kilogram) that may occur in a food or feed commodity following Good Agricultural Practice. The estimated maximum residue level is considered by JMPR to be suitable for establishing Codex maximum residue limits (MRLs) and is considered by the Codex Committee on Pesticide Residues as the basis when recommending the Codex MRLs.

Maximum residue limit (MRL)

Veterinary drugs: The maximum concentration of residue resulting from the use of a veterinary drug that is acceptable in or on a food. It is based on the type and amount of residue considered to be without toxicological hazard for human health as expressed by the acceptable daily intake (ADI) or on the basis of a temporary ADI that utilizes an additional safety factor. It also takes into account other relevant public health risks as well as food technological aspects and estimated food intakes. When establishing an MRL, consideration is also given to residues that occur in food of plant origin or the environment. The MRL may be reduced to be consistent with Good Practice in the Use of Veterinary Drugs and to the extent that practical analytical methods are available. MRLs are expressed in terms of milligrams per kilogram tissue or milligrams per litre milk. The MRLs elaborated by the Joint FAO/WHO Expert Committee on Food Additives are "recommended MRLs" that are forwarded to the Codex Committee on Residues of Veterinary Drugs in Foods for consideration.

Pesticides: The maximum concentration of a pesticide residue (expressed as milligrams per kilogram) recommended by the Codex Alimentarius Commission to be legally permitted in or on food commodities and animal feed. MRLs are based on Good Agricultural Practice data, and food derived from commodities that comply with the respective MRLs are intended to be toxicologically acceptable. Consideration of the various dietary residue intake estimates and determinations, at both the national and international level, in comparison with the acceptable daily intake should indicate that foods complying with Codex MRLs are safe for human consumption.

Maximum residue limit (MRL) "not specified"

Available data on the identity and concentration of residues of a veterinary drug in animal tissues indicate a large margin of safety for consumption of residues in food when the drug is used according to Good Practice in the Use of Veterinary Drugs. For that reason, and for

the reasons stated in the individual evaluation, the Committee has concluded that the presence of drug residues in the named animal product does not present a health concern and that there is no need to specify a numerical MRL.

Mean
The arithmetic average of all the values in the data set, computed by adding all the individual values together and dividing by the number in the group.

Mechanism of action
The specific biochemical interaction through which a substance produces an effect on a living organism or in a biochemical system. *Related term*: Mode of action.

Median
The midpoint value obtained by ranking all values from highest to lowest and choosing the value in the middle. The median divides a population into two equal halves.

Model
A set of constraints restricting the possible joint values of several quantities; a hypothesis or system of beliefs regarding how a system works or responds to changes in its inputs. The purpose of a model is to represent as accurately and precisely as necessary with respect to particular decision objectives a particular system of interest.

Model diets
A type of screening method used in dietary exposure assessments that assumes fixed default consumption levels, usually for categories of foods and beverages. Model diets can be based on hypothetical consumption data assuming maximum consumption amounts for broad food groups (e.g. the budget method) or can be derived from national food supply or consumption data (e.g. Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme consumption cluster diets or total diet studies).

Mode of action
A biologically plausible sequence of key events leading to an observed effect supported by robust experimental observations and mechanistic data. A mode of action describes key cytological and biochemical

events—that is, those that are both measurable and necessary to the observed effect—in a logical framework. *Related term*: Mechanism of action.

Monitoring data
Continuous or repeated observation, measurement and evaluation of health and/or environmental or technical data for defined purposes, according to prearranged schedules in space and time, using comparable methods for sensing and data collection. Evaluation requires comparison with appropriate reference values based on knowledge of the probable relationship between ambient exposures and adverse effects.

Mutagenicity
The capacity to give rise to mutations.

No acceptable daily intake (ADI) allocated
Terminology used by the Joint FAO/WHO Expert Committee on Food Additives in situations where an ADI is not established for a substance under consideration because 1) insufficient safety information is available, 2) no information is available on its food use or 3) specifications for identity and purity have not been developed. The evaluation should be consulted to learn why an ADI was not allocated.

Non-traditional foods
Foods that do not have a history of significant human consumption by the broad community for several generations as part of the ordinary diet.

No-observed-adverse-effect level (NOAEL)
Greatest concentration or amount of a substance, found by experiment or observation, that causes no adverse alteration of morphology, functional capacity, growth, development or lifespan of the target organism distinguishable from those observed in normal (control) organisms of the same species and strain under the same defined conditions of exposure.

No-observed-effect level (NOEL)
Greatest concentration or amount of a substance, found by experiment or observation, that causes no alteration of morphology, functional

capacity, growth, development or lifespan of the target organism distinguishable from those observed in normal (control) organisms of the same species and strain under the same defined conditions of exposure.

Novel food
A food or food ingredient produced from raw materials not normally used for human consumption or food that is severely modified by the introduction of new processes not previously used in the production of food.

Nutrient
Any element or compound necessary for or contributing to an organism's metabolism, growth or other function. Six nutrient groups exist, classifiable as those that provide energy and those that otherwise support metabolic processes in the body. Some of them are essential because they cannot be synthesized in the body and must be obtained from a food source.

Pesticide
Any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances that may be administered to animals for the control of insects, arachnids or other pests in or on their bodies. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant or agent for thinning fruit or preventing the premature fall of fruit, and substances applied to crops either before or after harvest to protect the commodity from deterioration during storage or transport.

Pesticide residue
See Residues of pesticides.

Pharmacodynamics
The study of the physiological effects of drugs on the body or on microorganisms or parasites within or on the body, the mechanisms of drug action and the relationship between drug concentration and effect. *Related term*: Toxicodynamics.

Pharmacokinetics
Description of the fate of drugs in the body, including a mathematical account of their absorption, distribution, metabolism and excretion. *Related term*: Toxicokinetics.

Point estimate
A single numerical value resulting from calculations. *Synonymous with*: Deterministic estimate.

Post-regulation dietary exposure assessment
Calculation of dietary exposure based on the chemical levels found in foods following implementation of regulatory limits or levels.

Poundage data
Estimates of the amount of a food chemical available for use in food manufacturing in a country during a specific period of time (usually 1 year). The total poundage is sometimes divided by the total population size in order to obtain an estimate of per capita availability of a specific chemical substance.

Precision
A measure of the reproducibility of the predictions of a model or repeated measurements, usually in terms of the standard deviation or other measures of variation among such predictions or measurements.

Probabilistic analysis
Analysis in which distributions are assigned to represent variability or uncertainty in quantities. The form of the output of a probabilistic analysis is likewise a distribution. *Related term*: Probabilistic distribution.

Probability distribution (e.g. normal, lognormal, gamma, logistic, log-logistic)
A mathematical description of a function that relates probabilities with specified intervals of a continuous quantity, or values of a discrete quantity, for a random variable. Probability distribution models can be non-parametric or parametric. A non-parametric probability distribution can be described by rank ordering continuous values and estimating the empirical cumulative probability associated with each. Parametric probability distribution models can be fit to data sets by

estimating their parameter values based upon the data. The adequacy of the parametric probability distribution models as descriptors of the data can be evaluated using goodness of fit techniques. Distributions such as normal, lognormal and others are examples of parametric probability distribution models.

Problem formulation
A process that describes the food safety problem and its context, in order to identify those elements of hazard or risk associated with a chemical that are relevant to potential risk management decisions.

Processing aid
Any substance or material, not including apparatus or utensils, and not consumed as a food ingredient by itself, intentionally used in the processing of raw materials, food or its ingredients, to fulfil a certain technological purpose during treatment or processing and that may result in the non-intentional but unavoidable presence of residues or derivatives in the final product.

Processing factor
For a specified pesticide residue, commodity and food process, the residue level in the processed product divided by the residue level in the starting commodity, usually a raw agricultural commodity.

Provisional maximum tolerable daily intake (PMTDI)
The reference value, established by the Joint FAO/WHO Expert Committee on Food Additives, used to indicate the safe level of intake of a contaminant with no cumulative properties. Its value represents permissible human exposure as a result of the natural occurrence of the substance in food and drinking-water. In the case of trace elements that are both essential nutrients and unavoidable constituents of food, a range is expressed, the lower value representing the level of essentiality and the upper value the PMTDI. The tolerable intake is generally referred to as "provisional" as there is often a paucity of data on the consequences of human exposure at low levels, and new data may result in a change to the tolerable level. *Related term*: Tolerable daily intake.

Provisional tolerable monthly intake (PTMI)
An end-point used by the Joint FAO/WHO Expert Committee on Food Additives for a food contaminant with cumulative properties that has

a very long half-life in the human body. Its value represents permissible human monthly exposure to a contaminant unavoidably associated with otherwise wholesome and nutritious foods.

Provisional tolerable weekly intake (PTWI)
The end-point used by the Joint FAO/WHO Expert Committee on Food Additives for food contaminants such as heavy metals with cumulative properties. Its value represents permissible human weekly exposure to those contaminants unavoidably associated with the consumption of otherwise wholesome and nutritious foods.

Quality assurance
A set of activities whose purpose is to demonstrate that an entity meets all quality requirements. These activities are carried out in order to inspire the confidence of both customers and managers that all quality requirements are being met.

Quality control
A set of activities or techniques whose purpose is to ensure that all quality requirements are being met. In order to achieve this purpose, processes are monitored and performance problems are solved.

Random error
Processes that are random or statistically independent of each other, such as imperfections in measurement techniques that lead to unexplainable but characterizable variations in repeated measurements of a fixed true value. Some random errors could be reduced by developing improved techniques. *Related term*: Error.

Random sampling
A sample selected from a statistical population such that each individual has an equal probability of being selected.

Reference dose
An estimate of the daily exposure dose that is likely to be without deleterious effect even if continued exposure occurs over a lifetime. *Related terms*: Acceptable daily intake, Health-based guidance value, Provisional maximum tolerable daily intake, Tolerable daily intake.

Regional diets
See GEMS/Food regional diets.

Reproductive testing
Tests covering several reproductive cycles to study reproductive toxicity associated with exposure to a chemical. In the three-generation test, the animals are exposed through three complete reproductive cycles (starting with the F_0 generation at weaning). These tests include exposure in utero and through the milk.

Reproductive toxicity
Adverse effects or abnormalities in, for example, gamete production, reproductive cycle (e.g. menstrual disorders), sexual behaviour (as seen in animals), fertility, gestation, parturition and/or lactation, pregnancy outcomes (spontaneous abortion, stillbirth, etc.) and premature reproductive senescence (i.e. early menopause). *Related term*: Developmental toxicity.

Residues of pesticides
Any specified substances in or on food, agricultural commodities or animal feed resulting from the use of a pesticide. The term includes any derivatives of a pesticide, such as conversion products, metabolites, reaction products and impurities considered to be of toxicological significance. The term "pesticide residue" includes residues from unknown or unavoidable sources (e.g. environmental) as well as known uses of the chemical. The definition of a residue for compliance with maximum residue limits (MRLs) is that combination of the pesticide and its metabolites, derivatives and related compounds to which the MRL applies.

Residues of veterinary drugs
The parent compounds and/or their metabolites in any edible portion of the animal product. They include residues of associated impurities of the veterinary drug concerned.

Response
Change developed in the state or dynamics of an organism, system or (sub)population in reaction to exposure to an agent.

Risk
The probability of an adverse effect in an organism, system or (sub)-population caused under specified circumstances by exposure to an agent.

Risk analysis
A process for controlling situations where an organism, system or (sub)population could be exposed to a hazard. The risk analysis process consists of three components: risk assessment, risk management and risk communication.

Risk assessment
A process intended to calculate or estimate the risk to a given target organism, system or (sub)population, including the identification of attendant uncertainties, following exposure to a particular agent, taking into account the inherent characteristics of the agent of concern as well as the characteristics of the specific target system. The risk assessment process includes four steps: hazard identification, hazard characterization (*Related term*: Dose–response assessment), exposure assessment and risk characterization. It is the first component in a risk analysis process. *Related term*: Safety assessment.

Risk characterization
The qualitative and, wherever possible, quantitative determination, including attendant uncertainties, of the probability of occurrence of known and potential adverse effects of an agent in a given organism, system or (sub)population, under defined exposure conditions. Risk characterization is the fourth step in the risk assessment process.

Risk communication
Interactive exchange of information about (health or environmental) risks among risk assessors, managers, news media, interested groups and the general public.

Risk estimation
Quantification of the probability, including attendant uncertainties, that specific adverse effects will occur in an organism, system or (sub)population due to actual or predicted exposure.

Risk management
Decision-making process involving considerations of political, social, economic and technical factors with relevant risk assessment information relating to a hazard so as to develop, analyse and compare regulatory and non-regulatory options and to select and implement appropriate regulatory response to that hazard.

Safety
Practical certainty that adverse effects will not result from exposure to an agent under defined circumstances. It is the reciprocal of risk.

Safety assessment
An approach that focuses on the scientific understanding and measurement of chemical hazards as well as chemical exposures, and ultimately the risks associated with them. Often (and in this monograph) used synonymously with risk assessment. *Related term*: Risk assessment.

Safety factor
A composite (reductive) factor applied by the risk assessment experts to the no-observed-adverse-effect level (NOAEL) or other reference point, such as the benchmark dose or benchmark dose lower confidence limit, to derive a reference dose that is considered safe or without appreciable risk, such as an acceptable daily intake or tolerable daily intake (the NOAEL or other reference point is divided by the safety factor to calculate the reference dose). The value of the safety factor depends on the nature of the toxic effect, the size and type of population to be protected, and the quality of the toxicological information available. *Related terms*: Assessment factor, Uncertainty factor.

Sample preparation
Includes actions taken to prepare the analytical sample from the laboratory (bulk) sample, such as reducing the size of a large bulk sample by subsampling or removing foreign materials and parts of the sample material that are not analysed (e.g. stones, withered leaves, stones of fruits, bones of meat). Sample preparation may include, for instance, washing, peeling, cooking, etc. so that foods are prepared as for normal consumption (i.e. table ready). Sample preparation may also involve compositing of food samples taken from different regions, brands and even food types before homogenization and analysis.

Sample processing
Includes physical operations performed to prepare a well-mixed or homogeneous matrix to form the analytical sample, from which the test portions for the analysis are taken.

Sampling procedure (protocol)
Operational requirements and/or instructions relating to the use of a particular sample plan (i.e. the instructions for the implementation of the plan).

Screening methods
In exposure assessment, methods used as the first step in estimating the dietary exposure to a food chemical in order to target those chemicals that might pose a health concern. Screening methods use conservative assumptions for both food consumption and chemical concentration. If the estimated exposure exceeds its toxicological reference value, a more accurate method of dietary exposure assessment is used; if it is below the reference value, no further assessment is conducted.

Sensitivity analysis
In risk assessment, a technique that tests the sensitivity of an output variable to the possible variation in the input variables of a given model. The purpose of sensitivity analysis is to quantify the influence of input variables on the output variable and develop bounds on the model output. This comes from dose–response modelling and application of statistical methods.

In exposure assessment, a study of how the variation in the outputs of a model can be attributed to, qualitatively or quantitatively, different sources of variation in model inputs.

Short-term exposure
Multiple or continuous exposure to an agent for a short period of time, usually about 10% of the animal's lifespan (e.g. 90 days in rat, 1 year in dog).

Short-term toxicity study
An animal study (sometimes called a subacute or subchronic study) in which the effects produced by the test material, when administered in repeated doses (or continuously in food or drinking-water) over a period of about 10% of the animal's lifespan (e.g. 90 days in rat, 1 year in dog), are studied.

Single-blind placebo-controlled food challenge
A study in which only the patient is unaware of the food or placebo being tested. In the test, the patient ingests a food that has been

disguised so that the patient is unaware of the contents of the challenge. *Related term*: Double-blind placebo-controlled food challenge.

Standard portion sizes
Quantities (weights) assigned to individual foods (e.g. glass of juice, cookie and banana) that represent amounts that are typically consumed. These values can be used as default values in food consumption surveys and for calculating dietary exposure.

Statistical uncertainty
See Uncertainty.

Steady state
The state where the body eliminates an amount of a xenobiotic that is the same as that absorbed during an exposure interval.

Stratified sampling
A method that selects values at regular intervals throughout each distribution. Calculating the result using the average or median value for each distribution may be thought of as the simplest example of a stratified sampling process, where each distribution has a single stratum.

Subchronic exposure
A contact between an agent and a target of intermediate duration between acute and chronic. (Other terms, such as "less-than-lifetime exposure", are also used.) *Related term*: Short-term exposure.

Supervised trials
Scientific studies in which pesticides are applied to crops or animals according to specified conditions intended to reflect commercial practice, after which harvested crops or tissues of slaughtered animals are analysed for pesticide resides. Specified conditions are usually those that approximate existing or proposed Good Agricultural Practice.

Supervised trials for estimating maximum residue levels
Scientific studies in which pesticides are applied to crops or animals according to specified conditions intended to reflect commercial practice, after which harvested crops or tissues of slaughtered animals are analysed for pesticide residues.

Supervised trials median residue (STMR)
The expected residue level in the food commodity (expressed in milligrams of residue per kilogram of commodity) when a pesticide has been used according to maximum Good Agricultural Practice (GAP) conditions. The STMR is estimated as the median of the residue values (one from each trial) from supervised trials conducted according to maximum GAP conditions and includes residue components defined by the Joint FAO/WHO Meeting on Pesticide Residues for estimation of dietary intake. For some commodities, such as banana, STMR levels may be determined directly from levels measured in the edible portion when data are available.

Supervised trials median residue – processed (STMR-P)
The expected residue in a processed food commodity when a pesticide has been used according to maximum Good Agricultural Practice conditions and the commodity is processed according to the main practice used to prepare the food prior to consumption. It is calculated by multiplying the STMR of the raw agricultural commodity by the corresponding processing factor or derived directly from a series of processing trials. The STMR-P is expressed in units of milligrams per kilogram of commodity.

Susceptibility factors
Characteristics thought to increase the susceptibility of an individual to adverse health outcomes.

Systematic error
See Error.

Target tissue or organ
For veterinary drugs, the edible animal tissue (muscle, fat, liver or kidney) selected to monitor for the total residue in the target animal. It is usually, but not necessarily, the tissue with the slowest depletion rate of residues. For food additives, contaminants and pesticides, the target tissue/organ means the biological tissue(s) or organ(s) where the biological activity/toxicity of the substance is exerted in the body.

Temporary acceptable daily intake (ADI)
Used when data are sufficient to conclude that use of the substance is safe over the relatively short period of time required to generate and evaluate further safety data, but are insufficient to conclude that use

of the substance is safe over a lifetime. A higher than normal safety factor is used when establishing a temporary ADI, and an expiration date is established by which time appropriate data to resolve the safety issue should be submitted for evaluation. The temporary ADI is listed in units of milligrams per kilogram of body weight.

Temporary maximum residue limit (MRL)
Used when a temporary acceptable daily intake has been established and/or when it has been found necessary to provide time to generate and evaluate further data on the nature and quantification of residues. Temporary MRLs are expressed in terms of milligrams per gram of tissue or milligrams per litre of milk.

"Tentative" specifications
Term used by the Joint FAO/WHO Expert Committee on Food Additives only in cases where data on the purity and identity of the substance (food additive) are required. The assignment "tentative" will require submission and re-evaluation of data within a specified period of time (usually 2 years).

Teratogen
An agent that, when administered prenatally, induces permanent abnormalities in structure.

Teratogenicity
The property of producing or potential to produce structural malformations or defects in an embryo or fetus.

Test portion
Quantity of material, of proper size for measurement of the concentration or other property of interest, removed from the test sample.

Theoretical added maximum daily intake (TAMDI)
A conservative estimate of potential exposure to a specific flavouring substance on the basis of proposed or allowed maximum (upper use) levels (UULs) in the different categories of foods and beverages that could be flavoured. The resulting exposure estimate is that of a hypothetical consumer who consumes every day one standard portion of food/beverage from each of these categories, and those foods/beverages always contain the specific flavouring at

its specified UUL. The TAMDI is calculated by summing the exposures estimated for each individual food/beverage category to estimate total daily intake.

Theoretical maximum daily intake (TMDI)
A prediction of the maximum daily intake of, for example, a pesticide residue, assuming that residues are present at the maximum residue levels/limits and average daily consumption of foods per person (e.g. as represented by Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme diets). The TMDI can be calculated for the various regional or consumption cluster diets and is expressed in milligrams of residue per person.

Threshold
Dose or exposure concentration of an agent below which a stated effect is not observed or expected to occur.

Threshold dose
The dose at which an effect just begins to occur—that is, at a dose immediately below the threshold dose, the effect will not occur, and immediately above the threshold dose, the effect will occur. For a given chemical, there can be multiple threshold doses, in essence one for each definable effect. For a given effect, there may be different threshold doses in different individuals. Further, the same individual may vary from time to time as to his or her threshold dose for any effect. For certain chemicals and certain toxic effects, a threshold dose may not be demonstrable. The threshold dose will fall between the experimentally determined no-observed-(adverse-)effect level and the lowest-observed-(adverse-)effect level, both of which have been used by different scientific groups as a surrogate for the threshold dose in the performance of risk assessments.

Tolerable daily intake (TDI)
Analogous to acceptable daily intake. The term tolerable is used for agents that are not deliberately added, such as contaminants in food. Note that the Joint FAO/WHO Expert Committee on Food Additives uses the term provisional maximum tolerable daily intake. *Related terms*: Acceptable daily intake, Health-based guidance value, Provisional maximum tolerable daily intake.

Tolerable intake
Estimated maximum amount of an agent, expressed on a body mass basis, to which each individual in a (sub)population may be exposed over a specified period without appreciable risk.

Total diet study
A study that determines levels of various food additives, pesticide and veterinary drug residues, contaminants and nutrients in foods, so that dietary intakes of those analytes by the population of interest can be estimated.

Total organic solids (TOS)
The difference between the total solids content and the ash, water, diluent and carrier contents. TOS is used when estimating dietary exposure to enzyme preparations. The estimated dietary exposure is expressed in terms of milligrams of TOS per kilogram of body weight.

Toxicity
The potential of a substance to cause injury (adverse reaction) to a living organism.

Toxicodynamics
The process of interaction of chemical substances with target sites and the subsequent reactions leading to adverse effects. *Related term*: Pharmacodynamics.

Toxicokinetics
The process of the uptake of potentially toxic substances by the body, the biotransformation they undergo, the distribution of the substances and their metabolites in the tissues, and the elimination of the substances and their metabolites from the body. Both the amounts and the concentrations of the substances and their metabolites are studied. The term has essentially the same meaning as pharmacokinetics, but the latter term should be restricted to the study of pharmaceutical substances. *Related term*: Pharmacokinetics.

Toxicological reference value
See Acceptable daily intake, Acute reference dose, Health-based guidance value, Provisional maximum tolerable daily intake, Provisional tolerable weekly intake, Provisional tolerable monthly intake, Reference dose, Tolerable daily intake.

Traditional foods
Foods that have a history of significant human consumption by the broad community for several generations as part of the ordinary diet at the global, regional or local level or as a part of an ethnic diet.

Transgenic
Referring to an experimentally produced organism in which deoxyribonucleic acid (DNA) has been artificially introduced and incorporated into the organism's germline, usually by injecting the foreign DNA into the nucleus of a fertilized embryo.

Transgenic animal
A fertile animal that carries an introduced gene in its germline.

Transplacental carcinogenesis
The appearance of neoplasia in the progeny of females exposed to chemical agents during pregnancy.

Uncertainty
In risk assessment, imperfect knowledge concerning the present or future state of an organism, system or (sub)population under consideration.

In exposure assessment, lack of knowledge regarding the "true" value of a quantity, lack of knowledge regarding which of several alternative model representations best describes a system of interest or lack of knowledge regarding which probability distribution function and its specification should represent a quantity of interest.

Uncertainty analysis
A process in which the sources of uncertainty in an estimate are identified and an estimate is made of the magnitude and direction of the resulting error.

Uncertainty factor
Reductive factor by which an observed or estimated no-observed-adverse-effect level or other reference point, such as the benchmark dose or benchmark dose lower confidence limit, is divided to arrive at a reference dose or standard that is considered safe or without appreciable risk. *Related terms*: Assessment factor, Safety factor.

Unit weight
This represents the typical weight of a commodity unit (e.g. a single apple, a single banana) that is used in the calculation of acute dietary exposure estimates.

Use pattern
The combination of all factors involved in the use of a pesticide, including the concentration of active ingredient in the preparation being applied, rate of application, time of treatment, number of treatments, use of adjuvants and methods and sites of application, which determine the quantity applied, timing of treatment and interval before harvest.

Validation
Process by which the reliability and relevance of a particular approach, method, process or assessment is established for a defined purpose. Different parties define "reliability" as establishing the reproducibility of the outcome of the approach, method, process or assessment over time. "Relevance" is defined as establishing the meaningfulness and usefulness of the approach, method, process or assessment for the defined purpose.

Variability
Heterogeneity of values over time, space or different members of a population. Variability implies real differences among members of that population. For example, in exposure assessment, different individuals have different intakes and susceptibilities. In relation to human exposure assessment, differences over time for a given individual are referred to as intraindividual variability; differences over members of a population at a given time are referred to as interindividual variability.

Veterinary drug
Any substance applied or administered to any food-producing animal, such as meat- or milk-producing animals, poultry, fish or bees, whether for therapeutic, prophylactic or diagnostic purposes or for modification of physiological functions or behaviour.

Veterinary drug residues
See Residues of veterinary drugs.

Weight of evidence
A process in which all of the evidence considered relevant to a decision is evaluated and weighted.

Withdrawal period
The interval between the time of the last administration of a veterinary drug and the time when the animal can be safely slaughtered for food or when milk or eggs can be safely consumed.

* * *

Main sources of definitions in glossary of terms:

FAO/WHO (2008) Codex Alimentarius Commission procedural manual, 18th ed. Rome, Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission (ftp://ftp.fao.org/codex/Publications/ProcManuals/Manual_18e.pdf).

IPCS (1986) Principles of toxicokinetic studies. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 57; http://www.inchem.org/documents/ehc/ehc/ehc57.htm).

IPCS (1987) Principles for the safety assessment of food additives and contaminants in food. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 70; http://www.inchem.org/documents/ehc/ehc/ehc70.htm).

IPCS (1990) Principles for the toxicological assessment of pesticide residues in food. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 104; http://www.inchem.org/documents/ehc/ehc/ehc104.htm).

IPCS (1994) Assessing human health risks of chemicals: derivation of guidance values for health-based exposure limits. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 170; http://www.inchem.org/documents/ehc/ehc/ehc170.htm).

IPCS (2004) IPCS risk assessment terminology. Geneva, World Health Organization, International Programme on Chemical Safety (Harmonization Project Document, No. 1; http://www.who.int/ipcs/methods/harmonization/areas/ipcsterminologyparts1and2.pdf).

IPCS (2008) Uncertainty and data quality in exposure assessment. Geneva, World Health Organization, International Programme on Chemical Safety (Harmonization Project Document, No. 6; http://www.who.int/ipcs/publications/methods/harmonization/exposure_assessment.pdf).

IPCS (2009) Principles for modelling dose–response for the risk assessment of chemicals. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 239; http://whqlibdoc.who.int/publications/2009/9789241572392_eng.pdf).

WHO (1997) Food consumption and exposure assessment of chemicals. Report of an FAO/WHO Consultation on Food Consumption and Exposure Assessment of Chemicals, Geneva, 10–14 February 1997. Geneva, World Health Organization (WHO/FSF/FOS/97.5).

ANNEX 2: DOSE CONVERSION TABLE

Where accurate doses cannot be calculated on the basis of measured body weights and food consumption, approximate doses can be estimated using the dose conversion factors in the following table, taken from EHC 70.

Table A-1. Approximate relation of mg/kg in the diet to mg/kg body weight per day

Species	Weight (kg)	Food consumed per day (g) (liquids omitted)	Type of diet	1 mg/kg in food = *x* mg/kg body weight per day	1 mg/kg body weight per day = *x* mg/kg of diet
Mouse	0.02	3	Dry laboratory chow diets	0.150	7
Chick	0.40	50		0.125	8
Rat (young)	0.10	10		0.100	10
Rat (old)	0.40	20		0.050	20
Guinea-pig	0.75	30		0.040	25
Rabbit	2.0	60		0.030	33
Dog	10.0	250		0.025	40
Cat	2	100	Moist, semi-solid diets	0.050	20
Monkey	5	250		0.050	20
Dog	10	750		0.075	13
Human	60	1 500		0.025	40
Pig or sheep	60	2 400	Relatively dry grain forage mixtures	0.040	25
Cow (maintenance)	500	7 500		0.015	65
Cow (fattening)	500	15 000		0.030	33
Horse	500	10 000		0.020	50

RESUME

Le Comité mixte FAO/OMS d'experts des additifs alimentaires (JECFA) et la Réunion conjointe FAO/OMS sur les résidus de pesticides (JMPR) suivent les mêmes principes généraux et les mêmes méthodes générales pour procéder aux évaluations des risques chimiques, qui sont publiées dans les rapports des deux comités. Pour donner suite aux recommandations formulées par le JECFA et la JMPR dans les années 80 concernant la nécessité d'examiner la validité des procédures d'évaluation utilisées à cette époque, le Programme international relatif à la sécurité des produits chimiques (IPCS) a parrainé l'élaboration de monographies sur les Critères de santé de l'environnement (EHC), sur les Principes d'évaluation de la sécurité des additifs et des contaminants dans les aliments (EHC 70) et sur les Principes d'évaluation toxicologique des résidus de pesticides dans les aliments (EHC 104). Les évaluations effectuées par le JECFA et la JMPR se fondent sur ces monographies et sur les principes formulés dans les rapports ultérieurs.

Une grande partie des indications fournies dans l'EHC 70 et l'EHC 104 sont encore valables, mais des progrès importants ont été accomplis depuis l'élaboration de ces monographies dans l'analyse chimique, la toxicologie, l'évaluation de l'exposition alimentaire et les méthodes d'évaluation des risques dus à la présence de substances chimiques dans les aliments. La FAO et l'OMS ont donc amorcé un projet visant à actualiser, harmoniser et consolider les principes et les méthodes utilisés par le JECFA et la JMPR pour évaluer les risques que présentent les additifs alimentaires, les contaminants alimentaires, les toxiques naturels et les résidus de pesticides et de médicaments vétérinaires. La présente monographie est le résultat de ce projet.

L'objectif de cette monographie est double: 1) fournir au JECFA et à la JMPR des directives destinées à s'assurer que les évaluations, par les experts, des données scientifiques utilisées pour procéder aux évaluations de risques chimiques dans les aliments continueront d'être menées de manière appropriée et transparente; et 2) donner aux utilisateurs des informations sur les conclusions du JECFA et de la JMPR (gestionnaires des risques et autres instances nationales et

locales chargées de l'évaluation de risques dans les États membres, par exemple).

La monographie porte sur les principales questions examinées par le JECFA et la JMPR lors de l'évaluation des risques liés aux substances chimiques dans les aliments. Ces questions sont résumées ci-après.

Le rôle de l'évaluation des risques dans l'analyse des risques

L'analyse des risques comprend trois volets: l'évaluation des risques, la gestion des risques et la communication sur les risques. L'évaluation des risques est l'élément central de l'analyse des risques et constitue le fondement scientifique sur lequel s'appuient les décisions concernant la prise de mesures adaptées pour protéger la santé publique. Elle tient compte de toutes les données scientifiques disponibles pertinentes et identifie toutes les incertitudes dans la base des connaissances. L'évaluation des risques comporte quatre étapes: l'identification des dangers, la caractérisation des dangers (y compris l'évaluation de la relation dose-réponse), l'évaluation de l'exposition et la caractérisation des risques. Il s'agit d'un cadre conceptuel qui, dans le contexte de l'évaluation de l'innocuité des substances chimiques contenues dans les aliments, fournit un mécanisme pour examiner les informations pertinentes de manière structurée afin d'estimer les différents effets susceptibles de se répercuter sur la santé en raison d'une exposition aux substances chimiques contenues dans les aliments.

L'évaluation des risques chimiques dans les produits alimentaires ou à leur surface constitue l'activité fondamentale du JECFA et de la JMPR. Sur les conseils de ces deux comités, des mesures sont prises en matière de sécurité sanitaire des aliments dans le cadre de la gestion des risques mise en œuvre par les États au niveau national et par la Commission du Codex Alimentarius au niveau international. Alors que le JECFA et la JMPR basent leurs évaluations sur des principes scientifiques adaptés et assurent la cohérence voulue dans la détermination de l'évaluation des risques, la Commission du Codex Alimentarius et ses comités respectifs, dont les travaux portent sur les substances chimiques dans les aliments, sont responsables, en qualité de gestionnaires des risques, de la prise des décisions finales

concernant l'établissement de limites maximales pour les résidus de pesticides, les résidus de médicaments vétérinaires, les contaminants et les additifs dans les aliments, ainsi que l'adoption d'autres mesures applicables.

Même s'il est souhaitable, pour garantir une indépendance scientifique, de séparer les activités fonctionnelles propres à l'évaluation des risques de celles liées à la gestion des risques, il est reconnu que les gestionnaires des risques doivent communiquer et interagir avec les évaluateurs des risques tout au long du processus afin de définir la portée de l'analyse, notamment lors de la formulation du problème. La relation entre l'évaluation des risques et la gestion des risques est donc un processus interactif, souvent itératif.

Caractérisation chimique, méthodes analytiques et élaboration de spécifications

La présente section de la monographie décrit les données chimiques nécessaires à l'évaluation des risques. La disponibilité de ces données est également une condition préalable à la surveillance et au contrôle des substances chimiques dans les aliments.

Le JECFA et la JMPR examinent les méthodes analytiques proposées pour vérifier qu'elles peuvent être utilisées par la communauté internationale. Il est nécessaire de recourir à des méthodes analytiques, par exemple, pour spécifier les contaminants, déterminer les concentrations d'une substance chimique et de ses métabolites dans les études pharmacocinétiques, toxicocinétiques et les études de déplétion de résidus, ainsi que pour établir les concentrations de contaminants et de résidus de médicaments vétérinaires et de pesticides dans les aliments de manière fiable. La monographie décrit les principales caractéristiques des méthodes analytiques appropriées et les critères de validation de ces méthodes.

Spécifications relatives aux additifs alimentaires

Des spécifications d'identité et de pureté sont nécessaires au JECFA pour évaluer l'innocuité des additifs alimentaires. Les évaluations d'additifs alimentaires effectuées par le JECFA reposent sur des études réalisées avec une substance ou un produit chimique ayant une identité, une pureté et une forme physique bien définies. L'évaluation de

l'innocuité n'est valable que pour les produits dont le profil d'identité et de qualité n'est pas très différent de celui du matériel utilisé pour générer les données utilisées dans le cadre de l'évaluation.

Pesticides

La réunion conjointe FAO/OMS sur les spécifications relatives aux pesticides (JMPS) établit des spécifications pour les produits techniques et les préparations. La JMPR prend en compte les spécifications de la JMPS durant l'évaluation de l'innocuité. La JMPR évalue les méthodes analytiques utilisées pour produire les données sur les résidus afin de vérifier qu'elles sont appropriées pour les types de substances à analyser et d'échantillons traités. La JMPR fournit également des informations sur les méthodes adaptées pour appliquer des limites maximales de résidus (LMR) et indique si certains composants se prêtent à une analyse par des méthodes multi-résidus.

Résidus de médicaments vétérinaires

Le JECFA doit avoir la garantie que tous les médicaments vétérinaires entrant dans le cadre de ses évaluations sont bien caractérisés et leurs propriétés chimiques et physiques, ainsi que la nature et les concentrations des principales impuretés doivent lui être fournies. Le processus de fabrication doit être également décrit, et la cohérence et la qualité des produits finaux doivent être prouvées.

La forme et la distribution des résidus résultant de chaque type d'application autorisée dans chaque espèce doivent être déterminées et il faut vérifier qu'il n'existe pas de transfert de résidus dans les tissus comestibles ou les produits d'origine animale. Il est également nécessaire de définir un résidu marqueur qui correspond généralement à la forme du médicament (composé souche ou métabolite) que l'on trouve en plus forte concentration pendant la période de temps la plus longue. Le rapport entre le résidu marqueur et le total des résidus du médicament doit être déterminé.

Contaminants

Les données nécessaires pour caractériser un contaminant doivent inclure ses concentrations dans les aliments et le régime alimentaire total de pays dont le nombre sera aussi grand que possible. Les

données doivent être formatées à l'aide du Système mondial de surveillance – Surveillance de la contamination des aliments et programme d'estimation (GEMS/Alimentation) afin de faciliter la collecte des données et le contrôle de leur qualité. Les données doivent être accompagnées d'informations détaillées sur les plans d'échantillonnage et sur les méthodes analytiques utilisées pour produire ces données.

Substances consommées en grandes quantités

Des analyses chimiques détaillées des substances consommées en grandes quantités, telles que les additifs de masse, doivent être réalisées pour identifier les éventuelles impuretés et pour fournir des informations sur l'adéquation nutritionnelle, en particulier lorsque ces substances remplacent des aliments conventionnels. Étant donné l'exposition potentiellement élevée à des impuretés indésirables (telles que les métaux lourds) résultant de l'ingestion de substances consommées en grandes quantités, des efforts particuliers doivent être réalisés pour identifier et quantifier ces impuretés.

Identification et caractérisation des dangers: études toxicologiques et humaines

Étendue et choix des méthodes de test

Les études toxicologiques peuvent être divisées en deux grandes catégories: 1) les études in vitro, qui ont recours à la culture d'organismes ou de cellules, ou à la préparation de tissus provenant d'animaux de laboratoire ou de l'homme; 2) les études in vivo sur des animaux de laboratoire ou sur l'homme. Ces études ont plusieurs objectifs, dont: l'identification des effets nocifs potentiels (identification des dangers), la définition des conditions d'exposition nécessaires à la production des effets nocifs, et l'évaluation de la relation dose-réponse en cas d'effet nocif (caractérisation des dangers). Le JECFA et la JMPR examinent les données issues de ces deux types d'étude lors de l'évaluation des risques.

Il est largement admis que les tests effectués sur les animaux doivent être réduits, affinés ou remplacés dans toute la mesure du possible, ce qui a conduit à une utilisation croissante de méthodes alternatives et à une amélioration de la conception des études. Il est également important que des méthodes et des approches reposant sur une solide

base scientifique soient utilisées pour tester l'innocuité des substances chimiques dans les aliments. Ainsi, même si des progrès ont été réalisés pour développer des méthodes in silico et in vitro, il n'est pas encore possible de remplacer les essais sur les animaux pour déterminer la plupart des effets négatifs concernés. Aucune espèce animale d'expérimentation n'est un modèle idéal pour l'homme, mais des indications montrent que les études effectuées sur des animaux sont généralement un moyen efficace pour évaluer la toxicité potentielle des substances contenues dans les aliments, à condition que les données soient interprétées de manière critique.

Plusieurs organisations de réputation mondiale, comme l'Organisation de la coopération et du développement économiques (OCDE), fournissent des orientations sur les normes minimales pour la conception et la conduite des études toxicologiques. Toutes les études utilisées pour évaluer les risques de présence d'une substance dans les aliments sont passées en revue afin de s'assurer de l'adéquation de leur conception et de leur réalisation, et ces études doivent être de préférence menées dans le respect des principes des Bonnes pratiques de laboratoire. La monographie examine également les récents développements prometteurs concernant les protocoles d'essai qui n'ont pas été encore officiellement acceptés par l'OCDE.

L'étude de l'absorption, de la distribution, du métabolisme et de l'excrétion (ADME) d'une substance aux premiers stades des essais est important pour aider à sélectionner les espèces animales et les doses d'essai entrant dans le cadre des études de toxicité. Lorsque cela est possible, l'investigation de toute différence qualitative ou quantitative d'ADME entre les espèces testées et l'homme fournira des renseignements importants pour caractériser les dangers.

L'étendue des tests toxicologiques nécessaires dépend de la nature et de l'utilisation de la substance étudiée. Il ne sera pas obligatoirement nécessaire de procéder à l'ensemble des tests décrits dans la monographie pour parvenir à une conclusion concernant l'évaluation des risques liés à une substance donnée. Des méthodes progressives sont également abordées; ces méthodes permettent de procéder à des essais de sélection ou à un nombre restreint d'études standards de toxicité, ce qui peut être suffisant pour évaluer les risques ou décider d'entreprendre des recherches complémentaires.

Des essais à court et long terme sont généralement réalisés pour déterminer la toxicité systémique générale. Ces essais identifient des organes cibles pour la toxicité et peuvent révéler la nécessité de conduire des essais supplémentaires ou plus spécifiques (pour la neurotoxicité ou l'immunotoxicité, par exemple). Les effets de la substance testée sont examinés sur une large gamme d'indicateurs de toxicité, comprenant des événements cibles observationnels, fonctionnels, biochimiques et pathologiques. Les études sont généralement conduites sur deux espèces, l'une appartenant à l'espèce des rongeurs et l'autre à une espèce différente, et sur les deux sexes, afin d'optimiser les possibilités de déceler des effets (identification des risques). Les essais à long terme comprennent également souvent des tests de carcinogénicité sur deux espèces de rongeurs. L'application d'une autre méthode peut être acceptable au cas par cas pour remplacer l'utilisation de l'une ou l'autre espèce de rongeurs; divers autres tests de carcinogénicité accentuant les réponses tumorigènes et diminuant la durée des essais biologiques ont été introduits, dont des modèles d'initiation/promotion, le modèle de rongeur à tumorigénicité néonatale et des modèles de souris transgéniques.

Les essais doivent être effectués de manière à se rapprocher le plus possible des scénarios d'exposition humaine. La sélection des doses doit tenir compte de l'exposition humaine projetée, de la fréquence de l'exposition et de la durée de l'exposition. Dans le cadre des études sur animaux à dose répétée, les substances présentes dans les aliments, sont généralement administrées par le biais de l'alimentation, du gavage ou de l'eau potable. Dans l'idéal, les niveaux de dose sélectionnés sont tels que le niveau le plus élevé produit des effets toxiques, mais sans provoquer la mort ou une grave souffrance, alors que les niveaux plus faibles engendrent des réactions progressives et que les niveaux de dose les plus faibles ne produisent aucun effet nocif. La conception de l'étude doit être adéquate afin de pouvoir déterminer un point de référence pour caractériser les dangers, ce qui est également connu sous le nom de point de départ (POD); il peut s'agir, par exemple, d'une concentration sans effet nocif observé (CSENO) ou d'une dose de référence (BMD en anglais) produisant un effet nocif faible, mais mesurable.

Il est important que toutes les conceptions d'étude accordent une attention particulière à l'espacement des doses et au nombre de

groupes d'études, à la dose maximale utilisée, au nombre d'animaux par sexe dans chaque groupe de dose, au choix des contrôles, au schéma de dosage, à la confirmation de la dose administrée par rapport à la dose nominale, ainsi qu'à la dose ingérée (acceptabilité du goût et gaspillage de nourriture, par exemple).

Outre les tests effectués pour déterminer une toxicité systémique générale, la génotoxicité potentielle d'une substance doit être évaluée à l'aide d'une gamme de tests adéquats in vitro et, si nécessaire, in vivo. Pour déterminer de manière détaillée la génotoxicité potentielle d'une substance, il est nécessaire de disposer d'informations relatives à la capacité d'induire des mutations génétiques, des anomalies de la structure chromosomique et une aneuploïdie. Un petit nombre d'essais in vitro est généralement choisi pour couvrir différents indicateurs d'effets génétiques. Un test de mutation génétique sur bactéries (essai de *Salmonella*/microsome) et un ou deux tests sur cellules mammaliennes visant à détecter les points de mutations ou les mutations chromosomiques (clastogenicité/aneugénicité) sont les test les plus fréquemment utilisés.

Les effets de la substance sur la performance reproductive, tant des mâles que des femelles, et sur le développement pré et post natal des descendants sont aussi déterminés. L'objectif des études de toxicité sur la reproduction et le développement est d'évaluer: 1) les effets susceptibles de s'exprimer par une baisse de la fertilité ou de la fécondité de l'un ou de l'autre des parents ou des descendants due à des anomalies morphologiques, biochimiques, génétiques ou physiologiques, et 2) la croissance et le développement normal des descendants. Toutefois, les tests de toxicité sur la reproduction et le développement ne couvrent pas nécessairement toute la gamme des effets susceptibles d'être induits par des substances chimiques interférant avec le système endocrinien. La mise au point d'une batterie de tests de sélection destinés à évaluer les substances chimiques qui interfèrent avec les œstrogènes, les androgènes et la thyroïde était toujours en cours au moment de la publication de la présente monographie.

Il faut en outre tenir compte de la nécessité de procéder à des tests de toxicité aiguë. Certaines substances (certains métaux, mycotoxines, résidus de médicaments vétérinaires, résidus de pesticides, par exemple) peuvent provoquer une intoxication aiguë après de courtes

périodes d'ingestion. Lors des évaluations auxquelles il procède, le JECFA inclut une évaluation des effets aigus et, lorsque cela est approprié, la possibilité d'effets aigus sur des individus sensibles. La JMPR considère aujourd'hui qu'il est normal d'établir une dose aiguë de référence (DARf) pour tous les pesticides qu'elle évalue. La JMPR a défini des orientations dans le cadre d'une étude sur des animaux de laboratoire auxquels ont été administrés une dose unique afin d'estimer les DARf plus précisément; ces orientations servent de fondement aux lignes directrices que l'OCDE est en cours d'établir sur les essais.

Des essais supplémentaires peuvent être également nécessaires pour déterminer les effets nutritionnels et neurotoxiques, y compris les effets neurocomportementaux, à la fois chez les adultes et au cours du développement, ainsi que les effets immunotoxicologiques. Les résultats des tests standards décrits ci-dessus peuvent rendre évidente la nécessité de procéder à des essais supplémentaires. Des études spécifiques sur le mécanisme de la toxicité ou le mécanisme d'action peuvent fournir des données supplémentaires utiles à l'évaluation.

Interprétation des résultats

L'évaluation critique de la conception des études et de leurs conclusions, ainsi que l'interprétation des résultats sont les étapes les plus importantes de l'évaluation des risques. Les résultats issus des groupes traités sont généralement comparés à ceux issus de contrôles concomitants. La comparaison des données des tests avec les données historiques, notamment dans le cas de la carcinogénicité et de la toxicité développementale, peut également être utile pour comprendre la portée d'une conclusion donnée.

L'évaluation de nombreux points finaux toxicologiques doit faire intervenir une méthode par évidence reposant sur les données issues de toutes les études disponibles portant sur l'examen de fluides, cellules, tissus ou organes identiques ou fonctionnellement liés. Des conclusions similaires tirées par diverses études et la preuve de relations dose-effet donnent plus de poids à la caractérisation des dangers.

Afin de déterminer si un composant est génotoxique ou ne l'est pas, les données disponibles doivent être évaluées globalement. Des résultats entièrement négatifs obtenus par une batterie de tests in vitro suffisent normalement pour conclure qu'une substance est dénuée

d'un potentiel génotoxique, sauf s'il faut tenir compte de circonstances particulières préoccupantes (niveau d'exposition humaine élevé ou soutenu, considérations structurelles, par exemple). Réciproquement, un ou plusieurs tests in vitro positifs nécessitent habituellement un suivi à l'aide d'un essai de génotoxicité in vivo. Les résultats des essais de génotoxicité peuvent être alors rapprochés des résultats expérimentaux tirés des essais de carcinogénicité chez les rongeurs, les résultats des essais à court terme à eux seuls ne fournissant pas une prévision fiable du caractère carcinogène d'une substance chez les rongeurs. Des études de génotoxicité positive renseignent bien sur le mécanisme d'action des substances carcinogènes et influent sur l'approche utilisée lors de la caractérisation ultérieure des risques. Les conclusions positives provenant des essais biologiques de carcinogénicité chez les rongeurs doivent être soigneusement interprétées en tenant compte du mécanisme d'action, des différences possibles dans l'effet de fond et dans la réponse en fonction des espèces, ainsi que de la question de l'extrapolation entre forte dose et faible dose. L'IPCS a développé un cadre conceptuel afin d'évaluer le mécanisme d'action de la carcinogénèse chimique chez des espèces animales de laboratoire; ce cadre a été ensuite élargi pour traiter de la question de la pertinence, chez l'homme, des données cancérologiques issues de tests sur des animaux. Les mécanismes pertinents pour l'homme incluent la réactivité à l'acide déoxyribonucléique ou la génotoxicité. Il a été déterminé que certains mécanismes ne sont pas pertinent pour l'homme, dont la néphropathie induite chez le rat par l'α2u-microglobuline et la prolifération de peroxisomes.

Lors de l'interprétation des données des études de toxicité sur la reproduction et le développement, il est important de rechercher les schémas de réponse biologiquement liés, ainsi que les relations existant entre les résultats aux différents points finaux, et de comparer les constatations aux données toxicologiques disponibles fournies par d'autres études. Étant donné que les conceptions des études standards requièrent que la dose la plus élevée produise une indication minimale de présence de toxicité maternelle, il peut s'avérer difficile d'évaluer dans quelle mesure l'effet provoqué par cette dose sur le développement est directement lié à l'action de la substance chimique sur l'embryon ou sur le fœtus, ou s'il est indirectement lié à l'homéostasie maternelle altérée. Bien qu'il existe plusieurs exemples de ce dernier type, il est important de ne pas conclure à une causalité sur la base de

l'association de la toxité sur la croissance et de la toxicité maternelle sans procéder à des essais et à une évaluation supplémentaires.

Allergies et autres hypersensibilités alimentaires

Les allergies alimentaires sont la conséquence de la réponse immunitaire non désirée ou non contrôlée envers un antigène dans l'alimentation chez des individus sensibles. Elles se fondent sur l'interprétation aberrante du corps à certaines protéines alimentaires qu'il considère comme « corps étrangers », ce qui déclenche une réaction exagérée du système immunitaire. Les allergies se développent par le biais d'un processus de sensibilisation. Durant la phase de sensibilisation, l'exposition à l'allergène alimentaire stimule la production de l'immunoglobuline E qui se forme pour lutter contre un antigène alimentaire.

L'évaluation des risques d'allergie alimentaire est une discipline relativement nouvelle et aucun consensus n'a été dégagé sur la manière de procéder, même si plusieurs approches ont été proposées. Ainsi, il n'existe actuellement pas de consensus sur une dose seuil en deçà de laquelle la sensibilisation aux allergénes alimentaires ne se produirait pas. Pour prévoir l'allergénicité potentielle des nouvelles protéines alimentaires, telles que les aliments génétiquement modifiés, des approches stratégiques fondées sur un arbre décisionnel ont été décrites.

Principes généraux relatifs aux études chez l'homme

Les données issues d'études sur l'homme sont potentiellement importantes pour identifier et caractériser les dangers, ainsi que pour apprécier les risques que présentent les additifs alimentaires, les contaminants et les résidus de médicaments vétérinaires et de pesticides. Ces données peuvent provenir d'expériences contrôlées sur des volontaires humains, d'études de surveillance, d'études épidémiologiques (études écologiques, études de cas-témoins, études de cohortes, études analytiques ou sur le terrain) réalisées sur des populations dont le niveau d'exposition diffère, d'études expérimentales ou épidémiologiques sur des sous-groupes spécifiques d'individus, de rapports cliniques (empoisonnement, par exemple) ou d'études de cas sur des individus. Les points finaux peuvent inclure l'examen de l'innocuité

ou de la tolérance, les effets nutritionnels et fonctionnels des aliments ou des composants alimentaires, le métabolisme et la toxico-cinétique de la substance, le mécanisme d'action en ayant éventuellement recours à des biomarqueurs pour les effets identifiés dans les études sur animaux, et les effets nocifs sur la santé suite à des expositions accidentelles (à un contaminant, par exemple).

Les aspects critiques de toute étude expérimentale sur l'homme concernent les contrôles éthiques, professionnels et juridiques fondamentaux qui déterminent de la nécessité de procéder à une étude sur l'homme et les circonstances dans lesquelles elle peut être menée de manière adéquate. Le nombre de sujets inclus dans une étude doit être suffisant pour atteindre les objectifs de la recherche. Il faut évaluer les cas dans lesquels il peut être suffisant d'utiliser des tissus humains ex vivo ou in vitro. L'expérimentation effectuée sur des cellules ou des tissus humains ou utilisant d'autres préparations contenant ou représentant des enzymes humains, des récepteurs et d'autres facteurs infracellulaires in vitro sont fondamentalement différents des études sur l'homme car elles ne tiennent pas compte de l'absorption, de la distribution, des aspects de métabolisme intégré et d'excrétion. Elles permettent toutefois de réaliser des études mécanistes dans des conditions contrôlées qui ne sont pas réalisables en clinique; ces techniques sont donc très précieuses pour aider à déterminer les voies métaboliques et les mécanismes de réponse susceptibles de revêtir une importance chez l'homme et qui méritent d'être étudiés en tant que biomarqueur de l'exposition ou de l'effet.

Considérations liées au tractus gastro-intestinal, y compris aux effets sur la microflore intestinale

Les interactions susceptibles de se produire entre les substances chimiques contenues dans les aliments et la flore bactérienne du tractus gastro-intestinal doivent être étudiées sur le plan tant des effets de la microflore intestinale sur les substances chimiques que des effets des substances chimiques sur la flore intestinale.

Les méthodes in vivo utilisées pour étudier le rôle de la microflore intestinale dans le métabolisme d'une substance sont les suivantes: 1) l'administration parentérale du composé, qui devrait déterminer une diminution du métabolisme microbien des composés polaires

mal absorbés par rapport à l'administration d'une dose par voie orale; 2) les études sur animaux consistant à réduire la flore bactérienne à l'aide d'antibiotiques; et 3) les études sur des animaux axéniques et sur des animaux (anciennement) axéniques auxquels des souches bactériennes connues ont été innoculées (animaux gnotobiotiques). Plusieurs facteurs peuvent influer sur l'activation métabolique des substances chimiques étrangères par la microflore hôte, dont l'espèce-hôte, le régime alimentaire, la médication et l'adaptation métabolique. Diverses méthodes in vitro et in vivo existent également pour tester le potentiel d'une substance à induire une résistance dans la microflore intestinale, suite à l'ingestion de substances ou de résidus dotés de propriétés anti-microbiennes.

Évaluation de la relation dose-réponse

L'évaluation de la relation dose-réponse est une étape principale de la caractérisation des dangers, qui est un volet du paradigme de l'évaluation des risques. L'évaluation de la relation dose-réponse permet de fournir des avis sur l'évaluation des risques et d'établir des valeurs recommandées en fonction de critères sanitaires.

Les approches se présentent généralement sous l'une des deux formes suivantes: 1) les analyses donnant une estimation quantitative ou qualitative du risque; et 2) les analyses établissant des valeurs recommandées en fonction de critères sanitaires, telles que la dose journalière admissible (DJA) ou la dose journalière tolérable (DJT) qui correspondent à des niveaux d'exposition considérés ne produire aucun « risque appréciable pour la santé de l'homme ». La DJT approche est utilisée pour les contaminants. La DJA approche s'utilise généralement lorsque l'exposition peut être contrôlée, comme dans le cas, par exemple, des additifs alimentaires et des résidus de pesticides et de médicaments vétérinaires dans les aliments. La monographie EHC 239 intitulée Principes de la modélisation dose-réponse pour l'évaluation des risques d'origine chimique examine les approches permettant d'évaluer la relation dose-réponse appliquées aux données issues d'études sur les animaux.

La détermination de la présence ou de l'absence d'une relation de cause à effet est l'une des premières composante d'une évaluation des risques. Si des données suffisantes rendent plausible l'existence d'une telle relation, il est indispensable de disposer de données sur la

relation dose-réponse. Les données relatives à la dose-réponse peuvent être issues des études in vivo sur des animaux de laboratoire ou sur l'homme, sur lesquelles se fonde normalement la caractérisation des risques. Dans chaque cas, afin de pouvoir interpréter les données concernant les effets, il faut généralement déterminer les niveaux d'exposition qui ne produisent pas d'effet mesurable et établir la relation entre la hausse de l'incidence et la sévérité ou la nature de l'effet résultant de l'augmentation de l'incidence.

La modélisation de la relation dose-réponse peut être décrite en six étapes principales. Les quatre premières étapes (sélection des données, sélection du modèle, liaisons statistiques et estimation des paramètres) concernent les données sur la relation dose-réponse. Dans cette analyse, les données observées de dose-réponse sont modélisées de manière à prévoir l'intensité probable de la réponse à une dose donnée soit à l'intérieur, soit à l'extérieur de la gamme observée de dose-réponse, ou la dose probable générant une intensité donnée de l'effet. Les deux dernières étapes se rapportent à la mise en application et à l'évaluation des résultats de l'analyse.

L'extrapolation est une composante nécessaire de l'évaluation des risques. Dans la plupart des cas examinés par le JECFA et la JMPR, les données utilisées pour évaluer la relation dose-réponse proviennent des expériences effectuées sur des animaux de laboratoire auxquels ont été administrées des doses sensiblement plus élevées que l'exposition humaine potentielle. Dans les analyses dose-réponse de ce type, deux aspects doivent être pris en considération pour l'extrapolation: 1) l'extrapolation à l'homme en partant des espèces testées; et 2) la prise en compte de différences humaines possibles dans la réponse. Les méthodes utilisées pour traiter de ces extrapolations sont abordées dans la monographie et sont diverses, allant de l'utilisation de facteurs d'incertitude à des schémas plus complexes de modélisation fondés sur les différences de réponses toxico-cinétiques et toxico-dynamiques entre l'homme et les animaux de laboratoire, ainsi que sur la variabilité entre différentes personnes.

Détermination de valeurs recommandées en fonction de critères sanitaires

La détermination de valeurs recommandées en fonction de critères sanitaires fournit des données quantitatives issues de l'évaluation

des risques pour permettre aux gestionnaires des risques de prendre des décisions en matière de protection de la santé publique. Les valeurs recommandées en fonction de critères sanitaires découlent de l'évaluation de la relation dose-réponse pour les points finaux les plus pertinents dans les espèces les plus pertinentes. La première approche, qui reste celle la plus utilisée par le JECFA et la JMPR pour dériver les valeurs recommandées afin de protéger contre les effets nocifs qui se manifestent au-delà d'un certain seuil, consiste à définir une CSENO ou parfois la plus basse concentration produisant des effets nocifs (LOAEL en anglais) en tant que POD. Une autre approche appliquée par le JECFA et la JMPR consiste à utiliser la borne inférieure de l'intervalle de confiance de la BMD (BMDL en anglais) comme POD pour déduire la valeur recommandée ou pour calculer une marge d'exposition (MOE). L'évaluation de la relation dose-réponse est quelquefois utilisée pour définir la dose associée à une augmentation négligeable de la réponse (1 pour 1 million, par exemple) par rapport au niveau de fond.

En ce qui concerne les additifs alimentaires et les résidus de pesticides et de médicaments vétérinaires dans les aliments, la valeur recommandée est exprimée par la dose journalière admissible (DJA). Les DJA fixées par le JECFA et la JMPR se fondent sur tous les faits connus au moment de l'évaluation. Le JECFA définit généralement les DJA en se basant sur la CSENO déterminée chez l'espèce animale la plus appropriée, c'est à dire en général la plus sensible. La DJA est exprimée en quantité (en milligramme, par exemple), par kilogramme de poids corporel, dans une plage généralement comprise entre zéro et une valeur limite supérieure. Les DJA sont normalement exprimées par une valeur numérique utilisant un seul chiffre significatif. Lorsque cela est approprié, le JECFA et la JMPR déterminent des DARf, qui fournissent une estimation de la quantité d'une substance contenue dans les aliments et/ou dans l'eau potable pouvant être ingérée pendant une journée ou moins, sans risque appréciable sur la santé du consommateur, sur la base de tous les faits connus au moment de l'évaluation. Les DARf sont habituellement exprimées en fonction du poids corporel.

S'agissant des contaminants alimentaires qui ne peuvent être généralement évités, le JECFA applique le terme « admissible » pour la valeur recommandée en matière de santé. Cela signifie que

l'absorption des contaminants inévitablement associés à la consommation d'aliments par ailleurs sains et nutritifs est admissible. Les principes régissant les niveaux d'ingestion admissibles sont identiques à ceux des DJA: les approches CSENO ou BMD peuvent être utilisées comme POD pour fixer les valeurs recommandées pour les contaminants. Les contaminants alimentaires comprennent les métaux lourds, les produits toxiques naturels (tels que les dioxines et les mycotoxines), les impuretés contenues dans les additifs alimentaires, les solvants utilisés lors de la transformation des denrées alimentaires, d'autres substances liés aux procédés alimentaires tels que la cuisson, les substances migrant des ustensiles et équipements qui entrent en contact avec les aliments, et les résidus provenant des additifs utilisés dans l'alimentation animale ou les composants non actifs des formules de médicaments vétérinaires. Les valeurs recommandées peuvent être exprimées par l'estimation d'une dose journalière admissible, DJA), d'une dose journalière admissible maximale temporaire (DJAMT), d'une dose hebdomadaire tolérable provisoire (DHTP) ou d'une dose mensuelle tolérable provisoire (DMTP). Le qualificatif « provisoire » indique qu'il s'agit d'une évaluation indicative, compte tenu de la rareté des données disponibles sur les conséquences de l'exposition humaine à des concentrations proches de celles auxquelles s'intéresse le JECFA. Les DJMT sont utilisées dans le cas des contaminants qui peuvent s'accumuler dans l'organisme. Le JECFA utilise les DHTP et les DMTP pour les contaminants qui ne semblent pas s'accumuler dans l'organisme avec le temps.

La sélection des données appropriées et la détermination de la CSENO représentent les étapes essentielles de l'approche CSENO pour calculer les valeurs recommandées en fonction de critères sanitaires. Lors du calcul de ces valeurs, un facteur de sécurité ou d'incertitude est appliqué à la CSENO afin d'attribuer une marge de sécurité prudente liée aux incertitudes inhérentes à l'extrapolation à l'homme des données de toxicité issues des animaux de laboratoire, ainsi qu'aux variations au sein de l'espèce humaine. Les termes « facteur de sécurité » et « facteur d'incertitude » sont souvent utilisés de façon interchangeable; le terme « facteur de sécurité » a été privilégié dans le passé, mais on lui préfère aujourd'hui celui de « facteur d'incertitude ». Le concept de facteurs d'ajustement spécifique à des substances chimiques a été introduit pour permettre l'utilisation de données spécifiques relatives aux différences entre espèces ou à la variabilité au sein de l'espèce

humaine en toxicocinétique ou en toxicodynamique; ce concept permet de calculer des facteurs d'incertitude fondés sur les données au lieu d'utiliser des facteurs par défaut, lorsque cela est possible.

L'approche BMD a été introduite comme une alternative à l'approche CSENO. Cette méthode définit un niveau d'exposition ou une dose donnant un effet faible, mais mesurable, comme POD afin d'évaluer les risques. La méthode BMD présente de nombreux avantages, dont l'utilisation de données complètes dose-effet dans l'analyse statistique, qui permettent de quantifier l'incertitude dans les données. Le degré d'incertitude dans les données dû, par exemple, aux tailles réduites des groupes ou à une forte variation au sein d'un groupe, se traduit par des valeurs recommandées d'un niveau plus faible.

Il est des circonstances où le JECFA et la JMPR estiment que l'utilisation d'une DJA numérique ne convient pas. Ce cas se présente lorsqu'il apparaît que la consommation estimée de l'additif sera bien inférieure à toute valeur numérique qui devrait normalement lui être attribuée. Dans de telles circonstances, l'expression DJA « non spécifiée » est utilisé.

Parfois, certains aspects sont insuffisamment couverts par l'ensemble des données disponibles sur une substance particulière ou de nouvelles données remettent en question la DJA précédemment déterminée par le JECFA ou la JMPR. Le Comité établit souvent une dose journalière admissible « temporaire » lorsqu'il a la certitude qu'une substance peut être employée sans danger pendant la période de temps relativement courte nécessaire pour produire et évaluer de nouvelles données sur son innocuité, mais qu'elle peut présenter un danger si elle est utilisée pendant toute une vie; cette dose sera modifiée, le cas échéant, lorsque les données nécessaires auront été fournies dans les délais impartis.

En ce qui concerne les médicaments vétérinaires et les pesticides, la DJA est utilisée pour confirmer la sécurité des limites maximales de résidus (LMR) proposées lorsque les substances sont utilisées conformément aux bonnes pratiques. La toxicité du médicament parent ou de ses principales métabolites est prise en considération lorsqu'une DJA est établie pour un résidu de médicament vétérinaire ou de pesticide; la DJA se fonde sur le point final toxicologique du composant le plus préoccupant.

Lorsqu'un médicament vétérinaire peut avoir une incidence sur la microflore intestinale de l'homme à des expositions plus faibles que celles provoquant des effets toxicologiques, la DJA est déterminée en fonction de ce point final. Le système d'arbre de décision, harmonisé au niveau international et faisant l'objet des directives de la Coopération internationale pour l'harmonisation des exigences techniques s'appliquant à l'enregistrement des produits pharmaceutiques vétérinaires (VICH en anglais), permet de déterminer la nécessité d'établir une DJA microbiologique. Les trois premières étapes examinent: 1) si les résidus du médicament et/ou ses métabolites sont biologiquement actifs en regard des représentants de la flore intestinale humaine, 2) si les résidus atteignent le colon humain, et 3) si les résidus contenus dans le colon continuent d'être microbiologiquement actifs. Si l'on obtient un résultat négatif à l'une quelconque des trois étapes, il n'est pas nécessaire de déterminer une DJA. Toutefois, lorsque des résidus de cette nature sont présents, on considère que les deux points finaux préoccupants pour la santé publique sont: 1) la perturbation de la barrière de colonisation et 2) l'augmentation des populations de bactéries résistantes.

Lorsqu'il est envisagé d'utiliser plusieurs substances produisant des effets toxiques similaires ou ayant une métabolite toxique en tant qu'additifs alimentaires, pesticides ou médicaments vétérinaires, ou lorsque ces substances se présentent sous la forme de contaminants, il peut être approprié d'établir une valeur recommandée en fonction des critères sanitaires; ces substances seront alors considérées comme un groupe pour limiter leur ingestion globale. Pour que cette procédure soit réalisable, les substances doivent avoir un mécanisme d'action identique et un éventail similaire de toxicité potentielle.

Il est préférable de déterminer des valeurs recommandées pour l'ensemble de la population. Ces valeurs sont normalement fixées de manière à protéger le sous-groupe de la population la plus sensible, sur la base des résultats sanitaires critiques les plus sensibles. Il est toutefois reconnu que le résultat sanitaire le plus critique n'est pas toujours pertinent pour certains sous-groupes de la population. Il est particulièrement important, par exemple, de s'assurer que la valeur recommandée est adéquate pour protéger l'embryon ou le fœtus contre d'éventuels effets in utero. Dans certaines situations dans lesquelles un point final relatif au développement ou un point

final spécifique à une sous-population détermine la valeur recommandée pour une substance ne présentant aucune autre toxicité, il est possible d'émettre un avis concernant une deuxième valeur (plus élevée) fondée sur un autre point final pertinent pour le reste de la population.

Évaluation de l'exposition alimentaire aux substances chimiques contenues dans les aliments

Lors de l'évaluation de l'exposition alimentaire aux substances chimiques, les données de consommation alimentaire sont associées aux données de concentration des substances chimiques dans les produits alimentaires. L'estimation de l'exposition alimentaire qui en résulte peut alors être comparée à la valeur recommandée ou au POD toxicologique (CSENO; BMDL) pour la substance chimique alimentaire concernée lors de la caractérisation des risques. Des évaluations peuvent être effectuées dans le cas d'expositions aiguës ou chroniques. Les évaluations de l'exposition alimentaire doivent couvrir la population générale, ainsi que les groupes critiques qui sont vulnérables ou dont l'exposition risque d'être sensiblement différente de celle de la population générale (nourrissons, enfants, femmes enceintes, personnes âgées et végétariens, par exemple).

En principe, il est nécessaire de procéder à des évaluations de l'exposition alimentaire pour toutes les substances chimiques contenues dans les aliments qui ont été mises en évidence lors de l'évaluation des risques. L'évaluation des risques peut être également appliquée aux contaminants, aux résidus de pesticides et de médicaments vétérinaires, aux additifs alimentaires (y compris les aromatisants), aux auxiliaires technologiques et à d'autres substances chimiques contenues dans les aliments. Il est recommandé d'adopter une approche progressive: parmi le grand nombre de substances chimiques susceptibles d'être présentes, des méthodes de sélection peuvent être appliquées pour identifier celles qui ne posent pas de problème de sécurité, en utilisant un minimum de ressources dans les délais les plus brefs possibles. Dans ce cas, il n'est nécessaire de procéder à une évaluation plus poussée de l'exposition. Les étapes ultérieures qui visent à affiner l'évaluation de l'exposition alimentaire doivent être conçues de manière à ne pas sous-estimer une exposition alimentaire potentiellement plus élevée.

Les sources d'information sur les concentrations de substances chimiques dans les aliments comprennent les limites maximales (LM) ou les limites maximales de résidus (LMR), les niveaux d'utilisation proposés par les fabricants, les données de suivi et de surveillance, les études de l'alimentation totale (TDS en anglais), la base de données GEMS/Alimentation, les études de déplétion de résidus de médicaments vétérinaires, l'utilisation des niveaux de résidus moyens ou élevés issus d'essais contrôlés pour les pesticides, et des documents scientifiques. Les données les plus précises proviennent de la mesure des concentrations de substances chimiques présentes dans les aliments consommés. Les programmes visant à produire des données sur les concentrations de substances chimiques dans les aliments nécessitent des plans d'échantillonnage validés et des méthodes analytiques. Il existe deux principales approches pour analyser les aliments lors de la génération de données analytiques à partir d'enquêtes: 1) l'analyse de la composition de groupes d'aliments; et 2) l'analyse de produits alimentaires individuels (sous forme d'échantillons uniques ou de composés).

Les données de consommation alimentaire peuvent être tirées des données du bilan alimentaire, qui incluent les volumes d'alimentation disponibles pour la consommation humaine qui sont déduits des statistiques nationales concernant la production alimentaire, la consommation apparente ou l'utilisation. Ces données sont généralement disponibles pour la plupart des pays. Les groupes de régimes alimentaires GEMS/Alimentation établis par l'OMS se fondent sur les bilans alimentaires de la FAO et correspondent à la consommation alimentaire moyenne par habitant. Les modules de régimes alimentaires remplacent les cinq régimes régionaux précédemment élaborés par l'OMS.

Les données de consommation alimentaire doivent être fournies dans un format permettant de mettre en correspondance les données de consommation avec les données de concentration utilisées pour évaluer l'exposition alimentaire. Les données recueillies à l'aide de méthodes représentatives d'une population sont généralement compilées et enregistrées pour les produits ou denrées agricoles à l'état brut ou transformé, et représentent le volume annuel total d'un produit disponible pour la consommation intérieure par an. Les données issues des enquêtes sur des produits alimentaires individuels sont rarement

rendues publique sous leur forme non traitée (c'est à dire au niveau de chaque personne interrogée); les évaluateurs des risques doivent donc s'appuyer sur les statistiques résumées publiées. Des corrections des parts de marché peuvent être appliquées aux données de consommation alimentaire pour les produits ou denrées transformés ou pour un pourcentage des cultures traitées. Cette méthode est essentiellement utilisée lorsque la substance évaluée a été délibérément ajoutée au produit ou à la denrée.

Les méthodes disponibles pour estimer l'exposition alimentaire ont été divisées entre celles qui fournissent des estimations seules (ponctuelles) et celles qui caractérisent la distribution complète des expositions des consommateurs. Les données ponctuelles incluent: 1) les méthodes de sélection, 2) les méthodes d'exposition fondées sur les estimations brutes de la consommation, comme la dose journalière maximale ajoutée théorique (DJMAT) et d'autres modèles de régimes alimentaires, et 3) des méthodes d'exposition plus affinées basées sur les données réelles de consommation et de concentration de substances chimiques, telles que les TDS, les études sélectives sur des produits alimentaires individuels et sur des portions alimentaires dédoublées. Une estimation déterministe ou ponctuelle de l'exposition alimentaire est tout simplement une valeur unique décrivant certains paramètres de l'exposition des consommateurs (exposition moyenne de la population, par exemple). La caractérisation de la distribution complète des expositions des consommateurs est l'évaluation exigeant le plus de ressources; il faut en effet disposer de données caractérisant la gamme non seulement des habitudes de consommation, mais aussi que des concentrations de substances chimiques dans les aliments consommés. Le degré d'affinement des estimations de l'exposition alimentaire dépendra, en partie, de la nature de la substance et du profil de toxicité.

Les méthodes de sélection surestiment l'exposition alimentaire des gros consommateurs car elles se fondent sur des hypothèses prudentes de consommation alimentaire et de concentrations de substances chimiques. Leur objectif n'est pas d'évaluer l'exposition alimentaire réelle, mais d'identifier les substances chimiques dans les aliments qui nécessitent une évaluation plus approfondie de l'exposition alimentaire. Les méthodes de sélection incluent les données de poids (pour les additifs alimentaires, y compris les aromatisants), la méthode du

budget (utilisée pour évaluer l'exposition la dose journalière maximale ajoutée théorique pour certains additifs alimentaires) et les régimes modèles (établis à partir des données disponibles sur la consommation alimentaire et conçus pour représenter le régime alimentaire type de la population dont l'exposition est étudiée).

La modélisation d'estimation ponctuelle peut également être appropriée comme une deuxième étape d'une approche en trois temps. Le modèle sélectionné peut être plus ou moins prudent, en fonction de l'objectif et des données disponibles. Des régimes modèles pour les gros consommateurs peuvent être établis à partir des données publiées à partir d'enquêtes sur la consommation alimentaire pour remplacer la méthode du budget ou comme étape supplémentaire du processus de sélection. Les volumes de consommation alimentaire et les expositions alimentaires des gros consommateurs peuvent être également obtenus à partir des données de répartition. Il peut s'avérer nécessaire de tenir compte de la tendance des consommateurs à acheter et à consommer toujours les mêmes produits alimentaires, ce qui est parfois désigné par le terme « loyauté du consommateur »; il peut être également nécessaire d'utiliser une gamme de concentrations pour calculer les estimations de l'exposition alimentaire en fonction des divers scénarios de comportement des consommateurs.

Une analyse probabiliste de la variabilité de l'exposition peut être utilisée pour les substances nécessitant un traitement plus poussé au-delà des méthodes de sélection ou des estimations ponctuelles d'exposition. Des approches telles que l'estimation simple de la distribution empirique, l'établissement de modèles probabilistes à partir d'ensembles de données, les échantillonnages stratifiés, les échantillonnages aléatoires (simulation de Monte Carlo) et les hypercubes latins ont été utilisées pour élaborer des modèles probabilistes de l'évaluation de l'exposition alimentaire.

Pour une évaluation probabiliste de l'exposition, les répartitions des données de consommation alimentaire que l'on peut facilement obtenir proviennent des études à court terme et ne sont pas représentatives de la consommation réelle à long terme. Les approches utilisées pour estimer la consommation à long terme incluent des méthodes associant des données de fréquence de consommation alimentaires à des informations sur les volumes d'aliments consommés, ainsi que

des modèles statistiques utilisant des corrélations entre les jours de consommation afin d'estimer l'ingestion « habituelle » de la substance concernée.

L'exposition aux substances chimiques dans les aliments peut se produire par d'autres voies, et des expositions à des substances chimiques ou à des médicaments partageant le même mécanisme d'action (toxicité) peuvent également se produire. On appelle exposition totale les expositions conjuguées à une seule substance chimique par de multiples voies (orale, cutanée, par inhalation) et par de multiples vecteurs (aliments, eau de boisson, résidentiel). L'évaluation des risques liés à l'exposition à plusieurs résidus de pesticides présentant un même mécanisme de toxicité doit être pris en compte; dans ce cas, l'estimation de l'exposition est appelée exposition cumulative. Des directives pour l'estimation de l'exposition totale ont été formulées.

Caractérisation des risques

La caractérisation des risques est la quatrième étape du processus d'évaluation des risques; elle intègre les informations fournies par la caractérisation des dangers et l'évaluation de l'exposition afin de fournir des avis scientifiques aux gestionnaires des risques. Dans le passé, des méthodes différentes ont été utilisées pour caractériser le risque de toxicité d'une substance selon que son effet critique a un seuil ou non. Le JECFA et la JMPR établissent des valeurs recommandées en fonction de critères sanitaires pour les substances produisant des effets à seuil. Afin de caractériser les risques liés à ces types de substances, les valeurs recommandées sont comparées avec l'exposition humaine estimée ou mesurée.

Dans les cas où les expositions dépassent les valeurs recommandées, les valeurs elles-mêmes ne fournissent pas d'avis aux gestionnaires des risques sur le degré possible du risque qu'encourent les personnes exposées à ces valeurs plus élevées. Il faut en premier lieu tenir compte du fait que les valeurs recommandées elles-mêmes intègrent des facteurs de sécurité ou d'incertitude. Une exposition alimentaire restreinte ou occasionnelle, dépassant la valeur recommandée, fondée sur une étude chronique ou subchronique n'implique pas obligatoirement des effets nocifs sur la santé publique.

Lorsque les données ne sont pas suffisantes pour proposer une valeur recommandée pour une substance ou lorsqu'on ne peut pas assumer que le mécanisme d'action présente un seuil, le JECFA et la JMPR peuvent fournir des observations sur la marge d'exposition entre les doses pour lesquelles on constate des effets sur les animaux et l'exposition alimentaire estimée chez l'homme.

La caractérisation des dangers doit prendre en compte et décrire l'incertitude et la variabilité. L'incertitude est liée aux limitations des connaissances de l'évaluateur des risques concernant les données et les modèles utilisés. La variabilité reflète l'hétérogénéité biologique inhérente de l'exposition ou de la réponse. Ainsi, même si l'incertitude et la variabilité peuvent toutes deux être caractérisées à l'aide de distributions de probabilité, ce sont des concepts différents. L'incertitude peut être diminuée à fur et à mesure que la quantité et la qualité des données s'améliorent. La modélisation de la variabilité est un exercice de statistique descriptive permettant d'obtenir un modèle d'une population, plutôt que d'un individu. La caractérisation de la variabilité de l'exposition alimentaire d'une population, par exemple, peut être améliorée par des informations plus pertinentes, mais la variabilité ne peut être éliminée. La caractérisation des risques doit comprendre une évaluation descriptive de l'incertitude, tant au niveau de l'exposition que des effets sur la santé. L'analyse de la sensibilité renvoie à des techniques quantitatives que l'on peut utiliser pour identifier les aspects des données d'entrée (données sur la concentration ou la consommation alimentaire, par exemple) qui contribuent le plus à l'incertitude.

Les parties prenantes à l'évaluation des risques sont de plus en plus conscientes de la nécessité de tenir compte des risques, quels qu'ils soient, liés à l'exposition combinée de mélanges de substances. Les types d'effets combinés ou d'interactions sont au nombre de quatre: addition de la dose, addition de la réponse, synergisme et antagonisme. Des évaluations de mélanges ont été entreprises par le JECFA et la JMPR pour certains additifs alimentaires, des résidus de pesticides et des médicaments vétérinaires produits et testés en tant que mélanges, et certains mélanges co-ocurants de contaminants. Pour les pesticides et les médicaments vétérinaires qui sont des mélanges, le JMPR et la JECFA, respectivement, basent la DJA pour les résidus sur le mélange testé. Dans certains cas, une DJA de groupe est attribuée. Le JECFA a également utilisé la DJA de groupe pour certains additifs

alimentaires métabolisés en une métabolite commune potentiellement toxique et une DJA de groupe pour les contaminants étroitement liés qui se présentent sous forme de mélanges. Une approche tenant compte de l'additivité de la dose est celle des facteurs d'équivalence toxique (TEF en anglais). Cette approche mesure l'exposition pour chaque composant d'un mélange par rapport à la puissance d'un produit chimique de référence (pour les dioxines ou les substances apparentées aux dioxines, par exemple).

Pour les substances génotoxiques et cancérogènes, l'hypothèse traditionnelle est qu'il ne semble pas exister de dose seuil et qu'un risque est plausible à n'importe quel niveau d'exposition. Le JECFA n'a donc pas établi de valeurs recommandées pour les substances que l'on sait être à la fois génotoxiques et cancérogènes. Certaines substances induisent toutefois un cancer chez les animaux de laboratoire par le biais de mécanismes non génotoxiques présentant un seuil, et des valeurs recommandées en fonction de critères sanitaires peuvent être déterminées.

Les substances qui sont à la fois génotoxiques et cancérogènes ne sont normalement pas considérées comme acceptables dans les additifs alimentaires, les pesticides ou les médicaments vétérinaires. Le JECFA a examiné plusieurs contaminants dont les propriétés à la fois génotoxiques et cancérogènes ont été prouvées, et a examiné plusieurs approches possibles pour formuler des avis permettant de mieux informer les gestionnaires des risques sur la gravité possible des problèmes sanitaires à divers niveaux d'ingestion chez l'homme. L'évaluation de l'exposition (ingestion) pour un composant présentant des propriétés à la fois génotoxiques et cancérigènes n'est pas différente de celle des autres types de contaminants. La caractérisation des risques peut se présenter sous différentes formes: 1) le calcul de la MOE entre la dose provoquant une incidence faible, mais définie, sur le cancer (habituellement dans des essais biologiques sur des animaux) et l'estimation de l'exposition humaine; 2) l'analyse de la relation dose–response hors de la gamme des doses observées dans les essais biologiques chez les animaux afin de calculer l'incidence du cancer qui est théoriquement associée à l'exposition estimée pour les hommes ou à l'exposition liée à une incidence prédéterminée du cancer (augmentation du risque de cancer au cours d'une vie de 1 sur un million, par exemple); et 3) l'extrapolation linéaire de faibles doses à

partir d'un POD, comme la BMDL. Parmi ces trois options, la MOE et l'extrapolation linéaire de faibles doses à partir d'un POD sont les plus pragmatiques et les plus utilisables à l'heure actuelle. Le JECFA a décidé que les avis relatifs aux composants présentant des propriétés tant génotoxiques que cancérigènes doivent se fonder sur les MOE estimées. La monographie souligne que les points forts et les points faibles inhérents aux données utilisées pour calculer les MOE doivent être décrits dans les avis communiqués aux gestionnaires des risques et comprendre des avis concernant l'interprétation des MOE.

Limites maximales de résidus pour les pesticides et les médicaments vétérinaires

Les limites maximales de résidus (LMR) pour les pesticides et les médicaments vétérinaires correspondent aux teneurs maximales en résidus autorisées dans une denrée ou à sa surface. La Commission du Codex Alimentarius adopte des normes internationales en matière de LMR, sur recommandation des comités respectifs du Codex, du Comité du Codex sur les résidus de pesticides (CCPR) et du Comité du Codex sur les résidus de médicaments vétérinaires dans les aliments (CCRVDF). Ces recommandations reposent sur les avis fournis par le JECFA et la JMPR. Le JECFA et la JMPR ont les mêmes exigences en matière d'identification et de caractérisation des substances étudiées en vue d'établir une DJA, une DARf et des LMR.

La JMPR évalue les données relatives aux résidus de pesticides résultant de l'emploi des pesticides tel que préconisé par les Bonnes pratiques agricoles (BPA) afin d'estimer les teneurs maximales en résidus dans les produits d'alimentation humaine et animale. La JMPR évalue les études de métabolisme de plantes et d'animaux (d'élevage) en tant que facteurs déterminants de la définition des résidus dans les produits d'alimentation humaine et animale. Les taux maximums de concentrations de résidus recommandés dans diverses cultures vivrières sont essentiellement basés sur des données issues d'essais contrôlés effectués en respectant les niveaux maximums de pesticides employés recommandés par les Bonnes pratiques agricoles. Les essais doivent couvrir l'éventail des conditions prévisibles en pratique, y compris les méthodes d'application, les saisons, les pratiques agronomiques et la variété des cultures. Si les taux de résidus dans le produit transformé dépassent les taux de résidus dans la denrée agricole brute avec

un écart suffisant pour exiger une LMR plus élevée que celle prévue pour la denrée agricole brute, la JMPR estime un niveau maximum de résidus pour le produit transformé. La charge en résidu de pesticide dans l'alimentation des animaux d'élevage est calculée à partir des données obtenues au moyen de tests contrôlés sur les résidus dans l'alimentation animale et de régimes étalonnés fondés sur les tableaux d'alimentation animale de l'OCDE. Les niveaux maximums estimés de résidus ainsi que les niveaux de résidus les plus élevés (HR en anglais) résultant des essais contrôlés et les concentrations médianes de résidus en essai contrôlé (MREC; STMR en anglais) calculées à partir de traitements animaux externes sont comparés à ceux obtenus à partir de l'exposition par le biais de l'alimentation. Les concentrations maximales de résidus recommandées, les HR et les MREC se fondent sur les valeurs les plus élevées résultant de cette comparaison. Les estimations de l'exposition chronique repose sur les MREC issues d'essais contrôlés et d'études de transformation des denrées alimentaires et de consommation alimentaire à long terme. Pour évaluer l'exposition à court terme, les estimations de forte ingestion des résidus de pesticide en un seul jour se fondent sur les HR produites en essai contrôlé.

En ce qui concerne les médicaments vétérinaires, le JECFA évalue des études sur la déplétion des résidus à l'aide d'une substance parentale radiomarquée, ainsi que des études supplémentaires utilisant une substance parentale non radiomarquée chez des espèces animales ciblées, afin de recommander des LMR dans les produits bruts d'origine animale. Les données fournies par les études utilisant une substance radiomarquée permettent d'estimer le temps d'absorption de la concentration du résidu total concerné et de déterminer un résidu marqueur. Les LMR dérivées sont définies sur la base du résidu marqueur. Le résidu marqueur peut être la substance parentale, un métabolite important, la somme de la substance parentale et de métabolites, ou un produit formé à partir des résidus de médicament pendant l'analyse. Il ne s'agit pas obligatoirement d'un résidu présentant un problème d'ordre toxicologique ou microbiologique, mais d'un résidu nécessaire à des fins de surveillance. Les données issues des études utilisant une substance non marquée sont utilisées pour estimer le temps d'absorption de la concentration du résidu marqueur dans les produits bruts d'origine animale dans des conditions de bonne pratique d'utilisation approuvées (comme les Bonnes pratiques

d'utilisation des médicaments vétérinaires, BPMV). Le rapport entre le résidu marqueur et le total des résidus permet de convertir les concentrations du résidu marqueur en concentrations de résidus totaux concernés afin d'estimer l'exposition alimentaire.

Les LMR sont généralement recommandées pour plusieurs tissus et produits animaux comestibles, en fonction de l'utilisation prévue comme, par exemple, pour les tissus musculaires, le foie, les rognons et la graisse (tissus adipeux) des animaux d'abattage, pour la graisse et la peau de volailles (et des porcins, le cas échéant) dans des proportions naturelles, pour les muscles et la peau du poisson dans des proportions naturelles, ainsi que pour le lait, les œufs et le miel.

Pour les médicaments vétérinaires, le JECFA formule à l'heure actuelle des recommandations pour les LMR qui se fondent sur les estimations d'ingestion chronique calculées sur les valeurs médianes de résidus et sur l'approche théorique d'un panier d'aliments (comprenant 300 g de tissu musculaire, 100 g de foie, 50 g de rognon, 50 g de graisse, 1500 g de lait, 100 g d'œufs et 20 g de miel). Ces valeurs visent à donner une estimation prudente de l'ingestion quotidienne de résidus, ce qui désigné sous le nom de dose journalière estimée (DJE). La dose journalière maximale théorique (DJMT) précédemment utilisée se fondait sur la LMR pour procéder à une estimation ponctuelle, ce qui correspond à une valeur unique représentant la limite supérieure du percentile de la distribution des teneurs en résidus. Le JECFA a conclu que cette méthode n'était pas réaliste et que toutes les concentrations dans la distribution des teneurs en résidus lors de l'estimation de l'ingestion chronique devaient être prises en compte. Lorsque la qualité des données n'est pas assez robuste pour pourvoir estimer un taux médian d'ingestion ou une médiane du résidu, on peut utiliser la DJMT pour obtenir une estimation prudente de l'ingestion.

Le JECFA peut formuler des recommandations complètes relatives aux LMR pour un médicament vétérinaire dans les tissus comestibles d'animaux précis sur la base d'une DJA et de données adéquates sur les résidus. Des LMR temporaires peuvent être recommandées soit lorsqu'une DJA a été fixée mais qu'il manque des données adéquates sur les résultats ou sur la performance des méthodes analytiques, soit lorsque la DJA est temporaire. Le Comité peut recommander des LMR « non spécifiées » ou « non nécessaires » lorsque la marge de

sécurité entre l'estimation de la consommation de résidus et la DJA est très importante.

Principes liés à des groupes de substances spécifiques

De nombreuses substances évaluées par le JECFA sont contenues dans les aliments en faibles concentrations, ce qui est le cas, par exemple, des aromatisants, des auxiliaires technologiques, des solvants d'extraction et des enzymes utilisés dans la production alimentaire. Pour évaluer ces substances, il peut être plus approprié d'appliquer les méthodes décrites ci-après.

Le concept de seuil de problème toxicologique (SPT; TTC en anglais) est l'une de ces approches. Ce concept repose sur le postulat que la toxicité est une fonction à la fois de la structure chimique et du degré d'exposition. Il permet aux évaluateurs des risques de fournir des avis scientifiques lorsque l'on peut affirmer qu'il existe une forte probabilité de dommages négligeable en se basant uniquement sur la faible exposition alimentaire et sur la structure chimique. Cela ne doit pas remplacer les procédures d'évaluation des risques appliquées par le JECFA et la JMPR pour les substances sur lesquelles on dispose de nombreuses données de toxicité.

L'approche SPT, telle qu'appliquée par le JECFA, utilise des valeurs seuils (valeurs SPT) pour trois classes structurelles de substances chimiques, en dessous desquelles le risque de générer des problèmes de santé à l'homme est très faible. Ces valeurs SPT sont établies à partir des données de toxicité existantes concernant les substances chimiques qui ont été classifiées dans l'une des trois classes structurelles. Les valeurs SPT pour les classes structurelles I, II et III sont 1800, 540 et 90 µg/personne par jour, respectivement. Étant donné que les valeurs seuil d'exposition humaine sont comparées à une exposition connue ou anticipée, l'approche SPT nécessite une estimation précise de l'exposition humaine.

Le JECFA a mis au point l'approche de l'arbre de décision (Procédure d'évaluation de l'innocuité des agents aromatisants) pour appliquer le concept SPT aux substances aromatisantes. Lorsque la procédure a été initialement adoptée, le JECFA a décidé que l'approche utilisée afin d'estimer les expositions d'origine alimentaire pour les

consommateurs d'agents aromatisants devait, pour être pratique et réaliste, s'appuyer sur des données relatives aux volumes de production annuelle pour différentes régions. Cette estimation, désignée sous le nom d'apport maximum dérivé d'une enquête (MSDI en anglais), était calculée à partir des chiffres de la production annuelle totale d'agents aromatisants, avec une correction pour tenir compte du fait que toutes les substances chimiques produites n'étaient pas déclarées et en supposant que l'agent aromatisant n'était consommé que par 10% de la population considérée.

Le JECFA a noté que l'utilisation du MSDI pouvait conduire à sous-estimer l'exposition alimentaire des consommateurs réguliers de certains aliments contenant un aromatisant. Une nouvelle méthode complémentaire a donc été établie pour estimer l'exposition alimentaire aux aromatisants, appelée technique d'exposition basée sur une portion unique (SPET en anglais). L'estimation SPET suppose une consommation journalière d'une portion unique d'un aliment contenant l'agent aromatisant, en fonction des niveaux additionnés fournis par l'industrie. La SPET identifie toutes les catégories d'aliments susceptibles de contenir l'agent aromatisant, attribue un niveau additionné à une portion unique "standard" de chacune de ces catégories, et identifie la catégorie individuelle d'aliments susceptible de contribuer à l'exposition alimentaire la plus élevée. La portion standard est censée représenter la consommation alimentaire moyenne pour les consommateurs de cette catégorie d'aliments, en assumant une consommation journalière sur une longue période de temps. La portion standard ne reflète pas les volumes élevés de consommation alimentaire signalés dans les enquêtes alimentaires nationales pour la catégorie d'aliments, et donne donc une prévision plus réaliste des habitudes alimentaires à long terme. Le JECFA a conclu que les estimations de l'exposition alimentaire du MSDI et de la SPET fournissent des informations différentes et complémentaires. La Procédure utilisera la valeur la plus élevée des deux estimations de l'exposition alimentaire (MSDI ou SPET).

Le JECFA a envisagé d'appliquer l'approche STP à la caractérisation des risques non seulement des substances aromatisantes, mais aussi d'autres substances présentes dans les aliments en petites quantités. Pour élargir la portée de l'approche STP, le Comité a noté qu'il conviendrait de l'utiliser conjointement aux estimations prudentes

de l'exposition alimentaire et qu'il serait sans doute nécessaire de recueillir des données supplémentaires sur la toxicité de substances structurellement liées. Le Comité a également recommandé que des orientations soient formulées sur l'application de l'approche STP aux substances contenues dans les aliments en petites quantités, telles que certains résidus d'auxiliaires technologiques, de matériaux d'emballage et de contaminants, afin de fournir des avis sur l'évaluation des risques de substances pour lesquelles des données toxicologiques complètes ne sont pas disponibles ou ne sont pas nécessaires.

L'évaluation de l'innocuité des matériaux d'emballage des produits alimentaires soulève des problèmes particuliers en raison du très grand nombre de matériaux utilisés, du faible niveau anticipé de migration des substances à partir de matériaux en contact avec les aliments, et de la faible exposition alimentaire résultante. En principe, il existe deux possibilités pour effectuer les évaluations du matériel entrant au contact des aliments. L'une consiste à requérir des données toxicologiques, quel que soit le niveau d'exposition alimentaire potentiel, afin de procéder à une évaluation de l'innocuité. L'autre consiste à appliquer une approche progressive dans laquelle la quantité de données toxicologiques requises est liée au degré d'exposition prévu, sur la base des études de migration.

Les auxiliaires technologiques se composent de diverses substances, y compris mais non de façon limitative, les solvants de support ou d'extraction et les enzymes utilisés lors de la transformation des denrées alimentaires. Le JECFA a élaboré des principes et des procédures pour évaluer l'innocuité des préparations enzymatiques qui sont régulièrement mis à jour.

L'évaluation de l'innocuité des substances consommées en assez grandes quantités, telles que les édulcorants de charge, les amidons transformés, les éléments nutritifs et substances apparentées, ainsi que les aliments complets non traditionnels, présente certains problèmes particuliers. L'évaluation de l'innocuité de ces substances diffère de celle des autres additifs alimentaires en raison d'un niveau élevé d'exposition alimentaire; les composants mineurs et les impuretés de transformation peuvent donc jouer un rôle plus d'important qu'ailleurs.

L'utilisation croissante d'aliments enrichis et de compléments alimentaires, en particulier d'aliments composés et d'aliments dits « fonctionnels », se traduit par une augmentation de l'ingestion de substances nutritives dans le monde entier. Le JECFA n'évalue que l'innocuité de ces ingrédients sur la base des principes et méthodes décrits dans la présente monographie et a précisé que les évaluations ne doivent pas être considérées comme une approbation de l'utilisation de ces substances pour leurs bénéfices revendiqués sur la nutrition ou la santé.

Les nutriments sont essentiels sur le plan biologique et il a été prouvé qu'ils sont bénéfiques pour la santé lorsqu'ils sont pris certains niveaux d'apport. Cette constatation influe sur les approches appliquées pour ajuster l'incertitude associée aux données utilisées pour estimer une valeur recommandée en fonction de critères sanitaires et nécessite de prendre en compte les mécanismes homéostatiques spécifiques aux substances nutritives essentielles. Il faut donc introduire des modifications dans l'approche classique d'évaluation des risques qui ne sont pas liés à des nutriments. Au niveau international, les orientations relatives à l'évaluation des risques des nutriments et des substances apparentées recommandent d'utiliser les apports maximums (UL en anglais), en sus d'un apport minimum pour divers segments de la population nécessaire pour éviter les carences nutritionnelles. La limite supérieure d'apport correspond à l'estimation du niveau d'apport régulier le plus élevé qui ne produit pas d'effets indésirables notables sur la santé. La limite supérieure d'apport peut être définie pour les nutriments en appliquant les mêmes principes d'évaluation des risques que ceux établis pour les agents biologiques et chimiques.

Les aliments provenant de nouvelles sources comprennent les aliments conventionnels et non conventionnels, les nouveaux aliments et les aliments utilisés à des fins alimentaires spécifiques. Il est nécessaire de disposer de spécifications pour garantir que la teneur des contaminants susceptibles de présenter un danger, tels que les mycotoxines et les métaux lourds, est maintenue à un niveau minimum. L'influence de l'introduction de la nouvelle substance dans la composition en nutriments du régime alimentaire dans son ensemble doit être déterminée, notamment pour les groupes composés d'enfants et de personnes âgées, ainsi que pour les personnes hospitalisées et les enfants en âge scolaire. La valeur nutritionnelle du nouvel élément doit être

évaluée initialement à partir de sa composition chimique en considérant à la fois les macronutriments et les micronutriments, en tenant compte des incidences des activités de transformation ou de stockage ultérieurs. Selon la nature et les utilisations prévues de l'aliment nouveau, il pourra être nécessaire d'effectuer des études sur des animaux de laboratoire pour compléter les études chimiques. Les études chez l'homme portant sur les aliments nouveaux doivent être déterminées au cas par cas. L'expérience sur l'homme est une partie essentielle de la collecte des données dans l'historique de l'utilisation. L'exposition, pour les aliments nouveaux, devra être déterminée en fonction des utilisations proposées. L'approche MOE, quant à elle, peut convenir à la caractériser les risques que présentent les nouveaux aliments.

RESUMEN

El Comité Mixto FAO/OMS (Organización de las Naciones Unidas para la Alimentación y la Agricultura/Organización Mundial de la Salud) de Expertos en Aditivos Alimentarios (JECFA) y la Reunión Conjunta FAO/OMS sobre Residuos de Plaguicidas (JMPR) aplican los mismos métodos y principios generales para determinar el riesgo de origen químico (publicados en los informes de ambos comités). En respuesta a las recomendaciones del JECFA y la JMPR formuladas en la década de 1980, de examinar la validez de los procedimientos de evaluación en uso en ese momento, el Programa Internacional de Seguridad de las Sustancias Químicas (IPCS) promovió la preparación de monografías (monografías EHC) sobre Principios para la evaluación de inocuidad de aditivos y contaminantes en los alimentos (EHC 70) y Principios para la evaluación toxicológica de residuos de plaguicidas en los alimentos (EHC 104). Estas monografías y los principios expuestos en informes posteriores han servido de base para las evaluaciones realizadas por el JECFA y la JMPR.

Si bien gran parte de las recomendaciones formuladas en las monografías EHC 70 y 104 siguen siendo válidas, desde su publicación se han producido avances importantes en los métodos de análisis químico y toxicológico, y en la determinación de la exposición alimentaria y el riesgo derivado de la presencia de sustancias químicas en los alimentos. Por esta razón, la FAO y la OMS lanzaron un proyecto para actualizar, armonizar y consolidar los principios y los métodos aplicados por el JECFA y la JMPR para la determinación del riesgo derivado de la presencia de aditivos, contaminantes, sustancias tóxicas naturalmente presentes, residuos de plaguicidas y de medicamentos de uso veterinario en los alimentos. Esta monografía es producto de ese proyecto.

El objetivo de esta monografía es doble: 1) proporcionar una guía descriptiva para que el JECFA y la JMPR continúen garantizando una evaluación transparente, fundamentada y realizada por expertos de los datos científicos, en las determinaciones del riesgo de origen químico en los alimentos y 2) brindar información a los usuarios de los productos del JECFA y la JMPR, como los gestores de riesgo y

otros organismos y autoridades que se ocupan de la determinación del riesgo en los Estados Miembros.

La monografía aborda los temas clave que el JECFA y la JMPR toman en cuenta en sus determinaciones de riesgos de origen químico en los alimentos, que se resumen a continuación.

Determinación del riesgo y su papel en el análisis del riesgo

El análisis de riesgo tiene tres componentes: la determinación, la gestión y la comunicación del riesgo. La determinación del riesgo es el componente más importante del análisis y proporciona la base científica para la adopción de decisiones de gestión del riesgo, medidas que pueden ser necesarias para proteger la salud humana. En la determinación del riesgo se toma en cuenta toda la información científica relevante disponible y se identifican incertidumbres en la base de conocimientos. La determinación del riesgo consta de cuatro pasos: identificación del peligro, caracterización del peligro (incluye la determinación de la relación dosis-respuesta), determinación de la exposición y caracterización del riesgo. Se trata de un marco conceptual que, en el contexto de la inocuidad química de los alimentos, provee un mecanismo de examen estructurado de la información relevante para establecer las posibles consecuencias para la salud de la exposición a sustancias químicas presentes en los alimentos.

El principal trabajo del JECFA y la JMPR es determinar el riesgo de origen químico en los alimentos. Siguiendo las recomendaciones de estos dos comités, los países (en el plano nacional) y la Comisión del Codex Alimentarius (en el plano internacional) toman medidas para garantizar la inocuidad de los alimentos. El JECFA y la JMPR basan sus evaluaciones en principios científicos y garantizan la necesaria coherencia de las determinaciones del riesgo, mientras que la CCA y sus comités pertinentes que se ocupan de las sustancias químicas en los alimentos son responsables, en su función de gestores del riesgo, de adoptar las decisiones finales sobre los límites máximos de residuos de plaguicidas y medicamentos de uso veterinario, contaminantes y aditivos admisibles en los alimentos, y de adoptar otras medidas relacionadas.

Si bien es conveniente separar las actividades de determinación del riesgo de las de gestión del riesgo para garantizar la independencia de

criterio científico, es sabido que en el proceso de determinación del alcance del análisis, en particular durante la formulación del problema, los gestores deben relacionarse e interactuar con quienes determinan el riesgo. Por lo tanto, existe una relación de interacción, y a menudo de iteración, entre la determinación y la gestión del riesgo.

Caracterización de sustancias químicas, métodos analíticos y elaboración de especificaciones

Esta sección de la monografía se refiere a la información sobre la sustancia química, necesaria para la determinación del riesgo. Esta información también es un prerrequisito para la vigilancia y el control de las sustancias químicas en los alimentos.

El JECFA y la JMPR revisan los métodos analíticos propuestos y deciden si son apropiados para uso internacional. Los métodos analíticos son necesarios, por ejemplo, para establecer en qué forma están presentes los contaminantes, determinar las concentraciones de una sustancia química y de sus metabolitos en los estudios farmacocinéticos, toxicocinéticos y de eliminación de residuos, y calcular las concentraciones de contaminantes y de residuos de medicamentos de uso veterinario y plaguicidas presentes en los alimentos. La monografía describe las principales características de los métodos analíticos apropiados y los criterios de validación de esos métodos.

Especificaciones para los aditivos alimentarios

Las especificaciones sobre identidad y pureza de los aditivos alimentarios son un producto necesario de las evaluaciones de inocuidad que realiza el JECFA. Las evaluaciones dependen de estudios realizados con una sustancia o producto químico de identidad, pureza y forma definidos. La evaluación de inocuidad es válida sólo para productos que no difieran sustancialmente, en términos de identidad y perfil de calidad, del material utilizado para generar los datos que se usan en la evaluación.

Plaguicidas

La Reunión Conjunta FAO/OMS sobre Especificaciones de Plaguicidas (JMPS) elaboró especificaciones para formulaciones y materiales destinados a aplicaciones técnicas (grado técnico). La

JMPR toma en cuenta las especificaciones del JMPS cuando valora la inocuidad. La JMPR examina los métodos analíticos utilizados para la generación de datos sobre residuos, con el fin de verificar que sean apropiados para los analitos y tipos de muestras relevantes. También difunde información sobre métodos apropiados para promover el cumplimiento de los límites máximos de residuos (LMR) y si algunos compuestos en particular se podrían analizar con métodos multi residuo.

Residuos de medicamentos de uso veterinario

En todos los casos, el JECFA debe asegurarse de que el medicamento de uso veterinario que va a evaluar está bien caracterizado, que se hayan detallado sus propiedades físicas y químicas, y la identidad y concentraciones de las principales impurezas. Además, debe contar con una descripción del proceso de fabricación y datos que demuestren la homogeneidad y calidad de los productos finales.

Se debe determinar la forma y distribución de los residuos derivados del método de aplicación autorizado en cada especie, y estudiar la eliminación de los residuos en los tejidos comestibles o alimentos de origen animal. Es preciso identificar un marcador, que habitualmente es la forma del fármaco (compuesto precursor o metabolito) que se halla en concentraciones más elevadas durante un periodo más prolongado. Se establece la relación entre este marcador y las concentraciones residuales totales del fármaco.

Contaminantes

Los datos requeridos para caracterizar un contaminante incluyen la concentración en los alimentos y en la dieta total de la mayor cantidad posible de países. Los datos se deben organizar de acuerdo con el Sistema Mundial de Vigilancia del Medio Ambiente/Programa de Vigilancia y Evaluación de la Contaminación de los Alimentos (SIMUVIMA/Alimentos), para facilitar las comparaciones y el control de calidad. Se deben incluir detalles adicionales sobre los planes de muestreo y los métodos analíticos utilizados para generar los datos.

Sustancias que se consumen en grandes cantidades

Se deben realizar análisis químicos exhaustivos de las sustancias de alto consumo, como los principales aditivos, para identificar posibles

impurezas y proporcionar información sobre su idoneidad nutricional, en particular cuando estas sustancias sustituyen a los alimentos tradicionales. Como es posible que la exposición a impurezas perjudiciales (como metales pesados) a raíz de la ingestión de sustancias que se consumen en grandes cantidades sea alta, se deben realizar esfuerzos especiales para identificar y cuantificar las impurezas.

Identificación y caracterización del peligro: estudios toxicológicos y en humanos

Alcance y elección de los métodos de análisis

En general, los estudios toxicológicos se pueden dividir en 1) estudios *in vitro*, en los que usan microorganismos cultivados, células o preparados de tejidos de animales de laboratorio o de humanos y 2) estudios *in vivo* en animales de laboratorio o humanos. Estos estudios se realizan con diferentes propósitos; entre ellos, identificar los posibles efectos adversos (identificación del peligro), definir las condiciones de exposición necesarias para producir esos efectos y determinar las relaciones dosis-respuesta en los efectos adversos (caracterización del peligro). El JECFA y la JMPR toman en cuenta ambos tipos de estudios en sus determinaciones del riesgo.

La idea de que se deben reducir, perfeccionar o sustituir las pruebas con animales toda vez que sea posible es ampliamente aceptada, y en consecuencia ha aumentado el uso de enfoques alternativos y se han mejorado los diseños de los estudios. Asimismo, es importante que se utilicen métodos y enfoques científicamente válidos para los ensayos de inocuidad química de alimentos. Por lo tanto, y aunque se han realizado avances en el desarrollo de pruebas *in silicio* e *in vitro,* en este momento no es posible prescindir de los ensayos con animales en relación con la mayoría de las variables de valoración relevantes. Si bien ninguna especie experimental es un modelo ideal, los datos indican que los estudios en animales por lo general son un medio apropiado para determinar la posible toxicidad para los humanos de las sustancias presentes en los alimentos, siempre que los datos se interpreten de forma crítica.

Algunas organizaciones internacionales reconocidas, como la Organización de Cooperación y Desarrollo Económicos (OCDE), han elaborado normas mínimas para el diseño y la realización de estudios

toxicológicos. Con este marco de referencia se evalúa la idoneidad del diseño y la realización de los estudios destinados a determinar el riesgo de la presencia de una sustancia en los alimentos, que preferiblemente se deben realizar de acuerdo con los principios de buenas prácticas de laboratorio. La monografía también analiza los recientes avances promisorios en protocolos de ensayos que aún no han sido formalmente aceptados por la OCDE.

El estudio de la absorción, la distribución, el metabolismo y la excreción (ADME) de una sustancia en una fase temprana del proceso es importante para seleccionar la especie apropiada y la dosis adecuada para los ensayos de toxicidad. Allí donde sea posible, la investigación de las diferencias cuantitativas y cualitativas de ADME entre la especie del ensayo y los seres humanos generará información importante para la caracterización del peligro.

El alcance de los ensayos toxicológicos depende de la naturaleza y el uso de la sustancia que se esté estudiando. No todos los ensayos que se mencionan en la monografía necesariamente se deben realizar para llegar a una conclusión en cuanto al riesgo de una sustancia en particular. También se analizan enfoques graduales, en los que se realizan pruebas de detección sistemática o un número limitado de estudios de toxicidad comunes, que pueden bastar para determinar el riesgo o señalar la necesidad de realizar otras investigaciones.

Por lo general, para determinar la toxicidad sistémica general se realizan ensayos de corto y largo plazo. Estos estudios permiten identificar los órganos diana de la toxicidad y pueden indicar la necesidad de realizar otras pruebas o pruebas más específicas (por ejemplo, de neurotoxicidad o inmunotoxicidad). Se examinan los efectos de la sustancia pertinente en relación con un amplio conjunto de parámetros de carácter observacional, funcional, bioquímico y patológico. Habitualmente, los estudios se realizan en dos especies, un roedor y un animal que no sea roedor o dos especies de roedores, y en animales de ambos sexos, para maximizar la posibilidad de hallar algún efecto (identificación del peligro). A menudo, los ensayos de largo plazo también incluyen pruebas de carcinogenicidad en dos especies de roedores. El uso de un método alternativo, para reemplazar a un roedor, puede ser aceptable según el caso. Se han incorporado diferentes pruebas alternativas de carcinogenicidad, en las cuales se

intensifican las respuestas carcinogénicas y, por lo tanto, se acorta la duración de las biovaloraciones; por ejemplo, el modelo del ratón neonato y modelos de iniciación-promoción y ratones transgénicos.

Los ensayos se deben llevar a cabo del modo que mejor permita relacionarlos con las condiciones de la exposición humana. En la selección de la dosis se debe tomar en cuenta la exposición humana prevista, su frecuencia y duración. En el caso de sustancias presentes en los alimentos, habitualmente se realizan ensayos en los que se administran dosis repetidas de la sustancia a los animales, en general con la comida, a través de una sonda o con el agua. Teóricamente, las dosis más altas de las seleccionadas producen efectos tóxicos pero no matan ni generan sufrimiento extremo, las dosis menores provocan respuestas graduadas y las más bajas no generan efectos adversos. El estudio debe estar diseñado de modo que permita obtener un punto de referencia para la caracterización del peligro, que también se llama punto de partida, que puede ser un nivel con el que no se observan efectos adversos (*no observed adverse effect level*, NOAEL) o una dosis de referencia (DR), que es la que provoca una respuesta adversa moderada pero medible.

En el diseño de todos los estudios se debe prestar especial atención al intervalo entre dosis, el número de grupos de estudio, la dosis máxima utilizada, la cantidad de animales de uno y otro sexo en cada grupo de dosis, la elección de los controles, la pauta de administración, la confirmación de la dosis administrada en relación con la nominal y la dosis ingerida (aceptabilidad, desperdicio de comida).

Además de los ensayos de toxicidad sistémica general, se debe evaluar la posible genotoxicidad de una sustancia mediante un conjunto de pruebas *in vitro* y, si es necesario, *in vivo*. El estudio completo de la posible genotoxicidad de una sustancia exige información sobre su capacidad de inducir mutaciones genéticas, aberraciones cromosómicas estructurales y aneuploidía. Por lo general se opta por un conjunto reducido de ensayos *in vitro* validados que cubren parámetros genéticos diferentes. La batería de pruebas comúnmente utilizadas incluye un ensayo de mutagenicidad en bacterias (prueba de Ames, *Salmonella*/microsoma) y una o dos pruebas en células de mamíferos que permiten detectar mutaciones puntuales o daño cromosómico (efecto clastogénico/aneugénico).

Usualmente, también se determinan los efectos de la sustancia en el rendimiento reproductivo de machos y hembras y en el desarrollo prenatal y posnatal de las crías. El propósito de los estudios de toxicidad para la función reproductora y el desarrollo es determinar 1) posibles efectos que se puedan manifestar en la reducción de la fertilidad o fecundidad de los progenitores o los descendientes, a causa de alteraciones morfológicas, bioquímicas, genéticas o fisiológicas y 2) si el crecimiento y el desarrollo de los descendientes es normal. No obstante, las pruebas para determinar la toxicidad para la función reproductora y el desarrollo no cubren necesariamente todos los efectos que podrían producir las sustancias químicas que interfieren con el sistema endocrino. En el momento de publicar esta monografía continuaba el desarrollo de una batería de pruebas de detección sistemática para evaluar las sustancias químicas que interactúan con las vías de señalización de los estrógenos, los andrógenos y la tiroides.

También es preciso considerar la necesidad de realizar pruebas de toxicidad aguda. Algunas sustancias (por ejemplo, determinados metales, micotoxinas, residuos de medicamentos de uso veterinario y residuos de plaguicidas) pueden tener efectos agudos relacionados con la ingestión durante periodos breves. El JECFA incluye en sus evaluaciones una determinación de efectos agudos y, cuando es pertinente, la posibilidad de que estos efectos agudos se produzcan en individuos sensibles. En la actualidad, habitualmente la JMPR también analiza la necesidad de fijar una dosis de referencia aguda (DRA) para todos los plaguicidas que evalúa. La JMPR has elaborado directrices para estudios en animales de experimentación con una sola dosis, para que el cálculo de la DRA fuera más preciso; la OCDE ha tomado estas directrices como base para una guía que está elaborando.

Algunas veces son necesarias otras pruebas sobre efectos nutricionales, neurotoxicidad (incluidos los efectos neuroconductuales en adultos y durante el desarrollo) e inmunotoxicidad. La necesidad de estas pruebas adicionales puede surgir de los resultados de las pruebas estándar descritas más arriba. Los estudios específicos de los mecanismos de toxicidad o los mecanismos de acción pueden proporcionar otros datos útiles para la evaluación.

Interpretación de los resultados

La evaluación crítica de los diseños y los datos de los estudios, y la interpretación crítica de los resultados, son los pasos más importantes de la determinación del riesgo. Habitualmente se comparan los datos obtenidos de los grupos tratados con los de los controles correspondientes. A veces, para comprender la significación de un hallazgo específico, también es preciso comparar los datos de la prueba con los de controles históricos, en particular cuando se trata de carcinogenicidad y toxicidad para el desarrollo.

La determinación de numerosos parámetros toxicológicos se basa en el peso de las pruebas y se utilizan los datos de todos los estudios disponibles en los que se hayan estudiado líquidos, células, tejidos u órganos iguales o funcionalmente relacionados. Los resultados similares en diferentes estudios y la demostración de relaciones dosis-respuesta le dan un valor agregado a la caracterización del peligro.

Cuando se trata de establecer si un compuesto es genotóxico o no lo es, es necesario realizar una evaluación general de los datos disponibles. Por lo general, se considera que resultados concluyentemente negativos en una batería de pruebas *in vitro* bastan para concluir que una sustancia no tiene poder genotóxico, a menos que haya elementos que preocupen especialmente (por ejemplo, exposición importante o continuada de los humanos, consideraciones de orden estructural). A la inversa, uno o más resultados positivos en las pruebas *in vitro* generalmente exigen un seguimiento mediante pruebas de genotoxicidad *in vivo.* El resultado de las pruebas de genotoxicidad se debe analizar junto con los resultados experimentales de los bioensayos de carcinogenicidad en roedores, porque los resultados de las pruebas de corto plazo por sí solos no so fiables para establecer si una sustancia química es carcinogénica en los roedores o no lo es. Los estudios de genotoxicidad positivos sí brindan información sobre el mecanismo de acción de las sustancias carcinógenas e influyen en el enfoque que de la caracterización del peligro posterior. Los resultados positivos en los bioensayos de cáncer en roedores exigen una interpretación cuidadosa respecto del mecanismo de acción, las posibles diferencias entre especies en relación con la incidencia de base y con la respuesta, y también de la extrapolación de los datos obtenidos con altas dosis a las dosis bajas. El IPCS ha elaborado un marco conceptual para la

evaluación del mecanismo de acción de la carcinogenia por sustancias químicas en especies de animales de laboratorio, que posteriormente se amplió para abordar el problema de la relevancia para los humanos de los datos sobre cáncer obtenidos en animales. Los mecanismos relevantes para los humanos son, entre otros, reactividad del ácido desoxirribonucleico y genotoxicidad. Se identificaron mecanismos que no son relevantes en los seres humanos, como nefropatía inducida por α2u-microglobulina y proliferación de peroxisomas en las ratas.

En la interpretación de los datos de estudios de toxicidad para la función reproductora y el desarrollo, es importante buscar patrones de respuesta biológicamente relacionados y la relación de los resultados con las distintas variables de valoración, y vincular cualquier hallazgo con los datos toxicológicos disponibles de otros estudios. Como los protocolos habituales de los estudios exigen que la dosis más alta tenga algún efecto mínimo de toxicidad materna, a veces es difícil determinar si el efecto en el desarrollo observado con esa dosis es el resultado directo de la acción de la sustancia química en el embrión o el feto, o un resultado indirecto de la alteración de la homeostasis materna. Si bien ha habido algunos ejemplos de esto último, es importante no deducir una relación causal de la asociación de toxicidad para el desarrollo y toxicidad materna sin realizar pruebas y evaluaciones adicionales.

Alergia a los alimentos y otros tipos de hipersensibilidad

Las alergias alimentarias son la consecuencia de una respuesta inmunitaria adversa o incontrolada a un antígeno alimentario en personas susceptibles. Ocurren cuando el organismo interpreta erróneamente que una proteína alimentaria es una sustancia "extraña", lo que genera un aumento de la respuesta del sistema inmunitario. Las alergias se desarrollan mediante un proceso de sensibilización. En la fase de sensibilización, la exposición al alérgeno alimentario estimula la producción de inmunoglobulina E específica para el antígeno.

La determinación del riesgo de alergia alimentaria es una disciplina relativamente nueva, y no hay consenso generalizado acerca de cómo se debería realizar, aunque se han sugerido diferentes enfoques. Por ejemplo, en la actualidad no hay consenso respecto del umbral de dosis por debajo del cual la sensibilización a los alérgenos no

ocurriría. Se han descrito abordajes del tipo árbol de decisiones para predecir los posibles efectos alergénicos de las nuevas proteínas alimentarias, como las de los alimentos genéticamente modificados.

Principios generales de los estudios en humanos

Los datos provenientes de estudios en humanos pueden ser importantes para identificar y caracterizar peligros y evaluar los riesgos derivados de los aditivos alimentarios, los contaminantes y los residuos de medicamentos de uso veterinario y plaguicidas. La información puede provenir de experimentos controlados con voluntarios, estudios de vigilancia, estudios epidemiológicos (por ejemplo, estudios ecológicos, estudios de casos y controles, de cohortes, analíticos o de intervención) realizados en poblaciones con distintos niveles de exposición, estudios experimentales o epidemiológicos en subgrupos particulares, informes clínicos (por ejemplo, de intoxicación) o estudios de casos individuales. Los criterios de valoración pueden ser la inocuidad o la tolerancia, los efectos nutricionales y funcionales de los alimentos o componentes de alimentos, el metabolismo y la toxicocinética de la sustancia, el mecanismo de acción, posiblemente usando biomarcadores para los efectos identificados en estudios de animales, y efectos adversos para la salud derivados de la exposición accidental (por ejemplo, a un contaminante).

Los controles éticos, profesionales y legales que establecen si un estudio en humanos es necesario y en qué circunstancias se puede realizar apropiadamente son fundamentales en cualquier estudio experimental en seres humanos. La cantidad de personas incluidas en un estudio debe ser suficiente para lograr los objetivos de la investigación. Se debe considerar si es posible utilizar sólo tejidos humanos *ex vivo* o *in vitro*. Los experimentos en células o tejidos humanos o en los que se usan otras preparaciones que contienen o expresan enzimas, receptores y otros factores subcelulares *in vitro* difieren sustancialmente de los estudios en personas, porque no permiten registrar la absorción, la distribución, ni aspectos del metabolismo integral y la excreción. No obstante, tienen una ventaja y es que permiten estudiar el mecanismo en condiciones controladas e imposibles en la clínica, y son considerablemente útiles para sugerir vías metabólicas y mecanismos de respuesta que podrían ser importantes en humanos y valdría la pena estudiar como biomarcadores de exposición o efecto.

Consideraciones sobre el tubo gastroentérico, incluidos los efectos sobre la microflora intestinal

Se deben tomar en cuenta las interacciones que puede haber entre las sustancias químicas presentes en los alimentos y la flora bacteriana del aparato gastrointestinal, en términos tanto de los efectos de la flora intestinal en la sustancia química como de dicha sustancia en la flora intestinal.

Los métodos de estudio *in vivo* de la función de la microflora intestinal en el metabolismo de una sustancia incluyen 1) administración parenteral del compuesto, que debería provocar una disminución del metabolismo microbiano de los compuestos polares escasamente absorbidos, en relación con la dosificación por vía oral; 2) estudios en animales en los que se ha reducido la flora bacteriana por el uso de antibióticos, y 3) estudios en animales exentos de microorganismos patógenos y en animales (anteriormente) exentos de microorganismos patógenos a los que se les han inoculado cepas bacterianas conocidas (animales notobióticos). Diferentes factores pueden influir en la activación metabólica de sustancias químicas extrañas por la microflora del hospedador, entre ellos la especie del hospedador, la alimentación, la medicación y la adaptación metabólica. Además, existen distintos métodos *in vivo* e *in vitro* para determinar la capacidad de una sustancia de generar resistencia en la microflora intestinal después de la ingestión de sustancias o residuos con propiedades antimicrobianas.

Determinación de la relación dosis-respuesta

La determinación de la relación dosis-respuesta es un componente fundamental de la caracterización del peligro en el marco del paradigma de la determinación del riesgo. La relación dosis-respuesta se usa para elaborar recomendaciones para la determinación del riesgo y deducir valores guía para la exposición basados en criterios de salud.

Por lo general, se utilizan dos enfoques: 1) análisis que proporcionan una estimación cuantitativa o cualitativa del riesgo, y 2) análisis que establecen valores guía para límites de exposición basados en criterios de salud, como la ingestión diaria admisible (IDA) o la ingestión diaria tolerable (IDT), que son niveles de exposición humana que se considera "no implican un riesgo apreciable para la salud". La IDT

generalmente se utiliza para los contaminantes; mientras que la IDA cuando se puede controlar la exposición, como es el caso de los aditivos alimentarios y los residuos de plaguicidas y medicamentos de uso veterinario. Los enfoques para la determinación de la relación dosis-respuesta aplicados a los datos de estudios en animales han sido analizados en Criterios de Salud Ambiental 239 (EHC 239) "Principios para modelar la relación dosis-respuesta en la determinación del riesgo de origen químico".

Establecer la presencia o ausencia de una relación causa-efecto es uno de los principales aspectos de la determinación del riesgo. Cuando esta relación es bastante probable, los datos sobre la relación dosis-respuesta son esenciales. Estos datos pueden provenir de estudios *in vivo* en animales de laboratorio o en humanos, que usualmente sientan las bases para la caracterización del riesgo. En todos los casos, generalmente la interpretación de los datos sobre efectos requiere conocer los niveles de exposición que no producen un efecto medible y la relación entre el aumento de la exposición y el aumento de la frecuencia o la gravedad del efecto.

El modelado de la relación dosis-respuesta se puede resumir en seis pasos básicos. Los primeros cuatro (selección de los datos, selección del modelo, coordinación estadística y estimación de parámetros) se relacionan con el análisis de los datos sobre la relación dosis-respuesta. En este análisis, los datos relativos a la relación dosis-respuesta observada se modelan de manera que permitan predecir la probable magnitud de la respuesta a una dosis determinada, dentro del intervalo de la dosis-respuesta observada o fuera de él, o establecer qué dosis probablemente causa una respuesta de una magnitud determinada. Los últimos dos pasos se relacionan con la aplicación y la evaluación de los resultados del análisis.

La extrapolación es necesaria en todas las determinaciones del riesgo. En la mayoría de los casos analizados por el JECFA y la JMPR, los datos para la determinación de la relación dosis-respuesta provienen de experimentos en animales de laboratorio a los que se les administran dosis considerablemente superiores a las posibles en caso de exposición humana. En este análisis de dosis-respuesta, se plantean dos cuestiones en relación con la extrapolación: 1) la extrapolación de los datos obtenidos de animales de experimentación a humanos,

y 2) las posibles diferencias entre humanos respecto de la respuesta. En la monografía se discuten los diferentes métodos empleados para abordar estas cuestiones, que van desde el uso de factores de incertidumbre hasta esquemas de modelado más complejos basados en las diferencias de orden toxicocinético y toxicodinámico entre humanos y animales de experimentación, y la variabilidad entre humanos.

Deducción de valores guía para límites de exposición basados en criterios de salud

Los valores guía para los límites de exposición basados en criterios de salud proporcionan información cuantitativa proveniente de la determinación del riesgo, que permite a los gestores de riesgos tomar decisiones para proteger la salud. Estos valores se calculan a partir de la determinación de la relación dosis-respuesta para las variables de valoración más importantes en las especies de mayor relevancia. El primer método, que es el que todavía utilizan más comúnmente el JECFA y la JMPR para calcular valores guía con el fin de proteger la salud contra efectos que se considera se producen cuando se rebasa un umbral de exposición, es definir el nivel sin efectos adversos observables (*no observed adverse effect level*, NOAEL) o, algunas veces, el nivel más bajo con efectos adversos observables (*lowest observed adverse effect level*, LOAEL) como punto de partida. El otro método es utilizar el límite inferior del intervalo de confianza unilateral de la dosis de referencia (*benchmark dose lower confidence limit*, BMDL) como punto de partida para calcular un valor guía para el límite de exposición basado en criterios de salud o un margen de exposición (ME). En ocasiones, la determinación de la relación dosis-respuesta se usa para definir la dosis asociada con un incremento insignificante de la respuesta (por ejemplo, de 1 en un millón) respecto de la referencia.

En el caso de los aditivos alimentarios y los residuos de plaguicidas y medicamentos de uso veterinario en los alimentos, el valor guía para los límites de exposición basado en criterios de salud se llama ingestión diaria admisible (IDA). El JECFA y la JMPR determinan la IDA sobre la base de los datos conocidos en el momento de la evaluación. Habitualmente, el JECFA establece la IDA en base al NOAEL más bajo relevante en las especies más sensibles. La IDA se expresa en cantidad (por ejemplo, mg) por kilogramo de peso corporal,

habitualmente como un intervalo que va de 0 a un límite superior. La expresión de la IDA por lo general es numérica y se usa sólo una cifra significativa. Cuando corresponde, la JMPR y el JECFA calculan una dosis de referencia aguda (DRA), una estimación de la cantidad de una sustancia presente en los alimentos, en el agua potable o en ambos (usualmente expresada en relación con el peso corporal) que se puede ingerir en un periodo de 24 hs o menos sin que se aprecie un riesgo para la salud del consumidor, de acuerdo con los datos conocidos en el momento de la evaluación.

En cuanto a los contaminantes de los alimentos que en general son inevitables, el JECFA ha usado para los valores guía el término "tolerable", que indica que la ingestión de contaminantes asociada con el consumo de alimentos por lo demás conformes a las normas de salubridad y nutritivos es aceptable. Los principios para calcular los niveles de ingestión tolerable son los mismos que para la IDA: se puede utilizar el método del NOAEL o de la DR como punto de partida para establecer valores guía para los límites de exposición basados en criterios de salud para los contaminantes. Los contaminantes de los alimentos pueden ser metales pesados, contaminantes medioambientales como dioxinas y micotoxinas, impurezas provenientes de los aditivos alimentarios, disolventes utilizados en el procesamiento de los alimentos, otras sustancias derivadas de procesos como el calentamiento, sustancias que migran de materiales en contacto con los alimentos y residuos derivados del uso de aditivos en los piensos o componentes inactivos de las formulaciones de uso veterinario. Los valores guía se pueden expresar en IDT, ingestión máxima diaria tolerable provisional (IMDTP), ingestión semanal tolerable provisional (ISTP) o ingestión mensual tolerable provisional (IMTP). El uso del término "provisional" indica que la evaluación es provisoria, cuando no hay suficientes datos fiables sobre las consecuencias de la exposición humana a niveles aproximados a los que el JECFA considera preocupantes. La IMDTP se fija para contaminantes alimentarios que se sabe no se acumulan en el organismo. En el caso de los contaminantes que sí se pueden acumular en el organismo con el tiempo, el JECFA ha utilizado la ISTP y la IMTP.

Los pasos clave del método del NOAEL para calcular valores guía para la exposición, basados en criterios de salud son la selección de los datos apropiados y la determinación del NOAEL. En el cálculo del

valor guía se aplica un factor de seguridad o incertidumbre al NOAEL, para dejar un margen de seguridad prudente por las incertidumbres inherentes a la extrapolación de los datos sobre toxicidad en animales de laboratorio a posibles efectos en humanos, y también por las variaciones entre humanos. Los términos "factor de seguridad" y "factor de incertidumbre" a menudo se utilizan de manera indistinta; históricamente se usó "factor de seguridad" pero en la actualidad se prefiere "factor de incertidumbre". Se ha incorporado el concepto de factor específico de ajuste químico para que se puedan usar datos específicos sobre las diferencias de orden toxicocinético o toxicodinámico entre distintas especies o entre humanos, para calcular, siempre que sea, posible factores de incertidumbre basados en los datos en lugar de aplicar factores por defecto.

El método del BMDL ha sido incorporado como alternativa al NOAEL. Con el BMDL se define un nivel de exposición que produce un efecto mínimo o un nivel de respuesta baja pero medible como punto de partida para la determinación del riesgo. Este método tiene algunas ventajas, entre ellas el uso de datos completos sobre la relación dosis-respuesta en el análisis estadístico, que permite cuantificar la incertidumbre de los datos. La mayor incertidumbre en los datos—por ejemplo por el tamaño reducido de los grupos o las importantes variaciones dentro de un mismo grupo—se reflejará en valores guía más bajos.

En ocasiones, el JECFA y la JMPR consideran que no es apropiado fijar una IDA en términos numéricos, por ejemplo cuando se prevé que el consumo estimado de un aditivo estará muy por debajo del valor numérico que comúnmente se le asignaría. En esas circunstancias, se utiliza el término "IDA no especificada".

Se pueden presentar situaciones en las que los datos disponibles sobre una sustancia son limitados en algunos aspectos o han surgido nuevos datos que ponen en duda la inocuidad de una sustancia química para la que el JECFA o la JMPR había establecido una IDA. Cuando el Comité está convencido de que el uso de una sustancia es seguro en el periodo relativamente corto necesario para generar y evaluar otros datos, pero no confía en que su uso sea seguro durante toda la vida, a menudo establece una IDA "temporaria", sujeta a la recepción de datos apropiados para resolver el problema de la inocuidad en un plazo establecido.

En el caso de los medicamentos de uso veterinario y los plaguicidas, la IDA se usa para confirmar la seguridad de los límites máximos de residuos (LMR) cuando las sustancias se utilizan de acuerdo con buenas prácticas. Cuando se fija la IDA para un medicamento de uso veterinario o un residuo de plaguicida, se toman en cuenta la toxicidad del compuesto precursor y de sus principales metabolitos, y la IDA se basa en el criterio de valoración toxicológica del compuesto más peligroso.

Cuando un medicamento de uso veterinario puede afectar la flora intestinal humana aunque el nivel de exposición sea inferior a aquél que produce efectos toxicológicos, esta variable se usa como base para establecer la IDA. Para decidir sobre la necesidad de fijar una IDA microbiológica se utiliza un enfoque de árbol de decisión internacionalmente armonizado y la guía pertinente elaborada por la Cooperación Internacional sobre la Armonización de Requisitos Técnicos para el Registro de Productos Medicinales Veterinarios (VICH). En los primeros tres pasos se analiza si 1) los residuos del fármaco o de sus metabolitos tienen actividad microbiológica contra la flora intestinal humana representativa, 2) los residuos entran al colon humano, y 3) la actividad microbiológica de los residuos que penetran en el colon humano persiste. Si la respuesta a cualquiera de los tres primeros pasos es "no", no es preciso determinar una IDA microbiológica. No obstante, si estos residuos estuvieran presentes, se analizan dos variables de importancia para la salud pública: 1) ruptura de la barrera que impide la colonización y 2) aumento de la población de bacterias resistentes.

Algunas veces, cuando se examina el uso como aditivo alimentario, plaguicida o fármaco de uso veterinario de sustancias que tienen efectos tóxicos similares o un metabolito tóxico común, o cuando las sustancias están presentes como contaminantes, conviene considerarlas en grupo para establecer un valor guía basado en criterios de salud, con el fin de limitar la ingestión de todas en general. Esto sólo es posible si las sustancias tienen mecanismos de acción y perfiles de toxicidad similares.

Es preferible establecer valores guía para límites de exposición basados en criterios de salud que abarquen a toda la población. Por lo general, estos valores se fijan para proteger a la subpoblación más

vulnerable, sobre la base de resultados sanitarios críticos en los más susceptibles. Sin embargo, se reconoce que los resultados sanitarios en los más vulnerables no siempre son relevantes para algunos subgrupos de población. Por ejemplo, es particularmente importante que todo valor guía basado en criterios de salud sea adecuado para proteger al embrión o al feto de posibles efectos en el útero. Por lo tanto, en algunas situaciones en las que un criterio de valoración del desarrollo o específico para una subpoblación determina el valor guía para una sustancia que no tiene otro efecto tóxico, se puede recomendar un segundo valor (más alto) sobre la base de otro criterio relevante para el resto de la población.

Determinación de la exposición a sustancias químicas presentes en los alimentos

En la determinación de la exposición alimentaria a sustancias químicas, los datos sobre consumo de alimentos se combinan con los de concentración de sustancias químicas en los alimentos. Luego, la estimación de la exposición alimentaria resultante se puede cotejar con el valor guía para límites de exposición basado en criterios de salud o con el punto de partida toxicológico (NOAEL, BMDL) para la sustancia química de que se trate, como parte de la caracterización del riesgo. Se puede determinar la exposición aguda o la exposición crónica. Las determinaciones de la exposición alimentaria abarcan a la población general y también a grupos vulnerables o en los que se prevé una exposición significativamente diferente de la de la población general (por ejemplo, lactantes, niños, embarazadas, ancianos, vegetarianos).

En principio, las determinaciones de exposición alimentaria se deben realizar para todas las sustancias químicas identificadas y presentes en los alimentos para las que se realiza la determinación de riesgos. Métodos similares son apropiados para los contaminantes y residuos de plaguicidas y medicamentos de uso veterinario, aditivos alimentarios (incluso saborizantes), coadyuvantes de elaboración y otras sustancias químicas presentes en los alimentos. Se recomienda un enfoque gradual en el que se pueden aplicar métodos de detección sistemática para identificar, entre la gran cantidad de sustancias que puedan estar presentes, aquellas que no plantean problemas de inocuidad, utilizando recursos mínimos y en la menor cantidad de tiempo

posible. No es necesario realizar una determinación de exposición más refinada en relación con estas sustancias. Los pasos para perfeccionar la determinación de la exposición alimentaria se deben formular de modo que no se subestime una posible exposición alimentaria elevada a una sustancia química dada.

Las fuentes de información sobre concentraciones de sustancias químicas en los alimentos incluyen los límites máximos (LM) propuestos o límites máximos de residuos (LMR), los niveles propuestos por el fabricante, los datos de seguimiento y vigilancia, estudios de la dieta total (EDT), la base de datos de SIMUVIMA/Alimentos, estudios de eliminación de residuos de medicamentos de uso veterinario, estudios de los niveles más altos y de la media de los niveles de residuos en ensayos supervisados de plaguicidas y la bibliografía científica. Los datos más precisos se obtienen de la medición de las concentraciones de sustancias químicas en los alimentos tal como se consumen. Los programas para generar datos sobre concentraciones de sustancias químicas en los alimentos exigen planes de muestreo y métodos analíticos convalidados. Dos son los principales enfoques para analizar los alimentos cuando se trata de generar datos analíticos a partir de encuestas: 1) análisis de grupos de alimentos y 2) análisis de alimentos individuales (muestras individuales o compuestas).

La información sobre consumo de alimentos se puede obtener de las hojas de disponibilidad de alimentos, que incluyen las cantidades de alimentos para consumo humano provenientes de las estadísticas nacionales de producción, desaparición o utilización de alimentos. Están disponibles en casi todos los países. Las dietas de grupos de consumo del SIMUVIMA/Alimentos elaboradas por la OMS se basan en hojas de disponibilidad de alimentos de la FAO seleccionadas y son representativas del consumo promedio por habitante. Las dietas de grupos de consumo reemplazan a las cinco dietas regionales elaboradas anteriormente por la OMS.

Los datos sobre consumo de alimentos deben estar disponibles en un formato congruente con los datos de concentraciones utilizados en la determinación de la exposición alimentaria. Por lo general, con los métodos basados en la población se compilan y difunden datos sobre materias primas o productos agrícolas crudos o semi procesados, e indican la cantidad anual total de productos disponibles para el

consumo nacional por año. Los datos de encuestas individuales sobre consumo de alimentos habitualmente no se difunden en bruto (con las respuestas de cada participante), y los evaluadores deben confiar en los resúmenes de datos estadísticos publicados. Se pueden realizar ajustes por cuota de mercado para corregir los datos sobre consumo de alimentos procesados o porcentaje de cultivos tratados. El método se usa principalmente cuando la sustancia que se está evaluando ha sido deliberadamente agregada a los alimentos.

Los métodos disponibles para estimar la exposición alimentaria han sido divididos en dos grupos: los que proveen una estimación (puntual) y los que permiten caracterizar la distribución completa de la exposición del consumidor. Las estimaciones puntuales incluyen 1) métodos de detección sistemática, 2) métodos de determinación de la exposición que dependen de datos brutos sobre consumo, como la ingestión diaria máxima teórica agregada (*theoretical added maximum daily intake*, TAMDI) y otras dietas modelo y 3) métodos más refinados basados en datos reales sobre consumo y concentraciones de sustancias químicas, como los estudios de dieta total, los estudios selectivos sobre alimentos individuales y los estudios con el método de muestreo de porciones duplicadas. Una estimación determinista o puntual de la exposición alimentaria es, simplemente, un valor único que describe algún parámetro de exposición del consumidor (por ejemplo, la exposición promedio de una población). La caracterización de la distribución completa de la exposición de los consumidores es el método más intensivo en términos de recursos, dado que exige datos que caractericen el rango de prácticas de consumo de alimentos y también el intervalo de concentraciones de sustancias químicas en los alimentos que se consumen. El grado de refinamiento necesario de las estimaciones de exposición alimentaria depende, en parte, de la naturaleza de la sustancia y el perfil de toxicidad.

Los métodos de detección sistemática sobreestiman la exposición alimentaria de los grandes consumidores al utilizar presunciones prudentes en términos de consumo de alimentos y concentraciones de sustancias químicas. Su objetivo no es determinar la verdadera exposición alimentaria sino identificar sustancias químicas presentes en los alimentos que exigen una evaluación más exhaustiva de la exposición alimentaria. Estos enfoques incluyen datos sobre peso (de los aditivos alimentarios, incluidos los saborizantess), método del presupuesto

(que ha sido usado para determinar la exposición a algunos aditivos alimentarios según la ingestión diaria máxima teórica) y dietas modelo (que se obtienen a partir de la información disponible sobre consumo de alimentos y se formulan para que sean representativos de la dieta típica de la población cuya exposición se va a analizar).

El modelo de estimación puntual también puede ser apropiado como un segundo paso en un abordaje con distintos niveles. El modelo elegido puede ser más o menos conservador, según el objetivo y la información disponible. Los modelos de dieta para grandes consumidores se pueden formular sobre la base de los datos publicados de encuestas sobre consumo de alimentos, como alternativa al método del presupuesto o como paso adicional del proceso de detección sistemática. Las cantidades de alimentos consumidos y la exposición alimentaria de los grandes consumidores también se pueden obtener de los datos de distribución. Es posible que sea necesario tomar en cuenta la tendencia de los consumidores a comprar y consumir repetidamente los mismos productos alimenticios, algunas veces llamada fidelidad del consumidor, y un intervalo de concentraciones, para generar estimaciones de exposición alimentaria que cubran diferentes escenarios de comportamiento de los consumidores.

Se puede realizar un análisis probabilístico de la variabilidad de la exposición para las sustancias que exigen un estudio más exhaustivo, más allá de los métodos de detección o las estimaciones puntuales de exposición. Los métodos para construir modelos probabilísticos para la determinación de la exposición alimentaria son, entre otros, estimación empírica simple de la distribución, formulación de modelos probabilísticos a partir de conjuntos de datos, muestreo estratificado, muestreo aleatorizado (simulación de Monte Carlo) e hipercubo latino.

Los datos de fácil acceso sobre la distribución del consumo de alimentos para una determinación probabilística de la exposición provienen de estudios de corto plazo y no son representativos del consumo real a largo plazo. Se ha recurrido a distintos métodos para estimar el consumo a largo plazo; entre ellos, métodos que combinan datos sobre frecuencia con información sobre cantidades consumidas y modelos estadísticos que utilizan las correlaciones entre los días de consumo para estimar la ingestión “habitual” de la sustancia que se está analizando.

La exposición a sustancias químicas presentes en los alimentos también es posible por otras vías, así como la exposición a sustancias químicas o fármacos que comparten un mecanismo de acción (toxicidad). Se llama exposición agregada a la exposición combinada a una sola sustancia química por múltiples vías (oral, dérmica, por inhalación) y a través de múltiples vehículos (alimentos, agua potable, viviendas). También se debe realizar la determinación del riesgo derivado de la exposición a múltiples residuos de plaguicidas que tienen un mecanismo de toxicidad común; se llama exposición acumulativa a la que se produce en esta situación. Se han publicado directrices para la estimación de la exposición agregada.

Caracterización del riesgo

La caracterización del riesgo es el cuarto paso del proceso de determinación del riesgo e integra información de la caracterización del peligro y la determinación de la exposición para generar recomendaciones científicas destinadas a los gestores del riesgo. Históricamente se han utilizado diferentes enfoques para la caracterización del riesgo de efectos tóxicos para los que se cree que existe un umbral (por encima del cual se observan los efectos adversos) y de efectos tóxicos para los que se cree que ese umbral no existe. El JECFA y la JMPR fijan valores guía para los límites de exposición basados en criterios de salud para sustancias que producen efectos por encima de un umbral. En la caracterización del riesgo de este tipo de sustancias, los valores guía se comparan con la exposición estimada o medida de los humanos.

En los casos en los que la exposición excede los valores guía establecidos para proteger la salud, los valores por sí solos no sirven para orientar a los gestores del riesgo respecto del posible alcance del riesgo para aquellos que están expuestos a estas cantidades más altas. En un primer análisis se debe tomar en cuenta el hecho de que los valores guía para los límites de exposición basados en criterios de salud incorporan factores de seguridad o incertidumbre. No necesariamente una exposición alimentaria breve u ocasional que supere el valor guía basado en un estudio de toxicidad subcrónica o crónica tendrá como consecuencia efectos adversos para la salud.

Cuando los datos no son suficientes para proponer un valor guía para los límites de exposición a una sustancia, o cuando no se puede

suponer que existe un umbral sobre la base del mecanismo de acción, el JECFA y la JMPR pueden realizar un comentario sobre el margen de seguridad entre las dosis a las cuales se observan efectos en animales y la exposición alimentaria humana estimada.

La caracterización del riesgo debe incluir el análisis y la descripción de la incertidumbre y la variabilidad. Por incertidumbre se entiende el conocimiento limitado del evaluador de riesgos sobre los datos y modelos utilizados. La variabilidad refleja la heterogeneidad biológica inherente en la exposición o la respuesta. En consecuencia, aunque tanto la incertidumbre como la variabilidad se pueden caracterizar utilizando distribuciones de probabilidades, son conceptos diferentes. La incertidumbre se puede reducir mejorando la cantidad o calidad de la información disponible. El modelado de la variabilidad es un ejercicio de estadística descriptiva cuyo producto es un modelo de una población y no de un individuo. La caracterización de la variabilidad en la exposición alimentaria de la población, por ejemplo, se puede perfeccionar mejorando la información, pero no es posible eliminar la variabilidad. La caracterización del riesgo debe incluir una evaluación descriptiva de la incertidumbre en relación con la exposición y los efectos en la salud. Los análisis de sensibilidad son técnicas cuantitativas que se pueden usar para identificar aquellos aspectos de los datos (por ejemplo concentraciones o consumo de alimentos) que más contribuyen a la incertidumbre.

Quienes se ocupan de la determinación de riesgos son cada vez más conscientes de la necesidad de tomar en cuenta todo riesgo asociado con la exposición combinada a mezclas de sustancias. Hay cuatro tipos de efectos combinados o interacciones: suma de dosis, suma de respuestas, sinergismo y antagonismo. El JECFA y la JMPR han realizado evaluaciones de mezclas de algunos aditivos alimentarios, plaguicidas y medicamentos de uso veterinario que se producen y ensayan como mezclas, y de mezclas de algunos contaminantes que están presentes simultáneamente. Para los plaguicidas y los medicamentos de uso veterinario que son mezclas, la JMPR y el JECFA basan la IDA para los residuos en la mezcla. En algunos casos se ha asignado una IDA para el grupo. El JECFA también ha utilizado la IDA conjunta para determinados aditivos alimentarios que se metabolizan en un metabolito común potencialmente tóxico y una IDT para el grupo de contaminantes estrechamente relacionados presentes como

mezclas. Un abordaje que toma en cuenta la suma de dosis es el factor de equivalencia de toxicidad (FET), que ajusta la exposición para cada componente de una mezcla en relación con la potencia de una sustancia química índice (por ejemplo, para dioxinas y sustancias químicas similares a dioxinas).

En el caso de sustancias que son genotóxicas y carcinógenas, tradicionalmente se ha supuesto la inexistencia de un umbral de dosis y que con cualquier nivel de exposición hay algún grado de riesgo. Por lo tanto, el JECFA no ha establecido valores guía para límites de exposición basados en criterio de salud para las sustancias con efectos genotóxicos y carcinógenos conocidos. No obstante, algunas sustancias químicas provocan cáncer en animales de experimentación por mecanismos que no son genotóxicos y por encima de un umbral, y es posible establecer valores guía para estas sustancias.

Por lo general, se considera que no es admisible el uso de sustancias genotóxicas y cancerígenas como aditivos alimentarios, plaguicidas o medicamentos de uso veterinario. El JECFA ha estudiado una cantidad de contaminantes con efectos genotóxicos y cancerígenos demostrados y analizado los posibles enfoques para la elaboración de materiales de orientación destinados a informar mejor a los gestores de riesgos sobre la posible magnitud de los problemas para la salud humana con diferentes niveles de ingestión. La determinación de la exposición (ingestión) en relación con un compuesto que es genotóxico y cancerígeno es similar a la de otros tipos de contaminantes. La caracterización del riesgo puede asumir formas diferentes: 1) cálculo del margen de seguridad respecto de la dosis con la que se observa una incidencia baja pero definida de cáncer (habitualmente en bioensayos con animales) y la exposición humana estimada; 2) análisis de la relación dosis-respuesta fuera del intervalo de dosis de los bioensayos con animales, para calcular la incidencia de cáncer teóricamente asociada con la exposición estimada para los humanos o la exposición asociada con una incidencia predeterminada de cáncer (por ejemplo, un aumento del riesgo de cáncer en la vida de 1 en un millón) y 3) extrapolación lineal de dosis bajas desde un punto de partida como el BMDL. De estas tres opciones, el método del margen de seguridad y la extrapolación lineal de dosis bajas desde un punto de partida son los más pragmáticos y aplicables en este momento. El JECFA ha decidido que las recomendaciones sobre compuestos genotóxicos y carcinógenos se

debe basar en márgenes de seguridad. La monografía subraya que en las recomendaciones a los gestores de riesgos se deben describir los puntos fuertes y las limitaciones propias de los datos empleados para calcular el margen de seguridad, y proporcionar orientación para la correcta interpretación de los márgenes de seguridad.

Límites máximos de residuos de plaguicidas y medicamentos de uso veterinario

Los límites máximos de residuos (LMR) de plaguicidas y medicamentos de uso veterinario son las concentraciones máximas de residuos admisibles en los alimentos. La CCA ha adoptado normas internacionales sobre los LMR de acuerdo con las recomendaciones de los comités pertinentes, el Comité del Codex sobre Residuos de Plaguicidas y el Comité del Codex sobre Residuos de Medicamentos de Uso Veterinario en los Alimentos. Estas recomendaciones se basan en la orientación proporcionada por la JMPR y el JECFA. Los requisitos establecidos por el JMPR y el JECFA para la identificación y caracterización de una sustancia para la que se va definir la IDA, la DRA y los LMR son similares.

La JMPR evalúa los datos sobre residuos de plaguicidas sobre la base del uso según las buenas prácticas agrícolas (BPA) en la utilización de plaguicidas, para calcular los niveles máximos de residuos en los alimentos y piensos. La JMPR evalúa estudios metabólicos en animales (ganado) y cultivos como los principales determinantes de la definición de residuos en alimentos y piensos. Los niveles máximos recomendados en distintos cultivos dependen principalmente de los datos de ensayos supervisados realizados de acuerdo con los usos máximos registrados en el marco de las BPA. Los ensayos deben cubrir todas las situaciones que se espera que se presenten en la práctica, incluidos los métodos de aplicación, las estaciones, las prácticas culturales y las variedades de cultivos. Si las concentraciones de residuos en el producto procesado superan los niveles en el producto agrícola en bruto por un margen suficiente como para que sea necesario un LMR más alto que el LMR para el producto agrícola en bruto, la JMPR debe calcular un nivel máximo de residuos para el producto procesado. La carga alimentaria de residuos de plaguicidas para el ganado se calcula a partir de datos de ensayos supervisados de residuos en piensos, multiplicados por la alimentación estándar de los animales, según las

tablas de alimentación de ganado de la OCDE. Los niveles máximos de residuos estimados, los residuos presentes en niveles más altos en los ensayos supervisados (en la porción comestible de un producto en ensayos realizados para estimar el nivel máximo de residuos en el producto) y la mediana de residuos obtenida en ensayos supervisados de tratamiento de animales externos se comparan con los obtenidos de la exposición a través del pienso. Los niveles máximos recomendados, los niveles más altos y la mediana de los niveles se basan en los valores más altos derivados de esta comparación. Las estimaciones de exposición crónica se basan en las medianas de residuos obtenidas de ensayos supervisados, en estudios de procesamiento de alimentos y en el consumo a largo plazo. Para la exposición a corto plazo, las estimaciones de ingestión de gran cantidad de residuos de plaguicidas en un día se basan en los niveles más altos encontrados en los ensayos supervisados.

Respecto de los medicamentos de uso veterinario, para recomendar LMR en productos crudos de origen animal el JECFA evalúa los estudios de eliminación de residuos con fármacos precursores radiomarcados y también otros con fármacos precursores no marcados en determinadas especies animales. Los datos de estudios con sustancias radiomarcadas se usan para estimar la evolución en el tiempo de las concentraciones totales del residuo en estudio y para determinar un residuo marcador. Los LMR se definen sobre la base del residuo marcador. El residuo marcador puede ser el compuesto precursor, un metabolito importante, una suma de fármaco precursor y metabolitos o un producto de reacción formado a partir de residuos del fármaco durante el análisis. Puede que no tenga importancia en términos toxicológicos o microbiológicos, pero es útil para el seguimiento. Los datos de estudios con sustancias no marcadas se usan para estimar la evolución en el tiempo de la concentración del residuo marcador en productos crudos de origen animal en prácticas de uso aprobadas (por ejemplo, buenas prácticas en el uso de medicamentos veterinarios). La relación entre el residuo marcador y el total de residuos se usa para convertir las concentraciones del residuo marcador en concentraciones totales de residuos de importancia para estimar la exposición alimentaria.

Por lo general, se recomiendan LMR para algunos tejidos y productos comestibles conforme al uso que se les vaya a dar; por ejemplo,

para músculo, hígado, riñón y grasa de animales de sacrificio, para la grasa y la piel de los animales de corral (y, si corresponde, de cerdos) en proporciones naturales, para músculo y piel de pescado en proporciones naturales y también para leche, huevos y miel.

En el caso de los medicamentos de uso veterinario, en la actualidad el JECFA elabora recomendaciones para los LMR basadas en estimaciones de ingestión crónica calculadas a partir de la mediana de los niveles de residuos y una canasta teórica de alimentos (compuesta de 300 g de músculo, 100 g de hígado, 50 g de riñón, 50 g de grasa, 1500 g de leche, 100 g de huevo y 20 g de miel), para calcular una ingestión diaria moderada de residuos, a la que se llama ingestión diaria estimada (IDE). La ingestión diaria máxima teórica que se utilizaba antes usaba como estimación puntual el LMR propiamente dicho, que es un valor único que representa el límite superior de un porcentil alto de la distribución de los residuos. El JECFA concluyó que este método no era realista y que en el cálculo de la ingestión aguda se deben tomar en cuenta todas las concentraciones en la distribución de los residuos. Cuando la calidad de los datos no permite calcular una mediana del nivel de residuos o de ingestión, se puede usar la ingestión máxima diaria teórica para obtener una estimación prudente de la ingestión.

El JECFA puede formular recomendaciones definitivas para los LMR de un medicamento de uso veterinario en especies y tejidos de animales comestibles sobre la base de una IDA y de datos sobre residuos adecuados, y puede realizar recomendaciones temporales cuando se ha calculado la IDA pero faltan datos apropiados sobre los residuos o sobre la eficiencia de los métodos analíticos, o cuando la IDA es provisoria. El Comité puede establecer LMR "no especificados" o "innecesarios" cuando el margen de seguridad entre el consumo de residuos estimado y la IDA es muy amplio.

Principios relativos a grupos particulares de sustancias

Muchas de las sustancias que evalúa el JECFA están presentes en los alimentos en concentraciones bajas; por ejemplo, los saborizantes, los coadyuvantes de proceso, los disolventes de extracción y las enzimas que se usan en la producción de alimentos. Los métodos que se mencionan en esta sección de la monografía pueden ser más apropiados para evaluar estas sustancias.

Uno de estos métodos es el del umbral de importancia toxicológica (UIT). La base de este concepto es el conocimiento de que la toxicidad es una función de la estructura química y del alcance de la exposición. El UIT permite que los evaluadores de riesgos formulen recomendaciones basadas en datos científicos en los casos en los que existe una alta probabilidad de que el perjuicio sea insignificante, tomando en cuenta solamente la escasa exposición alimentaria y la estructura química. La intención no es reemplazar los procedimientos de determinación del riesgo establecidos que utilizan el JECFA y la JMPR en el caso de sustancias para las que se dispone de numerosos datos sobre su toxicidad.

El enfoque del UIT, como lo aplica el JECFA, utiliza valores de umbral de exposición humana a tres clases estructurales de sustancias químicas, por debajo del cual la probabilidad de algún riesgo apreciable para la salud es sumamente baja. Estos valores se han obtenido de datos existentes sobre la toxicidad de sustancias químicas que se han clasificado en una de tres clases estructurales. Los valores del umbral de exposición humana para las clases estructurales I, II y III son, respectivamente, 1800, 540 y 90 µg por persona por día. Como los valores del umbral para la exposición humana se comparan con los de la exposición conocida o prevista, este enfoque exige estimaciones válidas de la exposición humana.

El JECFA ha elaborado un árbol de decisiones (el Procedimiento para la evaluación de inocuidad de los saborizantes) para aplicar el concepto de UIT a las sustancias saborizantes. Cuando el procedimiento se adoptó por primera vez, el JECFA decidió que un enfoque realista y práctico para calcular la exposición alimentaria estimada de los consumidores de saborizantes era utilizar datos sobre el volumen de producción anual en diferentes regiones. Esta estimación, llamada ingestión maximizada derivada de encuestas (IMDE), se calculó a partir de cifras de producción anual total de saborizantes, tomando en cuenta el hecho de que probablemente la información no incluía todas las sustancias químicas producidas y que sólo el 10% de la población considerada consumía el saborizante.

El JECFA señaló que el uso de la IMDE podría llevar a subestimar la exposición alimentaria a los saborizantes de los consumidores habituales de determinados alimentos que los contienen. En consecuencia,

se desarrolló un nuevo método para calcular la exposición alimentaria a sustancias saborizantes, la "técnica de la porción única para evaluar la exposición" (TPUE). En esta técnica se presume un consumo diario de una porción del alimento que contiene el saborizante, sobre la base de los niveles de uso agregados proporcionados por la industria. La TPUE identifica todas las categorías de alimentos que probablemente contienen un saborizante, asigna un nivel de uso agregado a una sola porción de tamaño "normal" de cada una de esas categorías y luego identifica la categoría de alimentos que probablemente contribuye a la mayor exposición alimentaria. Se presume que la porción de tamaño normal es representativa de la media de la cantidad de alimento consumida por los consumidores de esa categoría de alimento, suponiendo un consumo diario durante un periodo prolongado. La porción de tamaño normal no refleja el consumo de grandes cantidades publicado en encuestas nacionales para la categoría de alimento y, por lo tanto, es una predicción más realista de los patrones de consumo a largo plazo. El JECFA ha llegado a la conclusión de que los valores de IMDE y TPUE proporcionan información diferente y complementaria. En el Procedimiento se usará el valor más alto de las dos estimaciones de exposición alimentaria (IMDE o TPUE).

El JECFA has analizado la posibilidad de aplicar el método del UIT para la caracterización del riesgo no sólo de las sustancias saborizantes sino también de otras presentes en los alimentos en cantidades pequeñas. El Comité señaló que para extender la aplicación del método, el UIT debería usarse junto con estimaciones prudentes de exposición alimentaria y que podrían ser necesarios datos adicionales sobre toxicidad de sustancias estructuralmente relacionadas. Además, recomendó la elaboración de guías para la aplicación del enfoque en la evaluación de sustancias presentes en pequeñas cantidades en los alimentos, como determinados residuos de coadyuvantes de elaboración, materiales de envasado y contaminantes, para proporcionar orientación sobre la determinación del riesgo de sustancias para las cuales no se dispone de datos toxicológicos completos o esos datos no son necesarios.

La evaluación de la inocuidad de los materiales de envasado presenta problemas especiales a causa de la gran cantidad de materiales en uso y de la baja tasa de migración prevista de las sustancias a los alimentos en contacto con los envases y, en consecuencia, la baja

exposición alimentaria. En principio, existen dos alternativas para evaluar la inocuidad de los materiales en contacto con los alimentos. Una es solicitar datos toxicológicos independientemente de la magnitud de la posible exposición alimentaria, de modo de poder realizar una evaluación de la inocuidad. Otra opción es aplicar un enfoque gradual en el cual la cantidad de datos toxicológicos requeridos se relacione con el grado de exposición previsto, de acuerdo con los datos de los estudios de migración.

Los coadyuvantes de elaboración están compuestos de diversas sustancias, entre ellas, pero no exclusivamente, de portadores o disolventes de extracción y enzimas utilizadas en el procesamiento de los alimentos. El JECFA ha elaborado y actualizado periódicamente principios y procedimientos para determinar la inocuidad de las preparaciones con enzimas.

La determinación de la inocuidad de las sustancias que se consumen en cantidades relativamente grandes, como edulcorantes, almidones modificados, nutrientes y sustancias relacionadas, y alimentos orgánicos no tradicionales plantea algunos problemas particulares. La evaluación de la inocuidad de estas sustancias es diferente de la de otros aditivos alimentarios, porque la exposición alimentaria es elevada y los componentes menores y las impurezas del procesamiento adquieren una importancia inusual.

El consumo cada vez más difundido de alimentos fortificados, suplementos o alimentos dietéticos, alimentos especialmente formulados y alimentos supuestamente “funcionales” ha incrementado la ingestión de sustancias nutrientes en todo el mundo. El JECFA evalúa solamente la inocuidad de estos ingredientes de acuerdo con los principios y métodos expuestos en esta monografía, y ha expresado que no se debe interpretar que el JECFA aprueba el uso de estas sustancias que prometen beneficios en términos de salud o nutrición.

Las sustancias nutritivas son esenciales para la biología y la ingestión de cantidades determinadas es beneficiosa para la salud. Esta consideración influye en los enfoques que se adoptan para ajustar por la incertidumbre asociada con los datos utilizados para estimar un valor guía para la exposición basado en criterios de salud y exige que se tomen en cuenta los mecanismos homeostáticos específicos de los

nutrientes. En consecuencia, es necesario modificar el enfoque clásico de determinación del riesgo de las sustancias no nutritivas. En el plano internacional, las guías para la determinación del riesgo derivado de nutrientes y sustancias relacionadas recomiendan el uso del nivel superior de ingestión (NS), además de una ingestión mínima para distintos estratos de la población, necesaria para evitar las carencias nutricionales. El NS es la estimación del máximo nivel de ingestión regular que no implica un riesgo apreciable de efectos nocivos para la salud. El NS se puede calcular para los nutrientes de acuerdo con los principios de la determinación del riesgo que se han formulado para agentes químicos y biológicos.

Los alimentos de nuevas fuentes incluyen alimentos tradicionales y no tradicionales, alimentos nuevos y alimentos para dietas especiales. Son necesarias especificaciones para garantizar que las concentraciones de contaminantes posiblemente peligrosos, como micotoxinas y metales pesados se mantengan al mínimo. Se debe identificar la influencia de la incorporación de la nueva sustancia en la composición nutritiva de la dieta en su conjunto, en particular en lo que se refiere a grupos como los niños, los ancianos y las poblaciones "cautivas" (como pacientes hospitalizados o escolares). El valor nutritivo de los nuevos alimentos se debe evaluar inicialmente a partir de la composición química de sus macronutrientes y micronutrientes, tomando en cuenta los efectos de todo procesamiento o almacenamiento posterior. Según las características o el uso previsto del nuevo alimento, serán necesarios estudios en animales de laboratorio para complementar los estudios químicos. Es preciso diseñar estudios en humanos con criterio individual. La experiencia humana es una parte esencial de la recolección de datos en la historia de uso. Para los alimentos nuevos, será necesario estimar la exposición de acuerdo con el uso previsto. Es posible que el enfoque de ME sea apropiado para la caracterización del riesgo de los alimentos nuevos.

The **International Programme on Chemical Safety (IPCS)**, established in 1980, is a joint venture of the United Nations Environment Programme (UNEP), the International Labour Organization (ILO) and the World Health Organization (WHO). The overall objectives of the IPCS are to establish the scientific basis for assessment of the risk to human health and the environment from exposure to chemicals, through international peer review processes, as a prerequisite for the promotion of chemical safety, and to provide technical assistance in strengthening national capacities for the sound management of chemicals.

The **Inter-Organization Programme for the Sound Management of Chemicals (IOMC)** was established in 1995 by UNEP, ILO, the Food and Agriculture Organization of the United Nations, WHO, the United Nations Industrial Development Organization, the United Nations Institute for Training and Research and the Organisation for Economic Co-operation and Development (Participating Organizations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen cooperation and increase coordination in the field of chemical safety. The purpose of the IOMC is to promote coordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

WHO Library Cataloguing-in-Publication Data

Principles and methods for the risk assessment of chemicals in food.

(Environmental health criteria ; 240)

1. Risk assessment. 2. Hazard assessment. 3. Exposure assessment. 4. Dose-response assessment. 5. Chemicals. 6. Food safety. 7. Food additives. 8. Contaminants. 9. Pesticide residues. 10. Veterinary drug residues. I.World Health Organization. II.Food and Agriculture Organization of the United Nations.

ISBN 978 92 4 157240 8 (NLM classification: WA 712)
ISSN 0250-863X

This document was technically and linguistically edited by Marla Sheffer, Ottawa, Canada.

Printed by Wissenchaftliche Verlagsgesellschaft mbH, Stuttgart, Germany.

B

C

D

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Z