

# Evaluation of certain food additives and contaminants

Eightieth report of the Joint  
FAO/WHO Expert Committee on  
Food Additives



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*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*



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United Nations**



**World Health  
Organization**

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Rome, 16–25 June 2015

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## List of abbreviations

ADI	acceptable daily intake
AhR	aryl hydrocarbon receptor
BGF	beta-glucanase fungique
BMD	benchmark dose
BMD <sub>10</sub>	benchmark dose for a 10% response
BMDL	lower 95% confidence limit on the benchmark dose
BMDL <sub>10</sub>	lower 95% confidence limit on the benchmark dose for a 10% response
BMR	benchmark response
BMU	betamyl units
bw	body weight
CAR	constitutive androstane receptor
CCCF	Codex Committee on Contaminants in Foods
CCFA	Codex Committee on Food Additives
CCNFSDU	Codex Committee on Nutrition and Food for Special Dietary Uses
CIFOCosS	Chronic Individual Food Consumption Database – Summary statistics
CITREM	citric and fatty acid esters of glycerol
CYP	cytochrome P450
DHP	6,7-dihydro-7-hydroxy-1-hydroxymethyl-5H-pyrrolizine
DL-PCB	dioxin-like polychlorinated biphenyl
DMF	dimethyl formamide
DNA	deoxyribonucleic acid
EFSA	European Food Safety Authority
EHC	Environmental Health Criteria
ELISA	enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization of the United Nations
GC-MS	gas chromatography – mass spectrometry
GEGR	glycerol ester of gum rosin
GEMS/Food	Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme
GEWR	glycerol ester of wood rosin
GSFA	General Standard for Food Additives (Codex)
GST	glutathione S-transferase
HFCS	high-fructose corn syrup
HPLC	high-performance liquid chromatography
HPLC-MS/MS	high-performance liquid chromatography – tandem mass spectrometry
HUB	highest upper bound



IARC	International Agency for Research on Cancer
ICBA	International Council of Beverages Associations
IgE	immunoglobulin E
INS	International Numbering System for Food Additives
IPCS	International Programme on Chemical Safety
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC-MS/MS	liquid chromatography – tandem mass spectrometry
LD <sub>50</sub>	median lethal dose
LHG	Lo han guo (monk fruit extract)
LLB	lowest lower bound
LOD	limit of detection
LOQ	limit of quantification
ML	maximum level
MOE	margin of exposure
NA	not available
NCI	National Cancer Institute (USA)
ND	not detected
NDL-PCB	non-dioxin-like polychlorinated biphenyl
NOAEL	no-observed-adverse-effect level
NS	not specified
NTP	National Toxicology Program (USA)
OSA	octenyl succinic acid
PA	pyrrolizidine alkaloid
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo- <i>p</i> -dioxin
PCDF	polychlorinated dibenzofuran
PEG	polyethylene glycol
POP	persistent organic pollutant
PVA	polyvinyl alcohol
PXR	pregnane X receptor
QC	quality control
RIVM	National Institute for Public Health and the Environment (the Netherlands)
RNA	ribonucleic acid
RyR	ryanodine receptor
S9	9000 × <i>g</i> supernatant fraction from rat liver homogenate
SCF	Scientific Committee on Food (European Union)
SULT	sulfotransferase
T <sub>3</sub>	triiodothyronine
T <sub>4</sub>	thyroxine
TCDD	2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin

TDI	tolerable daily intake
TEF	toxic equivalency factor
TIPU	titratable phospholipase units
TOS	total organic solids
TSH	thyroid stimulating hormone
UGT	uridine disphosphate-glucuronosyltransferase
USA	United States of America
WHO	World Health Organization
w/w	weight per weight
ww	wet weight
XVU	xylanase viscosity units



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. 71, 2015 (and supplements).

Specifications are issued separately by FAO under the title:

*Compendium of food additive specifications.* FAO JECFA Monographs 17, 2015.





# 1. Introduction

The eightieth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) was held in Rome from 16 to 25 June 2015. The meeting was opened by Dr Renata Clarke, Head of the Food Safety and Quality Unit of the Food and Agriculture Organization of the United Nations (FAO), who welcomed participants on behalf of the Directors-General of FAO and the World Health Organization (WHO).

Dr Clarke thanked all participants for placing their valuable time and expertise at the disposal of the two organizations and commented that JECFA was one of the most successful joint undertakings of FAO and WHO, playing a critical role in the development of international food safety standards by the Codex Alimentarius Commission. The Committee was informed that the funding of joint FAO/WHO activities on scientific advice related to food safety would be discussed at the Thirty-eighth Session of the Codex Alimentarius Commission (to be held in Geneva on 6–11 July 2015) and that both organizations are actively involved in these discussions to ensure sustainable resources for the work of JECFA and the other FAO/WHO expert committees.

It was noted that the eightieth meeting of JECFA, which is dedicated to food additives and contaminants, would go through a challenging agenda, with new food additives to evaluate, others to review as follow-up work from previous meetings and two important groups of contaminants to assess.

Dr Clarke reminded participants that they have been invited to this meeting as independent experts and not as representatives of their countries or organizations. She also reminded them of the confidential nature of this meeting, which allows experts to freely express their opinions.

She closed by reiterating her sincere gratitude to participants for providing their time and expertise to this core component of both FAO and WHO work, providing the science that underpins international norms and standards.

## 1.1 Declarations of interests

The Secretariat informed the Committee that all experts participating in the eightieth meeting had completed declaration of interest forms and that no conflicts of interest had been identified.

The following declared interests were brought to the attention of the Committee. Ms Astrid Bulder, from the National Institute for Public Health and the Environment (RIVM) in the Netherlands, has worked on pyrrolizidine alkaloids (PAs) in the context of the Codex Committee on Contaminants in Foods (CCCCF), and RIVM has received funding from the Dutch government for the

risk assessment of PAs, also in support of the JECFA work. Dr Suzanne Jeurissen from the same institute was involved in the risk assessment of PAs undertaken at RIVM. Professor Helen Håkansson, Professor of Toxicology and Chemicals Health Risk Assessment at the Karolinska Institutet in Sweden, has received funds from and provided scientific advice to the Swedish Chemicals Agency and the European Food Safety Authority (EFSA) on developmental neurotoxicity. She has received European Union research grants as coordinator for the ATHON project on non-dioxin-like polychlorinated biphenyls (NDL-PCBs). Professor Martin van den Berg, Institute for Risk Assessment Sciences at the University of Utrecht, has received research grants from the European Union for a project related to polychlorinated biphenyls (PCBs) and from the United Nations Environment Programme related to NDL-PCBs.

## 1.2 Modification of the agenda

The Committee made the following modifications to the agenda (see original agenda in [Annex 3](#)):

- No data were submitted on monk fruit extract (Lo han guo) (LHG) *Siraitia grosvenorii* Swingle, so the food additive was removed from the agenda (agenda item 7.1).
- No data were submitted on aspartame for revision of specifications, so the food additive was removed from the agenda (agenda item 7.2).
- “Beta-glucanase, cellulase and xylanase from *Talaromyces emersonii*” was renamed as “Mixed  $\beta$ -glucanase, cellulase and xylanase from *Rasamsonia emersonii*”. This modification to the title reflects the reclassification of the production organism. It also reflects that the production of the enzymes by *R. emersonii* is simultaneous and that the enzymes are not mixed after manufacture.
- “Beta-glucanase and xylanase from *Disporotrichum dimorphosporum*” was renamed as “Mixed  $\beta$ -glucanase and xylanase from *Disporotrichum dimorphosporum*”. This modification to the title reflects that the production of the enzymes by *D. dimorphosporum* is simultaneous and that these enzymes are not mixed after manufacture.
- “Lipase from *Fusarium heterosporum* expressed in *Hansenula polymorpha*” was renamed as “Lipase from *Fusarium heterosporum* expressed in *Ogataea polymorpha*”. This modification to the title reflects the new name of the production organism.
- “Maltotetrahydrolase from *Pseudomonas saccharophila* expressed in *Bacillus licheniformis*” was renamed as “Maltotetrahydrolase from *Pseudomonas stutzeri* expressed in *Bacillus licheniformis*”. This



modification to the title reflects the reclassification of the donor organism.

- “Sodium aluminosilicate” was renamed as “Sodium aluminium silicate” to ensure consistency with the other silicate additives (e.g. Potassium aluminium silicate).

The Committee noted that monk fruit extract and aspartame had been assigned a high priority for evaluation during the Forty-sixth Session of the Codex Committee on Food Additives (CCFA). However, the Secretariat was not informed until late in the process that the requested data for these food additives were not going to be submitted. The Committee reiterated that respecting the commitment to submit data is extremely important for the work of the Committee and its efficiency.



## 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 79 previous meetings of the Committee (Annex 1). The present meeting was convened on the basis of a recommendation made at the seventy-ninth meeting (Annex 1, reference 220).

The tasks before the Committee were to:

- elaborate further principles for evaluating the safety of food additives (section 2);
- undertake safety evaluations and/or dietary exposure assessments of certain food additives (section 3 and Annex 2);
- undertake toxicological evaluations of certain contaminants in food (section 4 and Annex 2);
- review and prepare specifications for certain food additives (section 3 and Annex 2).

### 2.1 Report from the Forty-seventh Session of the Codex Committee on Food Additives (CCFA)

The Codex Secretariat provided the Committee with an update on the work of CCFA since the seventy-ninth meeting of JECFA.

The Forty-seventh Session of CCFA noted the conclusions of the seventy-ninth meeting of JECFA on the safety of Benzoe tonkinensis and on the use of carrageenan (INS 407), citric and fatty acid esters of glycerol (CITREM) (INS 472c), octenyl succinic acid (OSA)-modified starch (starch sodium octenyl succinate) (INS 1450) and pectin (INS 440) in infant formula or formula for special medical purposes intended for infants.

The Forty-seventh Session of CCFA encouraged Codex Members to submit relevant data to JECFA to complete the safety evaluation of octenyl succinic acid (OSA)-modified gum (INS 423); endorsed the provision of carrageenan in the *Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants* (CODEX STAN 72-1981); and agreed to request comments on uses and use levels of paprika extract (INS 160c(i)) for inclusion in the Codex General Standard for Food Additives (GSFA).

The Forty-seventh Session of CCFA agreed that lower purity limits for lead in specifications of food additives for use in infant formulas should be established in existing specifications on a case-by-case basis when needed and requested that JECFA take action with regard to the three food additives

evaluated at the seventh-ninth meeting of JECFA and for future evaluations of food additives that could be proposed for use in infant formula.

Work on more than 600 provisions of the GSFA was finalized; the adoption of specifications for the identity and purity of 10 previously evaluated food additives and 25 flavouring agents, prepared by the seventy-ninth meeting of JECFA, was recommended, as was the revocation of the specification of 2,5-dimethyl-3-acetylthiophene (No. 1051); and new INS numbers were assigned to polyvinylpyrrolidone–vinyl acetate copolymer (INS 1208) and lutein esters from *Tagetes erecta* (INS 161b(iii)).

The Forty-seventh Session of CCFA agreed on a revised priority list of substances for evaluation (or re-evaluation) by JECFA, which includes 24 substances for which data are immediately available or will be available by December 2015 as well as 63 flavouring agents. CCFA included the six colours scheduled for re-evaluation in a separate list, with the understanding that JECFA would re-evaluate two colours per year and that the remaining four colours would be included on a reserve list in the call for data, with the goal to evaluate them if other dossiers on the main list were not submitted on time.

The Forty-seventh Session of CCFA discussed, but did not support, a proposal of the Codex Committee on Nutrition and Food for Special Dietary Uses (CCNFSDU) to include, in the Preamble to the GSFA, a specific requirement for an assessment from JECFA stating that additives for use in CODEX STAN 72-1981 are safe for use in infants less than 12 weeks of age. It was noted that the JECFA Secretariat will check the JECFA assessments related to food additives used in infant formulas and report back at the next session of CCFA.

The Forty-seventh Session of CCFA continued work on the alignment of food additive provisions in the Codex standards with the corresponding provisions of the GSFA. It agreed to a working definition for secondary food additives and will examine the impact of the definition on the GSFA; it also prepared a proposal for new work on the revision of the *General Standard for the Labelling of Food Additives When Sold As Such* (CODEX STAN 107-1981) to align the terminology related to flavouring agents with that of the *Guidelines for the Use of Flavourings* (CAC/GL 66-2008).

## 2.2 Report from the Ninth Session of the Codex Committee on Contaminants in Foods (CCCF)

The Codex Secretariat provided the Committee with an update on the work of CCCF since the seventy-seventh meeting of JECFA (Cadmium: Assessment of exposure from cocoa and cocoa products) and in particular on the two contaminants scheduled for evaluation by the eightieth meeting of JECFA – i.e. PAs and NDL-PCBs.

Following the outcome of the seventy-seventh JECFA meeting, CCCF is currently working on the establishment of maximum levels (MLs) for cadmium in cocoa and cocoa products (including chocolate).

CCCF first considered the presence of PAs in food and feed at its Fourth Session in 2010 (2). The Fifth Session of CCCF in 2011 (3) agreed to include PAs in the Priority List of Contaminants and Naturally Occurring Toxicants proposed for Evaluation by JECFA and to request JECFA to identify which PAs in food and feed (as carry-over from feed to animal products) were of key interest to human health. CCCF also requested JECFA to perform a full risk assessment based on the available data for the identified PAs and to identify data gaps if a full risk assessment was not possible. In view of this, CCCF also agreed not to start work on MLs for PAs in food and feed but to develop a code of practice for the prevention and reduction of contamination of food and feed with PAs.

The Code of Practice for Weed Control to Prevent and Reduce Pyrrolizidine Alkaloid Contamination in Food and Feed (CAC/RCP 74-2014) was adopted by the Thirty-seventh Session of the Codex Alimentarius Commission in 2014 (4) and is available for consultation on the Codex website (<http://www.codexalimentarius.org/standards/en/>). Further work on “management practices to reduce exposure of animals to PAs”, “management practices to reduce exposure of food-producing animals to PA-containing plants – livestock and bees” and “management practices to reduce presence of PAs in commodities – raw and processed” would be included in the code of practice in the future when more data on existing practices and their efficacy to contain PA contamination become available.

NDL-PCBs were included in the Priority List by CCCF in 2011 for JECFA to carry out a full risk assessment. Both PAs and NDL-PCBs were removed from the Priority List at the last session of CCCF in 2015 (5) in view of their scheduled evaluation by the eightieth meeting of JECFA.

## 2.3 Food additive specifications

### 2.3.1 Update on the draft specifications monographs for 16 modified starches

Modified starches comprise a group of substances that differ with respect to processing, the functional groups introduced thereby and any resulting impurities. All these substances are described and included in a single specifications monograph, which was prepared by the thirty-fifth meeting of JECFA in 1989 ([Annex 1](#), reference 88); it was republished in the *Compendium of Food Additive Specifications* in 1992 ([Annex 1](#), reference 103) and the *Combined Compendium of Food Additive Specifications* in 2005 ([Annex 1](#), reference 180). Since then, the specifications monograph has been revised six times, and further revisions are to be expected.

At the seventy-ninth meeting ([Annex 1](#), reference 220), the Committee made the following recommendation:

The existing specifications monograph for modified starches includes 16 different modified starches, which complicates revisions of the specifications for any individual modified starch. Therefore, the Committee recommended that the specifications monograph for the modified starches be split into 16 individual specifications monographs. The Committee, as noted at its seventy-sixth meeting, considered that it would also be necessary to revise the specifications for all the modified starches, including test methods, at future meetings.

At the present meeting, the Committee was informed of the steps taken in response to this recommendation. As a first step, the 16 specifications have been separated into stand-alone documents based on the current content of the adopted monograph, without adding, deleting or modifying any information. Some of the resulting single draft specifications monographs are incomplete; in some cases, essential information is missing, in particular information that would normally be needed to serve the purpose of a specification to unambiguously characterize the additive. Therefore, a revision of at least some of these individual draft specifications monographs is required.

In order to facilitate the submission of comments by interested parties, the draft specifications monographs have been made available on the FAO JECFA website ([http://www.fao.org/fileadmin/user\\_upload/agns/pdf/jecfa/2015\\_02\\_22\\_Modified\\_Starches.pdf](http://www.fao.org/fileadmin/user_upload/agns/pdf/jecfa/2015_02_22_Modified_Starches.pdf)). This posting has been communicated via the Codex mailing list, and the Forty-seventh Session of CCFA was informed accordingly ([ftp://ftp.fao.org/codex/meetings/CCFA/ccfa47/fa47\\_03e Add. 1.pdf](ftp://ftp.fao.org/codex/meetings/CCFA/ccfa47/fa47_03e_Add.1.pdf)).

The Committee recommended continuing with the approach taken. As the next step, it was recommended that the data and information necessary to complete and revise the 16 individual draft specifications monographs be requested through a call for data. It was also noted that in addition to the missing information (highlighted in the individual draft specifications monographs currently posted on the JECFA website; see reference above), data relevant to the method of manufacture, detection methods, product characterization and levels of contaminants present (if any) should be requested as well.

### 2.3.2 HPLC method in the adopted specifications of cassia gum

The Committee was informed that a note had been sent to the Secretariat indicating that the high-performance liquid chromatography (HPLC) method for the determination of anthraquinones within the full specifications of cassia

gum (INS 427) was not fit for purpose and required revision. This issue was also brought to the attention of CCFA at its Forty-seventh Session (6).

The Committee noted that it does not develop analytical methods but considers and evaluates the information provided by the sponsors. It further noted that if any changes to the adopted specifications related to the determination of anthraquinones were to be requested, supporting data would have to be submitted in response to a call for data.

The Committee recommended that the data to revise the HPLC method for the determination of anthraquinones in cassia gum be requested through a call for data. Based on the information and data submitted, the Committee will consider revising the specifications as appropriate.

## 2.4 Principles governing the toxicological evaluation of compounds on the agenda

In making recommendations on the safety of food additives, the Committee took into consideration the principles established and contained in the publication *Principles and Methods for the Risk Assessment of Chemicals in Food* (Environmental Health Criteria [EHC] 240), published in 2009 (7).

### 2.4.1 Potential allergenicity of enzymes: change to the number of amino acids in segments used in allergen database searches

As there is no conclusive test that will predict a likely human immunoglobulin E (IgE) response to a genetically modified enzyme following oral exposure, an important first step involves undertaking a comparison of the amino acid sequence with those of established allergens. This amino acid sequence comparison is intended to detect both global similarities and short contiguous amino acid sequences that may represent linear IgE epitopes (8). For the short amino acid sequences, it is recognized that the 2001 FAO/WHO expert consultation (8) suggested moving from eight to six amino acid segments in searches. However, experience gained with a large number of enzymes at JECFA indicates that searches involving six amino acid segments result in positive matches that are of no biological relevance. The Committee recommends that such searches should consider only eight amino acid sequences.

## 2.5 Application of systematic review to the work of the Committee

A systematic review is the process of collecting and evaluating literature using prespecified and standardized methods to answer specific research questions. The process is aimed at identifying, selecting, evaluating, interpreting and

synthesizing all available research so that conclusions are drawn free of bias in a transparent and reproducible manner.

Systematic reviews use a well-defined approach to identify and evaluate all literature in a specific subject area. The conduct of the systematic review and the manner in which it is reported must be objective and transparent.

Systematic review has been applied to clinical medicine, but it is applicable to all areas of science and is now a matter of broad interest. Despite the common use of systematic reviews in areas of human health research, formal systematic reviews have rarely been used in the area of food safety.

For the present meeting, the Committee undertook a systematic review of the literature on PAs to identify all information relevant to their biochemistry, toxicology and epidemiology. This approach was taken to gain experience in the application of systematic review methodology to the work of the Committee.

The Committee concluded that the approach, as a general concept, has merit in the area of chemical food safety. The Committee concluded that elements of the systematic review process, such as defining clear questions to be addressed, searching and selecting the literature systematically, documenting search strategies and using predefined inclusion and exclusion criteria, can improve the transparency of the work of the Committee, as well as the reproducibility of the assessment. The Committee recommended that guidance on incorporating these elements into the work of the Committee should be developed for future consideration.

However, systematic review requires considerable resources. Systematic reviews may not be the most efficient approach in evaluating the safety of a food chemical for which a comprehensive body of standardized toxicology tests is available (e.g. when a new food additive is first evaluated). The Committee noted that systematic review may be most useful when it is possible to address a food-related matter of public health concern with one or just a few sharply focused questions.

The Committee concluded that the application of a systematic review should be considered on a case-by-case basis and that the approach is not appropriate for routine use by the Committee at this time. The Committee further concluded that methodological standards for food-related systematic review and criteria for determining when a systematic review is appropriate are needed.

## 2.6 Revised guidance for WHO JECFA monographers and reviewers

The Committee was provided with drafts of the two revised guidance documents for WHO monographers and reviewers evaluating food additives (excluding flavouring agents) and evaluating contaminants in food and feed. These guidance



documents are intended primarily for WHO Experts (monographers) who prepare monographs for JECFA and for Members (reviewers) who have been assigned to peer review them. The guidance will also be useful to manufacturers who submit dossiers to WHO and to other parties interested in understanding the process followed in the evaluation of food additives or contaminants in food and feed by JECFA.

The Committee was asked to provide written comments to the Secretariat so that the documents can be finalized soon after the meeting.

The Committee requested that a separate guidance document on enzymes be prepared.

The final guidance documents will be published on the WHO website at <http://www.who.int/foodsafety/chem/jecfa/guidelines/en/>.

## 2.7 Update on FAO and WHO databases related to the work of the Committee

The current FAO JECFA databases (one for food additives, one for flavouring agents and one for residues of veterinary drugs) were developed in early 2000 and are based on outdated underlying system technology. The Secretariat has therefore started a project to modernize these databases, starting with the one on flavouring agents.

While the major features and output will not differ significantly from the current version, the project aims to develop an online platform that allows the Secretariat to manage the process from receiving data for proposed new or revised specifications to adding records to the database or updating records already in the database and to publishing the adopted specifications. The new database on flavouring agents will also allow for improved interconnectivity with other databases – i.e. the Codex inventories of adopted flavouring specifications and the WHO summary of JECFA evaluations.

The new database on flavouring agents is currently being finalized. Upon completion, the databases on food additives and on residues of veterinary drugs will also be updated following the same approach.

The Committee was also updated on the latest developments on several WHO databases now available on a dedicated website (<http://www.who.int/foodsafety/databases/en/>). The searchable JECFA summary database (<http://apps.who.int/food-additives-contaminants-jecfa-database/search.aspx>) provides concise information and direct links to the JECFA reports and monographs for each compound evaluated by JECFA, including contaminants, providing details on critical studies and end-points and estimated dietary exposures. FOSCOLLAB combines information from several databases (e.g. JECFA, Global Environment Monitoring System – Food Contamination Monitoring and Assessment

Programme [GEMS/Food], Codex) and provides the key information from each in one overview page (dashboard). Such dashboards have been developed for contaminants ([https://extranet.who.int/sree/Reports?op=vs&path=/WHO\\_HQ\\_Reports/G7/PROD/EXT/chemical\\_overview&userid=G7\\_ro&password=inetsoft123](https://extranet.who.int/sree/Reports?op=vs&path=/WHO_HQ_Reports/G7/PROD/EXT/chemical_overview&userid=G7_ro&password=inetsoft123)) and for pesticides; another dashboard for veterinary drugs is under development.

To further improve the data used for dietary exposure assessment, FAO and WHO initiated a project to collect national individual food consumption data, detailed by different age groups and consumers only. Summary statistics from (currently) 37 surveys (only those with a duration of 2 days or more) from 26 countries are published in the FAO/WHO Chronic Individual Food Consumption Database – Summary statistics (CIFOCOs).

## 3. Specific food additives

The Committee evaluated five food additives for the first time and re-evaluated one other. In addition, the Committee evaluated dietary exposure to one previously evaluated food additive and considered nine food additives for revision of specifications only. Information on the safety evaluations, dietary exposure assessment and specifications is summarized in [Annex 2](#). Details of further toxicological studies and other information required for certain substances are summarized in [section 5](#).

### 3.1 Safety evaluations

#### 3.1.1 Benzoates: dietary exposure assessment

##### *Explanation*

At the request of CCFA at its Forty-sixth Session (9), the Committee evaluated dietary exposure to benzoic acid salts (benzoates). The Twenty-seventh Session of the Codex Alimentarius Commission (10) adopted the maximum level of benzoates (600 mg/kg) in GSA food category 14.1.4 on an interim basis with the understanding that a review would be conducted within 3 years. The safety of benzoates had been reviewed at the forty-sixth meeting of the Committee ([Annex 1](#), reference 122), and the group was assigned an acceptable daily intake (ADI) of 0–5 mg/kg body weight (bw), expressed as benzoic acid.

The fifty-first meeting of the Committee ([Annex 1](#), reference 137) assessed dietary exposure to benzoates from all categories of food based on maximum limits specified in national standards and in the GSA. The estimates of national exposures for consumers at the mean, based on national maximum limits, were below the upper bound of the ADI, ranging from 0.18 mg/kg bw per day (in Japan) to 2.3 mg/kg bw per day (in the USA). The estimated exposures at higher percentiles, based on food additive levels in national standards, exceeded the upper bound of the ADI in some cases (7.3 mg/kg bw per day, 150% of the upper bound of the ADI, in the USA; or 14 mg/kg bw per day, 280% of the upper bound of the ADI, in China). The Committee stated, “Because diets differ among countries, the foods that contribute most to benzoate intake would be expected to vary” (the present Committee noted that varying use levels in similar products across countries would also affect the order of importance of their contribution to dietary exposure to benzoates). For Australia, France, New Zealand, the United Kingdom and the USA, the GSA food category that contributed the most to dietary exposure to benzoates was carbonated water-based flavoured drinks (GSA food category 14.1.4.1). In Finland, 40% of the benzoates used in food was in soft drinks. Soya sauce was the main source in China and the second most important source in Japan.

**Data submitted or available to the Committee**

The Committee received data on “average typical” levels of benzoates in foods (796 individual branded products) for seven subcategories of GSFA food category 14.1 from six countries – Australia, Brazil, China, Mexico, South Africa and the USA – through the International Council of Beverages Associations (ICBA) as well as use level data for 86 products from Norway. The average typical and maximum reported use levels of benzoates in GSFA food category 14.1.4 (water-based flavoured drinks) ranged from 83 to 209 mg/L and from 131 to 627 mg/L, respectively.

The Committee additionally evaluated published data on dietary exposures to benzoates from all foods at a national level. Information published since 2000 from 15 countries was considered. The estimates were made by combining mean analytically measured levels (Australia, Austria, Belgium, Brazil, China, Denmark, Lebanon, New Zealand, the Republic of Korea, Saudi Arabia and Serbia) or means from use level surveys (France, Ireland, Italy and the United Kingdom) of benzoates in food with their consumption levels from national consumption surveys (24-hour recall or intake record and/or food frequency questionnaire). Norway also submitted an estimate of benzoate exposure from soft drinks, saft (a concentrate produced from fruit juice) and flavoured water.

**Assessment of dietary exposure**

The Committee reviewed dietary exposure estimates submitted by the ICBA for four countries (Brazil, Mexico, South Africa and the USA), performed by combining individual consumption data with maximum reported use levels or national maximum permitted levels for non-alcoholic beverages. The Committee concluded that the information would not be appropriate for this assessment because maximum reported use levels or national maximum permitted levels were used in place of measured or average typical use levels. However, the Committee decided to make its own exposure estimates for these countries using consumption figures from the submitted data combined with average typical use levels. The Committee also prepared exposure estimates using food consumption data for non-alcoholic beverages from the FAO/WHO CIFOcOs database. These estimates are summarized in [Table 1](#). A total of 131 consumption data from 25 countries belonging to 10 GEMS/Food clusters were used. Additionally, as previously noted, the Committee also evaluated published estimates of total dietary exposure to benzoates (including non-beverage uses). These are summarized in [Table 2](#).

Overall, the largest contributions to total estimated dietary exposure to benzoates were from non-alcoholic beverages (up to 80% for the general population of Brazil) for most countries.

Table 1

**Benzoate exposure for consumers-only from non-alcoholic (“soft”) beverages (CIFOCOs data plus Committee-prepared estimates)**

Age group	Mean exposure (mg/kg bw per day)	95th percentile exposure (mg/kg bw per day)
Toddlers and young children (1–7 years)	0.3–4.1	1.7–10.9 <sup>a</sup>
Other children, including adolescents (8–17 years)	0.2–2.7	0.5–7.0
Adults (18+ years)	0.1–1.7	0.2–4.2

<sup>a</sup> 97.5th percentile exposure from South Africa.

Table 2

**Literature-derived dietary exposure estimates for benzoates from all foods (including non-beverage uses)**

Age group	General population exposure (mg/kg bw per day)		Consumers-only exposure (mg/kg bw per day)	
	Mean	95th percentile	Mean	95th percentile
Toddlers and young children (1–7 years)	1.5	3.9	0.9–6.8	2.0–9
Other children, including adolescents (8–17 years)	0.1–1.0	0.4–2.6	0.2–4	1.1–8
Adults (18+ years)	0.04–1.3	0.2–2.9	0.1–6.2	0.7–5.5

**Evaluation**

Based on the available data set (reported use levels from industries and analytical measurements from the literature), the Committee noted that there is consistency in the average typical range of concentration levels for benzoates used in the GSFA food subcategory for non-alcoholic (“soft”) beverages (GSFA food category 14.1). For example, typical reported concentration levels from industries ranged from 83 to 209 mg/L for water-based flavoured drinks (food category 14.1.4), and analytically quantified measurements ranged from 63 to 259 mg/L for non-alcoholic beverages (food category 14.1). These levels are lower than national maximum limits (150–400 mg/L) or limits for GSFA food category 14.1.4 (600 mg/L). The Committee also noted that most of the reported estimates for mean and high-percentile per capita benzoate exposure were below the upper bound of the ADI, despite different methodologies and assumptions applied in the preparation of the exposure estimates.

None of the mean exposure estimates for consumers of non-alcoholic (“soft”) beverages exceeded the upper bound of the ADI: 0.3–4.1 mg/kg bw per day for toddlers and young children, 0.2–2.7 mg/kg bw per day for other children, including adolescents, and 0.1–1.7 mg/kg bw per day for adults. However, the

Committee noted that the 95th percentile exposures for the consumers-only group exceeded the upper bound of the ADI in some cases: up to 10.9 mg/kg bw per day for toddlers and young children and up to 7.0 mg/kg bw per day for other children, including adolescents. Additionally, the Committee noted that in some countries, the overall dietary exposure to benzoates for toddlers, young children and adolescents also exceeds the upper bound of the ADI at the high percentiles. Reduction of those exposures exceeding the upper bound of the ADI would require consideration of dietary patterns for both beverage and non-beverage foods containing benzoates and typical/allowed benzoate use levels in those countries.

A dietary exposure monograph was prepared.

### 3.1.2 Lipase from *Fusarium heterosporum* expressed in *Ogataea polymorpha*

#### **Explanation**

At the request of CCFA at its Forty-sixth Session (9), the Committee evaluated the safety of lipase (triacylglycerol lipase; Enzyme Commission No. 3.1.1.3) from *Fusarium heterosporum* expressed in *Ogataea polymorpha*, which it had not previously considered. *Ogataea polymorpha* was recently renamed from *Hansenula polymorpha* based on genetic analyses. Lipase hydrolyses ester bonds in the 1- and 3-positions of fatty acids in triglycerides. The enzyme also has activity towards sn-1 ester bonds in other lipid components, including diacyl-phospholipids and diacyl-galactolipids. In this report, the expression “lipase” refers to the lipase enzyme and its amino acid sequence, the expression “lipase liquid enzyme concentrate” refers to the test material used in the toxicity studies evaluated, and the expression “lipase enzyme preparation” refers to the preparation formulated for commercial use. The lipase enzyme preparation is used as a processing aid in the manufacture of bakery products, pasta and noodles, in egg yolk and in the degumming of edible oil.

#### **Genetic background**

The host microorganism, *Ogataea polymorpha*, is a non-pathogenic and non-toxicogenic yeast commonly used in commercial food enzyme production. A uracil auxotroph of the wild-type *O. polymorpha* strain ATCC 34438, designated as RB11, was further genetically modified via plasmid transformation to produce a lipase originating from *F. heterosporum*. The transformation vector was created from a modified *Escherichia coli* pBR322 in which the genes encoding ampicillin resistance (*Apr*) and tetracycline resistance (*TCr*) had been removed. The synthetic lipase gene, containing a codon sequence optimized for maximum production in *O. polymorpha* of the native *F. heterosporum* lipase, combined with a promoter and a terminator from native *O. polymorpha*, was inserted into the

vector. The *Saccharomyces cerevisiae* orotidine-5'-phosphate decarboxylase gene (*URA3*) was also inserted into the vector as a selectable marker. The resulting vector was used to transform the host strain RB11 to obtain the lipase production strain *O. polymorpha* GICC03251. The genetic construction was verified by Southern blot analysis to confirm that only the intended genetic modification to the *O. polymorpha* strain had been made. The production strain is stable with respect to the introduced DNA.

### **Chemical and technical considerations**

Lipase is produced by submerged straight-batch or fed-batch pure culture fermentation of a genetically modified strain of *O. polymorpha* containing a synthetic gene that encodes the same amino acid sequence as the native lipase gene from *F. heterosporum*. The fermentation broth carrying the enzyme is separated from the biomass by filtration and/or centrifugation. The liquid filtrate containing the enzyme is then concentrated by ultrafiltration, followed by polish filtration. Food-grade preservatives are added to the enzyme concentrate before spray drying or agglomeration, and the product is formulated to the desired activity with food-grade ingredients. The lipase enzyme preparation conforms to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing ([http://www.fao.org/ag/agn/jecfa-additives/docs/enzymes\\_en.htm](http://www.fao.org/ag/agn/jecfa-additives/docs/enzymes_en.htm)).

Lipase activity is measured in titratable phospholipase units (TIPU). One TIPU is defined as the amount of enzyme liberating 1  $\mu\text{mol}$  free fatty acid from a lecithin substrate per minute under the assay conditions. The mean activity of lipase from three batches of the lipase enzyme concentrate was approximately 14 000 TIPU/g. The mean total organic solids (TOS) content of these three batches was 15%. TOS includes the enzyme of interest and residues of organic materials, such as proteins, peptides and carbohydrates, derived from the production organism during the manufacturing process. The final commercial formulations can vary widely in activity and TOS content, depending on the use. The lipase enzyme preparation is used at concentrations up to 220 mg TOS/kg raw material, depending on the proposed food application. Lipase is expected to be inactivated in food or removed from the oil.

### **Assessment of potential allergenicity**

Lipase from *F. heterosporum* was evaluated for potential allergenicity using the bioinformatics criteria recommended by FAO/WHO (8, 11), but modified at the present meeting (see [section 2.4.1](#)). The amino acid sequence of lipase from *F. heterosporum* was compared with the amino acid sequences of known allergens in publicly available databases. A search for matches with greater than 35%

identity over a window of 80 amino acids and a search for sequence identity of eight contiguous amino acids produced no match. Therefore, the Committee considered that dietary exposure to lipase from *F. heterosporum* is not anticipated to pose a risk of allergenicity.

### **Toxicological data**

An acute oral toxicity study using a freeze-dried powdered form of a lipase liquid enzyme concentrate demonstrated no sign of toxicity at 1.33 g/kg bw in rats. In a 13-week oral toxicity study in rats, no treatment-related adverse effects were observed when the lipase liquid enzyme concentrate was administered by gavage at doses up to 669 mg TOS/kg bw per day (12). The results from an in vitro bacterial reverse mutation assay and an in vitro chromosomal aberration assay in human lymphocytes using the powdered form of the lipase enzyme concentrate were both negative. The Committee concluded that the lipase enzyme preparation is unlikely to be genotoxic.

### **Assessment of dietary exposure**

An estimate of the theoretical dietary exposure to this lipase enzyme preparation was made by the Committee based on the level of TOS in the lipase enzyme preparation and its maximum use levels in bakery products, pasta and noodles (44 mg TOS/kg flour) and egg yolk (220 mg TOS/kg egg) and in the degumming of edible oil (22 mg TOS/kg crude oil). The combination of these maximum levels with per capita food consumption data from the USA (supplied by the sponsor) or from the GEMS/Food cluster diets results in a potential total dietary exposure of 0.5 mg TOS/kg bw per day for a 60 kg individual. The Committee noted that the enzyme will be inactivated in baking and cooking steps and will be removed from the refined oil.

### **Evaluation**

No treatment-related adverse effects were seen at the highest dose tested (669 mg TOS/kg bw per day) in the 13-week study of oral toxicity in rats (12). A comparison of the dietary exposure estimate of 0.5 mg TOS/kg bw per day with the highest dose tested of 669 mg TOS/kg bw per day results in a margin of exposure (MOE) of at least 1300. The Committee established an ADI “not specified” for lipase from *F. heterosporum* expressed in *O. polymorpha* when used in the applications specified and in accordance with good manufacturing practice.

A toxicological monograph was prepared.

New specifications and a Chemical and Technical Assessment were prepared.



### 3.1.3 Magnesium stearate

#### **Explanation**

The commercial product called magnesium stearate is composed mainly of magnesium salts of stearic and palmitic acids, obtained from edible fats and oils.

In 2010, at the Forty-second Session of CCFA (13), the deletion of magnesium salts of fatty acids from the INS had been proposed. The International Alliance of Dietary/Food Supplement Associations offered technological justification for the use of this additive. CCFA at its Forty-third Session in 2011 (14) assigned the new INS number 470(iii) to magnesium stearate and asked the Committee to conduct a safety assessment, assess dietary exposure and set specifications for magnesium stearate.

Magnesium salts of fatty acids, previously included in the INS as number 470 (salts of fatty acids), have been evaluated by the Committee at its seventeenth, twenty-ninth, forty-ninth and seventy-sixth meetings (Annex 1, references 32, 70, 131 and 211). At the seventeenth meeting (in 1973), the Committee evaluated salts of palmitic and stearic acids and established ADIs “not limited”<sup>2</sup> with notes that palmitic and stearic acids are normal products of the metabolism of fats and that their metabolic fate is well established. Provided that the contribution of cations such as magnesium does not add excessively to the normal body load, there would be no need to consider the use of these substances in any different light to that of dietary fatty acids.

At its twenty-ninth meeting (in 1985), the Committee was of the opinion that “ADIs for ionizable salts should be based on previously accepted recommendations for the constituent cations and anions”. The Committee listed ADIs for a number of combinations of cations and anions, including those of magnesium stearate and magnesium palmitate (ADI “not specified”). The Committee was concerned that dietary exposure resulting from the use of magnesium salts as food additives may have a laxative effect. It was also noted that infants are particularly sensitive to the sedative effects of magnesium salts and that individuals with chronic renal impairment retained 15–30% of administered magnesium, which could cause toxicity. The Committee stated that fatty acids are normal constituents of coconut oil, butter and other edible oils and that they do not represent a toxicological problem. As the Committee had no information on the manufacture or use of the food-grade materials at that time, an ADI for magnesium stearate was not established.

At its forty-ninth meeting (in 1997), the Committee evaluated the safety of palmitic acid and stearic acid when used as flavouring agents and concluded that they would not present a safety concern under the proposed conditions of use.

<sup>2</sup> This term is no longer used by JECFA. It has the same meaning as ADI “not specified”.

At its seventy-sixth meeting (in 2012), the Committee established an ADI “not specified” for a number of magnesium-containing food additives and recommended that total dietary exposure to magnesium from food additives and other sources in the diet should be assessed. This was in the context of the evaluation of magnesium dihydrogen diphosphate, in which the estimated chronic dietary exposure to magnesium from the proposed uses was up to twice the background exposures from food previously noted by the Committee and may be in the region of the minimum laxative effective dose.

For the present evaluation, a range of published studies together with three reports on genotoxicity testing of magnesium stearate were submitted to the Committee.

### ***Chemical and technical considerations***

Magnesium stearate is an off-white to white, very fine powder that is greasy to the touch and practically insoluble in water. It is used as an anticaking agent, emulsifier and binder in food supplement tablets, capsules and powders, compressed and granulated mints and candy, chewing gum, herbs and spices, and bakery ingredients. According to the industry, the use levels in these categories range from 0.05% to 3% weight per weight (w/w).

The commercial product is manufactured by either a direct process, called fusion, in which fatty acids are directly reacted with a magnesium source, such as magnesium oxide, to form magnesium salts of the fatty acids; or an indirect process, called precipitation, in which a sodium soap is produced by the reaction of fatty acids with sodium hydroxide in water and the product is precipitated by adding magnesium salts to the soap.

The final product contains not less than 4.0% and not more than 5.0% magnesium, on a dried basis, and the fatty acid fraction contains not less than 40.0% stearic acid and not less than 90.0% of the sum of stearic acid and palmitic acid. Specifications for unsaponifiable matter are set to not more than 2%. In addition, the limits for cadmium, lead and nickel are specified.

According to the data provided by industry, magnesium stearate is a stable product for which no decomposition products are expected under normal storage conditions.

### ***Toxicological data***

The oral median lethal dose ( $LD_{50}$ ) for magnesium stearate of unknown composition administered to rats was greater than 10 g/kg bw. The Committee reviewed a 90-day study in which rats were fed a diet containing 0%, 5%, 10% or 20% of a commercial product of magnesium stearate of unknown composition. The Committee concluded that this study was not relevant for the evaluation

given the high concentrations tested, which might lead to dietary imbalances, and the lack of information on the composition of the material tested.

Magnesium stearate was not genotoxic in bacterial reverse mutation assays and did not induce chromosomal aberrations in mammalian cells. Magnesium stearate was also not genotoxic in an in vivo mouse micronucleus assay.

### ***Assessment of dietary exposure***

An estimate of the theoretical dietary exposure to magnesium stearate was made by the Committee based on the proposed maximum use levels. The combination of these levels with consumption data using EFSA's Comprehensive European Food Consumption Database for consumption groups "Other confectionery", "Chewing gum", "Bakery wares", "Herbs, spices, seasonings" and "Food supplements" results in a potential total dietary exposure to magnesium stearate of 44 mg/kg bw per day for children and 83 mg/kg bw per day for adults, corresponding to 2 and 4 mg/kg bw per day expressed as magnesium, respectively. This would contribute up to an additional 240 mg/day to the background exposure to magnesium from food of 180–480 mg/day.

The Committee noted that the consumption of the food additive may lead to an additional dietary exposure to stearic and palmitic acids in the order of 5 g/day.

### ***Evaluation***

An ADI "not specified" has been established for a number of magnesium salts used as food additives. The Committee concluded that there are no differences in the evaluation of the toxicity of magnesium stearate compared with other magnesium salts and confirmed the ADI "not specified" for magnesium salts of stearic and palmitic acids. However, the Committee was concerned that the use of magnesium salts in many food additives may result in combined exposure that may lead to a laxative effect.

### ***Recommendation***

Based on the present dietary exposure assessment, the Committee reiterates its earlier recommendation that total dietary exposure to magnesium from food additives and other sources in the diet should be assessed. This is important, as a large number of magnesium-containing food additives have been evaluated individually, but not collectively, in relation to their laxative effects.

A toxicological monograph was prepared.

New specifications and a Chemical and Technical Assessment were prepared.

### 3.1.4 Maltotetraohydrolase from *Pseudomonas stutzeri* expressed in *Bacillus licheniformis*

#### **Explanation**

At the request of CCFA at its Forty-sixth Session (9), the Committee evaluated the safety of maltotetraohydrolase (glucan 1,4- $\alpha$ -maltotetraohydrolase; Enzyme Commission No. 3.2.1.60) from *Pseudomonas stutzeri* expressed in *Bacillus licheniformis*, which it had not considered previously. The donor organism was recently reclassified from *Pseudomonas saccharophila*. Maltotetraohydrolase catalyses the hydrolysis of 1,4- $\alpha$ -D-glucosidic linkages in amylaceous polysaccharides. The reaction removes successive maltotetraose residues from the non-reducing chain ends. In this report, the expression “maltotetraohydrolase” refers to the modified maltotetraohydrolase enzyme and its amino acid sequence, the expression “maltotetraohydrolase liquid enzyme concentrate” refers to the test material used in the toxicity studies evaluated, and the expression “maltotetraohydrolase enzyme preparation” refers to the preparation formulated for commercial use. The maltotetraohydrolase enzyme preparation is commonly used as a processing aid in bakery products such as bread, bread buns, tortillas and crackers, as well as in the starch processing industry for the manufacture of corn sweeteners, such as high-fructose corn syrup (HFCS).

#### **Genetic background**

Maltotetraohydrolase is produced from a genetically modified strain of *B. licheniformis*, a non-pathogenic and non-toxigenic microorganism commonly used in commercial food enzyme production. Prior to the introduction of the maltotetraohydrolase gene from *P. stutzeri*, the host *B. licheniformis* strain was genetically modified through a series of deletions of genes encoding  $\alpha$ -amylase, chloramphenicol acetyltransferase, subtilisin, glutamic acid-specific protease and the *spoIIAC* gene responsible for sporulation. The resulting strain was transformed using an expression cassette containing the maltotetraohydrolase SAS3 gene, obtained from genetic cloning and a series of site-directed mutagenesis events. The maltotetraohydrolase SAS3 gene encodes a variant of the wild-type *P. stutzeri* maltotetraohydrolase, with the C-terminal starch-binding domain removed, 16 amino acids changed and one methionine residue added at the N-terminus of the enzyme. These changes improved thermostability, baking performance and fermentation yield. Upon transformation, the maltotetraohydrolase expression cassette was integrated into the host *B. licheniformis*, and the rest of the plasmid was deleted by recombinant excision. The final production strain was tested and found to be genetically stable.

### **Chemical and technical considerations**

Maltotetrahydrolase is produced by submerged straight-batch or fed-batch pure culture fermentation of the genetically modified strain of *B. licheniformis*. The fermentation broth carrying the enzyme is separated from the biomass by filtration and/or centrifugation. The liquid filtrate containing the enzyme is then concentrated by ultrafiltration, followed by polish filtration. The resulting enzyme concentrate is either spray-dried and standardized to the desired activity with food-grade ingredients (powdered form) or treated with sodium benzoate and potassium sorbate to the desired activity (liquid form). The maltotetrahydrolase enzyme preparation conforms to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing ([http://www.fao.org/ag/agn/jecfa-additives/docs/enzymes\\_en.htm](http://www.fao.org/ag/agn/jecfa-additives/docs/enzymes_en.htm)).

The activity of the maltotetrahydrolase enzyme is measured in betamyl units (BMU). One BMU is defined as the activity degrading 0.0351 mmol of blocked *p*-nitrophenyl- $\alpha$ -D-maltoheptaoside per minute in the presence of amyloglucosidase and  $\alpha$ -glucosidase at 25 °C in a reaction mix for 5 minutes. The mean activity of maltotetrahydrolase from three batches of the enzyme concentrate prior to formulation was approximately 300 000 BMU/g.

A typical commercial formulation of the maltotetrahydrolase enzyme preparation will contain 32% of enzyme as TOS. TOS includes the enzyme of interest and residues of organic materials, such as proteins, peptides and carbohydrates, derived from the production organism during the manufacturing process. The maltotetrahydrolase enzyme preparation is used at levels up to 23 mg TOS/kg raw material, depending on the proposed food application. The maltotetrahydrolase enzyme is expected to be inactivated during processing.

### **Assessment of potential allergenicity**

Maltotetrahydrolase was evaluated for potential allergenicity using the bioinformatics criteria recommended by FAO/WHO (8, 11), but modified at the present meeting (see [section 2.4.1](#)). The amino acid sequence of the enzyme was compared with the amino acid sequences of known allergens in publicly available databases. A search for matches with greater than 35% identity over a window of 80 amino acids and a search for sequence identity of eight contiguous amino acids produced no match. Therefore, the Committee considered that dietary exposure to maltotetrahydrolase is not anticipated to pose a risk of allergenicity.

### **Toxicological data**

Maltotetrahydrolase liquid enzyme concentrate administered to rats at 2 g/kg bw in an acute oral toxicity study demonstrated no sign of toxicity. In a 13-week repeated-dose oral toxicity study in rats, no treatment-related adverse effects

were observed when the maltotetraohydrolase liquid enzyme concentrate was administered by gavage at doses up to 93.4 mg TOS/kg bw per day (15). The results of an in vitro bacterial reverse mutation assay and an in vitro chromosomal aberration assay in human lymphocytes using the maltotetraohydrolase liquid enzyme concentrate were both negative. The Committee concluded that maltotetraohydrolase enzyme preparation is unlikely to be genotoxic.

### **Assessment of dietary exposure**

An estimate of the theoretical dietary exposure to maltotetraohydrolase enzyme preparation was made by the Committee based on the maximum level of TOS in the enzyme preparation and its maximum use levels in bakery products (23 mg TOS/kg flour) and HFCS production (20 mg TOS/kg starch). The combination of these levels with per capita food consumption data from the USA (supplied by the sponsor; corroborated with data from the GEMS/Food cluster diets) results in a potential dietary exposure of 0.1 mg TOS/kg bw per day for a 60 kg individual. The Committee noted that the enzyme will be inactivated in food processing and also removed from the HFCS final product during production.

### **Evaluation**

No treatment-related adverse effects were seen at the highest dose tested (93.4 mg TOS/kg bw per day) in the 13-week study of oral toxicity in rats (15). A comparison of the dietary exposure estimate of 0.1 mg TOS/kg bw per day with the highest dose tested of 93.4 mg TOS/kg bw per day results in an MOE of at least 900. The Committee established an ADI “not specified” for maltotetraohydrolase from *P. stutzeri* expressed in *B. licheniformis* when used in the applications specified and in accordance with good manufacturing practice.

A toxicological monograph was prepared.

New specifications and a Chemical and Technical Assessment were prepared.

#### **3.1.5 Mixed $\beta$ -glucanase, cellulase and xylanase from *Rasamsonia emersonii***

##### **Explanation**

At the request of CCFA at its Forty-sixth Session (9), the Committee evaluated the safety of mixed  $\beta$ -glucanase (3-(1,3;1,4)- $\beta$ -D-glucan 3(4) glucanohydrolase; Enzyme Commission No. 3.2.1.6), cellulase (4-(1,3;1,4)- $\beta$ -D-glucan 4-glucanohydrolase; Enzyme Commission No. 3.2.1.4) and xylanase (1,4- $\beta$ -D-xylan xylanohydrolase; Enzyme Commission No. 3.2.1.8) from *Rasamsonia emersonii*. This enzyme preparation has not been evaluated previously by the Committee. *Rasamsonia emersonii* was recently renamed from *Talaromyces emersonii* based on genetic analyses. The Committee evaluated several other

enzyme preparations of  $\beta$ -glucanase, cellulase or xylanase at its thirty-first, thirty-fifth, thirty-ninth, sixty-first and sixty-third meetings and established an ADI “not specified” for their use in several applications, such as the preparation of beer and baking products (Annex 1, references 78, 88, 101, 167 and 174). An exception was cellulase from *Penicillium funiculosum*, for which no ADI was established, as no safety data were submitted (Annex 1, reference 77). In this report, the expression “mixed  $\beta$ -glucanase, cellulase and xylanase liquid enzyme concentrate” is used when referring to the material tested in the toxicological studies evaluated; the expressions “ $\beta$ -glucanase”, “cellulase” and “xylanase” are used when referring to the enzymes and their amino acid sequences; and the expression “mixed  $\beta$ -glucanase, cellulase and xylanase enzyme preparation” is used when referring to the commercial enzyme product.

$\beta$ -Glucanase is an enzyme that catalyses the hydrolysis of 1,3- or 1,4- $\beta$ -D-glucosidic linkages in  $\beta$ -D-glucans. Cellulase is an enzyme that catalyses the endo-hydrolysis of 1,4- $\beta$ -D-glucosidic linkages in cellulose, lichenin and cereal  $\beta$ -D-glucans and the hydrolysis of 1,4-linkages in  $\beta$ -D-glucans that also have 1,3-linkages. Xylanase is an enzyme that catalyses the hydrolysis of 1,4- $\beta$ -xylosidic linkages in xylans.

The mixed  $\beta$ -glucanase, cellulase and xylanase enzyme preparation is intended to be used as a processing aid in brewing, potable alcohol (spirits) production and grain processing.

### **Genetic background**

The  $\beta$ -glucanase, cellulase and xylanase enzymes are simultaneously produced at high levels from a strain of *R. emersonii*. *Rasamsonia emersonii* has been taxonomically identified to be from the genus *Rasamsonia* by the Dutch culture collection, the Centraalbureau voor Schimmelcultures. *Rasamsonia emersonii* is a filamentous eukaryotic thermostable fungus that is capable of growing at pH 3.5–5.5 and 45–50 °C. *Rasamsonia emersonii* is also referred to in the literature as *Penicillium emersonii*, *Geosmithia emersonii* and *Talaromyces emersonii*. *Rasamsonia emersonii* is a non-pathogenic microorganism with a history of use in commercial food enzyme production. The *R. emersonii* production strain has been demonstrated to be genetically stable under laboratory conditions, with no significant decrease in yield or change in appearance of morphological variants. It is derived from the original wild-type strain that has been used for large-scale production of the mixed  $\beta$ -glucanase, cellulase and xylanase enzyme preparation since 1985. The production strain is a modification of the wild-type *R. emersonii* strain for increased enzyme production by classical mutagenesis and selection for higher enzyme productivity. Data indicate that the production strain does not



produce mycotoxins under large-scale fermentation conditions, indicating that the production strain is non-toxigenic.

### **Chemical and technical considerations**

The  $\beta$ -glucanase, cellulase and xylanase enzymes are produced by a controlled aerobic submerged fed-batch fermentation of a pure culture of *R. emersonii*. The enzymes are secreted into the fermentation broth and subsequently purified and concentrated. The enzyme concentrate is formulated with glycerol and sodium benzoate to achieve the desired activity and stability. The mixed  $\beta$ -glucanase, cellulase and xylanase enzyme preparation contains commonly used food-grade materials and conforms to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing ([http://www.fao.org/ag/agn/jecfa-additives/docs/enzymes\\_en.htm](http://www.fao.org/ag/agn/jecfa-additives/docs/enzymes_en.htm)).

The  $\beta$ -glucanase and cellulase activity is expressed in beta-glucanase fungique (BGF) units, as defined in the specific assay that measures a change in viscosity of a glucan substrate solution in the presence of  $\beta$ -glucanase and cellulase. The xylanase activity is expressed in xylanase viscosity units (XVU), as defined in the specific assay that measures a change in viscosity of a xylan substrate solution in the presence of xylanase; however, the method described to determine this activity is proprietary and non-transferable. The mean activities of  $\beta$ -glucanase and cellulase and of xylanase from three batches of the mixed  $\beta$ -glucanase, cellulase and xylanase, prior to formulation, were reported to be approximately 500 000 BGF/g and 3800 XVU/g, respectively.

A typical commercial formulation of the mixed  $\beta$ -glucanase, cellulase and xylanase enzyme preparation will contain 5.4–17% TOS, depending on the use. TOS includes the enzymes of interest and residues of organic materials, such as proteins, peptides and carbohydrates, derived from the production organism during the manufacturing process. The mixed  $\beta$ -glucanase, cellulase and xylanase enzyme preparation is used in brewing, potable alcohol (spirits) production and grain processing (production of non-alcoholic beverages [including soft drinks] and bakery ingredients) to reduce viscosity and improve filterability, yield and product consistency; it will be used at levels up to 25.5 mg TOS/kg raw material. The  $\beta$ -glucanase, cellulase and xylanase enzymes are expected to be inactivated during processing.

### **Assessment of potential allergenicity**

$\beta$ -Glucanase, xylanase and cellulase from *R. emersonii* have commonly been found in food, and there are no indications for allergic reactions due to their ingestion. In addition, two  $\beta$ -glucanases, a xylanase and a cellulase from *R. emersonii* have been evaluated for potential allergenicity using the bioinformatics



criteria recommended by FAO/WHO (8, 11), but modified at the present meeting (see section 2.4.1). A search for matches with greater than 35% identity over a window of 80 amino acids and a search for sequence identity of eight contiguous amino acids produced no match. Based on these data, the Committee concluded that dietary exposure to the  $\beta$ -glucanase, cellulase and xylanase enzymes from *R. emersonii* is not anticipated to pose a risk of allergenicity.

### **Toxicological data**

In a 13-week study of oral toxicity in rats, no treatment-related adverse effects were seen when the mixed  $\beta$ -glucanase, cellulase and xylanase liquid enzyme concentrate was administered by gavage at doses up to 84.8 mg TOS/kg bw per day (16). The mixed  $\beta$ -glucanase, cellulase and xylanase liquid enzyme concentrate gave negative results in a bacterial reverse mutation assay and an in vitro chromosomal aberration assay, and the Committee concluded that the mixed  $\beta$ -glucanase, cellulase and xylanase enzyme preparation is unlikely to be genotoxic.

### **Assessment of dietary exposure**

The Committee estimated the theoretical dietary exposure to the mixed  $\beta$ -glucanase, cellulase and xylanase enzyme preparation based on the estimated maximum levels in final food products (3.5 mg TOS/L in beer, 3 mg TOS/L in non-alcoholic beverages [including soft drinks], 3 mg TOS/kg in bakery ingredients [starch, fibres, flour] and 0 mg TOS/L in potable alcohol [spirits]). The combination of these maximum levels with per capita food consumption data from the USA (supplied by the sponsor) and data from the GEMS/Food consumption cluster diets results in a potential dietary exposure of 0.08 mg TOS/kg bw per day for a 60 kg person. The Committee noted that the enzymes will be inactivated in processed food and that the exposure estimate is conservative.

### **Evaluation**

No treatment-related adverse effects were seen at the highest dose tested (84.8 mg TOS/kg bw per day) in the 13-week study of oral toxicity in rats (16). A comparison of the dietary exposure estimate of 0.08 mg TOS/kg bw per day with the highest dose tested of 84.8 mg TOS/kg bw per day results in an MOE of at least 1000. The Committee established an ADI “not specified” for the mixed  $\beta$ -glucanase, cellulase and xylanase enzyme preparation from *R. emersonii*, used in the applications specified and in accordance with good manufacturing practice.

A toxicological monograph was prepared.

New tentative specifications were prepared, with a request for the following information:

- method to determine the identity for  $\beta$ -glucanase, including data from a minimum of five batches using the method described;
- method to determine the identity for cellulase, including data from a minimum of five batches using the method described;
- a non-proprietary method to determine the identity and activity for xylanase that can be used by control laboratories and data from a minimum of five batches using the method described.

The above-requested information should be submitted by December 2016 in order for the tentative specifications to be revised; failure to provide this information may lead to a withdrawal of the specifications, with a possible impact on the ADI.

A Chemical and Technical Assessment was prepared.

### 3.1.6 Mixed $\beta$ -glucanase and xylanase from *Disporotrichum dimorphosporum*

#### **Explanation**

At the request of CCFA at its Forty-sixth Session (9), the Committee evaluated the safety of mixed  $\beta$ -glucanase (3-(1,3;1,4)- $\beta$ -D-glucan 3(4) glucanohydrolase; Enzyme Commission No. 3.2.1.6) and xylanase (1,4- $\beta$ -D-xylan xylanohydrolase; Enzyme Commission No. 3.2.1.8) from *Disporotrichum dimorphosporum*. This enzyme preparation has not been evaluated previously by the Committee. The Committee evaluated several other enzyme preparations of  $\beta$ -glucanase or xylanase at its thirty-first, thirty-fifth, thirty-ninth, sixty-first and sixty-third meetings and established an ADI “not specified” for their use in several applications, such as the preparation of beer and baking products (Annex 1, references 78, 88, 101, 167 and 174). In this report, the expression “mixed  $\beta$ -glucanase and xylanase liquid enzyme concentrate” is used when referring to the material tested in the toxicological studies evaluated; the expressions “ $\beta$ -glucanase” and “xylanase” are used when referring to the enzymes and their amino acid sequences; and the expression “mixed  $\beta$ -glucanase and xylanase enzyme preparation” is used when referring to the commercial enzyme preparation.

$\beta$ -Glucanase is an enzyme that catalyses the hydrolysis of 1,3- or 1,4- $\beta$ -D-glucosidic linkages in  $\beta$ -D-glucans. Xylanase is an enzyme that catalyses the hydrolysis of 1,4- $\beta$ -D-xylosidic linkages in xylans.

The mixed  $\beta$ -glucanase and xylanase enzyme preparation is intended to be used as a processing aid in brewing, potable alcohol (spirits) production and grain processing.

#### **Genetic background**

The  $\beta$ -glucanase and xylanase enzymes are simultaneously produced at high levels from a strain of *D. dimorphosporum*. *Disporotrichum dimorphosporum* has

been taxonomically identified to be from the genus *Sporotrichum* by the Dutch culture collection, the Centraalbureau voor Schimmelcultures. *Disporotrichum dimorphosporum* is a saprophyte and a basidiomycete fungus; it is capable of growing at pH 3.5–5.5 and 28–32 °C. *Disporotrichum dimorphosporum* is a non-pathogenic microorganism with a history of use in commercial food enzyme production.

The *D. dimorphosporum* production strain is derived from the original wild-type strain that has been used for large-scale production of the mixed  $\beta$ -glucanase and xylanase enzyme preparation since 1999 after reisolation and subculturing. It has been demonstrated to be genetically stable under laboratory conditions, with no significant decrease in yield or change in appearance of morphological variants. Data indicate that the production strain does not produce mycotoxins under large-scale fermentation conditions, indicating that the production strain is non-toxicogenic.

#### **Chemical and technical considerations**

The  $\beta$ -glucanase and xylanase enzymes are produced by a controlled aerobic submerged fed-batch fermentation of a pure culture of *D. dimorphosporum*. The enzymes are secreted into the fermentation broth and subsequently purified and concentrated. Sodium benzoate and glycerol are added to the liquid enzyme concentrate, to standardize and stabilize the enzyme preparation. The mixed  $\beta$ -glucanase and xylanase enzyme preparation contains commonly used food-grade materials and conforms to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing ([http://www.fao.org/ag/agn/jecfa-additives/docs/enzymes\\_en.htm](http://www.fao.org/ag/agn/jecfa-additives/docs/enzymes_en.htm)).

The  $\beta$ -glucanase activity is expressed in BGF units, as defined in the specific assay that measures a change in viscosity of a glucan substrate solution in the presence of  $\beta$ -glucanase and cellulase. The xylanase activity is expressed in XVU, as defined in the specific assay that measures a change in viscosity of a xylan substrate solution in the presence of xylanase; however, the method described to determine this activity is proprietary and non-transferable. The mean activities of  $\beta$ -glucanase and xylanase from three batches of the mixed  $\beta$ -glucanase and xylanase, prior to formulation, were reported to be approximately 520 000 BGF/g and 3300 XVU/g, respectively.

A typical commercial formulation of the mixed  $\beta$ -glucanase and xylanase enzyme preparation will contain 11–17% TOS, depending on the use. TOS includes the enzymes of interest and residues of organic materials, such as proteins, peptides and carbohydrates, derived from the production organism during the manufacturing process.

The mixed  $\beta$ -glucanase and xylanase enzyme preparation is used in brewing, potable alcohol (spirits) production and grain processing (production of non-alcoholic beverages [including soft drinks] and bakery ingredients) to reduce viscosity and improve filterability, yield and product consistency; it will be used at levels up to 36.5 mg TOS/kg raw material. The  $\beta$ -glucanase and xylanase enzymes are expected to be inactivated during processing.

#### **Assessment of potential allergenicity**

$\beta$ -Glucanase and xylanase from *D. dimorphosporum* have commonly been found in food, and there are no indications for allergic reactions due to their ingestion. As these enzymes are not products of genetic modification, an assessment of their potential allergenicity is not required.

#### **Toxicological data**

In a 13-week study of oral toxicity in rats, no treatment-related adverse effects were seen when the mixed  $\beta$ -glucanase and xylanase liquid enzyme concentrate was administered by gavage at doses up to 199 mg TOS/kg bw per day (17). The mixed  $\beta$ -glucanase and xylanase liquid enzyme concentrate was not genotoxic in a bacterial reverse mutation assay. In an in vitro chromosomal aberration assay, the liquid enzyme concentrate induced a small, but statistically significant, increase in chromosomal aberrations (chromatid-type breaks) after exposure of the cells to the highest concentration tested for 20 hours, in the absence of S9 only. However, as the effect was small, a mitotic inhibition of 58% was observed at the highest concentration tested and the level of statistical significance was related to the fact that no aberrations were observed in the controls, the Committee considered these results not to be of toxicological relevance. In combination with the negative results of the in vitro reverse mutation assay, the Committee did not have concerns with respect to the genotoxicity of the mixed  $\beta$ -glucanase and xylanase enzyme preparation.

#### **Assessment of dietary exposure**

The Committee estimated the theoretical dietary exposure to the mixed  $\beta$ -glucanase and xylanase enzyme preparation based on the estimated maximum levels in final food products (6.2 mg TOS/L in beer, 28 mg TOS/L in non-alcoholic beverages [including soft drinks], 28 mg TOS/kg in bakery ingredients [starch, fibres, flour] and 0 mg TOS/L in potable alcohol [spirits]). The combination of these maximum levels with per capita food consumption data from the USA (supplied by the sponsor) and data from the GEMS/Food cluster diets results in a potential dietary exposure of 0.7 mg TOS/kg bw per day for a 60 kg person. The

Committee noted that the enzymes will be inactivated in processed food and that the exposure estimate is conservative.

### **Evaluation**

No treatment-related adverse effects were seen at the highest dose tested (199 mg TOS/kg bw per day) in the 13-week study of oral toxicity in rats (17). A comparison of the dietary exposure estimate of 0.7 mg TOS/kg bw per day with the highest dose tested of 199 mg TOS/kg bw per day gives an MOE of at least 280. The Committee established an ADI “not specified” for the mixed  $\beta$ -glucanase and xylanase enzyme preparation from *D. dimorphosporum*, used in the applications specified and in accordance with good manufacturing practice.

A toxicological monograph was prepared.

New tentative specifications were prepared, with a request for the following information:

- a method to determine the identity for  $\beta$ -glucanase, including data from a minimum of five batches using the method described;
- a non-proprietary method to determine the identity and activity for xylanase that can be used by control laboratories and data from a minimum of five batches using the method described.

The above-requested information should be submitted by December 2016 in order for the tentative specifications to be revised; failure to provide this information may lead to a withdrawal of the specifications, with a possible impact on the ADI.

A Chemical and Technical Assessment was prepared.

### **3.1.7 Polyvinyl alcohol (PVA) – polyethylene glycol (PEG) graft copolymer**

#### **Explanation**

At the request of CCFA at its Forty-sixth Session (9), the Committee evaluated the safety of polyvinyl alcohol (PVA) – polyethylene glycol (PEG) graft copolymer, which it had not evaluated previously. The individual components of the copolymer have been evaluated previously by the Committee. The use of PVA as a coating, binder, sealing and surface finishing agent in food products such as dairy-based desserts, confectionery, cereal products and food supplements was evaluated at the sixty-first meeting of the Committee, and the Committee established an ADI of 50 mg/kg bw for PVA (Annex 1, reference 167). At the twenty-third meeting, the Committee established an ADI of 10 mg/kg bw for polyethylene glycols (Annex 1, reference 51).

PVA-PEG graft copolymer is a synthetic branched graft copolymer primarily intended for use in aqueous film coatings in the preparation

and formulation of food supplements. It is currently approved for use for pharmaceutical applications in several regions, including the European Union, the USA and Japan. Recently, PVA-PEG graft copolymer was authorized as an additive for use in solid food supplements in the European Union, based on the evaluation by EFSA.

### ***Chemical and technical considerations***

Graft copolymers are a form of copolymer where the side-chains are structurally different from the main chain. PVA-PEG graft copolymer is a synthetic, branched graft copolymer consisting of side-chains of PVA on a main chain of PEG. It consists of approximately 75% vinyl alcohol units ( $-\text{CH}_2\text{CH}_2(\text{OH})-$ ) and 25% ethylene glycol units ( $-\text{CH}_2\text{CH}_2\text{O}-$ ).

PVA-PEG graft copolymer is a white to pale yellow free-flowing powder. It is manufactured by grafting polyvinyl acetate side-chains onto a PEG backbone that has an average molecular weight of 6000 Da. The polyvinyl acetate side-chains are then hydrolysed to form PVA side-chains. Based on the manufacturing conditions, PVA-PEG graft copolymer has an average of 2–3 PVA side-chains per PEG backbone. It has a weight-average molecular weight ranging from 40 000 to 50 000 Da. In the specifications, maximum limits have been set for a number of impurities, including vinyl acetate (20 mg/kg) and ethylene glycol and diethylene glycol (400 mg/kg, singly or in combination).

The predominant use in food supplements is that of glazing agent or, more specifically, as an aqueous film coating for food supplement tablets at a use level of up to 5% (w/w). PVA-PEG graft copolymer also has minor uses in food supplements as a stabilizer and binder for tablets at a use level of up to 10% (w/w).

### ***Toxicological data***

The toxicokinetic properties of  $^{14}\text{C}$ -labelled PVA-PEG graft copolymer were investigated in rats following administration of a single oral dose of 10 or 1000 mg/kg bw by gavage. The cumulative percentage of radioactivity recovered in the faeces of males and females at 48 hours post-dosing was approximately 100%. Excretion via urine, exhaled air and bile was negligible at both dose levels. The bioavailability was calculated to be less than 1%. No study on toxicokinetics in humans was available, but the Committee concluded that in humans, PVA-PEG graft copolymer would also be expected to be mainly eliminated via the faeces and that the bioavailability of PVA-PEG graft copolymer would be negligible.

PVA-PEG graft copolymer had an  $\text{LD}_{50}$  of greater than 2000 mg/kg bw in an acute oral toxicity study in rats.

In a 90-day oral toxicity study in rats, the only treatment-related effect observed was an increase in water consumption in high-dose males and females. In the absence of other treatment-related findings, this was not considered an adverse effect. The no-observed-adverse-effect level (NOAEL) was 1610 mg/kg bw per day, the highest dose tested.

In a 9-month oral toxicity study in dogs, an increase in mean absolute ovary weights was reported in all female treatment groups. These values were within the historical control range of the testing facility (range: 979–2258 mg; mean: 1378 mg), whereas the value of the control group was below the historical control range. In the absence of changes in the reproductive organs in rats in the above-described study and because no effects were seen in the reproductive toxicity study (see below), it was concluded that these changes were not treatment related. Therefore, the NOAEL was 780 mg/kg bw per day, the highest dose tested.

PVA-PEG graft copolymer was tested for genotoxicity in a bacterial reverse mutation assay, a gene mutation assay in mouse lymphoma L5178Y TK<sup>+/−</sup> cells and an in vivo micronucleus assay in mice using intraperitoneal administration. All three tests gave negative results. Therefore, the Committee concluded that PVA-PEG graft copolymer is unlikely to be genotoxic.

PVA-PEG graft copolymer has not been tested in a long-term toxicity and carcinogenicity study. Given the negative genotoxicity studies, the lack of adverse effects in the short-term studies and the negligible bioavailability of PVA-PEG graft copolymer, the Committee did not consider a study of long-term toxicity or carcinogenicity to be necessary for the safety evaluation of PVA-PEG graft copolymer.

In a two-generation reproductive toxicity study in rats, no treatment-related effects were observed. The NOAEL for parental, offspring and reproductive toxicity was 1000 mg/kg bw per day, the highest dose tested.

In two developmental toxicity studies, no treatment-related maternal or developmental effects were observed when rats or rabbits were given PVA-PEG graft copolymer at dose levels up to 1000 mg/kg bw per day during gestation. In both studies, the NOAELs for maternal toxicity and for embryo/fetal toxicity were 1000 mg/kg bw per day, the highest dose tested.

The Committee noted that the use of PVA-PEG graft copolymer that complies with the proposed specifications could lead to exposure to vinyl acetate, ethylene glycol and diethylene glycol. The Committee has not previously evaluated the safety of vinyl acetate. In oral long-term studies of the toxicity and carcinogenicity of vinyl acetate in rats and mice (18–20), statistically significant increases in incidences of tumours, mainly in the upper gastrointestinal tract, were observed at dose levels starting from 50 mg/kg bw per day (19).

Diethylene glycol was evaluated by the Committee at its twenty-third meeting ([Annex 1](#), reference 50). At that meeting, the Committee concluded



that diethylene glycol was not suitable as a food additive because it produces renal damage, calcium oxalate stones and liver damage in a number of species, including humans, and is associated with bladder tumours in rats at higher levels. In view of the secondary nature of the bladder tumours produced and the relatively high levels of the substance required to produce kidney stones or liver damage, the Committee concluded that its presence as an impurity in food additives at low levels may be tolerated and that this should be evaluated on a case-by-case basis. Ethylene glycol has not been evaluated previously by the Committee. The Scientific Committee on Food of the European Union (SCF) derived a group tolerable daily intake (TDI) of 0.5 mg/kg bw for ethylene glycol and diethylene glycol in 1986 (21) and confirmed it in 2002 (22).

### **Assessment of dietary exposure<sup>3</sup>**

An estimate of the theoretical dietary exposure to PVA-PEG graft copolymer was made by the Committee based on the estimated levels in food supplements (50 mg PVA-PEG graft copolymer for a 1 g tablet) from its primary use as a coating for tablets and the assumption that the exposures to PVA-PEG graft copolymer from pharmaceutical products and food supplements are the same. The exposure from the stated minor uses as a stabilizer and binder for tablets at levels up to 10% is expected to be covered by the conservative estimates below. If the levels of PVA-PEG graft copolymer in food supplements are combined with high consumption data for food supplements from the USA (supplied by the sponsor) and from the United Kingdom Food Standards Agency and if exposure to PVA-PEG graft copolymer from pharmaceutical products is included, potential total dietary exposures of 25 mg/kg bw per day for adults and 40 mg/kg bw per day for children can be calculated.

If it is assumed that the impurity vinyl acetate is present in PVA-PEG graft copolymer at a concentration up to 20 mg/kg, dietary exposure to vinyl acetate from both food supplements and pharmaceutical products for high consumers could be up to 0.0005 mg/kg bw per day for adults and 0.0008 mg/kg bw per day for children.

If it is assumed that the impurities ethylene glycol and diethylene glycol are present in PVA-PEG graft copolymer at a concentration up to 400 mg/kg, singly or in combination, dietary exposure to the glycols (singly or in combination) from both food supplements and pharmaceutical products for high consumers could be up to 0.010 mg/kg bw per day for adults and 0.016 mg/kg bw per day for children.

<sup>3</sup> In this section and in the Evaluation, "dietary exposure" refers to exposure from both food supplements and pharmaceutical products.



The theoretical maximum daily intakes estimated here are conservative, owing to the assumption that all food supplements and pharmaceutical products are coated with PVA-PEG graft copolymer and the fact that the exposure estimates are for high consumers of both food supplements and pharmaceutical products.

### ***Evaluation***

On the basis of the available studies, in which no treatment-related effects were seen at the highest doses tested, the Committee considered PVA-PEG graft copolymer to be a substance of low oral toxicity in rats, rabbits and dogs. The bioavailability of PVA-PEG graft copolymer in rats is negligible, and PVA-PEG graft copolymer is unlikely to be genotoxic and is not associated with reproductive or developmental toxicity. Therefore, the Committee concluded that calculation of an MOE for PVA-PEG graft copolymer would not be meaningful.

Based on these data, the Committee would normally establish an ADI “not specified”. However, the Committee decided not to establish an ADI “not specified” for PVA-PEG graft copolymer in view of the impurities present, some of which may also be impurities in other food additives. The Committee had concerns that establishing an ADI “not specified” could lead to additional uses beyond those considered in the present evaluation and consequently could increase exposure to the impurities.

The use of PVA-PEG graft copolymer that complies with the proposed specifications could lead to a dietary exposure to ethylene glycol and diethylene glycol from both food supplements and pharmaceutical products up to 0.016 mg/kg bw per day for children (high consumers). This is 3% of the TDI of 0.5 mg/kg bw per day derived by the SCF, and therefore the exposure to ethylene glycol and diethylene glycol from the use of PVA-PEG graft copolymer that complies with the specifications established at the current meeting is not of safety concern when the food additive is used in the applications specified.

The use of PVA-PEG graft copolymer that complies with the proposed specifications could lead to dietary exposure to vinyl acetate from both food supplements and pharmaceutical products up to 0.0008 mg/kg bw per day for children. This dietary exposure estimate is at least 62 500 times lower than the dose levels at which increases in tumour incidences are observed in oral long-term studies of toxicity and carcinogenicity in rats and mice. Therefore, the exposure to vinyl acetate from the use of PVA-PEG graft copolymer that complies with the specifications established at the current meeting is not of safety concern when the food additive is used in the applications specified.

The Committee concluded that the use of PVA-PEG graft copolymer that complies with the specifications established at the current meeting is not of safety concern when the food additive is used as a glazing agent (aqueous film

coating), stabilizer and binder for tablets in the preparation and formulation of food supplements and in accordance with good manufacturing practice.

### **Recommendation**

The Committee noted that ethylene glycol and diethylene glycol may also be present as impurities in other food additives, such as polyethylene glycols and polysorbates, and the total exposure to these compounds from food additives may be higher than from PVA-PEG graft copolymer alone. Currently, only the specifications monograph for polyethylene glycols contains maximum limits for ethylene glycol and diethylene glycol (2500 mg/kg, singly or in combination). The Committee recommends setting and/or revising maximum limits for ethylene glycol and diethylene glycol that may occur as impurities in food additives at a future meeting.

A toxicological monograph was prepared.

New specifications and a Chemical and Technical Assessment were prepared.

## **3.2 Revision of specifications**

### **3.2.1 Advantame**

The specifications of advantame prepared at the seventy-seventh meeting of the Committee ([Annex 1](#), reference 214) were made tentative pending submission of the following information: suitability of the headspace gas chromatography method (using appropriate dissolution solvent) for determination of residual solvents, an alternative/improved HPLC method for the assay of advantame and acid of advantame using a standard curve, additional data and analytical methods for determination of palladium and platinum, and information on the purity and availability of the commercial reference standards used in the assay of advantame and acid of advantame.

At the present meeting, the Committee received and reviewed the above-requested data. The existing tentative specifications were revised, and the tentative status was removed. The Chemical and Technical Assessment was revised.

### **3.2.2 Annatto extracts (solvent-extracted bixin and solvent-extracted norbixin)**

At its seventy-seventh meeting ([Annex 1](#), reference 214), the Committee considered the suitability of the general method for the determination of residual solvents published in Volume 4 of the *Combined Compendium of Food Additive Specifications* ([Annex 1](#), reference 180) for the analysis of residual solvents in the preparation of solvent-extracted annatto extracts of bixin and norbixin.

The Committee concluded that neither solvent listed in the method is suitable for the analysis of solvent-extracted bixin and norbixin by headspace gas chromatography and considered a submitted method using dimethyl formamide (DMF) as dilution solvent. This method was published as tentative in FAO JECFA Monograph No. 14 (2013) and included in the online version of Volume 4 of the *Combined Compendium of Food Additive Specifications*. In order to evaluate the suitability of the method for the determination of residual solvents in annatto extracts dissolved in DMF, the Committee recommended that manufacturers provide results from the analysis of samples of solvent-extracted bixin and norbixin products using both methods.

At the present meeting, the Committee considered data submitted in response to the call for data. The Committee decided that sufficient information was provided to support the use of DMF as a suitable solvent for the determination of residual solvents in solvent-extracted bixin and norbixin.

The method for residual solvents by headspace gas chromatography in Volume 4 (2006) was revised at the present meeting to include a statement referring to the acceptable use of DMF or other solvents in headspace determination of residual solvents. This revised method will replace the current method in Volume 4 (2006) and will also replace the tentative method for the determination of residual solvents in annatto extracts published online and in FAO JECFA Monographs 14 (2013). The revised method will also be published in FAO JECFA Monographs 17 (2015).

The two existing specifications for solvent-extracted bixin and norbixin were revised to reflect the modification of the method in Volume 4 and to include sample and standard preparation information.

### 3.2.3 Food additives containing aluminium and/or silicon

The Committee at its seventy-seventh meeting ([Annex 1](#), reference 214) reviewed the specifications of food additives containing aluminium and/or silicon – namely, aluminium silicate; calcium aluminium silicate; calcium silicate; silicon dioxide, amorphous; and sodium aluminium silicate. The Committee found that information on composition and methods of manufacture, functional uses other than anticaking agent, data on loss on drying and loss on ignition, impurities soluble in 0.5 M hydrochloric acid, and assay was either not available or incomplete. Consequently, the specifications were made tentative, and data/information was requested. At the seventy-seventh meeting, the Committee agreed that “the tentative specifications will be withdrawn unless the requested information becomes available by the end of 2014”.

These additives were on the agenda of the present meeting to revise their tentative specifications.

### 3.2.3.1 Aluminium silicate

At the present meeting, the Committee did not receive any information. Consequently, the Committee decided to withdraw the tentative specifications.

### 3.2.3.2 Calcium aluminium silicate

At the present meeting, the Committee did not receive any information. Consequently, the Committee decided to withdraw the tentative specifications.

### 3.2.3.3 Calcium silicate

At the present meeting, the Committee received the requested information. The specifications were revised to include information on functional uses, pH, loss on drying, loss on ignition, impurities soluble in 0.5 M hydrochloric acid and the assay. The tentative status was removed. A Chemical and Technical Assessment was prepared.

### 3.2.3.4 Silicon dioxide, amorphous

Different forms of silicon dioxide (pyrogenic silica, precipitated silica, hydrated silica, silica aerogel and colloidal silica) are in use as food additives. At the present meeting, the Committee received limited information on some forms of silicon dioxide. The specifications were revised to include information on pH, loss on drying, loss on ignition, impurities (lead and arsenic) soluble in 0.5 M hydrochloric acid and the assay for some forms of silicon dioxide. The tentative status was maintained, and the following information was requested:

- raw materials used and methods of manufacture for different forms of silicon dioxide (pyrogenic silica, precipitated silica, hydrated silica, silica aerogel and colloidal silica);
- identification methods allowing the differentiation between the above forms of silicon dioxide;
- functional uses of different forms, and information on the types of products in which it is used and the use levels in these products;
- data on solubility using the procedure documented in Volume 4 (Analytical methods) of the *Combined Compendium of Food Additive Specifications* ([Annex 1](#), reference 180);
- data on the impurities soluble in 0.5 M hydrochloric acid for all forms of silicon dioxide used as food additives, from a minimum of five batches. If a different extraction and determination method is used, data should be provided along with details of the method and quality control (QC) data;

- suitability of the analytical method for the determination of aluminium, silicon and sodium using the proposed “Method of assay” along with data from a minimum of five batches. If a different method is used, data should be provided along with details of the method and QC data;
- in addition to the above information, data on pH, loss on drying and loss on ignition for hydrated silica, silica aerogel and colloidal silica.

The tentative specifications will be withdrawn unless the requested information is provided by December 2016.

#### 3.2.3.5 Sodium aluminium silicate

At the present meeting, the Committee received limited information. Specifications were revised to include information on Chemical Abstracts Service number, chemical formula, pH, loss on drying, loss on ignition and limits on impurities (lead and arsenic) soluble in 0.5 M hydrochloric acid. The tentative status was maintained, and the following information was requested:

- functional uses other than anticaking agent, if any, and information on the types of products in which it is used and the use levels in these products;
- data on solubility using the procedure documented in Volume 4 (Analytical methods) of the *Combined Compendium of Food Additive Specifications* ([Annex 1](#), reference 180);
- data on the impurities soluble in 0.5 M hydrochloric acid, from a minimum of five batches. If a different extraction and determination method is used, data should be provided along with details of the method and QC data;
- suitability of the analytical method for the determination of aluminium, silicon and sodium using the proposed “Method of assay”, along with data, from a minimum of five batches, using the proposed method. If a different method is used, data should be provided along with details of the method and QC data.

The tentative specifications will be withdrawn unless the requested information is provided by December 2016.

#### 3.2.4 Glycerol ester of gum rosin

The Committee has previously considered glycerol ester of gum rosin (GEGR), for use as an emulsifier/density adjustment agent for flavouring agents in non-alcoholic beverages and cloudy spirit drinks, at its seventy-first (2009), seventy-

fourth (2011) and seventy-seventh (2013) meetings ([Annex 1](#), references 196, 205 and 214). For each meeting, a public call for data was published requesting necessary information for the evaluation and to answer questions raised by the Committee.

At the seventy-first meeting, the Committee reviewed toxicological and chemical data and established a group ADI of 0–25 mg/kg bw for glycerol ester of wood rosin (GEWR) and GEGR and prepared new tentative specifications for GEGR. GEGR was evaluated based on the toxicity data for GEWR, the absence of toxicological effects of their corresponding non-esterified rosins and the qualitative similarity of the chemical components of GEGR and GEWR. However, the available toxicological data were very limited, and key studies (two 90-day oral toxicity studies in rats) were available only as summaries. The Committee requested

that it be provided with full reports of the two 90-day toxicity studies with GEGR in rats fed dietary concentrations of up to 1.0% to confirm the validity of the comparison of GEWR with GEGR. The Committee considered that although GEWR and GEGR are chemically similar, they are produced from different sources, processed using different procedures and conditions, and not identical in composition. The Committee therefore developed separate specifications for GEGR. The specifications were made tentative pending the submission of infrared spectra that correspond to the commercially available products, data on the resin acid composition obtained with updated chromatographic techniques, and additional information on methods that enable the identification of the individual rosin esters and their differentiation. This information should be submitted by the end of 2010. [[Annex 1](#), reference 196]

At the seventy-fourth meeting, the Committee reviewed GEGR:

The Committee noted that the requested full reports of the 90-day studies on GEGR had not been provided and that the validity of evaluating GEGR on the basis of toxicological data on GEWR still requires confirmation. The Committee noted that the temporary group ADI will be withdrawn if the compositional information on GEWR as well as the full reports of the 90-day toxicity studies on GEGR are not submitted by the end of 2012. [[Annex 1](#), reference 205]

Furthermore, the Committee at the seventy-fourth meeting noted

that the information on the composition and ester distribution of GEGR was incomplete and therefore could not confirm the claimed similarities to GEWR. No description of the methods used to generate the data on the GEGR composition was provided. [[Annex 1](#), reference 205]

The Committee at the seventy-fourth meeting maintained the specifications for GEGR as tentative and requested the following information:

To complete the evaluation of GEGR, additional data are required to characterize GEGR in commerce in relation to the composition of 1) the refined gum rosin currently used as the source rosin for the production of GEGR, 2) the glycerol ester of gum rosin, 3) the total glycerol esters of resin acids and 4) the neutrals. Validated methods for the determination of the substances considered in the specifications are also required. [Annex 1, reference 205]

At the seventy-seventh meeting, the Committee reported that

the requested two unpublished 90-day oral toxicity studies on GEGR in rats were not submitted. Furthermore, complete information on the composition of GEGR was not submitted. As the requested data were not submitted, the Committee withdrew the temporary group ADI of 0–12.5 mg/kg bw for GEGR and GEWR. [Annex 1, reference 214]

The Committee also noted the following:

Although the submitted analytical data included summarized information in relation to the composition of free resin acids and neutrals (non-acidic saponifiable and unsaponifiable substances) in GEGR, the Committee noted that the information on the composition and ester distribution of GEGR was incomplete and therefore could not confirm the claimed similarities to GEWR. [Annex 1, reference 214]

Therefore,

The specifications were maintained as tentative pending the submission of additional information by the end of 2014. Additional data are requested to characterize GEGR in commerce in relation to the composition of 1) the refined gum rosin currently used as the source rosin with regard to the levels (%) of resin acids and neutrals, 2) the glycerol ester of gum rosin with regard to the levels (%) of a) glycerol esters, b) free resin acids and c) neutrals and 3) the total glycerol esters of resin acids with regard to the levels (%) of a) glycerol monoesters and b) the sum of glycerol diesters and triesters (assay). Validated methods for the determination of the substances considered in the specifications are also required. [Annex 1, reference 214]

GEGR was again on the call for data for the present meeting. However, in response to this call, the Secretariat was informed that no further data would be provided to the Committee. Nonetheless, the Secretariat received information

from a sponsor on the first morning of the present meeting of the Committee. Despite the late submission, the Committee reviewed the documents, which refer to EFSA and JECFA assessments, but found that they do not contain any of the data required to complete the evaluation started at previous meetings.

The Committee agreed to withdraw the tentative specifications for GEGR.



## 4. Contaminants

### 4.1 Non-dioxin-like polychlorinated biphenyls

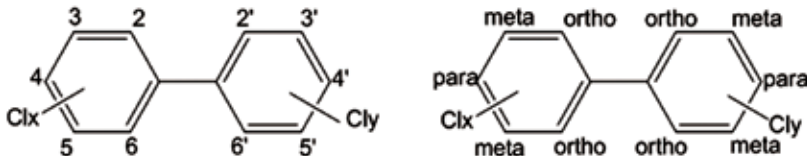
#### Explanation

The Committee was requested to undertake an assessment of NDL-PCBs by CCCF. The Committee has not previously evaluated NDL-PCBs specifically. The Committee reviewed PCBs at its thirty-fifth meeting ([Annex 1](#), reference 88). Additionally, WHO evaluated PCBs as part of EHC 2 in 1976 (23) and again as part of EHC 140 in 1993 (24). Dioxin-like PCBs (DL-PCBs), together with polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), were also reviewed by the Committee at its fifty-seventh meeting ([Annex 1](#), reference 154).

Two linked benzene rings in which 1–10 chlorine atoms substitute the hydrogen atoms on the benzene rings comprise the class of chemicals known as PCBs ([Fig. 1](#)). There are a total of 209 possible PCB congeners, based on the substitution positions along the phenyl rings. PCBs were intentionally produced in considerable amounts between the 1930s and 1970s and were used for a wide range of applications. Although there are 209 possible PCB congeners, of which 197 are NDL-PCBs, only about 130 have been reported in commercial mixtures. The congener profiles observed in commercial mixtures are not reflective of the congener profiles present in environmental compartments, food or human tissues. PCBs are thermally stable, persist in the environment and are found at large distances from their area of release. PCBs are lipophilic compounds and accumulate in the tissues of living organisms; they are taken up by humans primarily through the consumption of food, with foods of animal origin being the primary source of human exposure.

Fig. 1

#### Generic chemical structure of PCBs



where  $x + y = 1-10$

PCBs exhibit different toxicological effects depending on the site of chlorine substitution on the phenyl rings. The position of chlorine substitution on the ring structure is important, because the receptor interaction profile is highly dependent on it. Congeners having chlorine substitution in both *para* and at least two *meta* positions and also having zero or one chlorine atom present in an *ortho* position have the highest binding affinity for the aryl hydrocarbon receptor (AhR) and induce typical dioxin-like toxicity. These congeners, of which there are 12, are known as the DL-PCBs and have been assigned WHO toxic equivalency factors (TEFs). Congeners with two or more chlorine atoms in the *ortho* position are generally considered to be NDL-PCBs. The NDL-PCBs have a different spectrum of toxicological activity relative to DL-PCBs and PCDDs/PCDFs. International bodies have identified seven PCBs that can be used to characterize the presence of PCB contamination. Six of these seven are NDL-PCBs (PCB 28, PCB 52, PCB 101, PCB 138, PCB 153 and PCB 180), and one is a DL-PCB (PCB 118). The six NDL-PCBs are often called “indicator PCBs”.

For this evaluation, the Committee decided to focus on the six indicator PCBs, as there were sufficient data (toxicity, biomonitoring, occurrence and dietary exposure) available for review. Other NDL-PCBs were also considered to identify any for which adequate data were available to conduct a risk characterization, as was found for PCB 128.

### ***Toxicokinetics and mode of action***

The main determinants of the fate and behaviour of PCB congeners in the body are their lipid solubility and their rate of metabolism. In general, PCBs are lipid soluble and are well absorbed from the gastrointestinal tract in mammalian species. They are rapidly distributed to all body compartments, especially the liver and muscle. The highest amounts of PCBs are usually found in the liver, fat, skin and breast milk. Rates of metabolism of PCBs vary greatly across species and also vary with the number and position of the chlorine atoms in the different congeners. In all species studied, PCB congeners with adjacent unsubstituted carbon atoms in the *meta* and *para* positions are more readily metabolized, whereas congeners without such adjacent unsubstituted carbon atoms are generally metabolized and cleared very slowly. PCBs with higher numbers of chlorine atoms are generally metabolized more slowly than those with lower numbers of chlorine atoms. Some PCBs with few chlorine atoms have apparent half-lives in blood as short as a week in experimental animals. However, many highly chlorinated PCB congeners have half-lives in humans that are much longer and therefore accumulate in the body. Depending on the species, half-lives vary from several months (e.g. rat) or a year or more (e.g. monkey) to over a decade (humans). The long half-lives in humans compared with rodents have been attributed to the poor metabolism of these

compounds, and this, combined with the high lipid solubility of PCBs, results in high PCB content in the adipose tissue of humans. In terms of PCB metabolites, the hydroxy metabolites tend to be polar and more readily excreted, whereas the methyl sulfone metabolites are lipophilic and can be retained in adipose tissue.

PCBs are potent inducers of both phase I and phase II enzymes in the liver, and there are several routes of metabolism for PCBs. Biotransformation involves initial phase I oxidation by cytochrome P450 (CYP) enzymes. PCBs can be oxidized across the aromatic ring to one or more unstable intermediate arene oxides, which can spontaneously rearrange to produce hydroxy metabolites. PCBs that oxidize to more stable arene oxides are subsequently reduced to dihydroxy metabolites. Dihydroxy metabolites can then be dehydrogenated to form catechols, which are in equilibrium with their oxidized forms, the corresponding hydroquinones and quinones. In these metabolic processes, dechlorination and shift of chlorine atoms may also occur. Thus, lower chlorinated NDL-PCB congeners can be metabolized to reactive intermediates, such as epoxides, quinones and reactive oxygen species, that can form adducts with macromolecules, such as proteins, DNA, RNA and lipids. PCB congeners lacking adjacent unsubstituted hydrogen atoms do not easily form arene oxides but can be metabolized by an alternative pathway of direct insertion of a hydroxyl group to form a monohydroxy metabolite. Hydroxy metabolites are excreted as such, or the lower chlorinated PCBs can be conjugated with glucuronide or sulfate by the phase II enzymes uridine diphosphate-glucuronosyltransferase (UGT) and sulfotransferase (SULT). Another route of metabolism for PCBs, which involves glutathione S-transferase (GST), is the formation of methyl sulfones. Methyl sulfones are the final product of the most rapidly metabolized PCBs.

Approximately 40 of the hundreds of potential hydroxy metabolites have been identified in human blood. Of these, only five persist in the blood, and these are hydroxy metabolites that are substituted in the *para* position, with chlorine atoms on each side of the hydroxyl group. In blood, they are bound to transthyretin, which normally binds thyroxine ( $T_4$ ). Concentrations of hydroxy metabolites in human blood are approximately 5–10 times lower than those of the most persistent parent PCB congeners. Methyl sulfones undergo enterohepatic recirculation, and this, together with their lipophilicity, may account for some of the long retention times for methyl sulfone metabolites. Fifty or more methyl sulfone metabolites have been detected in human blood, but their concentrations in blood are low (generally less than 1% of the concentration of the most persistent PCB congeners), and much lower than those of the hydroxy metabolites. For both hydroxy and methyl sulfone metabolites, it may be more relevant to assess the effects of those metabolites with the highest retention potential than to assess the effects of the parent congener.

PCBs can interact with several cellular receptors, including the constitutive androstane receptor (CAR), pregnane X receptor (PXR) and AhR. The induction profile for these receptors differs between NDL-PCBs and DL-PCBs: NDL-PCBs most typically activate CAR and PXR, whereas DL-PCBs induce a pronounced activation of AhR, but not of CAR or PXR. Activation of either CAR and PXR or AhR results in different cytochrome P450 enzyme induction profiles. PXR and CAR activation induces CYP3A and CYP2B isoforms, respectively, whereas AhR activation induces CYP1A1, CYP1A2 and CYP1B1 isoforms. These differing cytochrome P450 induction profiles have traditionally been used to differentiate between NDL-PCB and DL-PCB congeners for toxicological purposes.

Interactions with CAR and PXR are crucial in the biotransformation and elimination of NDL-PCBs, because they can induce the relevant cytochrome P450 enzymes that metabolize NDL-PCBs. The activation of CAR and PXR also has potential toxicological implications, as these receptors play a significant role in the metabolism of endogenous molecules, such as hormones and vitamins. For example, induction of cytochrome P450 and conjugation enzymes by NDL-PCBs can influence hormonal homeostasis, as demonstrated in animal experiments for thyroid and steroid hormones, corticosteroids and retinoids. Recent studies have also indicated that CAR and PXR play an important role in the development of diabetes and inflammation.

NDL-PCBs also induce conjugation enzymes, such as UGTs, SULTs and GSTs. All these enzymes play important roles in the metabolism of NDL-PCBs, which is not only an important detoxification process, but also a route for the formation of transient reactive intermediates and more persistent hydroxy and methyl sulfone metabolites that are toxicologically relevant. Hydroxy metabolites can be agonists or antagonists for estrogen receptors, can interfere with thyroid hormone homeostasis and have neurotoxic potential. Methyl sulfone metabolites also interfere with thyroid hormone homeostasis and have been shown *in vitro* to have anti-estrogenic activity and to act as antagonists for glucocorticoid receptors. Based on the above mechanisms, a sustained exposure to NDL-PCBs may have toxicological implications.

In addition, there is cross-talk between PXR or CAR and other nuclear receptors, but the full scope of these interactions has not yet been fully elucidated. NDL-PCBs also activate the ryanodine receptor (RyR), which plays a crucial role in calcium ( $\text{Ca}^{2+}$ ) signalling and in the decrease of brain dopamine levels. These mechanisms are thought to be major pathways leading to the observed neurobehavioural toxicity of NDL-PCBs in experimental animals. In general, the interactions of NDL-PCBs with these receptors and the enzyme activation reported in animal studies are considered to have human relevance.

### **Toxicological data**

#### *Acute toxicity and short-term studies of toxicity*

There is no information on the acute toxicity of individual NDL-PCB congeners. The available data are mainly on rats and include 28-day studies on PCB 52 and PCB 180 and 90-day studies on PCB 28, PCB 128 and PCB 153. There are no short-term studies on two of the indicator PCBs, PCB 101 and PCB 138. In the two 28-day studies, the doses were expressed as total doses administered by oral gavage over the entire study period; the total dose comprised four (PCB 52) or six (PCB 180) higher daily loading doses during week 1, followed by three lower maintenance doses given 3 times per week during weeks 2–4. The total doses over the entire study period ranged from 3 to 3000 mg/kg bw for PCB 52 and from 3 to 1700 mg/kg bw for PCB 180. In the 90-day studies, fixed concentrations were administered in the diet and expressed as daily doses, which were similar for all three congeners, ranging from approximately 0.003 to 4.4 mg/kg bw per day. The main effects observed in these repeated-dose studies were on liver and thyroid and were fairly consistent across studies. Body weight was not affected in any of the 90-day studies.

In the liver, one of the most sensitive responses to exposure to NDL-PCBs is induction of phase I and phase II enzymes. In the short-term studies, this was reflected in structural changes, such as hepatocyte hypertrophy, cellular vacuolation and alterations in cytoplasm density and homogeneity, which are well-recognized adaptive signs of increased liver activity following exposure to xenobiotics. The pattern of effects was similar for the five congeners tested, with effects being observed at most doses in both the 28-day and 90-day studies, usually from the lowest dose tested, and with males tending to be more sensitive than females. In many instances, there was little or no dose–response relationship in either incidence or severity of these minimal changes over the entire dose range covering 3 orders of magnitude (whether expressed as administered dose or as adipose tissue concentration at the end of dosing). In the 28-day studies, liver weight was increased at the highest total dose of 3000 mg/kg bw for PCB 52 and with a dose–response relationship for PCB 180 at doses of 300 mg/kg bw and higher. In the 90-day studies, liver weight was increased at the highest dose of 4.4 mg/kg bw per day for PCB 128 and at the highest dose of 4.1 mg/kg bw per day for PCB 153.

Studies on individual NDL-PCB congeners have shown effects on thyroid histology and/or circulating thyroid hormone concentrations in adults. Effects on thyroid histology were seen in all the 28-day and 90-day studies from the lowest doses tested. The effects included reductions in the size of large follicles, collapsed follicles, increases in epithelial height and cytoplasmic vacuolation. Blood thyroid hormone levels were measured in the two 28-day studies, with

reductions in  $T_4$  levels at and above 300 mg/kg bw total dose for PCB 52 and dose-related reductions at and above 100 mg/kg bw total dose for PCB 180. Effects on the thyroid are potentially important, particularly because of the sensitivity of the developing brain to reductions in maternal and early postnatal thyroid hormone levels. It should be noted that both DL-PCBs and NDL-PCBs (and their hydroxy metabolites) can have effects on the thyroid and/or circulating thyroid hormone levels. In rodents, commercial mixtures of PCBs reduce circulating total  $T_4$  and free  $T_4$ , but have little or no effect on total or free triiodothyronine ( $T_3$ ) or on thyroid stimulating hormone (TSH). Studies on NDL-PCBs also show effects on  $T_3$ , but these are usually less marked than those on  $T_4$ . The precise mechanisms underlying these changes and their relative contributions to NDL-PCB-induced thyroid effects are not yet clear.

#### *Long-term studies of toxicity and carcinogenicity*

The only NDL-PCB congener studied for long-term toxicity and carcinogenicity is PCB 153 (25). Analytical checks of the test material for purity showed no contamination with DL-PCBs and only minor contamination with PCB 101 (0.21%) and PCB 180 (0.002%). Male animals were not used. Female rats were administered PCB 153 by oral gavage at 0, 10, 100, 300, 1000 or 3000  $\mu\text{g}/\text{kg}$  bw per day, 5 days/week, for up to 105 weeks (equivalent to 0, 7, 70, 200, 700 or 2000  $\mu\text{g}/\text{kg}$  bw per day when adjusted for 5 days/week dosing). These doses resulted in a linear increase in concentrations in fat; at the end of the study, concentrations were approximately 440, 20 000, 160 000, 520 000, 1 600 000 and 4 300 000 ng/g lipid for the 0, 10, 100, 300, 1000 and 3000  $\mu\text{g}/\text{kg}$  bw per day dose groups, respectively. There was equivocal evidence of carcinogenic activity of PCB 153, based on the occurrence of a small number of cholangiomas of the liver in two animals in each of the two highest-dose groups. The study authors considered that the occurrence of bile duct hyperplasia at doses of 300  $\mu\text{g}/\text{kg}$  bw per day and above could have contributed to cholangioma formation, and so the tumours may have been treatment related. The Committee noted that "bile duct hyperplasia" might better be described as atypical tubular epithelial cell hyperplasia.

It is notable that in this study, there was no increase in hepatic cell proliferation or any increases in hepatocellular adenomas or carcinomas. This is despite dose-related increases in hepatocyte hypertrophy, which were seen from the lowest dose of 10  $\mu\text{g}/\text{kg}$  bw per day, equivalent to 7  $\mu\text{g}/\text{kg}$  bw per day when adjusted for 5 days/week dosing, and which became statistically significant at doses of 300  $\mu\text{g}/\text{kg}$  bw per day and above during the first year of the study and at all dose levels by the end of the study. There were also statistically significant increases in absolute and/or relative liver weights at 1000 and 3000  $\mu\text{g}/\text{kg}$  bw per day at weeks 14 and 31 and at doses of 100  $\mu\text{g}/\text{kg}$  bw per day and above at

week 53 of the study; there was also evidence of diffuse fatty change in the liver at doses of 300 µg/kg bw per day and above at the end of the study. Concerning the thyroid, there were no increases in thyroid tumours, despite statistically significant reductions in serum thyroid hormone concentrations (total T<sub>4</sub>, free T<sub>4</sub>, free T<sub>3</sub>) in the 3000 µg/kg bw per day group during the first year of the study and a significant increase in follicular cell hypertrophy in the mid-dose group (300 µg/kg bw per day) and highest-dose group (3000 µg/kg bw per day) at the end of the study. There were no effects on thyroid weight. The observations from this long-term study support the view that the liver and thyroid changes observed in the short-term studies on PCB 153 and the four other NDL-PCBs, at lower dose levels than those used in this study, are unlikely to lead to major pathological changes over the long term.

NDL-PCBs may have weak tumour promotion effects in the liver, based on studies in rodents using diethylnitrosamine as an inducer.

#### *Genotoxicity*

Genotoxicity studies on individual NDL-PCB congeners have produced both positive and negative results. Some in vitro tests on PCB 3/PCB 3 metabolites, PCB 52/PCB 52 metabolites, PCB 101, PCB 138 and PCB 153 were positive for genotoxicity. In vivo studies have been conducted only on PCB 52 and PCB 153, and these were negative. The positive results in vitro may be due to the formation of reactive intermediates and induction of oxidative stress. It seems likely that some NDL-PCBs may be indirect-acting genotoxicants.

#### *Reproductive and developmental toxicity*

There are no oral reproductive toxicity studies on individual NDL-PCB congeners.

In developmental toxicity studies on individual NDL-PCB congeners (PCB 28, PCB 153 and PCB 180) in rodents, reduced birth weight and increases in offspring liver weight were seen only at high doses of 32 mg/kg bw per day and above. The majority of developmental toxicity studies have focused on neurodevelopmental outcomes. Rodent studies have shown that prenatal and/or postnatal exposures to NDL-PCBs cause effects on end-points such as spontaneous (locomotor) activity, habituation capability, spatial learning and anxiety-like behaviour at maternal doses ranging from 0.2 to 1000 mg/kg bw per day. A limitation of many of the neurobehavioural studies is that they used only one or two dose levels and effects were seen at the lowest dose or at the only dose tested, which does not allow derivation of a NOAEL. The Committee noted that the administered doses used in the developmental neurobehavioural studies were considerably higher than those used in the short- and long-term studies of toxicity and that the lowest effect level in the developmental neurobehavioural



studies was at least 2 orders of magnitude higher than the lowest doses that induced minimal changes in liver and thyroid in the short-term studies of toxicity. Internal dose data (concentrations in fat) are not available for the developmental toxicity studies. In vitro studies also indicate a potential role of hydroxylated metabolites of NDL-PCBs in neurodevelopmental mechanisms of toxicity, but support from in vivo studies is very limited. These results from developmental neurobehavioural studies in rodents indicate similar patterns of effects, albeit with congener-specific differences. Based on in vivo studies, a distinct mechanism of action for the neurodevelopmental effects of NDL-PCBs cannot be established. However, results from in vitro or ex vivo studies using neuronal cells indicate mechanistic pathways that involve disruption of intracellular calcium or thyroid hormone homeostasis. Based on available data, the Committee concluded that neurodevelopmental outcomes are not the most sensitive end-points for the toxicity of NDL-PCBs in rodents.

#### *Immunological studies*

In vitro and in vivo studies using doses ranging from 0.1 mg/kg bw per day up to several hundred milligrams per kilogram body weight per day have shown that NDL-PCBs can exert immunological effects. In these studies, a comparison was often made between 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and an NDL-PCB, such as PCB 153. Results from in vivo studies in mice indicate that the mechanism of action for immunological effects differs between dioxin-like and non-dioxin-like compounds. For example, PCB 153 significantly enhanced splenic plaque-forming cell responses to sheep red blood cells injected into mice, whereas TCDD induced an opposite response. Moreover, in studies of the effects of co-administration of TCDD and PCB 153, it has been shown that PCB 153 can counteract the AhR-mediated effects of TCDD on plaque-forming cell responses. The results of repeated-dose studies in rodents suggest that PCB 153 can induce a proinflammatory response in various tissues (e.g. liver, spleen, lungs and uterus), which is not seen with TCDD. From the doses used in the immunological studies, such effects seem unlikely to be the most sensitive end-points for NDL-PCBs, but the data relate mostly to PCB 153.

#### **Observations in humans**

##### *Biomonitoring and modelling of body burden*

The most commonly used biomarkers of PCB exposure in humans are PCB concentrations in adipose tissue, serum, plasma and milk. These mainly reflect exposure from the diet. There is a strong correlation between serum and adipose tissue concentrations, when expressed on a lipid basis, and both are widely regarded as useful biomarkers of PCB body burden. In general, concentrations



in blood lipids reflect more recent exposures, as well as the full spectrum of PCB congeners to which a person has been exposed, whereas the pattern of PCB congeners in adipose tissue reflects long-term exposure and, to a lesser degree, the extent to which a particular congener is metabolized. Concentrations in human milk largely reflect the pattern and amounts of PCB congeners present in maternal adipose tissue and blood, when expressed on a lipid basis.

Numerous publications confirm that PCB 138, PCB 153 and PCB 180 are the most consistently detected and quantitatively dominant PCB congeners found in human blood and tissues, accounting for 65–80% of total PCBs in human serum. If only one congener is to be used as a marker of total PCB exposure, then PCB 153 is a good choice, because it is very stable and often the most abundant congener. PCB 153 has been shown to have a high correlation with the total amount of PCBs in human milk, plasma and serum. However, if a more complete profile of congeners is considered, the correlations are lower, and either total PCBs or PCB 153 as a marker of the total could be misleading indicators of the differential exposure to other individual or groups of congeners of toxicological significance (26).

The total body burden of PCBs and their metabolites generally increases with age, and this is reflected in blood and adipose tissue biomarker concentrations. With the phasing out of the production of PCBs since the 1980s, results of studies conducted since that time show clear trends for decreasing blood and human milk concentrations of PCBs: in Europe, mean blood concentrations of PCB 138, PCB 153 and PCB 180 appear to have decreased by approximately 80% in 20 years; in WHO surveys on human milk, concentrations of the six indicator PCBs have steadily decreased by approximately 10-fold over the decade 2000–2010.

Biomonitoring results from numerous countries over the preceding decade illustrate a wide range of concentrations for individual congeners in human serum, adipose tissue and milk. The results are summarized in [Table 3](#), together with equivalent and modelled body burdens. The concentrations of hydroxy metabolites of PCBs in human serum are not shown, but are in a similar range to the concentrations of many parent PCB congeners, except for those PCBs that are the most prevalent or persistent. Human milk, serum and adipose tissue are all considered to be relevant matrices for assessment of body burdens of PCBs. Human milk has been recognized by WHO as the preferred matrix for monitoring levels of environmental contaminants. In [Table 3](#), ranges of equivalent body burdens were derived using the available range of mean PCB concentrations reported in human milk, as the Committee considered that the milk values were more representative, reflecting both adult (maternal) and infant exposures.

Table 3  
**Summary of human biomonitoring results on selected NDL-PCBs from studies published in the preceding decade and body burden estimates**

NDL-PCB congener	Range of mean concentrations (ng/g lipid)			Range of equivalent body burden <sup>c</sup> (µg/kg bw) across studies	Range of modelled body burden <sup>d</sup> (µg/kg bw) across countries
	Serum <sup>a</sup>	Adipose tissue	Milk <sup>b</sup>		
28	1–14	0.7–39	0.6–7.8	0.12–1.6	0.05–1.7
52	0.2–26	0.3–72	0.2–15.8	0.04–3.6	0.06–0.7
101	0.1–5.2	0.2–58	0.3–8.3	0.06–1.7	0.14–3.7
128 <sup>e</sup>	NA	NA	0.2–4.0	0.04–0.8	NA
138	3.6–186	3.6–181	1.6–98	0.32–20.1	0.49–10.0
153	11–423	0.9–310	3.4–130	0.68–26.7	0.66–14.9
180	6–374	1.7–245	1.6–96	0.32–19.7	0.25–4.2

NA: not available

<sup>a</sup> The majority of serum samples were at the low end of the range.

<sup>b</sup> Excludes results from upper end of range in Czech Republic, as data set included results from an industrial area with a former PCB-based paint production plant.

<sup>c</sup> Body burdens based on reported range of concentrations in human milk; 65 kg bw adult; 20% lipid.

<sup>d</sup> Model-based estimates using upper-bound mean dietary exposure in China, Czech Republic, Finland, France, Germany, Ireland, Italy, Japan, the Netherlands, the Republic of Korea, Sweden and the United Kingdom.

<sup>e</sup> Based on limited human milk analysis (29–31).

The Kinetic Dietary Exposure Model (27) was used to simulate body burdens in adult populations in the countries from which dietary exposure data (see below) were available (China, Czech Republic, Finland, France, Germany, Ireland, Italy, Japan, the Netherlands, the Republic of Korea, Sweden and the United Kingdom). Based on upper-bound mean dietary exposures, body burdens were also modelled for each congener and each country. Ranges of modelled body burden estimates across countries are reported in Table 3. No modelled body burdens for PCB 128 could be developed owing to the lack of half-life information.

The human body burden for each congener was predicted using a one-compartment model, an assumption of 20% body fat and information on dietary exposure (see below). Half-life estimates were drawn from Ritter et al. (28), and sensitivity analyses were performed to take into account the large variability in half-life data across other publications. The modelling predicted body burdens of the same order of magnitude as those reported in human biomonitoring data.

### *Epidemiology*

Humans are exposed to complex mixtures of PCBs. The epidemiological literature covers studies that have analysed outcomes according to both exposure to complex mixtures of PCBs and exposure to specific marker NDL-PCBs. The studies included a broad range of potential outcomes, including growth and development, neurodevelopment and neurobehaviour in childhood, neurotoxic

effects in adults, cancer, endocrine and metabolic effects (e.g. on thyroid hormone homeostasis, diabetes, obesity, insulin resistance and metabolic syndrome), reproductive effects in males and females, immunological effects and infections, respiratory diseases, cardiovascular diseases, hepatic effects, musculoskeletal effects and endometriosis. However, methodological issues and some study design features must be considered for a proper interpretation of human studies. Of particular concern is the extensive use of cross-sectional studies reporting associations between exposure and outcome, in which the exposure measurements are taken at the same time as the outcome is ascertained. A cross-sectional estimate of body burden may not reflect the exposure during the time period critical for the development of a particular outcome. Exceptions are in cases when the exposure and response are known to occur during a defined short period (e.g. prenatal exposure measured in cord blood in relation to effects in newborns). Other than this, cross-sectional studies are of little value. In case-control studies, major drawbacks arise from the fact that measurement may be affected by the disease and treatments, as well as the potential for selection and information bias in hospital-based studies. The degree of control of relevant confounders is highly variable across studies, including exposure to other contaminants, and the potential for confounding by factors not explicitly considered in the analysis cannot be ruled out. Finally, there is always co-exposure to dioxin-like congeners; given the strong collinearity between exposure to DL-PCBs and NDL-PCBs, this makes it very difficult to make a valid estimate of the independent effect of NDL-PCBs.

In spite of all the limitations, some well-designed and well-conducted studies have identified potential health effects associated with exposure to NDL-PCBs, including changes in thyroid hormone homeostasis, neurodevelopmental effects, immunological effects and some types of cancer. Some of the results offer support for the toxicological findings, especially regarding thyroid effects, identified as potentially relevant for NDL-PCBs in animal studies. The results of prospective and cross-sectional studies in newborns and children suggest that increasing concentrations of NDL-PCBs are correlated with lower levels of  $T_4$  and higher levels of TSH in the blood, although there are some inconsistencies between results across studies. Perinatal exposure to NDL-PCBs in birth cohorts was found to be associated with increased incidence of acute respiratory infections in children. Maternal and early postnatal exposure to NDL-PCBs in some birth cohorts was associated with impaired behavioural, cognitive and psychomotor development and with alteration of visual evoked potentials.

Regarding cancer, the recent evaluation by the International Agency for Research on Cancer (IARC) (32) reported an association between melanoma and PCB exposure, mainly based upon cohort studies of exposed workers in various industries, for whom exposure would be by multiple routes. IARC (32) also

considered studies in the general population with different study designs. Only one population-based case-control study reported specific results for NDL-PCBs, showing a significant increased risk of melanoma for a group of 11 NDL-PCBs, as well as for some individual congeners. In this study, a similar increased risk was also observed for two DL-PCB congeners. The association between NDL-PCBs and non-Hodgkin lymphoma has also been assessed in five prospective cohorts, but the results were not consistent.

### ***Analytical methods***

The methodology used for the analysis of NDL-PCBs is largely similar regardless of the laboratory performing the analysis. Although some agencies (e.g. AOAC International, International Organization for Standardization) have developed validated matrix-specific methods of analysis that may be followed for the analysis of NDL-PCBs and DL-PCBs, others have developed a set of performance criteria to ensure that laboratories develop data of acceptable quality. The use of automated solid-phase extraction systems for the extraction and/or cleanup of samples has increased, which has improved efficiency. Gas chromatography coupled to  $^{63}\text{Ni}$  electron capture detectors and mass spectrometers (including ion trap, low-resolution, high-resolution and tandem mass spectrometers) have been used in the analysis of NDL-PCBs.

The availability and use of stable isotope internal standards and certified reference materials lead to improved accuracy of analytical results. The use of analytical methods with satisfactory performance characteristics, as well as methods subjected to interlaboratory comparison studies, should be considered, as appropriate, to ensure that the data submitted for evaluation are of adequate quality.

### ***Sampling protocols***

Although there are no established protocols set specifically for the collection and storage of samples for NDL-PCB analysis, best practices have been established for other persistent organic pollutants (POPs) present at ultra-trace levels (e.g. PCDDs/PCDFs, DL-PCBs). These practices include the collection of samples using containers that are non-reactive (e.g. glass, aluminium) and that have been chemically cleaned or certified to be free of contaminants.

### ***Effects of processing***

NDL-PCBs are thermally stable and resistant to degradation. Studies on the impact of processing in relation to PCB concentrations have been largely focused on the cooking techniques used to prepare foods and techniques that change the fat content (e.g. PCB levels are lowered in skimmed milk, but

increased concentrations are found in foods with higher fat content, such as cheese or cream). Although the studies related to the impact of processing on PCB concentrations include both DL-PCBs and NDL-PCBs, the impact on the concentrations is similar for both groups. Ultimately, processing that results in the removal of lipids will lead to a decrease in PCB concentrations in the final food product.

### ***Prevention and control***

The focus of efforts related to preventing exposure to POPs, including NDL-PCBs, is on limiting contamination of the food-chain, including exposure of food-producing animals to PCBs. With the knowledge that fish, meat and dairy product consumption makes the most significant contribution to human PCB exposure, methods of PCB reduction in animals from which these foods are derived are of primary interest. Transfer of DL-PCBs and NDL-PCBs from feed to animal-based food products (e.g. milk) occurs; transfer of PCB 138, PCB 153 and PCB 180 is greater than that observed for PCB 28, PCB 52 and PCB 101. Adherence to good agricultural practices and good animal feeding practices will contribute to the efforts to reduce PCB concentrations in food for human consumption. PCB contamination of animal housing and/or buildings (e.g. silos) near animal pastures may contribute to animal exposure levels, as does the pasturing of animals on lands contaminated with PCBs. It is therefore important to identify contaminated pastures to ensure that they are not used for grazing. PCB contamination can be further reduced by establishing and adhering to soil guidelines for agricultural purposes, performing diligent monitoring programmes to confirm compliance and establishing critical control points for the feed manufacturing process where PCB concentrations can be reduced. Additionally, farming practices should include plans for isolation, among other procedures, if PCB contamination is detected.

### ***Levels and patterns of food contamination***

Thirty countries (Australia, Austria, Belgium, Canada, China, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Japan, Luxembourg, the Netherlands, New Zealand, Norway, Poland, the Republic of Korea, Romania, Singapore, Slovakia, Slovenia, Spain, Sweden and the United Kingdom) provided data to the Committee for its review of NDL-PCBs. The submissions from Europe were received through EFSA. Those data that were not in an acceptable format for evaluation by the Committee were removed from the data set. Submitted data included results from sample collection periods extending from 1995 to 2014. Most (90.5%) of the data were submitted from

Europe; the Pacific region contributed 8.5% of the submissions, and 1% of the data were provided from North America.

The food categories having elevated NDL-PCB concentrations were fish/fish products, meat/meat products, egg/egg products and milk/milk products. Concentrations of the six indicator PCBs in these food categories varied widely (Table 4). Few occurrence data were submitted for PCB 128, and lower concentrations of this congener were reported relative to the indicator congeners (Table 3). It was noted that concentrations of the more chlorinated indicator congeners (PCB 101, PCB 138, PCB 153 and PCB 180) were higher than those of the trichlorinated PCB 28 and tetrachlorinated PCB 52, particularly in fish/fish products (Fig. 2). The maximum concentration reported in fish/fish products exceeded 1 000 000 ng/kg expressed on a wet weight (ww) basis, whereas the median concentrations remained very low for all six congeners (PCB 28: 0.73 ng/kg ww; PCB 52: 2.6 ng/kg ww; PCB 101: 8.9 ng/kg ww; PCB 138: 30 ng/kg ww; PCB 153: 50 ng/kg ww; PCB 180: 7.5 ng/kg ww).

### ***Dietary exposure assessment***

Estimates of dietary exposure were evaluated by the Committee, focusing on the six indicator PCBs, singly and in combination. Only chronic dietary exposure assessments were included in the evaluation. Dietary exposure estimates from the literature were reviewed. Both national and international estimates of dietary exposure were made by the Committee based on consumption and concentration data available from the GEMS/Food database. The concentration data submitted (as shown at a major food group level in Table 4) were summarized by specific food type (e.g. for herring or cow's milk) for use in the exposure calculations conducted by the Committee. Estimates of dietary exposure for the six indicator PCBs were also calculated individually for countries that provided concentration and consumption data, for both body burden modelling and risk characterization purposes. Exposures to PCB 128 were also estimated, as relevant toxicity data were available for this congener for risk characterization purposes.

### ***National estimates of chronic dietary exposure***

Estimated national dietary exposures for the sum of the six indicator PCBs are shown in Table 5.

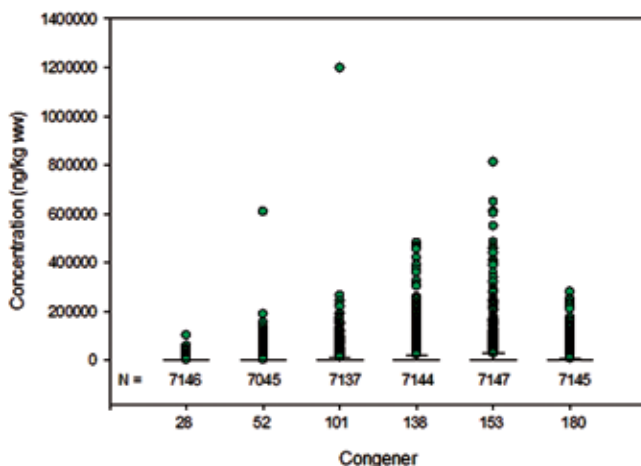
National estimates of dietary exposure were also calculated for each of the six indicator PCBs individually. Exposures were highly variable, depending on the congener, concentration data, country and population subgroup assessed. As a result of a high proportion of "non-detects" (concentrations below the limit of quantification [LOQ]) across a wide range of food groups, high LOQs and similar LOQs across congeners, the exposures based on data from one country

Table 4  
**Concentrations of the six indicator PCBs and PCB 128 in specific foods**

<b>Food category</b>	<b>n</b>	<b>n &lt; LOD</b>	<b>Concentration range (ng/kg ww)</b>
<b>PCB 28</b>			
Eggs	1 086	392	ND–6 800
Fish	7 146	2 119	ND–103 000
Meat	2 488	1 473	ND–16 100
Milk	6 510	736	ND–5 250
<b>PCB 52</b>			
Eggs	1 069	484	ND–6 710
Fish	7 045	1 875	ND–610 000
Meat	2 506	1 495	ND–9 440
Milk	6 513	716	ND–4 600
<b>PCB 101</b>			
Eggs	1 085	508	ND–8 410
Fish	7 137	1 762	ND–1 200 000
Meat	2 463	1 478	ND–240 000
Milk	6 481	741	ND–1 850
<b>PCB 138</b>			
Eggs	1 090	312	ND–34 800
Fish	7 144	1 543	ND–482 000
Meat	2 521	1 013	ND–12 900
Milk	6 531	524	ND–5 740
<b>PCB 153</b>			
Eggs	1 092	294	ND–31 000
Fish	7 147	1 463	ND–812 000
Meat	2 521	993	ND–17 300
Milk	6 534	429	ND–16 900
<b>PCB 180</b>			
Eggs	1 092	310	ND–34 700
Fish	7 145	1 777	ND–280 000
Meat	2 517	1 074	ND–198 000
Milk	6 528	571	ND–4 210
<b>PCB 128</b>			
Eggs	2	1	ND–0.002
Fish	356	2	ND–6.63
Meat	13	4	ND–0.017
Milk	15	3	ND–1.29

LOD: limit of detection; ND: not detected

Fig. 2  
**Concentrations of indicator PCB congeners in fish and fish products**



were excluded from the evaluation. The estimates are summarized in Table 6 for the remaining countries. Mean and high-percentile exposures, including lower-bound and upper-bound estimates, across all countries and population groups and individual congeners ranged between <1 and 4.7 ng/kg bw per day at the mean and 9.4 ng/kg bw per day at the high percentile.

Table 5  
**Overall range of estimated national dietary exposures to the sum of the six indicator PCBs**

Source	Population group	Lower-bound to upper-bound dietary exposures <sup>a</sup> (ng/kg bw per day)	
		Mean exposure	High-percentile exposure <sup>b</sup>
Literature	Children <sup>c</sup>	3–24	12–87
	Adults	1–18	8–45
Estimated by the Committee	Children <sup>c</sup>	<1–82	1–163
	Adults	<1–25	<1–51

<sup>a</sup> Lower bound where “non-detects” were assigned a zero concentration; upper bound where “non-detects” were assigned either the limit of detection or limit of quantification.

<sup>b</sup> Usually 90th or 95th percentile reported. The estimates calculated by the Committee were 90th percentiles.

<sup>c</sup> Includes infants consuming a mixed diet; excludes infants solely breastfed or formula fed.



Table 6

**Summary of the range of estimated dietary exposures for individual indicator PCBs for adults and children<sup>a</sup> from all countries assessed**

NDL-PCB	Population group <sup>b</sup>	Estimated dietary exposure (ng/kg bw per day)	
		Mean (lower bound – upper bound)	High percentile (lower bound – upper bound)
28	Children (infants to adolescents)	<1–2.8	<1–5.7
	Adults	<1–<1	<1–1.1
52	Children (infants to adolescents)	<1–2.6	<1–5.3
	Adults	<1–<1	<1–1.1
101	Children (infants to adolescents)	<1–3.4	<1–6.7
	Adults	<1–1.2	<1–2.5
138	Children (infants to adolescents)	<1–4.7	<1–9.4
	Adults	<1–1.8	<1–3.7
153	Children (infants to adolescents)	<1–3.5	<1–7.1
	Adults	<1–2.2	<1–4.3
180	Children (infants to adolescents)	<1–2.7	<1–5.4
	Adults	<1–<1	<1–1.5

<sup>a</sup> Includes infants consuming a mixed diet; does not include infants exclusively breastfed or formula fed.

<sup>b</sup> Where defined for the consumption survey, infants are <1 year, toddlers 1 to <3 years, children 3 to <10 years, adolescents 10–<18 years, adults 18+ years.

For PCB 128, dietary exposure estimates were available for only two countries (Finland and the United Kingdom), based on concentration data for limited food commodities. For this reason, the estimated dietary exposures were not used to determine the body burden modelled from external dose for PCB 128. An alternative approach was used, whereby the proportion that the concentration of PCB 128 represents of the six indicator PCBs considered individually was determined on the basis of data from GEMS/Food (the average was 16%).

To represent approximate PCB 128 exposure, 16% of the average of the upper-bound exposures for each individual indicator PCB for both mean and high percentile for adults was used (mean 0.2 ng/kg bw per day, high percentile 0.4 ng/kg bw per day).

Lower estimates of dietary exposure to the six indicator PCBs were determined where methods with lower limits of detection were used for the food analysis and low concentrations in foods were determined. This highlights the importance of using specific analytical techniques for this group of contaminants. Higher estimates of dietary exposure, particularly at the upper bound, were strongly influenced by the sensitivity of the analytical method and therefore the concentration assigned to “non-detects” for upper-bound scenarios.

Despite variations in methodologies used to estimate dietary exposure, the majority of national estimates of dietary exposure to the NDL-PCBs assessed

were in the same ranges, with slightly more variation in the estimates of upper-bound levels of dietary exposure than in the lower-bound estimates.

The main contributor to dietary exposure to the sum of the six indicator PCBs at the national level was fish and seafood, followed by meat or meat products. Milk and dairy also contributed for some populations and population subgroups, particularly children. The differences in the main contributors between countries depended on the importance of the food in the countries' diets as well as the concentration of the NDL-PCBs in the food used in the estimate.

#### *International estimates of dietary exposure*

Estimates of international mean dietary exposure per capita for the sum of the six indicator PCBs were calculated by the Committee using concentration data submitted to the GEMS/Food contaminants database and consumption data from the GEMS/Food cluster diets.

The range of estimated dietary exposure to the sum of the six indicator PCBs between clusters for the lower-bound scenario was 1–60 ng/kg bw per day. For upper-bound exposures, the range between clusters was 2–83 ng/kg bw per day. Fish was a major contributor to dietary exposure across the majority of the clusters, contributing up to about 90% of dietary exposure for one cluster. This was due to the higher concentration of NDL-PCBs in fish and/or the higher consumption of fish for the cluster.

#### *Dietary exposure of infants*

Mean dietary exposure of breastfed infants to the sum of the six indicator PCBs was reported for 11 European countries by EFSA (33) to be 1200 ng/kg bw per day. The most recent WHO study reported a mean dietary exposure for breastfed infants of 1600 ng/kg bw per day, with a wide range of means from around 200 to 7000 ng/kg bw per day (32). Estimated dietary exposures of breastfed infants to NDL-PCBs are up to 2 orders of magnitude higher than those for the rest of the population. Biomonitoring data indicate that breastfed infants have higher body burdens of NDL-PCBs compared with formula-fed infants.

#### *Contribution of individual congeners to total exposures from all sources*

For the sum of the six indicator PCBs, the contribution of each of the individual congeners differs between countries and population groups. However, for both dietary exposure and body burden estimates (which also take into consideration kinetics and half-lives), the main contributor is PCB 153 (41%), followed by PCB 138 (28%), PCB 180 (14%), then PCB 101 (8%) and PCB 28 (6%), with the lowest contribution from PCB 52 (3%).

With the exception of those individuals with high occupational exposure, total exposures from all sources are likely to be only slightly higher than those predicted from the diet alone. Dietary exposures to NDL-PCBs have been decreasing over time, as indicated in studies from a number of countries, owing primarily to the phasing out of the manufacture and use of PCBs.

### ***Estimation of margins of exposure***

For non-genotoxic substances, the Committee would normally develop health-based guidance values using the most sensitive adverse effect in the most sensitive species as a point of departure. The Committee therefore considered whether the toxicological information available for the six indicator PCBs (PCB 28, PCB 52, PCB 101, PCB 138, PCB 153, PCB 180) and PCB 128 was sufficient and appropriate for such an approach. It was noted that there are a large number of *in vivo* and *in vitro* studies available with respect to possible hepatotoxicity, thyroid toxicity, and neurodevelopmental or neurotoxic effects, but that there is a general lack of *in vivo* toxicity data for two of the indicator PCBs (PCB 101 and PCB 138). In addition, there is not enough information on relative potencies for each congener with respect to receptor interactions and the downstream consequences.

The Committee considered whether it would be possible to undertake a group evaluation for NDL-PCBs using the available information for the indicator congeners. Such an approach should be based on internal rather than external dose because of the species differences in half-lives for these congeners. Owing to the lack of relevant toxicological data for two congeners (PCB 101 and PCB 138), the Committee decided not to undertake such a group evaluation.

The Committee further concluded that none of the available short-term toxicity studies on four of the six indicator PCBs and PCB 128 were suitable for the derivation of health-based guidance values (e.g. provisional tolerable monthly intakes) or assessment of their relative potency compared with a reference compound, such as PCB 153. This conclusion was based on the lack of clear dose–response relationships, doubts about the toxicological relevance of the observed minimal effects on the liver and thyroid and the limited experimental time periods of most of the studies (28 or 90 days). The Committee considered whether the 2-year United States National Toxicology Program (NTP) study on PCB 153 might form a basis for deriving a benchmark dose lower confidence limit (BMDL) that could be used as a point of departure for a health-based guidance value for that congener. From a human exposure and risk assessment point of view, PCB 153 is highly relevant, as it represents up to 40% of the six indicator PCBs that are present in the diet or in human milk. The most sensitive end-point in the 2-year study was hepatocyte hypertrophy, observed at and above 7 µg/kg

bw per day. After critical evaluation of these results, the Committee concluded that the hepatocyte hypertrophy should not be modelled to derive a BMDL, as the end-point may not be toxicologically relevant. Hence, data from the long-term NTP study with PCB 153 were not considered suitable to derive a health-based guidance value.

Therefore, a comparative approach using the minimal effect doses from the available studies was developed in order to estimate MOEs to provide guidance on human health risk. The available toxicological data on individual congeners showed that minimal changes in liver and thyroid histopathology were evident from the lowest doses tested of 2.8–7 µg/kg bw per day in the 90-day studies (PCB 28, PCB 128, PCB 153) and 3 mg/kg bw total dose in the 28-day studies (PCB 52, PCB 180) and were similar across the short-term and long-term toxicity studies. Bearing in mind that, with the exception of PCB 153, the available studies on individual NDL-PCB congeners were of relatively short duration (28 or 90 days), the Committee decided to take the lower end of the range of test doses used for each congener at which these minimal changes occurred as a conservative point of departure for estimating MOEs. Given the major difference in dosing regimens between the 28-day and 90-day studies and the bioaccumulative nature of PCBs, MOEs have been estimated on the basis of both external dose and internal dose. The internal dose MOEs based on amounts present in fat are considered the most appropriate comparison, particularly because they also eliminate interspecies differences in toxicokinetics.

The MOEs obtained for adults are shown in [Table 7](#). The MOE comparisons for internal dose are based on the range of reported mean values for human milk expressed on a fat basis, which should reflect both fetal and adult (maternal) body burdens. The biomonitoring data on concentrations in human adipose tissue were not used because they represent far fewer, non-random samples derived from postmortem tissues from non-homogenous populations.

### **Evaluation**

The body burden MOEs for adults derived from the range of reported mean human milk concentrations range from 4.5 to 5000. The Committee noted that for some of the NDL-PCBs, these body burden estimates were developed from experimental studies with a less than chronic duration of exposure. For PCB 153, the lower end of the body burden MOE range based on human milk was 4.5 when derived from the short-term study, whereas in the long-term study it was 75, which is a 16-fold difference. The lower end of the range of body burden MOEs modelled from external dose also gave a 16-fold difference between long- and short-term studies on PCB 153. Thus, in the long-term study, similar hepatic effects to those seen in the short-term study were observed only at an internal

Table 7

**Estimated MOEs for adults from repeated-dose studies on individual NDL-PCB congeners in rats**

NDL-PCB congener / study duration / mode of administration (reference)	Minimal effect dose expressed as external dose ( $\mu\text{g}/\text{kg bw}$ per day)	Minimal effect dose expressed as body burden <sup>a</sup> (mg/kg bw)	External dose MOE <sup>b</sup>	Body burden MOE (based on human milk) <sup>c</sup>	Body burden MOE (modelled from external dose) <sup>c</sup>
PCB 28 / 90 days / diet (34)	2.8	0.07	2 500–5 600	44–580	41–1 400
PCB 52 / 28 days / gavage (unpublished data)	3.0 <sup>d</sup>	NA	97 000–210 000	NA	NA
PCB 101	NA	NA	NA	NA	NA
PCB 128 / 90 days / diet (35)	4.2	0.07	11 000–21 000	88–1 700	NA
PCB 138	NA	NA	NA	NA	NA
PCB 153 / 90 days / diet (36)	3.6	0.12	840–1 600	4.5–170	8–180
PCB 153 / 2 years / gavage (25)	7 <sup>e</sup>	2.0	1 600–3 100	75–2 900	130–3 000
PCB 180 / 28 days / gavage (37)	3.0 <sup>d</sup>	1.6	71 000–150 000	81–5 000	380–6 400

NA: not available

<sup>a</sup> Body burden based on reported concentration of NDL-PCB congener in adipose tissue; 350 g rat with 10% lipid.

<sup>b</sup> For MOEs expressed as a range, the lower end of the range relates to upper-bound adult high-percentile exposure, and the higher end of the range relates to upper-bound adult mean exposure (see Table 6). The dietary exposure estimate for PCB 128 is based on it making, on average, a contribution equal to 16% of the total exposure to the six indicator PCBs.

<sup>c</sup> See Table 3.

<sup>d</sup> Total dose (mg/kg bw) administered over the whole study.

<sup>e</sup> Dose adjusted to 7  $\mu\text{g}/\text{kg bw}$  per day from 10  $\mu\text{g}/\text{kg bw}$  per day to take account of 5 days/week dosing.

dose that was substantially higher. Use of the MOE value from the long-term study on PCB 153 would give lower-end MOEs for all congeners in the range 44–88 for adults. MOEs for breastfed infants, which may have a body burden up to 2-fold higher than that for adults, would be approximately half of the adult values. The MOEs for children would be expected to be intermediate between those for adults and those for breastfed infants, owing to the initial contribution from breastfeeding and the subsequent lower dietary contribution compared with human milk.

As the MOEs are based on minimal effect doses, they were considered to be adequate and to give some assurance that dietary exposures to NDL-PCBs are unlikely to be of health concern for adults and children, based on the available data. For breastfed infants, the MOEs would be expected to be lower. However, based on present knowledge, the benefits of breastfeeding (38) are considered to

outweigh the possible disadvantages that may be associated with the presence of NDL-PCBs in breast milk.

The Committee recognized that there are similarities in some of the reported effects for NDL-PCBs and that, ideally, risk estimates for combined exposure are desirable. The Committee concluded that this cannot be done on the basis of currently available data, but noted that the points of departure selected for derivation of the MOEs were particularly conservative, as they were based on effects on liver and thyroid that were not of clear toxicological significance, the changes were minimal and the lowest doses at which they were seen were used for the points of departure, combined with upper-bound estimates of body burden.

### **Recommendations**

A more complete toxicological database, including mechanistic studies on both parent congeners and hydroxy metabolites, would have allowed a more definitive assessment. The Committee recommended that further toxicological studies should be done, particularly in vivo studies for those congeners, such as PCB 101 and PCB 138, that contribute significantly to dietary exposure and human body burden.

There were some limitations with the exposure assessment where there was a limited range of countries and/or foods for which concentration data were provided. This meant that some assumptions needed to be made for estimating dietary exposures for national and international assessments. To ensure better estimates of dietary exposure with a lower degree of uncertainty, the Committee recommends the following:

- As the risk characterization is driven mainly by dietary exposure estimates from European countries, information from a broader range of countries would be desirable to provide a more globally representative conclusion.
- More countries should submit concentration data to the GEMS/Food contaminants database (with as specific details as possible, including unique sample identifiers, specific food details, lower limits of detection, form of the food, etc.).
- National food consumption data should also be submitted by more countries to CIFOcOss to allow a broader range of country-specific exposure assessments to be undertaken in the future.
- For analytical surveys on NDL-PCBs, it is important to ensure the generation of occurrence data for congeners beyond the six indicator PCBs for a broad range of foods and to include all key congeners and sources of dietary exposure to NDL-PCBs.

- More concentration data in infant formula as well as data from a broader range of countries would assist in determining more reliable estimates of dietary exposure for formula-fed infants.

A toxicological monograph was prepared.

## 4.2 Pyrrolizidine alkaloids

### *Explanation*

PAs are toxins produced by an estimated 6000 plant species. More than 600 different PAs, mainly 1,2-unsaturated PAs, and their associated nitrogen oxides (*N*-oxides) are known, and new PAs continue to be identified on a regular basis in both new and previously studied plant species. The main plant sources are the families Boraginaceae (all genera), Asteraceae (tribes Senecioneae and Eupatorieae) and Fabaceae (genus *Crotalaria*). Different plant species in these families produce characteristic mixtures of 1,2-unsaturated PAs and their saturated analogues and varying amounts of their corresponding *N*-oxides. The PAs present in these plants are esters of pyrrolizidine diols. The diols are referred to as necines, and the esterifying acids involved are necic acids. These PAs can be classified as open-chain monoesters, open-chain diesters and macrocyclic diesters.

The pyrrolizidine ring system consists of two fused, five-membered rings with a nitrogen atom at the bridgehead. Pyrrolizidines and pyrrolizidine alkaloids<sup>4</sup> are, by definition, fully saturated and have no double bonds. However, the term “saturated pyrrolizidine alkaloid” is sometimes used to emphasize the fact that there are no double bonds present. The terms “1,2-unsaturated” or “1,2-dehydro” pyrrolizidine alkaloids indicate that the alkaloids being referred to are modified pyrrolizidine alkaloids having a double bond between carbons 1 and 2. The term “free base” means that the nitrogen lone pair electrons on the alkaloids are not protonated by acids or oxidized to *N*-oxides. The *N*-oxide forms of PAs occur naturally together with the PA parent molecules.

In this report, the term “PAs” used by itself refers to all saturated and 1,2-unsaturated pyrrolizidine alkaloids and their associated *N*-oxides.

PAs have not been previously evaluated by JECFA, but they have been evaluated by a WHO Task Group on Environmental Health Criteria for Pyrrolizidine Alkaloids (coordinated by the International Programme on Chemical Safety [IPCS]) in 1988 (39) and IARC in 1976, 1983, 1987 and 2002 (40–43). IPCS (39) concluded that, based on animal data, a potential cancer risk for humans should be seriously considered; however, as no information was found

<sup>4</sup> The term “alkaloid” (alkali-like) refers to naturally occurring plant secondary chemicals with a basic nitrogen atom.

on the long-term follow-up of humans exposed to PAs, it was not possible to make an evaluation of the cancer risk of PAs for humans. IARC (40–43) evaluated several PAs and classified lasiocarpine, monocrotaline and riddelliine as Group 2B (possibly carcinogenic to humans) and hydroxysenkirkine, isatidine, jacobine, retrorsine, seneciphylline, senkirkine and symphytine as Group 3 (not classifiable as to its carcinogenicity to humans).

The Fifth Session of CCCF (3) discussed PAs and concluded that there is significant new information since the evaluation by IPCS in 1988 (39), warranting an updated assessment by JECFA. CCCF therefore placed PAs on its priority list and requested that JECFA:

- perform a full risk assessment;
- identify the most relevant PAs (in terms of both occurrence and toxicity) for human health;
- identify data gaps; and
- consider PAs in feed, as PAs can carry over from feed to animal products.

#### ***Process of literature gathering and assessment for toxicological evaluation***

As IPCS evaluated PAs in 1988 (39), the year 1988 was taken as the cut-off point for literature selection. As EFSA evaluated PAs in 2011 (44), it was decided to report EFSA's conclusions on non-critical studies in the JECFA evaluation and reassess only those studies considered critical for the risk assessment.

A systematic review approach was used to gather data. A systematic review is a literature review aimed at identifying, selecting, evaluating, interpreting and synthesizing all available research relevant to a particular research question by means of prespecified and standardized methods. The aim of a systematic review is to minimize bias and increase the transparency, objectivity and reproducibility of assessments. It is performed in subsequent steps of literature search, selection based on title/abstract, full text selection, data extraction from studies, quality assessment of studies, synthesizing included data (meta-analysis), presenting data and results, and interpreting results and drawing conclusions. The process uses a predefined protocol with defined selection and quality criteria. The method has been used in human health research, mainly on narrow clinical and epidemiological questions, and is being implemented increasingly for questions in the animal toxicity area. It was tested in this JECFA evaluation of PAs to determine its usefulness in a broad risk assessment.

A protocol was developed with six defined research questions that were used for the literature search in selected databases. Literature searches were performed in early 2015. More than 10 000 references were identified through title/abstract selection, which resulted in subgroups of included references relevant



for different toxicology-related parts of the evaluation. As working through six research questions proved to be very labour intensive and there was insufficient time left before the JECFA meeting, stages of the systematic review subsequent to the title/abstract selection were not performed according to the protocol, and full text selection was done using the critical appraisal method regularly used in the preparation of JECFA monographs. The Committee agreed that the protocol would be updated with these changes and made publicly available through the website or in the monograph.

Because of the narrow time frame between the work on the selection of references and the JECFA meeting, the evaluation could not be completed at the meeting, but the Committee considered the information sufficient to determine an approach for the evaluation. The Committee agreed that only preliminary findings would be reported in the current meeting report and that the full evaluation would be published subsequently. The results included in this meeting report are therefore preliminary and will need later confirmation when all studies have been quality assessed and described in detail.

The Committee also revisited the evaluations of IPCS (39) and EFSA (44) during the meeting to determine whether there were studies included that could provide more information on the relative potency of PAs. Other studies were also identified that were relevant to the assessment of PAs but had not been included in either of these evaluations.

### ***Preliminary findings for toxicological evaluation***

Preliminary findings on biochemical and toxicological aspects of saturated and 1,2-unsaturated PAs and their associated *N*-oxides include the following:

- The 1,2-unsaturated PAs are rapidly absorbed and distributed in the body.
- Ingested PA-*N*-oxides are efficiently reduced to PA free bases in the digestive tract.
- The 1,2-unsaturated PAs are metabolized primarily in the liver by three main pathways: cleavage of the ester bonds; *N*-oxygenation of the necine base of retronecine- and heliotridine-type PAs, leading to the more readily excreted *N*-oxides; and a cytochrome P450-mediated oxidative pathway, leading to the formation of reactive (+/-) 6,7-dihydro-7-hydroxy-1-hydroxymethyl-5*H*-pyrrolizine (DHP) esters (which are considered to be responsible for the toxicity of 1,2-unsaturated PAs via alkylation of DNA and proteins) in the liver. DHP ester metabolites can also undergo DHP conjugation with glutathione and other nucleophilic substances *in vivo*, and they are readily hydrolysed to DHP diols, which, being less reactive than DHP

esters, circulate widely in vivo and alkylate and form DHP adducts with nucleophilic groups in many tissues.

- Of the PAs investigated in acute toxicity studies in rats and mice, senecionine, retrorsine (and its *N*-oxide) and riddelliine are among the most acutely toxic, whereas echimidine and heliotrine appear to be relatively less toxic.
- Chronic liver lesions are induced by single doses of PAs, including lasiocarpine, heliotrine, retrorsine, riddelliine, seneciphylline, senkirkine and hydroxysenkirkine.
- The most common toxic effect in both short-term and long-term studies of 1,2-unsaturated PAs is hepatotoxicity, characterized by megalocytosis (enlarged hepatocytes containing hyperchromatic nuclei) and, to a lesser extent, centrilobular necrosis, fibrosis and bile duct hyperplasia.
- Carcinogenicity is considered to be the most critical end-point following long-term exposure of experimental animals to certain PAs. Riddelliine produces haemangiosarcomas in the liver in rats and mice and alveolar/bronchiolar neoplasms in female mice, and lasiocarpine produces hepatocellular tumours and angiosarcomas of the liver in male and female rats and haematopoietic tumours in female rats.
- The genotoxicity of PAs and PA-containing preparations has been extensively studied in a variety of in vitro and in vivo assays. Overall, these clearly demonstrate that those 1,2-unsaturated PAs that have been tested form DNA adducts and are mutagenic. The saturated PAs have not been tested, but because they are not metabolized to DHP esters, they are less likely to be genotoxic.
- PAs cross the placenta and induce toxicity in the fetus, although this may be related to maternal toxicity.

#### ***Long-term studies of toxicity and carcinogenicity***

The Committee considered in detail the two long-term toxicity and carcinogenicity studies on lasiocarpine and riddelliine, which were evaluated by IPCS (39) and/or EFSA (44). These are the studies performed by the United States National Cancer Institute (NCI) on lasiocarpine (45) and by the United States NTP on riddelliine (46). No new long-term studies published subsequent to those two evaluations were identified by the Committee.

#### ***Lasiocarpine (45)***

A carcinogenicity bioassay of lasiocarpine was conducted by administering the test chemical in the diet to male and female Fischer 344 rats.

Groups of 24 Fischer 344 rats of each sex were administered lasiocarpine (purity 97%) at 7, 15 or 30 mg/kg feed (equivalent to 350, 750 and 1500 µg/kg bw per day, respectively) for 104 weeks. Matched controls consisted of groups of 24 untreated rats of each sex. Feed and water were available ad libitum.

Body weights were lower in mid- and high-dose animals compared with controls at the end of the study. High mortality was noted in the mid- and high-dose groups of both sexes. None of the animals of the high-dose group survived until the end of the study. Survival rates in male animals were 88% in controls, 54% in the low-dose group and 17% in the mid-dose group; in females, survival rates were 92% in controls, 42% in the low-dose group and 4% in the mid-dose group. As more than 50% of the high-dose females died before week 52 on study, the statistical analysis of female rats was performed using only those animals surviving more than 52 weeks: 24 in the control group, 24 in the low-dose group, 23 in the mid-dose group and nine in the high-dose group.

Non-neoplastic lesions are not described in detail in the study report, but it was noted that nodular hyperplasia of the liver occurred in both male and female rats in all dose groups.

In male rats, there was a dose-related increase in the incidence of haemangiosarcoma of the liver (controls 0/24, low dose 5/24, mid dose 11/24, high dose 13/24). In females, the incidences in both the low- and mid-dose groups, but not in the high-dose group, were significantly higher than that in the controls (controls 0/24, low dose 8/24, mid dose 7/24, high dose 2/9). The study authors noted that the lower incidence in the high-dose females, compared with the low- and mid-dose animals, could be related to the increased mortality rate in the high-dose group. Furthermore, in high-dose females, there was a significant increase in the combined incidence of hepatocellular carcinoma and adenoma of the liver. A positive trend for these tumours was also observed in male rats, but incidences did not reach statistical significance. The combined incidence of lymphoma or leukaemia was significant in both the low- and mid-dose female groups, but not in the high-dose group, perhaps because of the early deaths in this group. The combined incidences of these tumours in the males were not significant.

NCI (45) concluded that “under the conditions of this bioassay, lasiocarpine was carcinogenic in Fischer 344 rats producing hepatocellular tumors and angiosarcomas of the liver in both sexes and hematopoietic tumors in female animals”.

#### *Riddelliine (46)*

The carcinogenic potential of riddelliine was studied by the NTP in B6C3F1 mice and F344/N rats of both sexes.

Groups of 50 male and 50 female rats were administered riddelliine (purity 92%) by gavage in sodium phosphate buffer at a dose of 0 or 1 mg/kg bw per day, 5 days/week, for 105 weeks (equivalent to 0 and 0.71 mg/kg bw per day on a 7 days/week basis); additional groups of 50 female rats received 0.01, 0.033, 0.1 or 0.33 mg/kg bw per day, 5 days/week, also for 105 weeks (equivalent to 0.007, 0.024, 0.071 and 0.236 mg/kg bw per day on a 7 days/week basis).

Mean body weights were reduced in high-dose rats (by 21% and 18% in males and females, respectively) compared with vehicle controls. Survival days were reduced in high-dose males and females, and only 3/50 male and 0/50 female animals survived until the end of the study. Because of their high mortality, male rats were terminated at week 72. The only clinical finding related to riddelliine administration was a general debilitation of the animals prior to death. Non-neoplastic effects observed in the liver of female rats consisted of dose-related increased incidences of hepatocyte cytomegaly, starting from 0.033 mg/kg bw per day, and further histopathological observations (among them, regenerative hyperplasia, eosinophilic foci, clear cell foci) at the two highest doses and necrosis and haemorrhage at the highest dose. All these effects were also evident in the only dose (high) investigated in male animals. Further severe non-neoplastic lesions, such as renal tubule necrosis, were observed in high-dose males and females.

Haemangiosarcomas of the liver were present in the liver of 86% of males and 76% of females in the 1 mg/kg bw per day group and 6% of female animals in the 0.33 mg/kg bw per day group. The incidences of hepatocellular adenoma and mononuclear cell leukaemia in 1 mg/kg bw per day males and females were also significantly increased.

In the study with mice, groups of 50 male and 50 female animals, 5–6 weeks of age, were administered riddelliine (purity 92%) by gavage at a dose of 0 or 3 mg/kg bw per day, 5 days/week, for 105 weeks (equivalent to 0 and 2.1 mg/kg bw per day on a 7 days/week basis). Additional groups of 50 male mice received 0.1, 0.3 or 1 mg/kg bw per day, 5 days/week, also for 105 weeks (equivalent to 0.071, 0.21 and 0.71 mg/kg bw per day on a 7 days/week basis).

Survival of males and females administered 3 mg/kg bw per day was significantly less than that of the vehicle controls. Twenty out of 50 male mice and 17 out of 50 female mice survived until study termination. At 3 mg/kg bw per day, body weights were reduced in both females and males (by 19% and 33%, respectively), compared with controls. In the 1 mg/kg bw per day male group, the body weight at termination was on average 6% lower than that of controls. There were no treatment-related clinical findings.

In males administered 0.3 mg/kg bw per day or above and females given 3 mg/kg bw per day, incidences of hepatocyte cytomegaly, karyomegaly and necrosis were significantly increased compared with controls. Focal haemorrhage in the

liver increased starting from 1 mg/kg bw per day onwards. Increased incidences of various kidney effects were observed at higher doses in males and females, such as glomerulosclerosis (two highest-dose groups in males and high-dose group in females), nephropathy (females) and renal tubule karyomegaly (males). Further effects observed in the high-dose females were alveolar epithelial hyperplasia and chronic arterial inflammation in various organs.

Increased incidences of liver haemangiosarcomas were restricted to the high-dose males (62% versus 4% in vehicle controls). In high-dose females, increased incidences of alveolar/bronchiolar adenoma or carcinoma were observed (26% versus 4% in vehicle controls). Decreased incidences of liver adenoma and carcinoma were noted in both male and female mice dosed with riddelliine.

The NTP concluded that “under the conditions of these studies, there was clear evidence of carcinogenic activity of riddelliine in male and female F344/N rats based on increased incidences of hemangiosarcoma in the liver”. They also considered the increased incidences of hepatocellular adenoma and mononuclear cell leukaemia in male and female rats to be treatment related.

The NTP also concluded that “there was clear evidence of carcinogenic activity of riddelliine in male B6C3F1 mice based on increased incidences of hemangiosarcoma in the liver” and “clear evidence of carcinogenic activity in female B6C3F1 mice based on increased incidences of alveolar/bronchiolar neoplasms”.

### ***Analytical methods***

The Committee reviewed and identified specific analytical issues associated with the screening and quantification of PAs – saturated and 1,2-unsaturated PAs and their *N*-oxides – and DHP adducts in various foods and feeds.

The main issues related to analytical methods include large variations in PA concentrations in food and feed samples, variation in PA profiles between plants in various regions of the world, the stability of PAs during storage and the issue of whether to quantify individual PAs or total necines.

PAs are extracted from plants and food samples with hot or cold methanol or ethanol or dilute aqueous acid. The alcoholic or aqueous acid extracts are then applied to prepared strong cation exchange solid-phase extraction cartridges, followed by washing of the cartridges with water and methanol to remove non-adsorbed impurities and then elution of the PAs and *N*-oxide components using a small volume of ammoniated methanol. Subsequent evaporation and reconstitution of the residue in methanol or another suitable solvent produce samples ready for the analysis of PAs.

Several screening methods are currently available, including thin-layer chromatography, electrophoresis, nuclear magnetic resonance and immunological methods. Thin-layer chromatography with colorimetric detection of 1,2-unsaturated PAs is inexpensive, but the results are qualitative rather than quantitative. Nuclear magnetic resonance has been used to determine the total alkaloid content, but it probably lacks the sensitivity required for food safety risk assessment purposes. Enzyme-linked immunosorbent assay (ELISA)-based screening methods for 1,2-unsaturated PAs and their *N*-oxides have been developed, but these are currently limited by a lack of antibodies that specifically bind all of the 1,2-unsaturated PAs and their *N*-oxides with comparable affinity. At the same time, antibodies developed for specific 1,2-unsaturated PAs or their *N*-oxides seem to lack specificity for other 1,2-unsaturated PAs and their *N*-oxides. The development of sensitive ELISAs for quantifying necines could be quite useful in summation analysis methods for quantifying total 1,2-unsaturated PAs and their *N*-oxides based on hydrolysis. However, results from ELISA should always be confirmed using quantitative reference methods, such as gas chromatography – mass spectrometry (GC-MS) and/or HPLC – tandem mass spectrometry (HPLC-MS/MS), as immunological methods have limitations in selectivity and reproducibility.

Recent developments have shown the need for validated methods to analyse DHP adducts in liver tissue and foods of animal origin. Methods for sulfur-bound DHP adducts, DHP-DNA adducts and DHP-protein adducts are available, but not validated, for foods. The significance of DHP adducts in foods is uncertain. Some DHP adducts are reversible and may be capable of transferring the DHP moiety to DNA *in vivo*; for example, DHP-glutathione has been shown to transfer DHP to DNA *in vitro*, and it and other DHP adducts in food could potentially cause mutations.

Quantitative analysis of PAs is based on the determination of individual PAs using liquid chromatography – tandem mass spectrometry (LC-MS/MS) or analysis of their common necine groups using GC-MS. Some issues of concern are related to the instability of *N*-oxides during sample preparation and analysis.

Challenges to all analytical methods are the lack of high-quality standards, internal standards and certified reference materials. Harmonized methods or performance criteria for PAs are currently not available, despite several proficiency tests carried out in the European Union, with promising results in tea, honey and several feedstuffs.

### **Sampling protocols**

PA contamination can be non-homogeneous owing to the uneven distribution of plant parts in a batch of feed or food. Sampling will therefore be critical.

Existing sampling protocols for other natural toxins such as mycotoxins should be followed in sampling for PAs in bulk commodities and in consumer products. DHP adducts are expected to be more evenly distributed in foods of animal origin, and common sampling protocols for contaminants should be applied.

### ***Effects of processing***

Reports on the effects of food and feed processing on the concentration of PAs in the subsequently produced foods have been evaluated by the Committee.

The main focus of the reports in the literature is on 1,2-unsaturated PAs and their *N*-oxides. The 1,2-unsaturated PAs and their *N*-oxides are stable during tea infusion making. The removal of co-harvested seeds and weeds from the raw materials will reduce the content of 1,2-unsaturated PAs and their *N*-oxides significantly. Intoxications of humans related to 1,2-unsaturated PAs and their *N*-oxides have involved consumption of tea, bread, yoghurt, kitchen herbs and spices. To a certain extent, this indicates the stability of PAs during various food preparation steps. The presence of PAs in foods and dietary supplements such as pollen and honey is further proof of the stability of PAs during food processing. However, details on the rate of possible degradation during food processing are not available, with the exception of tea infusion.

The occurrence of PAs in animal feed, compound feed and ensiled fodder shows that 1,2-unsaturated PAs and their *N*-oxides are fairly stable during their production, although reliable data on the rate of degradation and the metabolites that are formed are lacking.

### ***Prevention and control***

Management strategies to prevent PA-containing plants from entering the food-chain were evaluated by the Committee. Management practices currently focus on minimizing the occurrence of weeds containing 1,2-unsaturated PAs and their *N*-oxides in feed and food. Management practices to help prevent and reduce the levels of 1,2-unsaturated PAs and their *N*-oxides in food were indicated in a recently published Codex code of practice (47). Good agricultural practices, hazard analysis and critical control points, and good manufacturing practice strategies must be in place to prevent batches of food contaminated with PAs from entering the food-chain and co-mingling with uncontaminated products.

### ***Levels and patterns of contamination in food commodities and feeds***

Data on the content of 1,2-unsaturated PAs and their *N*-oxides in foods for evaluation by the Committee were obtained from the scientific literature and from submissions from Brazil, Germany, Hungary, Luxembourg and FoodDrinkEurope (formerly the Confederation of the Food and Drink Industries of the European

Union). The total number of analytical results evaluated at the current meeting was 21 793. Table 8 summarizes the prevalence and concentration range of 1,2-unsaturated PAs and their *N*-oxides by food category. The concentration range (minimum to maximum) is based only on analytical results that were quantified.

The majority of the evaluated data (99%) were from European countries. Patterns of contamination with 1,2-unsaturated PAs and their *N*-oxides are likely to differ between regions, and a better understanding of the worldwide situation would be aided by the submission of data from other regions.

Transfer of 1,2-unsaturated PAs and their *N*-oxides from feed to foods, in particular eggs, milk and meat, is reported from studies using high dose rates. DHP adducts can also be expected in these products. The analytical results evaluated by the Committee lacked information on 1,2-unsaturated PAs and their *N*-oxides and DHP adducts in retail samples of foods of animal origin (milk, eggs and meat). The information on 1,2-unsaturated PAs and their *N*-oxides in cereals and cereal products included data from surveys of heavily contaminated cereals in Afghanistan and the Islamic Republic of Iran. Further information on background levels of 1,2-unsaturated PAs and their *N*-oxides in cereals would aid the evaluation. Data on 1,2-unsaturated PAs and their *N*-oxides in non-herbal teas are inconsistent, and further data on these foods would be of benefit. Herbal medicines were found to contain high concentrations of 1,2-unsaturated PAs and their *N*-oxides ( $n = 103$ ,  $n < \text{LOD} = 38$ , concentration range 1–7 900 000  $\mu\text{g}/\text{kg}$ ).

Table 8  
Summary of available data on 1,2-unsaturated PAs and their *N*-oxides in food products

Food category	<i>n</i>	<i>n</i> < LOD	Concentration range ( $\mu\text{g}/\text{kg}$ )
Cereals and cereal products	1 368	1 304	0.12–98 000 <sup>a</sup>
Tea and herbal tea	377	60	0.021–8 500 <sup>b</sup>
Culinary herbs	21	6	0.9–74
Herbal dietary supplements	21	14	0.1–8.4 <sup>c</sup>
Miscellaneous food ingredients	34	25	4.3–4 800
Honey	19 698	5 672	0.3–5 600
Honey products	62	52	10–590
Bee pollen dietary supplements	174	86	11–38 000
Milk and milk products	27	21	1.5–87 <sup>a</sup>
Oils	11	8	0.08–0.6

LOD: limit of detection

<sup>a</sup> Includes results from an investigation of an outbreak of veno-occlusive disease.

<sup>b</sup> Includes results for dry tea and for tea infusions.

<sup>c</sup> Concentration data were not available. Results are in units of  $\mu\text{g}/\text{daily dose}$ .



Table 9

**Summary of available data on 1,2-unsaturated PAs and their *N*-oxides in animal feed**

<b>Food category</b>	<b><i>n</i></b>	<b><i>n</i> &lt; LOD</b>	<b>Concentration range (µg/kg)</b>
Forage and roughage	420	253	5.0–23 000
Compound feed	20	12	5.3–140
Other feed	99	64	4.9–3 300

LOD: limit of detection

Data on the content of 1,2-unsaturated PAs and their *N*-oxides in animal feeds, for evaluation by the Committee, were obtained from the scientific literature. The total number of analytical results evaluated at the current meeting was 539. Table 9 summarizes the prevalence and concentration range of 1,2-unsaturated PAs and their *N*-oxides by feed category. The concentration range (minimum to maximum) is based only on analytical results that were quantified.

***Food consumption and dietary exposure assessment***

Based on the preliminary toxicological evaluation by the Committee, both acute and chronic dietary exposures are relevant to PAs; therefore, both were considered for this assessment. No specific at-risk population groups were identified; therefore, all dietary exposure estimates for the general population were considered.

No estimates of dietary exposure were submitted to the Committee for review, and dietary exposure estimates summarized below were obtained from a search of the literature or from calculations carried out by the Committee. The PAs of most relevance were 1,2-unsaturated PAs and their *N*-oxides. Owing to analytical differences between studies, the number of compounds that the dietary exposure estimates represent may vary. All dietary exposure estimates performed to date relate to exposure to 1,2-unsaturated PAs and their *N*-oxides from a single food type, with the exception of a small ( $n = 63$ ) duplicate-diet study carried out in the Netherlands.

National and regional estimates of dietary exposure are summarized in Table 10.

No information was available on dietary exposure to 1,2-unsaturated PAs and their *N*-oxides from consumption of foods of animal origin, excluding honey. Using EFSA estimates of “worst-case” exposure of a “high-yielding dairy cow” to 1,2-unsaturated PAs and their *N*-oxides from consumption of contaminated feed and transfer information from animal feeding trials, it was estimated that the maximum likely exposure to 1,2-unsaturated PAs and their *N*-oxides from consumption of contaminated milk would be 0.089 µg/kg bw per day.

Table 10  
**Summary of estimates of dietary exposure to 1,2-unsaturated PAs and their *N*-oxides**

Study	Mean (95th percentile) dietary exposure ( $\mu\text{g}/\text{kg}$ bw per day) <sup>a</sup>			
	Acute <sup>b</sup>		Chronic	
	Children	Adults	Children	Adults
<b>Duplicate diet</b>				
Netherlands (48)	–	–	–	0.001 3
<b>Honey</b>				
Australia (current assessment)	0.20–0.24 (0.61–0.65)	0.10–0.12 (0.31–0.32)	0.011–0.013 (0.038–0.045) <sup>c</sup>	0.003 2–0.003 9 (0.022–0.026)
Brazil (current assessment)			0.000 20–0.000 21	
Europe (44) <sup>d</sup>				
Retail honey	0.003 3–0.11 (0.012–0.25)	0.000 9–0.049 (0.003 2–0.11)	0.000 01–0.005 (NS–0.057)	0.000 02–0.001 (NS–0.009)
Bulk honey	0.007 1–0.17 (0.031–0.55)	0.002 0–0.071 (0.008 5–0.24)	0.000 03–0.007 (NS–0.082)	0.000 05–0.002 (NS–0.014)
Germany (49) <sup>e</sup>	0.028–0.057 (0.092–0.11)	0.018–0.037 (0.060–0.072)	0.002–0.004 (0.006–0.012)	0.001–0.002 (0.005–0.017)
Ireland (50) <sup>f</sup>	0.19–0.20 (0.48–0.51)	0.048–0.051 (0.12–0.13)	–	–
<b>Tea</b>				
Germany (51)				
Tea	0.15–0.25 (0.69–0.78)	0.060–0.099 (0.27–0.31)	0.011–0.018 (0.046–0.076)	0.006–0.010 (0.030–0.050)
Herbal tea	0.13–0.21 (0.57–0.66)	0.060–0.099 (0.27–0.31)	0.005–0.009 (0.027–0.045)	0.002–0.003 (0.018–0.030)
Ireland (52) <sup>g</sup>	–	–	–	0.001 3–0.11
Netherlands (48)				
Herbal tea <sup>h</sup>	–	–	–	0.003–0.13 (0.01–0.26)
<b>Herbal medicines/supplements</b>				
Ireland (52) <sup>i</sup>	–	–	–	0.000 8–0.13
Netherlands (48) <sup>j</sup>	–	–	–	0.000 4–0.06 (0.001–0.60)
USA (53) <sup>k</sup>	–	–	–	0.001 7–0.14
<b>Culinary herbs</b>				
Germany (54) <sup>l</sup>	–	0.032–0.11	–	–

NS: not specified (some of the countries included in the European assessment did not provide 95th percentile estimates of honey consumption)

<sup>a</sup> All exposure estimates have been rounded to two significant figures and may appear different from the equivalent numbers in the original publications. Ranges show lower-bound to upper-bound estimates.

<sup>b</sup> Acute exposures summarized here are those based on a 95th or 97.5th percentile consumer consuming food containing 1,2-unsaturated PAs and their *N*-oxides at either the mean concentration or the 95th percentile concentration, unless otherwise stated.

<sup>c</sup> In this case, the high percentile is a 90th percentile, rather than a 95th percentile.

<sup>d</sup> The results for children are those for a toddler (1–3 years), rather than “other children” (3–10 years). Chronic exposures are those estimated for all respondents, rather than consumers only.

<sup>e</sup> In the German assessment, chronic exposures were calculated with and without “brand loyalty”. Brand-loyal consumers were considered to always consume honey at the 95th percentile of the concentration distribution. Results presented here are those based on the mean concentration.

<sup>f</sup> Based on mean consumption of 20 g honey/day or maximum consumption of 50 g honey/day.

<sup>g</sup> Based on daily consumption of three cups (600 mL) of herbal tea.

<sup>h</sup> Mean exposure estimates were based on consumption of three 150 mL cups of tea per day, each containing 3 g of tea. High percentile (97.5th percentile, consumer only) estimates of exposure were based on 11 × 150 mL cups, each containing 3 g of tea. For two of 11 teas, recommended dose information was used. Exposures were calculated using a 70 kg body weight.

<sup>i</sup> Exposure from use of herbal medicines at the recommended dose, assuming a 60 kg body weight.

<sup>j</sup> Exposure from use of herbal supplements at the recommended dose. Figures in parentheses are from use of supplements at a rate of 10 capsules or tablets per day. Exposures were calculated using a 70 kg body weight.

<sup>k</sup> The range represents exposure to 1,2-unsaturated PAs and their *N*-oxides from recommended use of PA-containing dietary supplements based on the common butterbur (*Petasites hybridus*), assuming a 60 kg body weight.

<sup>l</sup> Based on consumption of a traditional German meal in which surveyed commercial herb preparations may have been used, assuming a 60 kg body weight.

The available occurrence data for 1,2-unsaturated PAs and their *N*-oxides were deemed to be inappropriate for use in determining international estimates of chronic dietary exposure in combination with the GEMS/Food consumption cluster diets. There were insufficient or inappropriate data for most relevant foods or not enough information about the samples to enable data from different sources to be combined.

### ***Dose–response analysis***

Two long-term carcinogenicity studies were available, which provide information on the carcinogenic effects – considered to be the most critical end-point following long-term exposure – of certain PAs in experimental animals. These are the studies performed by the United States NCI (45) on lasiocarpine and by the United States NTP (46) on riddelliine, described in more detail above.

Haemangiosarcoma of the liver was the most prominent and frequent tumour type in both studies with rats and also in the riddelliine study with mice, but mice proved to be less sensitive than rats. High lethality and reduced survival rates were observed in the riddelliine study at the highest dose and in the lasiocarpine study at the two highest doses in both sexes. When comparing respective doses, (similarly) high mortality appears to be related to doses of approximately 0.7 mg/kg bw per day for both substances in these two studies with rats. Non-neoplastic lesions in the liver were observed in rats in the study with riddelliine at a dose of 0.033 mg/kg bw per day (0.024 mg/kg bw per day after correction for continuous exposure) and with lasiocarpine starting at the lowest dose of 0.35 mg/kg bw per day.

Whereas a clear dose–response relationship was observed after riddelliine exposure and high incidences of liver haemangiosarcoma (86% in males, 76% in females) were noted in the highest-dose group, incidences in the highest-dose group in the lasiocarpine study were lower (females) or only slightly higher (males) than those in the lower-dose groups. Therefore, it seems likely that tumour incidences in the highest-dose groups of the lasiocarpine study were influenced by the low survival rates and consequently the shorter period of time in which tumours could develop. Still, from the information obtained from the lower-dose

groups, it can be concluded that similar tumour numbers are induced at similar doses by lasiocarpine compared with riddelliine, although it must be noted that the different forms of administration in the two studies (administration by feed versus gavage) set limitations to a quantitative comparison.

Modelling was carried out for the induction of liver haemangiosarcoma by riddelliine in female rats using the software PROAST (version 38.9). Eight models (including two families of nested models) were fitted to the data. Using a *P*-value of 0.1 as the criterion, all models provided acceptable fits and were used to derive benchmark dose (BMD) and BMDL values for a benchmark response (BMR) of 10% extra risk (BMD<sub>10</sub> and BMDL<sub>10</sub>, respectively). BMD<sub>10</sub> values ranged from 208 to 363 µg/kg bw per day, and BMDL<sub>10</sub> values ranged from 182 to 299 µg/kg bw per day. The lowest BMDL<sub>10</sub> value for riddelliine, derived with the two-stage model, was 182 µg/kg bw per day, or 0.182 mg/kg bw per day.

### **Evaluation**

The Committee was unable to complete its toxicological evaluation at the present meeting. The full evaluation will be published at a future time.

Nevertheless, the Committee was able to agree on the following:

Exposure to PAs has been associated with a wide range of effects, with rats being the most sensitive species. Laboratory studies have identified the liver as the most sensitive organ in rats. Humans are also sensitive to PAs.

The Committee considered that the genotoxic mode of action did not allow derivation of a health-based guidance value and decided to use the BMDL<sub>10</sub> of 182 µg/kg bw per day for liver haemangiosarcoma in female rats from the NTP (46) study on riddelliine as the point of departure for use in an MOE approach.

The Committee calculated MOEs between the BMDL<sub>10</sub> of 182 µg/kg bw per day and mean and high-percentile (90th, 95th or 97.5th, depending on the study) chronic exposure estimates for children and adults from consumption of honey and tea, separately. As several national estimates of dietary exposure were available for each food, MOEs were calculated using a range from the lowest lower-bound mean or high-percentile dietary exposure to the highest upper-bound mean or high-percentile dietary exposures. This range includes consideration of the uncertainty in measurements of 1,2-unsaturated PAs and their *N*-oxides and the variability in their concentrations and national estimates of food consumption. MOEs are summarized in [Table 11](#).

It should be noted that estimates of dietary exposure to 1,2-unsaturated PAs and their *N*-oxides from tea consumption are likely to be overestimates, as concentration data from herbal teas have been combined with information on total tea consumption.

Table 11

**Summary of MOE calculations for exposure to 1,2-unsaturated PAs and their *N*-oxides from consumption of honey or tea or duplicate diets**

Population group	Consumption range (g/kg bw per day)	Dietary exposure to 1,2-unsaturated PAs and their <i>N</i> -oxides ( $\mu\text{g}/\text{kg}$ bw per day)		MOE	
		LLB	HUB	LLB	HUB
<b>Honey<sup>a</sup></b>					
Adults					
Mean	0.002–0.05	0.000 02	0.003 9	9 000 000	46 000
High percentile	0.20–0.28	0.005	0.026	36 000	6 900
Children					
Mean	0.001–0.14	0.000 01	0.013	18 000 000	14 000
High percentile	0.35–1.58	0.006	0.082	30 000	2 200
<b>Tea<sup>b</sup></b>					
Adults					
Mean	0.015–0.13	0.001 3	0.13	140 000	1 400
High percentile	0.09–0.47 <sup>c</sup>	0.010	0.26	18 000	700
Children					
Mean	0.026–0.055	0.005	0.018	36 000	10 000
High percentile	0.14–0.23	0.027	0.076	6 700	2 400
<b>Duplicate diet<sup>d</sup></b>					
Adults					
Mean	NS	0.001 3		140 000	

HUB: highest upper bound; LLB: lowest lower bound; NS: not stated

<sup>a</sup> From 44, 49 and the current Committee assessment.

<sup>b</sup> From 48 and 51.

<sup>c</sup> Tea consumption is expressed in terms of dry tea weight. Typical conversion rates are 100 mL of tea infusion for 1 g of dry tea.

<sup>d</sup> From 48. Exposure estimates are mean estimates, rather than high percentile.

There is currently insufficient information to determine MOEs for other food types or for the total diet. Although mean dietary exposures from a small duplicate-diet study in the Netherlands (0.001 28  $\mu\text{g}/\text{kg}$  bw per day) equates to an MOE of 140 000, no high-percentile exposure estimate from this study is available.

### Conclusions and future work

The Committee will complete its evaluation and publish the results as soon as possible.

The Committee noted the reports on acute poisoning cases in humans and will attempt to derive a critical dose from human case reports and poisonings in domestic animals.

The Committee will also investigate whether it is possible to identify potency factors for the different 1,2-unsaturated PAs and their *N*-oxides, in order to evaluate the possible effects of combined exposure. Although the preferred data for comparing potency would be carcinogenicity, the available data do not appear to be sufficient to distinguish between the potency of the PAs tested. Therefore, the Committee will investigate whether it is possible to use genotoxicity or acute toxicity data on PAs for this purpose.

Based on limited occurrence data, the Committee noted that the calculated MOEs for honey and tea for high consumers (and for average tea consumption by children) indicated a concern. It should be noted that PAs measured in these commodities might not be representative for all food groups and all regions; however, the calculation provided a conservative risk estimate, as it was compared with the BMDL<sub>10</sub> for the potent PA riddelliine.

Based on average exposure, the MOEs were of less concern (except for the above-mentioned average exposure of children from tea consumption). However, the Committee considered it of concern that exposure to a single food product could result in such low MOEs. The Committee noted that exposure to PAs resulted from other food items as well, and animal products such as milk might contribute to the total exposure as a result of the presence of PAs in feed. A first indication of total exposure could be taken from a small duplicate-diet study, from which an MOE of 140 000 could be derived, but it was unclear how representative these data were.

## **5. Future work and recommendations**

### **General considerations**

#### **Update on the draft specifications monographs for 16 modified starches**

Following the recommendation made by the seventy-ninth meeting of the Committee, the 16 specifications for modified starches have been separated into stand-alone documents without adding, deleting or modifying any information. Some of the resulting single draft specifications monographs are incomplete; in some cases, essential information is missing, in particular information that would normally be needed to serve the purpose of a specification to unambiguously characterize the additive. Therefore, a revision of at least some of these individual draft specifications monographs is required. As the next step, the Committee recommended that the data and information necessary to complete and revise the 16 individual draft specifications monographs be requested through a call for data. In addition to the missing information (highlighted in the individual draft specifications monographs currently posted on the JECFA website at [http://www.fao.org/fileadmin/user\\_upload/agns/pdf/jecfa/2015\\_02\\_22\\_Modified\\_Starches.pdf](http://www.fao.org/fileadmin/user_upload/agns/pdf/jecfa/2015_02_22_Modified_Starches.pdf)), data relevant to the method of manufacture, detection methods, product characterization and levels of contaminants present (if any) should be requested as well.

#### **HPLC method in the adopted specifications of cassia gum**

The Committee recommended that the data to revise the HPLC method for the determination of anthraquinones in cassia gum be requested through a call for data. Based on the information and data submitted, the Committee will consider revising the specifications as appropriate.

#### **Application of systematic review to the work of the Committee**

The Committee recommended that methodological standards for food-related systematic review and criteria for determining when a systematic review is appropriate should be developed. Guidance on incorporating elements of the systematic review process into the work of the Committee should also be developed for future consideration.

#### **Revised guidance for WHO JECFA monographers and reviewers**

The draft guidance documents for WHO monographers and reviewers evaluating 1) food additives (excluding flavouring agents) and 2) contaminants in food and feed will be revised based on written comments provided to the Secretariat by the

Committee after the meeting. A separate guidance document on enzymes will also be prepared.

## Specific food additives

### Magnesium stearate and other magnesium-containing food additives

Based on the present dietary exposure assessment, the Committee reiterated its earlier recommendation that total dietary exposure to magnesium from food additives and other sources in the diet should be assessed. This is important, as a large number of magnesium-containing food additives have been evaluated individually, but not collectively, in relation to their laxative effects.

### Mixed $\beta$ -glucanase, cellulase and xylanase from *Rasamsonia emersonii*

New tentative specifications were prepared, with a request for the following information:

- a method to determine the identity for  $\beta$ -glucanase, including data from a minimum of five batches using the method described;
- a method to determine the identity for cellulase, including data from a minimum of five batches using the method described;
- a non-proprietary method to determine the identity and activity for xylanase that can be used by control laboratories, and data from a minimum of five batches using the method described.

The above-requested information should be submitted by December 2016 in order for the tentative specifications to be revised; failure to provide this information may lead to a withdrawal of the specifications, with a possible impact on the ADI.

### Mixed $\beta$ -glucanase and xylanase from *Disporotrichum dimorphosporum*

New tentative specifications were prepared, with a request for the following information:

- a method to determine the identity for  $\beta$ -glucanase, including data from a minimum of five batches using the method described;
- a non-proprietary method to determine the identity and activity for xylanase that can be used by control laboratories, and data from a minimum of five batches using the method described.

The above-requested information should be submitted by December 2016 in order for the tentative specifications to be revised; failure to provide this information may lead to a withdrawal of the specifications, with a possible impact on the ADI.



### Ethylene glycol and diethylene glycol impurities in food additives

The Committee noted that ethylene glycol and diethylene glycol, which are impurities in PVA-PEG graft copolymer, may also be present as impurities in other food additives, such as polyethylene glycols and polysorbates, and the total exposure to these compounds from food additives may be higher than from PVA-PEG graft copolymer alone. Currently, only the specifications monograph for polyethylene glycols contains maximum limits for ethylene glycol and diethylene glycol (2500 mg/kg, singly or in combination). The Committee recommended setting and/or revising maximum limits for ethylene glycol and diethylene glycol that may occur as impurities in food additives at a future meeting.

### Silicon dioxide, amorphous

The tentative status of the specifications was maintained, and the following information was requested:

- raw materials used and methods of manufacture for different forms of silicon dioxide (pyrogenic silica, precipitated silica, hydrated silica, silica aerogel and colloidal silica);
- identification methods allowing the differentiation between the above forms of silicon dioxide;
- functional uses of different forms, and information on the types of products in which it is used and the use levels in these products;
- data on solubility using the procedure documented in Volume 4 (Analytical methods) of the *Combined Compendium of Food Additive Specifications*;
- data on the impurities soluble in 0.5 M hydrochloric acid for all forms of silicon dioxide used as food additives, from a minimum of five batches. If a different extraction and determination method is used, data should be provided along with details of the method and QC data;
- suitability of the analytical method for the determination of aluminium, silicon and sodium using the proposed “Method of assay” along with data from a minimum of five batches. If a different method is used, data should be provided along with details of the method and QC data;
- in addition to the above information, data on pH, loss on drying and loss on ignition for hydrated silica, silica aerogel and colloidal silica.

The tentative specifications will be withdrawn unless the requested information is provided by December 2016.

## Sodium aluminium silicate

The tentative status of the specifications was maintained, and the following information was requested:

- functional uses other than anticaking agent, if any, and information on the types of products in which it is used and the use levels in these products;
- data on solubility using the procedure documented in Volume 4 (Analytical methods) of the *Combined Compendium of Food Additive Specifications*;
- data on the impurities soluble in 0.5 M hydrochloric acid, from a minimum of five batches. If a different extraction and determination method is used, data should be provided along with details of the method and QC data;
- suitability of the analytical method for the determination of aluminium, silicon and sodium using the proposed “Method of assay”, along with data, from a minimum of five batches, using the proposed method. If a different method is used, data should be provided along with details of the method and QC data.

The tentative specifications will be withdrawn unless the requested information is provided by December 2016.

## Contaminants

### Non-dioxin-like polychlorinated biphenyls

A more complete toxicological database, including mechanistic studies on both parent congeners and hydroxy metabolites, would have allowed a more definitive assessment. The Committee recommended that further toxicological studies should be done, particularly in vivo studies for those congeners, such as PCB 101 and PCB 138, that contribute significantly to dietary exposure and human body burden.

There were some limitations with the exposure assessment where there was a limited range of countries and/or foods for which concentration data were provided. This meant that some assumptions needed to be made for estimating dietary exposures for national and international assessments. To ensure better estimates of dietary exposure with a lower degree of uncertainty, the Committee recommends the following:

- As the risk characterization is driven mainly by dietary exposure estimates from European countries, information from a broader range of countries would be desirable to provide a more globally representative conclusion.

- More countries should submit concentration data to the GEMS/Food contaminants database (with as specific details as possible, including unique sample identifiers, specific food details, lower limits of detection, form of the food, etc.).
- National food consumption data should also be submitted by more countries to CIFOCoSS to allow a broader range of country-specific exposure assessments to be undertaken in the future.
- For analytical surveys on NDL-PCBs, it is important to ensure the generation of occurrence data for congeners beyond the six indicator PCBs for a broad range of foods and to include all key congeners and sources of dietary exposure to NDL-PCBs.
- More concentration data in infant formula as well as data from a broader range of countries would assist in determining more reliable estimates of dietary exposure for formula-fed infants.

### **Pyrrrolizidine alkaloids**

The Committee will complete its evaluation and publish the results as soon as possible. The Committee will attempt to derive a critical dose from human case reports and poisonings in domestic animals and will investigate whether it is possible to identify potency factors for the different 1,2-unsaturated PAs and their *N*-oxides, in order to evaluate the possible effects of combined exposure.

Preliminary data gaps that have been identified to date include the following:

- the lack of a comprehensive range of high-quality standards, internal standards and certified reference materials;
- the need for improved sampling protocols;
- the effects of processing, taking into account possible metabolites formed during processing;
- occurrence data from areas other than the European Union and food products other than honey, particularly foods of animal origin, to improve the risk assessment;
- *in vivo* toxicity studies in which a comparison of multiple PAs is performed, for development of the relative potency of PAs;
- more information on the toxicity and occurrence of saturated PAs, as most available data are on the 1,2-unsaturated PAs, and as the saturated PAs may elicit toxicity by a different mode of action;
- more information on transfer from feed to food to estimate whether PA concentrations in food resulting from PAs in feed could be of concern for human health;

- epidemiological studies on long-term follow-up to incidents with PA contamination, with the aim to confirm the carcinogenic potential of PAs in humans.

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## Annex 1

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

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3. Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants) (Third report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, Vol. I. Antimicrobial preservatives and antioxidants, Rome, Food and Agriculture Organization of the United Nations, 1962 (out of print).
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## Annex 2

### Toxicological and dietary exposure information and information on specifications

#### *Food additives considered for specifications only*

Food additive	Specifications
Advantame	R <sup>a</sup>
Aluminium silicate	W <sup>b</sup>
Annatto extract (solvent-extracted bixin)	R <sup>c</sup>
Annatto extract (solvent-extracted norbixin)	R <sup>c</sup>
Calcium aluminium silicate	W <sup>b</sup>
Calcium silicate	R <sup>d</sup>
Glycerol ester of gum rosin	W <sup>b</sup>
Silicon dioxide, amorphous	R, T <sup>e</sup>
Sodium aluminium silicate	R, T <sup>f</sup>

R: existing specifications revised; T: tentative specifications; W: tentative specifications withdrawn

<sup>a</sup> The requested information was received, and the method of assay was revised. The tentative status of the specifications was removed.

<sup>b</sup> The requested information was not received.

<sup>c</sup> The specifications were revised to reflect the modification of the method for residual solvents by headspace gas chromatography and to include sample and standard preparation information.

<sup>d</sup> The requested information was received, and the specifications were revised to include information on functional uses, pH, loss on drying, loss on ignition, impurities soluble in 0.5 M hydrochloric acid and the assay. The tentative status of the specifications was removed.

<sup>e</sup> Limited information was received. The specifications were revised to include information on pH, loss on drying, loss on ignition, impurities (lead and arsenic) soluble in 0.5 M hydrochloric acid and the assay for some forms of silicon dioxide. The tentative status of the specifications was maintained, and information was requested in order for the tentative specifications to be revised.

<sup>f</sup> Limited information was received. The specifications were revised to include information on Chemical Abstracts Service number, chemical formula, pH, loss on drying, loss on ignition and limits on impurities (lead and arsenic) soluble in 0.5 M hydrochloric acid. The tentative status of the specifications was maintained, and information was requested in order for the tentative specifications to be revised.

#### *Food additives evaluated toxicologically and/or assessed for dietary exposure*

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations and dietary exposure information
Benzoates: dietary exposure assessment	NA	Based on the available data set, the Committee noted that there is consistency in the average typical range of concentrations for benzoates reported to be used or analysed in non-alcoholic ("soft") beverages (Codex General Standard for Food Additives [GSFA] food category 14.1). For example, typical reported concentrations from industries ranged from 83 to 209 mg/L for water-based flavoured drinks (food category 14.1.4), and analytically quantified measurements ranged from 63 to 259 mg/L for non-alcoholic beverages (food category 14.1). These levels are lower than national maximum limits (150–400 mg/L) or limits for GSFA food category 14.1.4 (600 mg/L). The Committee also noted that most of the reported estimates for mean and high-percentile benzoate exposure were below the upper bound of the ADI of 0–5 mg/kg body weight (bw), expressed as benzoic acid, despite different methodologies and assumptions applied in the preparation of the exposure estimates.

(continued)

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations and dietary exposure information
Lipase from <i>Fusarium heterosporum</i> expressed in <i>Ogataea polymorpha</i>	N	<p>None of the mean exposure estimates for consumers of non-alcoholic ("soft") beverages exceeded the upper bound of the ADI: 0.3–4.1 mg/kg bw per day for toddlers and young children, 0.2–2.7 mg/kg bw per day for other children, including adolescents, and 0.1–1.7 mg/kg bw per day for adults. However, the Committee noted that the 95th percentile exposures for the consumers-only group exceeded the upper bound of the ADI in some cases: up to 10.9 mg/kg bw per day for toddlers and young children and up to 7.0 mg/kg bw per day for other children, including adolescents. Additionally, the Committee noted that in some countries, the overall dietary exposure to benzoates for toddlers, young children and adolescents also exceeds the upper bound of the ADI at the high percentiles. Reduction of those exposures exceeding the upper bound of the ADI would require consideration of dietary patterns for both beverage and non-beverage foods containing benzoates and typical/allowed benzoate use levels in those countries.</p> <p>No treatment-related adverse effects were seen at the highest dose tested (669 mg total organic solids [TOS]/kg bw per day) in a 13-week study of oral toxicity in rats. A comparison of the dietary exposure estimate of 0.5 mg TOS/kg bw per day (for a 60 kg individual) with the highest dose tested of 669 mg TOS/kg bw per day results in a margin of exposure (MOE) of at least 1300.</p> <p><b>The Committee established an ADI "not specified"<sup>a</sup> for lipase from <i>F. heterosporum</i> expressed in <i>O. polymorpha</i> when used in the applications specified and in accordance with good manufacturing practice.</b></p>
Magnesium stearate	N	<p>The Committee estimated the potential total dietary exposure to magnesium stearate based on the proposed maximum use levels: 44 mg/kg bw per day for children and 83 mg/kg bw per day for adults, corresponding to 2 and 4 mg/kg bw per day, expressed as magnesium, respectively. These dietary exposures would contribute up to an additional 250 mg/day to the background exposure to magnesium from food of 180–480 mg/day. The Committee noted that the consumption of the food additive may lead to an additional dietary exposure to stearic and palmitic acids in the order of 5 g/day.</p> <p>An ADI "not specified"<sup>a</sup> has previously been established for a number of magnesium salts used as food additives. The Committee concluded that there are no differences in the evaluation of the toxicity of magnesium stearate compared with other magnesium salts. <b>The Committee confirmed the ADI "not specified" for magnesium salts of stearic and palmitic acids.</b> However, the Committee was concerned that the use of magnesium salts in many food additives may result in combined exposure that could lead to a laxative effect. Therefore, the Committee reiterated its previous recommendation to undertake an exposure assessment for magnesium from use of food additives.</p>



Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations and dietary exposure information
Maltotetraohydrolase from <i>Pseudomonas stutzeri</i> expressed in <i>Bacillus licheniformis</i>	N	<p>No treatment-related adverse effects were seen at the highest dose tested (93 mg TOS/kg bw per day) in a 13-week study of oral toxicity in rats. A comparison of the dietary exposure estimate of 0.1 mg TOS/kg bw per day (for a 60 kg individual) with the highest dose tested of 93 mg TOS/kg bw per day results in an MOE of at least 900.</p> <p><b>The Committee established an ADI “not specified”<sup>a</sup> for maltotetraohydrolase from <i>P. stutzeri</i> expressed in <i>B. licheniformis</i> when used in the applications specified and in accordance with good manufacturing practice.</b></p>
Mixed β-glucanase, cellulase and xylanase from <i>Rasamsonia emersonii</i>	N, T <sup>b</sup>	<p>No treatment-related adverse effects were seen at the highest dose tested (84.8 mg TOS/kg bw per day) in a 13-week study of oral toxicity in rats. A comparison of the dietary exposure estimate of 0.08 mg TOS/kg bw per day (for a 60 kg individual) with the highest dose tested of 84.8 mg TOS/kg bw per day results in an MOE of at least 1000.</p> <p><b>The Committee established an ADI “not specified”<sup>a</sup> for the mixed β-glucanase, cellulase and xylanase enzyme preparation from <i>R. emersonii</i> when used in the applications specified and in accordance with good manufacturing practice.</b></p>
Mixed β-glucanase and xylanase from <i>Disporotrichum dimorphosporum</i>	N, T <sup>b</sup>	<p>No treatment-related adverse effects were seen at the highest dose tested (199 mg TOS/kg bw per day) in a 13-week study of oral toxicity in rats. A comparison of the dietary exposure estimate of 0.7 mg TOS/kg bw per day (for a 60 kg individual) with the highest dose tested of 199 mg TOS/kg bw per day gives an MOE of at least 280.</p> <p><b>The Committee established an ADI “not specified”<sup>a</sup> for the mixed β-glucanase and xylanase enzyme preparation from <i>D. dimorphosporum</i> when used in the applications specified and in accordance with good manufacturing practice.</b></p>
Polyvinyl alcohol (PVA) – polyethylene glycol (PEG) graft copolymer	N	<p>On the basis of the available studies, in which no treatment-related effects were seen at the highest doses tested, the Committee considered PVA-PEG graft copolymer to be a substance of low oral toxicity in rats, rabbits and dogs. The bioavailability of PVA-PEG graft copolymer in rats is negligible, and PVA-PEG graft copolymer is unlikely to be genotoxic and is not associated with reproductive or developmental toxicity. Therefore, the Committee concluded that calculation of an MOE for PVA-PEG graft copolymer would not be meaningful. Based on these data, the Committee would normally establish an ADI “not specified”. However, the Committee decided not to establish an ADI “not specified” for PVA-PEG graft copolymer in view of the impurities present, some of which may also be impurities in other food additives. The Committee had concerns that establishing an ADI “not specified” could lead to additional uses beyond those considered at the current meeting and consequently could increase exposure to the impurities.</p>

(continued)

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations and dietary exposure information
		<p>The use of PVA-PEG graft copolymer that complies with the proposed specifications could lead to a dietary exposure to ethylene glycol and diethylene glycol from both food supplements and pharmaceutical products up to 0.016 mg/kg bw per day for children (high consumers). This is 3% of the tolerable daily intake (TDI) of 0.5 mg/kg bw per day derived by the Scientific Committee on Food of the European Union, and therefore the exposure to ethylene glycol and diethylene glycol from the use of PVA-PEG graft copolymer that complies with the specifications established at the current meeting is not of safety concern when the food additive is used in the applications specified.</p> <p>The use of PVA-PEG graft copolymer that complies with the proposed specifications could lead to a dietary exposure to vinyl acetate from both food supplements and pharmaceutical products up to 0.0008 mg/kg bw per day for children. This dietary exposure estimate is at least 62 500 times lower than the dose levels at which increases in tumour incidence are observed in oral studies of long-term toxicity and carcinogenicity in rats and mice. Therefore, the dietary exposure to vinyl acetate from the use of PVA-PEG graft copolymer that complies with the specifications established at the current meeting is not of safety concern when the food additive is used in the applications specified.</p> <p><b>The Committee concluded that the use of PVA-PEG graft copolymer that complies with the specifications established at the current meeting is not of safety concern when the food additive is used as a glazing agent (aqueous film coating), stabilizer and binder for tablets in the preparation and formulation of food supplements and in accordance with good manufacturing practice.</b></p>

N: new specifications; NA: not applicable (dietary exposure assessment only); T: tentative specifications

<sup>a</sup> ADI “not specified” is used to refer to a food substance of very low toxicity that, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary exposure to the substance arising from its use at the levels necessary to achieve the desired effects and from its acceptable background levels 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 expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice – i.e. it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal food of inferior quality or adulterated food, and it should not create a nutritional imbalance.

<sup>b</sup> Information is required in order for the tentative specifications to be revised (see section 5).

## Contaminants

### Non-dioxin-like polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) are lipophilic compounds that accumulate in the tissues of living organisms and are taken up by humans primarily through the consumption of food, with foods of animal origin being the primary source of human exposure. There are 209 possible PCB congeners, of which 197 are non-dioxin-like PCBs (NDL-PCBs). PCBs were reviewed at the thirty-fifth meeting of the Committee, and dioxin-like PCBs (DL-PCBs) were reviewed by

the Committee at its fifty-seventh meeting. NDL-PCBs have not previously been specifically evaluated by the Committee.

PCBs exhibit different toxicological effects depending on the site of chlorine substitution on the phenyl rings. Congeners with two or more chlorine atoms in the *ortho* position are generally considered to be NDL-PCBs, with a different toxicological spectrum from DL-PCBs, which have a high affinity for the aryl hydrocarbon receptor (AhR) and thus exhibit dioxin-like activity. International bodies have identified seven PCBs that can be used to characterize the presence of PCB contamination. Six of these are NDL-PCBs (PCB 28, PCB 52, PCB 101, PCB 138, PCB 153 and PCB 180), and one is a DL-PCB (PCB 118). The six NDL-PCBs are often called “indicator PCBs.” For this evaluation, the Committee decided to focus on the six indicator PCBs, as there were sufficient data (toxicity, biomonitoring, occurrence and dietary exposure) available for review. Other NDL-PCBs were also considered to identify any for which adequate data were available to conduct a risk characterization, as was found for PCB 128.

National estimates of dietary exposure to the sum of the six indicator PCBs ranged, for mean exposure, from <1 to 82 ng/kg bw per day and, for high-percentile exposure, from <1 to 163 ng/kg bw per day. International estimates based on Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) consumption cluster diets are in the same range. For the sum of the six indicator PCBs, the contribution of each of the individual congeners differs between countries and population groups. However, for both dietary exposure and body burden estimates (which also take into consideration kinetics and half-lives), the main contributor is PCB 153, followed by PCB 138, PCB 180, then PCB 101 and PCB 28, with the lowest contribution from PCB 52.

The Committee concluded that none of the available studies on the six indicator PCBs (PCB 28, PCB 52, PCB 101, PCB 138, PCB 153 and PCB 180) and PCB 128 was suitable for derivation of health-based guidance values or for assessment of the relative potency of the NDL-PCBs compared with a reference compound. Therefore, a comparative approach using the minimal effect doses was developed in order to estimate MOEs to provide guidance on human health risk. Based on the available toxicological data on individual congeners, minimal changes in liver and thyroid histopathology were evident from the lowest doses tested of 2.8–7 µg/kg bw per day in the 90-day studies (PCB 28, PCB 128, PCB 153) and 3 mg/kg bw total dose in the 28-day studies (PCB 52, PCB 180) and were similar across the short-term and long-term studies of toxicity. The Committee decided to take the lower end of the range of test doses used for each congener at which these minimal changes occurred as a conservative point of departure for estimating MOEs on the basis of both external dose and internal dose (based on amounts present in fat).

Owing to the long half-lives of NDL-PCBs and to eliminate interspecies differences in toxicokinetics, the Committee considered it appropriate to estimate body burdens rather than using external dose (dietary exposure) for the risk characterization. From human biomonitoring studies, the Committee derived equivalent body burdens based on the reported range of NDL-PCB concentrations in human milk for each congener.

Comparison of the human body burden estimates (derived from human milk concentrations) with the body burden estimates from animal studies derived as points of departure for each congener resulted in MOEs for adults ranging from 4.5 to 5000.

MOEs for breastfed infants, which may have a body burden up to 2-fold higher than that of adults, would be approximately half of the adult values. The MOEs for children would be expected to be intermediate between those for adults and those for breastfed infants, owing to the initial contribution from breastfeeding and the subsequent lower dietary contribution compared with human milk.

Because the MOEs are based on minimal effect doses, they were considered to give some assurance that dietary exposures to NDL-PCBs are unlikely to be of health concern for adults and children, based on the available data. For breastfed infants, the MOEs would be expected to be lower. However, based on present knowledge, the benefits of breastfeeding are considered to outweigh the possible disadvantages that may be associated with the presence of NDL-PCBs in breast milk.

The Committee recognized that there are similarities in some of the reported effects for NDL-PCBs and therefore that risk estimates for combined exposure are desirable. The Committee concluded that this cannot be done on the basis of currently available data. The Committee also noted that the end-point selected for derivation of the MOEs was particularly conservative, as it was not of clear toxicological significance, it was a minimal change, and the lowest doses at which it was seen were used for the point of departure, combined with upper-bound estimates of body burden.

### ***Pyrrolizidine alkaloids***

Pyrrolizidine alkaloids (PAs) are toxins produced by an estimated 6000 plant species. More than 600 different PAs, mainly 1,2-unsaturated PAs, and their associated nitrogen oxides (*N*-oxides) are known, and new PAs continue to be identified on a regular basis in both new and previously studied plant species. PAs have not been previously evaluated by the Committee, but they have been evaluated by a WHO Task Group on Environmental Health Criteria for Pyrrolizidine Alkaloids (coordinated by the International Programme on

Chemical Safety [IPCS]) and by the International Agency for Research on Cancer (IARC).

A systematic review approach was used for gathering the data. The year 1988, when IPCS evaluated PAs, was taken as the cut-off point for literature selection. As the European Food Safety Authority (EFSA) evaluated PAs in 2011, it was decided to report EFSA's conclusions on non-critical studies in the JECFA evaluation and reassess only those studies considered critical for the risk assessment.

A systematic review protocol was developed with six defined research questions that were used for the literature search in selected databases. More than 10 000 references were retrieved through title/abstract selection of the systematic review process. Because of time constraints, subsequent stages of the systematic review were not performed according to the protocol, and full text selection was done using the critical appraisal technique regularly used in the preparation of JECFA monographs.

Because of the narrow time frame between the work on the selection of references and the JECFA meeting, the evaluation could not be completed at the meeting, but the Committee considered the information sufficient to determine an approach for the evaluation. The Committee agreed that only preliminary findings would be reported in the current meeting report and that the full evaluation would be published subsequently. The results included in this meeting report are therefore preliminary and will need later confirmation when all studies have been quality assessed and described in detail.

The Committee concluded that exposure to PAs has been associated with a wide range of effects, with rats being the most sensitive species. Laboratory studies have identified the liver as the most sensitive organ in rats. Humans are also sensitive to PAs.

Two long-term carcinogenicity studies were available, which provided information on the carcinogenic effects – considered to be the most critical end-point following long-term exposure – of certain PAs in experimental animals. The Committee considered that the genotoxic mode of action did not allow derivation of a health-based guidance value and decided to use the  $BMDL_{10}$  of 182  $\mu\text{g}/\text{kg}$  bw per day for liver haemangiosarcoma in female rats from the study on riddelliine as the point of departure for use in an MOE approach.

The Committee calculated MOEs between the  $BMDL_{10}$  of 182  $\mu\text{g}/\text{kg}$  bw per day and mean and high-percentile (90th, 95th or 97.5th, depending on the study) chronic exposure estimates for children and adults from consumption of honey and tea, separately. Based on limited occurrence data, the Committee noted that the calculated MOEs for honey and tea for high consumers (and for average tea consumption by children) indicated a concern. It should be noted that PAs measured in these commodities might not be representative for all food

groups and all regions; however, the calculation provided a conservative risk estimate, as it was compared with the  $BMDL_{10}$  for the potent PA riddelliine.

Based on average exposure, the MOEs were of less concern (except for the above-mentioned average exposure of children from tea consumption). However, the Committee considered it of concern that exposure to a single food product could result in such low MOEs. The Committee noted that exposure to PAs resulted from other food items as well, and animal products such as milk might contribute to the total exposure as a result of the presence of PAs in feed. A first indication of total exposure could be taken from a small duplicate-diet study, from which an MOE of 140 000 could be derived, but it was unclear how representative these data were.

# Annex 3

## Meeting agenda



Food and Agriculture  
Organization of the  
United Nations



World Health  
Organization

80th JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES (JECFA)  
FAO Headquarters, Rome, 16–25 June 2015

**Opening:**  
Philippine Room (C 277), 16 June 2015, 9.30h

### Draft Agenda

1. Opening
2. Election of Chairperson and Vice-Chairperson, appointment of Rapporteurs
3. Adoption of Agenda
4. Declarations of Interests (information by the Secretariat on any declared interests and discussion)
5. Matters of interest arising from previous Sessions of the Codex Committee on Food Additives (CCFA) and the Codex Committee on Contaminants in Foods (CCCF)
  - a. Report from CCFA - questions for action
  - b. Report from CCCF - questions for action
6. Critical issues and questions from Working Papers (first brief round of discussion on all subjects to inform the full committee)
7. Evaluations

#### Food Additives

7.1. Toxicological Evaluation, Exposure Assessment, and Establishment of Specifications:

- Benzoates
- Beta-glucanase, cellulase and xylanase from *Talaromyces emersonii*
- Beta-glucanase and xylanase from *Disporotrichum dimorphosporum*

- Lipase from *Fusarium heterosporum* expressed in *Hansenula polymorpha*
- Magnesium stearate (INS 470(iii))
- Maltotetrahydrolase from *Pseudomonas saccharophila* expressed in *Bacillus licheniformis*
- Monk fruit extract/Lo han guo (LHG) *Siraitia grosvenorii* Swingle
- Polyvinyl alcohol (PVA)-polyethylene glycol (PEG) graft copolymer

7.2. Food additives for revision of specifications only:

- Advantame
- Annatto extracts (solvent-extracted bixin and solvent-extracted norbixin)
- Aspartame (INS 951)
- Food additives containing aluminium and/or silicon:
  - o Aluminium silicate (INS 559)
  - o Calcium aluminium silicate (INS 556)
  - o Calcium silicate (INS 552)
  - o Silicon dioxide, amorphous (INS 551)
  - o Sodium aluminosilicate (INS 554)
- Glycerol ester of gum rosin

Contaminants

7.3. Non-dioxin-like PCBs

7.4. Pyrrolizidine alkaloids

8. Other matters to be considered (general considerations):

a. For information:

- i. Update on the draft Specification Monographs for sixteen Modified Starches
- ii. Update on new FAO JECFA databases on additives and flavourings
- iii. Update on FAO/WHO CIFOCCOs database

b. For discussion:

- i. Revised JECFA guidance to monographers for additives and for contaminants
- i. HPLC method in the adopted specifications of Cassia Gum (INS 427)

9. Other matters as may be brought forth by the Committee during discussions at the meeting.

10. Adoption of the report.







## SELECTED WHO PUBLICATIONS OF RELATED INTEREST

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### **Evaluation of Certain Food Additives**

Seventy-ninth Report of the Joint FAO/WHO Expert Committee on Food Additives  
WHO Technical Report Series, No. 990, 2015 (124 pages)

### **Safety Evaluation of Certain Food Additives**

Seventy-ninth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)  
WHO Food Additives Series, No. 70, 2015 (369 pages)

### **Evaluation of Certain Veterinary Drug Residues in Food**

Seventy-eighth Report of the Joint FAO/WHO Expert Committee on Food Additives  
WHO Technical Report Series, No. 988, 2014 (127 pages)

### **Toxicological Evaluation of Certain Veterinary Drug Residues in Food**

Seventy-eighth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)  
WHO Food Additives Series, No. 69, 2014 (241 pages)

### **Evaluation of Certain Food Additives and Contaminants**

Seventy-seventh Report of the Joint FAO/WHO Expert Committee on Food Additives  
WHO Technical Report Series, No. 983, 2013 (75 pages)

### **Safety Evaluation of Certain Food Additives and Contaminants**

Seventy-seventh Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)  
WHO Food Additives Series, No. 68, 2013 (335 pages)

### **Evaluation of Certain Food Additives**

Seventy-sixth Report of the Joint FAO/WHO Expert Committee on Food Additives  
WHO Technical Report Series, No. 974, 2012 (190 pages)

### **Safety Evaluation of Certain Food Additives**

Seventy-sixth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)  
WHO Food Additives Series, No. 67, 2012 (335 pages)

### **Evaluation of Certain Veterinary Drug Residues in Food**

Seventy-fifth Report of the Joint FAO/WHO Expert Committee on Food Additives  
WHO Technical Report Series, No. 969, 2012 (108 pages)

### **Toxicological Evaluation of Certain Veterinary Drug Residues in Food**

Seventy-fifth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)  
WHO Food Additives Series, No. 66, 2012 (183 pages)

## Evaluation of certain food additives and contaminants

This report represents the conclusions of a Joint FAO/WHO Expert Committee convened to evaluate the safety of various food additives and contaminants and to prepare specifications for identity and purity.

The first part of the report contains a brief description of general considerations addressed at the meeting, including updates on matters of interest to the work of the Committee. A summary follows of the Committee's evaluations of technical, toxicological and/or dietary exposure data for seven food additives (benzoates; lipase from *Fusarium heterosporum* expressed in *Ogataea polymorpha*; magnesium stearate; maltotetrahydrolase from *Pseudomonas stutzeri* expressed in *Bacillus licheniformis*; mixed  $\beta$ -glucanase, cellulase and xylanase from *Rasamsonia emersonii*; mixed  $\beta$ -glucanase and xylanase from *Disporotrichum dimorphosporum*; polyvinyl alcohol (PVA) – polyethylene glycol (PEG) graft copolymer) and two groups of contaminants (non-dioxin-like polychlorinated biphenyls and pyrrolizidine alkaloids).

Specifications for the following food additives were revised or withdrawn: advantame; annatto extracts (solvent-extracted bixin and solvent-extracted norbixin); food additives containing aluminium and/or silicon (aluminium silicate; calcium aluminium silicate; calcium silicate; silicon dioxide, amorphous; sodium aluminium silicate); and glycerol ester of gum rosin.

Annexed to the report are tables or text summarizing the toxicological and dietary exposure information and information on specifications as well as the Committee's recommendations on the food additives and contaminants considered at this meeting.

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