WHO Technical Report Series
909

EVALUATION OF CERTAIN FOOD ADDITIVES AND CONTAMINANTS

Fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives

World Health Organization
Geneva

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization or of the Food and Agriculture Organization of the United Nations

WHO Technical Report Series
909

EVALUATION OF CERTAIN FOOD ADDITIVES AND CONTAMINANTS

Fifty-seventh report of the
Joint FAO/WHO Expert Committee on
Food Additives

World Health Organization
Geneva 2002

WHO Library Cataloguing-in-Publication Data

Joint FAO/WHO Expert Committee on Food Additives (2001 : Rome, Italy)
Evaluation of certain food additives and contaminants : fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives.

(WHO technical report series ; 909)

1.Food additives — toxicity 2.Food additives — analysis 3.Flavoring agents — analysis 4.Food contamination I.Title II.Series

ISBN 92 4 120909 7 (NLM classification: WA 712)
ISSN 0512-3054

Typeset in Hong Kong
Printed in Switzerland
2001/14227 — SNPBest-set/Schuler — 6800

The World Health Organization was established in 1948 as a specialized agency of the United Nations serving as the directing and coordinating authority for international health matters and public health. One of WHO's constitutional functions is to provide objective and reliable information and advice in the field of human health, a responsibility that it fulfils in part through its extensive programme of publications.

The Organization seeks through its publications to support national health strategies and address the most pressing public health concerns of populations around the world. To respond to the needs of Member States at all levels of development, WHO publishes practical manuals, handbooks and training material for specific categories of health workers; internationally applicable guidelines and standards; reviews and analyses of health policies, programmes and research; and state-of-the-art consensus reports that offer technical advice and recommendations for decision-makers. These books are closely tied to the Organization's priority activities, encompassing disease prevention and control, the development of equitable health systems based on primary health care, and health promotion for individuals and communities. Progress towards better health for all also demands the global dissemination and exchange of information that draws on the knowledge and experience of all WHO's Member countries and the collaboration of world leaders in public health and the biomedical sciences.

To ensure the widest possible availability of authoritative information and guidance on health matters, WHO secures the broad international distribution of its publications and encourages their translation and adaptation. By helping to promote and protect health and prevent and control disease throughout the world, WHO's books contribute to achieving the Organization's principal objective — the attainment by all people of the highest possible level of health.

The *WHO Technical Report Series* makes available the findings of various international groups of experts that provide WHO with the latest scientific and technical advice on a broad range of medical and public health subjects. Members of such expert groups serve without remuneration in their personal capacities rather than as representatives of governments or other bodies; their views do not necessarily reflect the decisions or the stated policy of WHO. An annual subscription to this series, comprising about 10 such reports, costs Sw. fr. 132.– (Sw. fr. 92.40 in developing countries).

Joint FAO/WHO Expert Committee on Food Additives

Rome, 5–14 June 2001

Members

Dr J. Alexander, Department of Environmental Medicine, National Institute of Public Health, Oslo, Norway

Ms J. Baines, Senior Nutritionist, Australia New Zealand Food Authority, Barton, ACT, Australia

Professor J.R. Bend, Chairman, Department of Pharmacology and Toxicology, Faculty of Medicine and Dentistry, University of Western Ontario, London, Ontario, Canada *(Joint Rapporteur)*

Professor S.M. Dagher, Department of Biology, American University of Beirut, Beirut, Lebanon

Dr D.G. Hattan, Director, Division of Health Effects Evaluation, Office of Premarket Approval, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC, USA

Dr Y. Kawamura, Section Chief, Division of Food Additives, National Institute of Health Sciences, Tokyo, Japan

Dr A.G.A.C. Knaap, Centre for Substances and Risk Assessment, National Institute of Public Health and the Environment, Bilthoven, Netherlands

Dr P.M. Kuznesof, Leader, Chemistry and Exposure Assessment Team, Division of Product Manufacture and Use, Office of Premarket Approval, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC, USA *(Joint Rapporteur)*

Dr J.C. Larsen, Head, Division of Biochemical and Molecular Toxicology, Institute of Food Safety and Toxicology, Danish Veterinary and Food Administration, Ministry of Food, Agriculture and Fisheries, Søborg, Denmark

Mrs I. Meyland, Senior Scientific Adviser, Institute of Food Research and Nutrition, Danish Veterinary and Food Administration, Ministry of Food, Agriculture and Fisheries, Søborg, Denmark *(Chairman)*

Dr G. Pascal, Scientific Director, Human Nutrition and Food Safety, National Institute for Agricultural Research, Paris, France

Dr M.V. Rao, Head, Chemistry Unit, Food and Environment Laboratory, Dubai, United Arab Emirates

Dr P. Sinhaseni, Deputy Director for Research, Institute of Health Research, Chulalongkorn University, Bangkok, Thailand

Professor R. Walker, Emeritus Professor of Food Science, School of Biological Sciences, University of Surrey, Guildford, Surrey, England *(Vice-Chairman)*

Mrs H. Wallin, Senior Food Control Officer, National Food Agency, Helsinki, Finland

Dr D.B. Whitehouse, Food Regulatory Affairs, Bowdon, Cheshire, England

Secretariat

Dr P.J. Abbott, Australia New Zealand Food Authority, Canberra, ACT, Australia *(WHO Temporary Adviser)*

Dr A.J. Baars, National Institute of Public Health and the Environment, Bilthoven, Netherlands *(WHO Temporary Adviser)*

Dr D. Benford, Food Standards Agency, London, England *(WHO Temporary Adviser)*

Dr R.A. Canady, Toxicologist, Office of Plant and Dairy Foods and Beverages, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC, USA *(WHO Temporary Adviser)*

Dr C.E. Cerniglia, Director, Division of Microbiology and Chemistry, National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR, USA *(WHO Temporary Adviser)*

Ms M.L. Costarrica, Senior Nutrition Officer, Food Quality Liaison Group, Food Quality and Standards Service, Food and Nutrition Division, FAO, Rome, Italy

Dr K. Crump, Ruston, LA, USA *(WHO Temporary Adviser)*

Dr M. DiNovi, Division of Product Manufacture and Use, Office of Premarket Approval, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC, USA *(WHO Temporary Adviser)*

Ms J. Eastwood, Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch, Health Canada, Ottawa, Ontario, Canada *(WHO Temporary Adviser)*

Dr C.E. Fisher, Head, Risk Analysis and International Coordination Branch, Ministry of Agriculture, Fisheries and Food, London, England *(FAO Consultant)*

Dr J. Freijer, National Institute of Public Health and the Environment, Bilthoven, Netherlands *(WHO Temporary Adviser)*

Dr J. Gry, Institute of Toxicology, National Food Agency of Denmark, Ministry of Food, Agriculture and Fisheries, Søborg, Denmark *(WHO Temporary Adviser)*

Mr E.F.F. Hecker, Chairman, Codex Committee on Food Additives and Contaminants, Department of Veterinary and Food Policy and Environmental Affairs, Ministry of Agriculture, Nature Management and Fisheries, The Hague, Netherlands *(WHO Temporary Adviser)*

Dr J.L. Herrman, Scientist, International Programme on Chemical Safety, WHO, Geneva, Switzerland *(Joint Secretary)*

Dr F. Kayama, Division of Environmental Immunology and Toxicology, Department of Health Science, Jichi Medical School, Tochigi, Japan *(WHO Temporary Adviser)*

Dr M. Kogevinas, Associate Professor, Respiratory and Environmental Health Research Unit, Municipal Institute of Medical Research, Barcelona, Spain *(WHO Temporary Adviser)*

Dr C.A. Lawrie, Food Standards Agency, London, England *(FAO Consultant)*

Dr R. Lorentzen, Office of Science, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC, USA *(WHO Temporary Adviser)*

Dr M. Luetzow, Nutrition Officer, Food Quality and Standards Service, Food and Nutrition Division, FAO, Rome, Italy *(Joint Secretary)*

Dr R. Malisch, State Institute for Chemical and Veterinarian Analysis of Food, Freiburg, Germany *(FAO Consultant)*

Dr A. Mattia, Division of Product Policy, Office of Premarket Approval, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC, USA *(WHO Temporary Adviser)*

Dr G. Moy, Food Safety, WHO, Geneva, Switzerland

Dr I.C. Munro, President, CanTox Health Sciences International, Mississauga, Ontario, Canada *(WHO Temporary Adviser)*

Dr A. Nishikawa, Division of Pathology, Biological Safety Research Centre, National Institute of Health Sciences, Tokyo, Japan *(WHO Temporary Adviser)*

Dr S.W. Page, Joint Institute of Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC, USA *(WHO Temporary Adviser)*

Dr J. Park, President, LabFrontier Co., Seoul, Republic of Korea *(WHO Temporary Adviser)*

Professor A.G. Renwick, Head, Clinical Pharmacology Group, University of Southampton, Southampton, England *(WHO Temporary Adviser)*

Dr J. Rice, Chief, Unit of Carcinogen Identification and Evaluation, International Agency for Research on Cancer, Lyon, France

Dr J. Schlatter, Swiss Federal Office of Public Health, Institute of Veterinary Pharmacology and Toxicology, University of Zurich, Zurich, Switzerland *(WHO Temporary Adviser)*

Professor P. Shubik, Senior Research Fellow, Green College, University of Oxford, Oxford, England (*WHO Temporary Adviser*)

Professor I.G. Sipes, Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ, USA *(WHO Temporary Adviser)*

Dr G.J.A. Speijers, Head, Section on Public Health, Centre for Substances and Risk Assessment, National Institute of Public Health and Environmental Protection, Bilthoven, Netherlands *(WHO Temporary Adviser)*

Dr P.J.P. Verger, Scientific Directorate on Human Nutrition and Food Safety, National Institute for Agricultural Research, Paris, France *(FAO Consultant)*

Dr J.D. Wilson, Senior Fellow, Center for Risk Management, Resources for the Future, Washington, DC, USA *(WHO Temporary Adviser)*

Dr M. Zeilmaker, Centre for Substances and Risk Assessment, National Institute of Public Health and the Environment, Bilthoven, Netherlands *(WHO Temporary Adviser)*

Dr N.W. Zeman, Triangle Biotechnology Consulting, Chapel Hill, NC, USA *(FAO Consultant)*

Monographs containing summaries of relevant data and toxicological evaluations are available from WHO under the title:

Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 48, 2002.

Specifications are issued separately by FAO under the title:

Compendium of food additive specifications, addendum 9. FAO Food and Nutrition Paper, No. 52, Add. 9, 2001.

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

The preparatory work for toxicological evaluations of food additives and contaminants by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is actively supported by certain of the Member States that contribute to the work of the International Programme on Chemical Safety (IPCS).

The International Programme on Chemical Safety (IPCS) is a joint venture of the United Nations Environment Programme, the International Labour Organization and the World Health Organization. One of the main objectives of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment.

Contents

1. Introduction 1

2. General considerations 1
   - 2.1 Modification of the agenda 2
   - 2.2 Principles governing the toxicological evaluation of compounds on the agenda 2
   - 2.3 Principles for the safety assessment of chemicals in food 2
   - 2.4 Flavouring agents evaluated by the Procedure for the Safety Evaluation of Flavouring Agents 3
   - 2.5 α,β-Unsaturated carbonyl compounds and aldehydes 3
   - 2.6 Minimum assay values for flavouring agents 5
   - 2.7 Requests for data relating to intake assessments 6
     - 2.7.1 Food additives 6
     - 2.7.2 Contaminants 7
   - 2.8 Principles governing the establishment and revision of specifications 7
     - 2.8.1 Inclusion of raw materials and manufacturing methods in specifications 7
     - 2.8.2 General specifications and considerations for enzyme preparations used in food processing 8

3. Specific food additives (other than flavouring agents) 9
   - 3.1 Safety evaluations 10
     - 3.1.1 Emulsifiers 10
       - 3.1.1.1 Diacetyltartaric and fatty acid esters of glycerol 10
       - 3.1.1.2 Quillaia extracts 14
     - 3.1.2 Enzyme preparation 18
       - 3.1.2.1 Invertase from *Saccharomyces cerevisiae* 18
     - 3.1.3 Food colours 19
       - 3.1.3.1 β-Carotene from *Blakeslea trispora* 19
       - 3.1.3.2 Curcumin 20
     - 3.1.4 Food salts 21
       - 3.1.4.1 Phosphates, diphosphates and polyphosphates 21
     - 3.1.5 Glazing agent 23
       - 3.1.5.1 Hydrogenated poly-1-decene 23
     - 3.1.6 Preservative 25
       - 3.1.6.1 Natamycin (pimaricin) 25
     - 3.1.7 Sweetening agent 29
       - 3.1.7.1 D-Tagatose 29
     - 3.1.8 Thickening agents 32
       - 3.1.8.1 Carrageenan and processed *Eucheuma* seaweed 32
       - 3.1.8.2 Curdlan 36
     - 3.1.9 Miscellaneous substances 38
       - 3.1.9.1 Acetylated oxidized starch 38
       - 3.1.9.2 α-Cyclodextrin 40
       - 3.1.9.3 Sodium sulfate 42
   - 3.2 Revision of specifications 43
     - 3.2.1 Acesulfame K 43
     - 3.2.2 Blackcurrant extract 43

3.2.3 L-Malic acid 44
3.2.4 Oxystearin 44
3.2.5 Pectins 44
3.2.6 Smoke flavourings 45
3.2.7 Tagetes extract 45
3.3 Revision of limits for metals in food additives 46

4. Flavouring agents 49
4.1 Substances evaluated by the Procedure for the Safety Evaluation of Flavouring Agents 49
4.1.1 Pyrazine derivatives 51
4.1.1.1 Estimated daily per capita intake 59
4.1.1.2 Absorption, distribution, metabolism and elimination 59
4.1.1.3 Application of the Procedure for the Safety Evaluation of Flavouring Agents 60
4.1.1.4 Consideration of combined intakes from use as flavouring agents 61
4.1.1.5 Conclusions 61
4.1.2 Aromatic substituted secondary alcohols, ketones and related esters 61
4.1.2.1 Estimated daily per capita intake 70
4.1.2.2 Absorption, distribution, metabolism and elimination 70
4.1.2.3 Application of the Procedure for the Safety Evaluation of Flavouring Agents 71
4.1.2.4 Consideration of combined intakes from use as flavouring agents 72
4.1.2.5 Conclusions 72
4.1.3 Benzyl derivatives 73
4.1.3.1 Estimated daily per capita intake 81
4.1.3.2 Absorption, distribution, metabolism and elimination 81
4.1.3.3 Application of the Procedure for the Safety Evaluation of Flavouring Agents 82
4.1.3.4 Consideration of combined intakes from use as flavouring agents 83
4.1.3.5 Conclusions 83
4.1.4 Hydroxy- and alkoxy-substituted benzyl derivatives 84
4.1.4.1 Estimated daily per capita intake 95
4.1.4.2 Absorption, distribution, metabolism and elimination 95
4.1.4.3 Application of the Procedure for the Safety Evaluation of Flavouring Agents 95
4.1.4.4 Consideration of combined intakes from use as flavouring agents 98
4.1.4.5 Conclusions 98
4.1.5 Aliphatic acyclic diols, triols and related substances 98
4.1.5.1 Estimated daily per capita intake 99
4.1.5.2 Absorption, distribution, metabolism and elimination 105
4.1.5.3 Application of the Procedure for the Safety Evaluation of Flavouring Agents 105
4.1.5.4 Consideration of combined intakes from use as flavouring agents 107
4.1.5.5 Conclusions 107

4.1.6 Aliphatic acyclic acetals 107
4.1.6.1 Estimated daily per capita intake 110
4.1.6.2 Absorption, distribution, metabolism and elimination 110
4.1.6.3 Application of the Procedure for the Safety Evaluation of Flavouring Agents 111
4.1.6.4 Consideration of combined intakes from use as flavouring agents 112
4.1.6.5 Conclusions 112
4.2 Revision of certain specifications for purity 112
4.2.1 Flavouring agents with specifications designated as "tentative" at previous meetings 112
4.2.2 Flavouring agents with minimum assay values less than 95% 113
4.2.3 Specifications for flavouring agents being reviewed for safety 113

5. Contaminants 114
5.1 Chloropropanols 114
5.1.1 3-Chloro-1,2-propanediol 114
5.1.1.1 Absorption, distribution, metabolism and excretion 114
5.1.1.2 Toxicological studies 114
5.1.1.3 Occurrence 116
5.1.1.4 Estimates of dietary intake 116
5.1.1.5 Evaluation 117
5.1.1.6 Impact of regulatory limits 118
5.1.2 1,3-Dichloro-2-propanol 118
5.1.2.1 Absorption, distribution, metabolism and excretion 118
5.1.2.2 Toxicological studies 118
5.1.2.3 Occurrence 119
5.1.2.4 Estimates of dietary intake 119
5.1.2.5 Evaluation 120
5.2 Polychlorinated dibenzodioxins, polychlorinated dibenzofurans and coplanar polychlorinated biphenyls 121
5.2.1 Introduction 121
5.2.2 Toxicokinetics 123
5.2.2.1 Absorption and biotransformation 123
5.2.2.2 Metabolism and excretion 125
5.2.2.3 Relationship between human intake and doses used in studies in laboratory animals 125
5.2.2.4 Determinants of dose received by fetuses in studies of developmental toxicity 127
5.2.3 Toxicological studies 128
5.2.3.1 Acute toxicity studies 128
5.2.3.2 Carcinogenicity studies 129
5.2.3.3 Genotoxicity studies 129
5.2.3.4 Developmental toxicity studies 129
5.2.4 Observations in humans 133
5.2.4.1 Effects other than cancer 133
5.2.4.2 Carcinogenicity 134
5.2.5 Sampling and analytical methods 135
5.2.6 Levels and patterns of contamination of food commodities 137
5.2.7 Estimated dietary intake 139

5.2.8 Evaluation 141
5.2.8.1 Background body burdens in laboratory animals 141
5.2.8.2 Identification of safety factors 142
5.2.8.3 Tolerable intake 143
5.2.8.4 Comparison of PTMI with estimated intake from food 144
5.2.8.5 Uncertainties 145
5.2.8.6 Effect of maximum limits on intake, risk and food availability 146

6. Future work 146

7. Recommendations 147

Acknowledgement 148

References 148

Annex 1
Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives 150

Annex 2
Acceptable Daily Intakes, other toxicological information and information on specifications 159

Annex 3
Further information required or desired 170

1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives met in Rome from 5 to 14 June 2001. The meeting was opened by Mr W. Clay, Chief, Nutrition Programmes Service, Food and Nutrition Division, FAO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization. Mr Clay reminded the Committee that one of its tasks was to provide scientific advice to Member States of the two organizations with respect to food regulations and control. He noted that dioxins and some related compounds were to be discussed by the Committee for the first time, almost 25 years after the accident in Seveso, Italy, in which large quantities of dioxins had been released into the environment. That event had raised awareness and concern both in the general population and among regulators, leading to a greater demand for global assessment, management and communication of risks relating to environmental contamination and food. The Committee's deliberations on the topic would therefore be important and should help to improve communication between those responsible for risk assessment and risk management. Mr Clay informed the Committee that its activities would be part of a wider effort by FAO and WHO to improve food safety. The two organizations were planning to establish a Global Forum for Food Safety Regulators, in order to promote the exchange of information about ways of dealing with issues of potential importance to public health and international food trade among those responsible for regulating food safety.

2. General considerations

As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives, held in September 1955 (*1*), there have been fifty-six previous meetings of the Committee (Annex 1). The present meeting was convened on the basis of a recommendation made at the fifty-fifth meeting (Annex 1, reference *149*).

The tasks before the Committee were:

— to elaborate further principles for evaluating the safety of food additives and contaminants (section 2);
— to undertake toxicological evaluations of certain food additives, flavouring agents and contaminants (sections 3–5 and Annex 2);
— to review and prepare specifications for selected food additives and flavouring agents (sections 3 and 4 and Annex 2).

2.1 Modification of the agenda

Annatto extracts were scheduled for evaluation at a future meeting, when the results of toxicological studies that were being performed would become available to the Committee for consideration. Amyloglucosidase from *Aspergillus oryzae*, var. had been included in the call for data erroneously.

Sodium ethyl *p*-hydroxybenzoate, sodium propyl *p*-hydroxybenzoate, sodium methyl *p*-hydroxybenzoate, calcium sulfite, sodium formate, calcium formate, synthetic γ-tocopherol, synthetic δ-tocopherol, calcium tartrate, sorbitan trioleate, dipotassium diphosphate and dimagnesium diphosphate have been removed from the draft Codex General Standard for Food Additives and were referred to the Committee for evaluation. There was no indication, however, that any of these substances are used as food additives, and consequently little information was provided that would permit the establishment of Acceptable Daily Intakes (ADIs) or the preparation of specifications.

Phenyl salicylate was removed from the agenda because no data were available.

2.2 Principles governing the toxicological evaluation of compounds on the agenda

In making recommendations on the safety of food additives and contaminants, the Committee took into consideration the principles established and contained in Environmental Health Criteria, No. 70, *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference *76*), as well as the principles elaborated subsequently at a number of its meetings (Annex 1, references *77*, *83*, *88*, *94*, *101*, *107*, *116*, *122*, *131*, *137*, *143* and *149*), including the present one. Environmental Health Criteria, No. 70 (Annex 1, reference *76*) contains the most important observations, comments and recommendations made, up to the time of its publication, by the Committee and associated bodies in their reports on the safety assessment of food additives and contaminants. At its present meeting, the Committee noted that the publication included recommendations that are still appropriate and indicated potential problems associated with those that are no longer valid in the light of technological changes.

2.3 Principles for the safety assessment of chemicals in food

The Committee was informed that FAO and WHO are intending to update and consolidate principles and methods for the safety assessment of chemicals in food, including food additives, contaminants, and residues of veterinary drugs and pesticides. The project was

initiated on the basis of a recommendation of the Conference on International Food Trade Beyond 2000 that was held in Melbourne, Australia, in October 1999 (*2*), and in view of the scientific advances, changes in procedures and the increasing complexity of assessments of chemicals in food that have occurred since the publication of Environmental Health Criteria, No. 70 (Annex 1, reference *76*) and Environmental Health Criteria, No. 104, *Principles for the toxicological assessment of pesticide residues in food* (*3*). The project would include consideration of all those aspects of the assessment of chemicals in food that are addressed by the Committee and by the Joint FAO/WHO Meeting on Pesticide Residues.

The Committee recognized the importance of this initiative and recommended that it be undertaken as soon as possible.

2.4 Flavouring agents evaluated by the Procedure for the Safety Evaluation of Flavouring Agents

The Committee questioned whether some of the substances included in the lists of flavouring agents that it had been asked to evaluate at its present meeting were in fact used as flavouring agents. The Committee noted that some of the substances were used extensively in food processing as solvents, emulsifiers or preservatives.

The Committee stressed that the Procedure for the Safety Evaluation of Flavouring Agents is intended for application to flavouring agents used to impart flavour to foods and not to other uses of these substances or to other chemicals that may be used in flavouring formulations. Consequently, the Committee was unable to finalize the evaluations of certain substances listed on the agenda,[1] pending confirmation of their use and intake as flavouring agents.

A clear definition of "flavouring agent" has not been elaborated by the Committee. Although Environmental Health Criteria, No. 70 (Annex 1, reference *76*) provides some guidance, the Committee recommended that this issue be addressed at a future meeting.

2.5 α,β-Unsaturated carbonyl compounds and aldehydes

The α,β-unsaturated carbonyl group is a reactive moiety that represents a potential structural alert for toxicity. Five flavouring agents containing such a group were considered by the Committee at its forty-ninth meeting (Annex 1, reference *131*), but their evaluation was postponed, pending consideration of other α,β-unsaturated carbonyl compounds. The safety of these five agents was reconsidered by

[1] See sections 4.1.3–4.1.5.

the Committee at its fifty-fifth meeting (Annex 1, reference *149*), when it also evaluated furfural, cinnamaldehyde, structural analogues of cinnamaldehyde, pulegone and esters of the corresponding alcohols, which are predicted to be metabolized by formation of α,β-unsaturated carbonyls. The available data on the toxicity of these compounds in experimental animals showed a number of adverse effects at high doses, and no-observed-effect-levels (NOELs) for these effects were identified. The presence of protective processes in cells, such as conjugation with glutathione, provides adequate capacity for detoxification at the low doses associated with the use of such compounds as flavouring agents. In consequence, the Committee concluded that the presence of an α,β-unsaturated carbonyl group in a flavouring agent, or its formation during metabolism, would not preclude assessment of that substance by the Procedure for the Safety Evaluation of Flavouring Agents. This conclusion was supported by data on the toxicokinetics of 4-phenyl-3-buten-2-one (No. 820), which was considered by the Committee at its present meeting. This α,β-unsaturated carbonyl compound undergoes complete first-pass metabolism in rats and mice after oral administration and is rapidly eliminated (with a half-life of 20 min in rats and 10 min in mice) after intravenous administration. A number of other α,β-unsaturated carbonyl compounds were also evaluated by the Committee at its present meeting (Nos 821, 826 and 829), as were several compounds predicted to be metabolized to an α,β-unsaturated carbonyl compound (Nos 819, 944, 946 and 948).

Aldehyde groups are also chemically reactive and can bind to soluble proteins and protein components of membranes. Several aldehydes were evaluated previously by the Committee, and the potential genotoxicity of furfural was considered in detail at the fifty-first meeting (Annex 1, reference *137*). Furfural was reported to be genotoxic in three of 16 assays for reverse mutation in *Salmonella typhimurium* and in one of three assays for *rec* gene mutation in *Bacillus subtilis*. A few chromosomal aberrations were seen in Chinese hamster ovary cells in culture when furfural was added at relatively high concentrations (Annex 1, reference *138*). Sister chromatid exchanges and forward mutations were induced in mouse lymphoma cells. The Committee concluded that the weak activity seen in vitro in some tests for genotoxicity might be explained by the reactivity of the aldehyde group. Various metabolic processes (i.e. oxidation, conjugation and condensation) effectively eliminate the reactive aldehyde functional group, when the metabolic pathways are not saturated by high, non-physiological doses. The flavouring agents evaluated at the present meeting included a number of aldehydes (Nos 22, 865, 866,

868, 869, 877–879, 889–893, 896–898 and 937) and compounds that are predicted to be metabolized to aldehydes, such as acetals (Nos 837–840, 867, 940–949 and 954). Metabolism of these flavouring agents is predicted to result in gradual formation of aldehydes, which undergo extensive biotransformation, resulting in only low concentrations of the aldehydes per se. The results of tests for reverse mutation in bacteria were positive for pyruvaldehyde (No. 937), but consistently negative for Nos 22, 80, 95, 98, 867, 868, 877–879, 889, 893, 896, 897 and 953; the results of assays for *rec* gene mutation in *B. subtilis* were negative (Nos 878, 889 and 893) or equivocal (Nos 22 and 896). Chromosomal aberrations were reported in vitro in some studies with Nos 22, 878, 889, 893, 896 and 937, but not with No. 80. Similarly, sister chromatid exchanges were reported in some studies with Nos 22, 80, 878, 889 and 937, but not with Nos 868, 893 and 897. Mutations were reported in mouse lymphoma cells exposed to some aldehydes (Nos 80, 877, 878 and 893), but not other aldehydes or acetate (Nos 867, 889 and 896). The results of studies in vivo did not indicate genotoxicity after oral administration in a variety of test systems: in *Drosophila melanogaster* (with Nos 22, 80, 879 and 893), in assays for micronucleus formation in mice (with Nos 879, 889 and 893) and in assays for dominant lethal mutations in mice (No. 896). Sister chromatid exchange was induced in mice and hamsters by intraperitoneal injection of acetaldehyde (No. 80), and weakly positive results were obtained in several tests in vivo with pyruvaldehyde (No. 937) at very high doses (>200 mg/kg of body weight). Pyruvaldehyde is a natural component of some foods, and the amount ingested due to its use as a flavouring agent would be much less than the estimated intake from natural sources. The Committee concluded that metabolic processes such as oxidation and conjugation effectively eliminate reactive aldehyde functional groups from such substances when they are consumed in the amounts that would arise from their use as flavouring agents.

2.6 Minimum assay values for flavouring agents

At its fifty-third meeting, the Committee developed criteria for establishing specifications for flavouring agents (Annex 1, reference *143*). The Committee noted that these criteria — chemical formula and relative molecular mass, identity test and the minimum amount that can be determined (minimum assay value) — constitute the core information required to establish acceptable specifications. At its present meeting, the Committee considered that a minimum assay value of 95% for an individual flavouring agent would apply to both the flavouring agent itself and to the agent plus its known secondary components. The minimum assay values of about 90% of the flavouring agents evaluated to date meet or exceed 95%, and the Committee received