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966

EVALUATION OF CERTAIN FOOD ADDITIVES AND CONTAMINANTS

Seventy-fourth report of the
Joint FAO/WHO Expert Committee on
Food Additives

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Seventy-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives

Rome, 14–23 June 2011

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

Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 65 in press.

Specifications are issued separately by FAO under the title:

Compendium of food additive specifications. FAO JECFA Monographs 11, 2011.

Dedication

Dr Ian C. Munro

It was with a sense of great loss and sadness that the Committee noted the passing of Dr Ian C. Munro, who had served the Committee for over 30 years. Ian first attended JECFA at the twenty-fourth meeting in 1980. At the forty-fourth meeting in 1995, he introduced a decision-tree for the safety evaluation of flavouring agents, based on his pioneering development of the threshold of toxicological concern. The decision-tree was applied at the following meeting and has since become the backbone for the safety assessment of flavouring agents by the Committee. To date, over 2000 flavouring agents have been evaluated, a task that would have been impossible without Ian's innovative approach and his deep knowledge and understanding of the principles of risk assessment. His work for the Committee combined dedication and drive with a sparkling sense of humour, all of which will be greatly missed.

In recognition of his services and contributions, the Committee has dedicated this report to the memory of Dr Ian C. Munro, a true friend and colleague.

1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) met in Rome from 14 to 23 June 2011. The meeting was opened by Mr Samuel Jutzi, Officer in Charge of the Nutrition and Consumer Protection Division of the Agriculture and Consumer Protection Department of the Food and Agriculture Organization of the United Nations (FAO), on behalf of the Directors-General of the World Health Organization (WHO) and FAO. Mr Jutzi provided information to the Committee on the reform process in FAO, in particular in relation to the recent adoption of a corporate results-based approach to programme planning and measuring achievements to better meet the demands of countries for improved efficiency. The current medium-term plan of programmes and resources has been aligned with defined strategic objectives and outcomes. The provision of scientific advice on food safety is part of the strategic objective named "improved quality and safety of food at all stages of the food chain". When it comes to food security and food safety, this strategic approach provides for new opportunities for cooperation between units in FAO and with WHO and other United Nations agencies involved in the farm-to-table food production to consumption continuum as well as in food safety control and standard setting. Mr Jutzi emphasized that the two organizations greatly appreciate the important contribution by experts in providing their expertise to the work of the Committee on provision of scientific advice on human health risks of chemicals in food. He expressed his sincere appreciation to the experts for taking time from their very busy daily work schedules to prepare for and participate in this meeting. He also noted the increasing need by countries and the Codex Alimentarius Commission to have access to objective advice on food safety matters and stated that this remains a high priority for the organizations.

1.1 Declarations of interests

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

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 73 previous meetings of the Committee (Annex 1). The present meeting was convened on the basis of a recommendation made at the seventy-third meeting (Annex 1, reference *202*).

The tasks before the Committee were:

— to elaborate further principles for evaluating the safety of food additives and contaminants in food (section 2);

— to undertake toxicological evaluations of certain food additives (section 3 and Annex 2);

— to review and prepare specifications for certain food additives (section 3 and Annex 2);

— to undertake toxicological evaluations of certain contaminants in food (section 4 and Annex 2).

2.1 Modification of the agenda

The following items were added to the agenda:

— revision of the tentative specifications for sucrose monoesters of lauric, palmitic or stearic acid, as additional information had been received;

— revision of test method for colouring matters for the specifications for aluminium lakes of colouring matters;

— withdrawal of the existing specifications for potassium bromate;

— consideration of deletion of a synonym in the specifications for sodium carboxymethyl cellulose;

— revision of specifications for octenyl succinic acid (OSA) modified starch in the specifications monograph for modified starches;

— request from the Codex Committee on Food Additives (CCFA) to consider modification of the names of certain caramel colours evaluated by the Committee.

2.2 Report from the Forty-third Session of the Codex Committee on Food Additives (CCFA) and the Fifth Session of the Codex Committee on Contaminants in Foods (CCCF)

The Codex Secretariat informed the Committee about the principal achievements and outputs of the Forty-third Session of CCFA and the Fifth Session of CCCF.

The Forty-third Session of CCFA had forwarded 193 food additive provisions of the Codex General Standard for Food Additives (GSFA) (*2*) to the Thirty-fourth Session of the Codex Alimentarius Commission for adoption, with amendments to the section on carry-over of food additives and to the descriptors of food categories related to confectionery products of the GSFA. In addition, CCFA recommended the adoption of 14 revised specifications for the identity and purity of food additives and 167 new and revised specifications for flavourings, prepared by the seventy-third meeting of JECFA, and amendments to the International Numbering System for Food Additives (INS) (*3*), including revised names for caramels.

CCFA agreed on a revised priority list of compounds for evaluation (or re-evaluation) by JECFA and to develop criteria to prioritize food additives for re-evaluation, starting with the prioritization of the 107 colour additives evaluated by JECFA.

The Fifth Session of CCCF considered the conclusions of the assessments of the seventy-second and seventy-third meetings of the Committee and agreed to initiate new work on maximum limits (MLs) for arsenic in rice and for lead in various foods.

CCCF agreed on a priority list of substances for evaluation by JECFA.

To a question on why a request had been made to also consider animal feed in the evaluation of fumonisins, the Codex Secretariat clarified that the Commission took risk management measures for animal feed as far as it had an impact on human health. The meeting was also informed of the re-establishment of an ad hoc Intergovernmental Task Force on Animal Feeding.

2.3 Principles governing the toxicological evaluation of compounds on the agenda

In making recommendations on the safety of food additives and contaminants, the Committee took into consideration the principles established and contained in the new publication, Environmental Health Criteria, No. 240, *Principles and methods for the risk assessment of chemicals in food*, published in 2009 (*4*).

2.3.1 *The use of P-values as an exclusion criterion for the selection of BMDLs derived by benchmark dose modelling of animal data*

In addition to providing parameter estimates, the United States Environmental Protection Agency's BMDS modelling program used by the Committee to generate benchmark dose (BMD) and lower limit on the benchmark dose (BMDL) estimates at the present meeting provides several quantitative "goodness-of-fit" measures that can be used to gauge how well a particular model describes a specific data set. One of these measures, the *P*-value, is often used to exclude certain models. For example, the guidance provided with the BMDS suggests a cut-off *P*-value of 0.05. JECFA used a value of 0.1 as a criterion in the evaluation of furan at the seventy-second meeting (Annex 1, reference *199*).

The problem that arises with the use of a fixed value as an exclusion criterion is that the size of the *P*-value is partly attributable to the data set itself, rather than to any particular model. For example, small data sets that are relatively easy to fit tend to generate *P*-values close to 1 (which indicates a perfect fit), regardless of which model is used. In contrast, data sets with many doses or non-monotonic characteristics may generate low *P*-values, regardless of which model is used.

The *P*-value can be used to compare models and to exclude models based on how well they fit relative to each other, rather than using a fixed cut-off value. With this approach, the exclusion criterion is relative rather than absolute, and therefore the role of the data set in determining model exclusion is minimized. The BMD analyses conducted for the evaluation of fumonisins at the present meeting, in which models with *P*-values differing by 3-fold or more relative to the highest *P*-value were excluded (see section 4.2), provide an example of the use of *P*-values as a relative exclusion criterion.

2.4 Food additive specifications

2.4.1 *Request from CCFA to modify names of certain food additives: caramel colours*

The Committee noted the request from the Forty-third Session of CCFA (*5*) to consider modifying the names of caramel colours to correspond to those included in the INS (*3*).

The Committee decided to change the names of two caramel colours in order to harmonize the names in the specifications with those included in Codex texts:

INS	*Class*	*Current name*	*Modified name*
150a	I	Plain caramel, caustic caramel	Plain caramel
150b	II	Caustic sulfite caramel	Sulfite caramel

The Committee noted that there was no need to modify the names of the caramel colours in Class III (INS 150c) or in Class IV (INS 150d), as these names are the same as those in Codex texts.

2.4.2 *Methods for analysis of propylene chlorohydrins*

While revising the test method for the determination of propylene chlorohydrins in the specifications monograph for hydroxypropylmethyl cellulose, the Committee noted that the specification limit for this impurity was also included in three other monographs (hydroxypropyl cellulose, hydroxypropyl starch and hydroxypropyl distarch phosphate). The Committee noted that these monographs include three different analytical techniques for the determination of propylene chlorohydrins.

The Committee recommends that the gas chromatography–mass spectrometric (GC-MS) method introduced at the present meeting into the specifications monograph for hydroxypropylmethyl cellulose be validated for use in the specifications monographs for the three other substances.

2.4.3 *Withdrawal of specifications*

2.4.3.1 *Potassium bromate*

The Committee noted that although the specifications for potassium bromate prepared at the forty-fourth meeting and published in FAO Food and Nutrition Paper No. 52, Addendum 3, were not republished and included in the Combined Compendium of Food Additives Specifications (FAO JECFA Monographs No. 1), they were never formally withdrawn.

As bromates are genotoxic carcinogens, the Committee reiterated the general principle that bromates should not be present in food as consumed and withdrew the specifications for potassium bromate.

2.5 General comment about data submissions

The Committee would like to emphasize that interested parties requesting an evaluation by the Committee need to be committed to providing all necessary data in a timely manner, as specified in the call for data. It is also important that they are prepared to respond to questions and to requests for clarifications or to provide additional data in a timely manner, both before and during the meeting. This refers to requests for safety assessments as well as to requests for the preparation or revision of specifications.

The Committee acknowledges that this requires a significant commitment and full cooperation on the part of those providing data. Such cooperation is, however, imperative to allow for complete evaluations without wasting the time and resources of the Committee.

3. Specific food additives

The Committee evaluated four food additives for the first time and re-evaluated a number of others. Information on the safety evaluations and on specifications is summarized in Annex 2. Details on further toxicological studies and other information required for certain substances are given in Annex 3.

3.1 Safety evaluations

3.1.1 *Aluminium-containing food additives*

Explanation

Aluminium can occur in food as a result of its natural occurrence in the environment, contamination from various sources, leaching from food contact materials and the use of aluminium-containing food additives.

Various aluminium compounds were evaluated by the Committee at its thirteenth, twenty-first, twenty-sixth, twenty-ninth, thirtieth, thirty-third and sixty-seventh meetings (Annex 1, references *20*, *44*, *59*, *70*, *73*, *83* and *184*). At its thirteenth meeting, the Committee established an acceptable daily intake (ADI) "not specified" for sodium aluminosilicate and aluminium calcium silicate (Annex 1, reference *20*). At its twenty-sixth meeting, the Committee established a temporary ADI of 0–0.6 mg/kg body weight (bw) for sodium aluminium phosphate (Annex 1, reference *59*). At its thirtieth meeting, the Committee noted concerns about a lack of precise information on the aluminium content of the diet and a need for additional safety data. The Committee extended the temporary ADI of 0–0.6 mg/kg bw expressed as aluminium to all aluminium salts added to food and recommended that aluminium in all its forms should be reviewed at a future meeting (Annex 1, reference *73*).

The Committee evaluated aluminium as a contaminant at its thirty-third meeting, placing emphasis on estimates of consumer exposure, absorption and distribution of dietary aluminium and possible neurotoxicity, particularly the relationship between exposure to aluminium and Alzheimer disease. The Committee established a provisional tolerable weekly intake (PTWI) of 0–7.0 mg/kg bw for aluminium, and a consolidated monograph was produced

(Annex 1, reference *84*). The Committee concluded that there was no need to set a separate ADI for the food additives sodium aluminium phosphate basic or sodium aluminium phosphate acidic, because the PTWI included aluminium exposure arising from food additive uses.

At its sixty-seventh meeting, the Committee re-evaluated aluminium used in food additives and from other sources and concluded that aluminium compounds have the potential to affect the reproductive system and developing nervous system at doses lower than those used in establishing the previous PTWI (Annex 1, reference *186*). The Committee noted that the lowest lowest-observed-effect levels (LOELs) for aluminium in a range of different dietary studies in mice, rats and dogs were in the region of 50–75 mg/kg bw per day. The Committee selected the lower end of this range of LOELs (50 mg/kg bw per day) and established a PTWI of 1 mg/kg bw by applying an uncertainty factor of 100 to allow for interspecies and intraspecies differences and an additional uncertainty factor of 3 for deficiencies in the database, notably the absence of no-observed-effect levels (NOELs) in the majority of the studies evaluated and the absence of long-term studies on the relevant toxicological end-points. The PTWI applied to all aluminium compounds in food, including food additives. The previously established ADIs and PTWI for aluminium compounds were withdrawn. The Committee noted that the PTWI was likely to be exceeded to a large extent by some population groups, particularly children, who regularly consume foods that include aluminium-containing food additives. The Committee also noted that dietary exposure to aluminium is expected to be very high for infants fed on soya-based formula. The Committee noted a need for:

- further data on the bioavailability of different aluminium-containing food additives;
- an appropriate study of developmental toxicity and a multigeneration study incorporating neurobehavioural end-points using relevant aluminium compounds;
- studies to identify the forms of aluminium present in soya-based formula and their bioavailability.

Aluminium-containing food additives were re-evaluated by the Committee at its present meeting, as requested by CCFA. The Committee was asked to consider all data necessary for safety evaluation (bioavailability, developmental toxicity and multigeneration reproductive toxicity) and data on actual use levels in food. In addition, the Committee was asked to consider all data necessary for the assessment of safety, dietary exposure and specifications for aluminium lactate and potassium aluminium silicate, which had not been evaluated previously by the Committee for use as food additives. Potassium

aluminium silicate is mined from natural sources and then further purified for use as a carrier substrate for potassium aluminium silicate–based pearlescent pigments. Potassium aluminium silicate–based pearlescent pigments are produced by reaction of potassium aluminium silicate with soluble salts of titanium and/or iron followed by calcination at high temperatures. The pigments can be produced with a variety of different pearlescent colour effects depending upon particle size and the combination of titanium dioxide and/or iron oxide deposited on the potassium aluminium silicate.

The Committee received submissions from a number of sponsors, including unpublished studies of bioavailability and toxicity and a review of the scientific literature. Additional information was identified from the scientific literature. No information was received on the forms of aluminium present in soya-based infant formula.

Toxicological data

As recommended by the Committee at its sixty-seventh meeting, new studies had been conducted on the bioavailability of aluminium compounds. The new data indicated that absorption of aluminium following the ingestion of various aluminium compounds by rats is generally in the region of 0.01–0.3% and support the assumption that the more water-soluble aluminium compounds are generally more bioavailable. As a result of limitations in the sensitivity of the analytical methods, inter-animal variation and methodological differences between studies, including the administered doses, it is not possible to draw firm conclusions on quantitative differences in absorption between different compounds. There are indications that there are sex differences in absorption in rats and that the proportion of the dose absorbed is lower following repeated administration than following single administration. The reported absorptions of the food additives for which data were available (sodium aluminium phosphate acidic, sodium aluminium phosphate basic, sodium aluminosilicate, aluminium sulfate, FD&C aluminium lake, aluminium metal, aluminium ammonium sulfate) are within the overall range of 0.01–0.3% in rats. A possible exception relates to potassium aluminium silicate–based pearlescent pigments. These products are marketed in particulate form. The solubility of the particulates is very low, and therefore it is likely that the bioavailability is lower than for other aluminium-containing food additives. However, direct data to support a conclusion that aluminium is appreciably less available from these pigments than from other aluminium compounds were not available.

In studies reviewed previously by the Committee, absorption of aluminium in human volunteers was within the same range as that in rats, with some indication of increased absorption in the elderly. The absorption can be modified

by substances in foods that bind to the aluminium ion, such as citrate, which increases absorption, and phosphate, which forms an insoluble aluminium salt, thereby decreasing absorption. The newly available data indicate that absorption in humans is likely to vary widely, but did not support an estimation of bioavailability.

New studies in rats have confirmed that absorbed aluminium is able to cross the placental barrier into the fetus and then into the fetal brain and that it is also transferred to the offspring via lactation. The new studies have also confirmed that administration of a number of aluminium salts to rats can result in increased concentrations of aluminium in bone, kidney and spinal cord. About 90% of Al^{3+} in plasma is bound to transferrin, and about 10% to citrate. Cellular uptake is thought to occur from the aluminium bound to transferrin by transferrin receptor–mediated endocytosis.

No new data on excretion were identified. Studies reviewed previously by the Committee have shown that urine is the primary route of excretion of absorbed aluminium in experimental animals and in humans. Initial half-lives of 2–5 hours have been reported in rats, mice, rabbits and dogs after intravenous administration and less than 1 day in humans after intravenous administration. In different studies and species, multiple half-lives have been reported, arising from slower rates of elimination from different tissues.

Based on the available data relating to the absorption, distribution and elimination of aluminium from a variety of different aluminium compounds, the Committee concluded that there was no basis for deriving a chemical-specific adjustment factor for either interspecies or intraspecies differences in toxicokinetics.

As recommended by the Committee at its sixty-seventh meeting, new multigeneration reproductive and developmental toxicity studies incorporating neurobehavioural end-points had been conducted.

The multigeneration reproductive studies conducted with aluminium sulfate and aluminium ammonium sulfate administered to rats in the drinking-water did not provide evidence of reproductive toxicity. The major developmental effects observed in both studies were delayed maturation of the female offspring, decreased body weight gain and changes in some organ weights. These effects are likely to have been related to the reported decrease in maternal fluid and feed consumption. Thus, it is not possible to attribute the findings to a direct effect of the aluminium. No effects on motor activity or learning ability were observed in these studies.

The available developmental toxicity studies include two published studies involving dosing of aluminium chloride by oral gavage to pregnant rats. These studies provided evidence of fetotoxicity, but it was unclear if the findings

were secondary to maternal toxicity. There were no effects on pregnancy outcome in a developmental study of aluminium chloride basic.

Cognitive deficits were observed in a number of new studies of neurotoxicity and neurobehavioural end-points. Most of these studies have limitations for use in risk assessment, such as administration of only one high dose level, failure to consider aluminium content in the diet, lack of assessment of other forms of toxicity and assessment of only a limited number of outcomes. The lowest aluminium dose linked with cognitive effects was 0.5 mg/kg bw per day administered to rats as aluminium chloride in the drinking-water, which was reported to be associated with impaired memory in old rats. In this study, the rats were given a restricted amount of feed twice weekly in order to reduce the rats' weight to approximately 85% of the free-feeding weight and hence prolong their lifespan. Typically, they ate the feed in the first 2–3 days and had a day or more with no feed. Whereas impaired cognitive function in old age is a potentially relevant observation, the impact of the restricted feeding regimen used in this study is unknown, and impaired cognitive function has been observed in other studies only at much higher levels of exposure, albeit in younger animals. The Committee therefore concluded that the results of this study require independent verification and were not suitable for use in the risk assessment.

In a developmental and chronic neurotoxicity study of aluminium citrate administered to rats in drinking-water, the major treatment-related effects were renal damage (hydronephrosis, urethral dilatation, obstruction and/or presence of calculi) and reduced grip strength, but not cognitive impairment, in the pups. Renal damage was not observed in a control group of rats given sodium citrate at the molar equivalent of the high-dose aluminium citrate, demonstrating that the effect was not due to the citrate ion. Dosing with both aluminium citrate and sodium citrate resulted in a significant increase in fluid consumption compared with control animals. The no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) for these effects were at target aluminium doses of 30 and 100 mg/kg bw per day. However, because the aluminium citrate was administered in the drinking-water, the actual dose was influenced by the water consumption, which varied in the different stages of the study. Mean doses at the NOAEL were 10–14% below target during gestation, up to 50% above target during lactation, up to about 30% above target in the weaned pups for the first few weeks, but then 15–45% of target for the remainder of the study. At the LOAEL, the mean dosage level was approximately at target during gestation, up to 90% above target during lactation and the first few weeks post-weaning, and then 25–50% of target for the remainder of the study. Hence, if the effects in the pups were mediated in utero, the NOAEL is slightly overestimated; conversely, however, if the effects were mediated during lactation

or the first few weeks after weaning, the NOAEL is underestimated. As the effect on grip strength was more pronounced in younger animals, exposure in utero and/or during lactation is likely to be more important than exposure during the later stages, when exposure was decreased due to decreased fluid consumption. The Committee concluded that, taking into account the greater bioavailability of aluminium from aluminium citrate than from other aluminium compounds, it was appropriate to assume that the NOAEL was 30 mg/kg bw per day. In view of the uncertainty regarding the doses at different times of this study as a result of changes in water consumption, the Committee decided not to model the dose–response data.

The Committee received a submission specifically on potassium aluminium silicate–based pearlescent pigments. No effects were observed in subchronic or chronic toxicity studies at doses of the test material up to 2500 mg/kg bw per day, equivalent to 360 mg/kg bw per day as aluminium, but no studies were available regarding reproductive or neurobehavioural effects.

Most epidemiological studies reviewed addressed the potential neurotoxicity of aluminium in drinking-water or antacids, by means of different designs: experimental, prospective cohort or case–control studies or ecological studies. The results of these studies were controversial; some of the drinking-water studies showed an association of aluminium with dementia or Alzheimer disease, whereas others reported an absence of neuropsychological effects measured in several ways. None of these studies took into account the ingestion of aluminium in food. The coincidental observation of neuropathological features of Alzheimer disease and aluminium in brain reported in some cases does not demonstrate a causal role of aluminium in Alzheimer disease. Occupational exposure to aluminium does not seem to have an impact on cognitive performance, motor performance or adverse reproductive outcomes in exposed workers. Although recent studies do not definitively rule out a positive association between aluminium in drinking-water and Alzheimer disease, the information available remains inconsistent and does not support a causal association. Neonates who were exposed to aluminium from solutions for parenteral nutrition had reduced lumbar spine and hip bone mass in adolescence. However, in elderly people, the aluminium content in bones was not associated with increased risk of hip fractures. There was no information from the epidemiological literature about the potential effects of oral exposure to aluminium in food. Given these limitations, no pivotal epidemiological studies are available for risk assessment.

Assessment of dietary exposure

Owing to their multiple functions, aluminium-containing food additives are permitted for use in a large variety of foods. At its present meeting, the

Committee was asked to evaluate the safety of potassium aluminium silicate–based pearlescent pigments based on the recommendation of the Forty-second Session of CCFA (*6*). This aluminium-containing food additive has not previously been evaluated by the Committee.

Potassium aluminium silicate (mica) is used as a carrier substrate for titanium dioxide and/or iron oxide. Potassium aluminium silicate is not intended to be placed on the market as such, but only when coated with the food colours titanium dioxide and/or iron oxide. In the European Union (EU), E555 potassium aluminium silicate is approved as a carrier for E171 titanium dioxide and E172 iron oxides and hydroxides (maximum 90% potassium aluminium silicate relative to the pigment) (*7*). In the United States of America (USA), pearlescent pigments consisting of potassium aluminium silicate coated with titanium dioxide are approved for use as a colour additive at levels up to 1.25% in cereals, confections and frostings, gelatine desserts, hard and soft candies (including lozenges), nutritional supplement tablets and capsules, and chewing gum (*8*). Potassium aluminium silicate–based pearlescent pigments are proposed to be used in confectionery, chewing gums and beverages at usage levels ranging from a minimum of 0.02% up to a maximum of 1.25%.

The Committee noted that no actual usage data were submitted for aluminium ammonium sulfate (INS 523), sodium aluminium phosphate basic (541(ii)), aluminium silicate (INS 559), aluminium powder or aluminium potassium sulfate (INS 522). Currently used aluminium-containing food additives are aluminium sulfate (INS 520), sodium aluminosilicate (INS 554), sodium aluminium phosphate acidic (INS 541(i)) and aluminium lakes of food colour.

At the sixty-seventh meeting, the Committee considered only consumer exposure to aluminium in the diet; occupational exposure and other routes or commodities were not considered. Dietary sources of exposure include natural dietary sources, drinking-water, migration from food contact materials and food additives. The potential range of exposure to aluminium from dietary sources reviewed at the sixty-seventh meeting by the Committee was 14–280 mg/week (Table 1).

For the evaluation of potassium aluminium silicate–based pearlescent pigments as a new food additive, the Committee evaluated an anticipated dietary exposure assessment based on food consumption data from the EU and the USA with the maximum proposed levels of use of potassium aluminium silicate–based pearlescent pigments. The Committee concluded that anticipated dietary exposure in the general population from the use of this food colour at the maximum proposed use levels (0.5% in beverages and 1.25% by weight in solid food) would range from 10 mg/kg bw per day at the mean to 323

Table 1
Estimated ranges of mean exposure of the adult population to aluminium from different dietary sources

Country/region	Estimated exposure from food additives used in cereals and cereal-based products (mg/person per week)	Estimated exposure from overall diet including natural sources, water consumption, food contact materials and food additives (mg/person per week)
WHO[a]	—	14–280
WHO[b]	2–124	11–136
Australia	4	17
Brazil	40–70	—
China	4–124	23–136
China, Hong Kong Special Administrative Region	30	36
Europe (EFSA)	2–46	11–91
Japan	—	84
USA	24–30	60

EFSA, European Food Safety Authority
[a] Estimated ranges from the sixty-seventh meeting of the Committee (Annex 1, reference *184*).
[b] Estimated ranges from the data reviewed at this meeting.

mg/kg bw per day for consumers with a high consumption of non-alcoholic beverages. When converted to an aluminium basis, this corresponds to an aluminium exposure from potassium aluminium silicate–based pearlescent pigments of 1.8 mg/kg bw per day up to 58 mg/kg bw per day.

The Committee recognizes that its estimates are conservative, as it is assumed that all processed foods and beverages contain the colour added at the maximum proposed use levels. The Committee noted that non-alcoholic flavoured drinks are the major contributor in these estimates, accounting for 20–70% of overall dietary exposure.

For other aluminium-containing food additives under re-evaluation, a tentative estimate of dietary exposure from food additive sources has been made, taking into account previous assessments and other publications or submissions reviewed by the Committee at the current meeting. The Committee noted, from the report of its sixty-seventh meeting and from a European Food Safety Authority (EFSA) scientific opinion, that the range of estimates was mainly based on dietary exposure calculated with the total diet study method, which takes into account water consumption. It is known from the literature that the main sources of migration of aluminium into food are from the use of cookware or aluminium utensils. It is also known that the design of total

diet studies generally tries to control any bias of additional contamination that may result from the use of containers, cookware or utensils containing aluminium during the preparation and storage of food as consumed.

The Committee noted that estimates of the contribution to overall mean dietary exposure from all sources (including natural sources, water consumption, food contact materials and food additives) were in the range of 10–140 mg/week in adult populations (0.2–2.3 mg/kg bw per week as aluminium, assuming a body weight of 60 kg; Table 1). Major contributors to these estimates were cereals and cereal-based food products, with a proportion of 20–90%, depending on the country, equivalent to a dietary exposure of approximately 2–120 mg/week (0.03–2 mg/kg bw per week as aluminium, assuming a body weight of 60 kg).

This assessment is consistent with previous evaluations made by the Committee in which cereal products were considered as potentially high contributors to dietary aluminium exposure. The Committee also noted from its review that high levels of the actual uses of aluminium-containing food additives were reported for cereals and cereal-based products, in particular for sodium aluminosilicate (INS 554) and sodium aluminium phosphate acidic (INS 541(i)). Based on this, the Committee concluded that aluminium from the consumption of cereals and cereal-based products could reasonably be assumed to be mainly from food additive sources.

The Committee noted that the estimated dietary exposures related to average adult populations and that high dietary exposures (e.g. 90th or 95th percentile) are generally assumed to be 2 times higher than the reported average. It also noted that children generally have higher food intake than adults when expressed on a body weight basis and therefore represent the highest potential exposure to aluminium per kilogram of body weight.

Evaluation

The new data submitted to the Committee and available in the published literature addressed some of the research needs identified previously, including studies of bioavailability and reproductive, developmental and neurobehavioural effects.

The absorption of aluminium compounds is generally in the region of 0.01–0.3%. Soluble aluminium compounds appear to be more bioavailable, but it is not possible to draw conclusions on quantitative differences in the overall toxicokinetics of different aluminium-containing food additives or between experimental animals and humans.

The recent evidence did not show effects of aluminium on reproductive outcomes. The new studies support previous observations of neurodevelop-

mental effects in experimental animals, but there continues to be a lack of consistency regarding the reported effects, and there are some limitations to all of the studies. Most of the studies involved administration of aluminium compounds in drinking-water, rather than in the diet.

At its current meeting, the Committee noted that the new data did not substantially change the LOAEL range of 50–75 mg/kg bw per day, but one of the studies also provided a NOAEL of 30 mg/kg bw per day. This NOAEL was identified from a study in which aluminium citrate was administered in drinking-water. Aluminium citrate is more soluble than many other aluminium compounds and is likely to be more bioavailable from drinking-water than from food. The Committee concluded that the NOAEL of 30 mg/kg bw per day was an appropriate basis for establishing a PTWI for aluminium compounds. Because long-term studies on the relevant toxicological endpoints had become available since the sixty-seventh meeting, there was no longer a requirement for an additional uncertainty factor for deficiencies in the database. The Committee therefore established a PTWI of 2 mg/kg bw from the NOAEL of 30 mg/kg bw per day by applying an uncertainty factor of 100 for interspecies and intraspecies differences. The previous PTWI of 1 mg/kg bw was withdrawn.

The data submitted on aluminium lactate and potassium aluminium silicate–based pearlescent pigments were insufficient to demonstrate that these food additives differ from other forms of aluminium in their bioavailability or toxicity. The PTWI applies to all aluminium compounds in food, including food additives. The Committee emphasized that whereas substances that have long half-lives and accumulate in the body are not generally considered suitable for use as food additives, consumption of aluminium-containing food additives would not be a health concern, provided that total dietary exposure to aluminium is below the PTWI.

The Committee concluded that, for adults, the estimates of mean dietary exposure to aluminium-containing food additives from consumption of cereals and cereal-based products are up to the PTWI of 2 mg/kg bw. Estimates of dietary exposure of children to aluminium-containing food additives, including high-level dietary exposure, can exceed the PTWI by up to 2-fold.

For potassium aluminium silicate–based pearlescent pigments at the maximum proposed use levels and using conservative estimates, the Committee noted that anticipated dietary exposure at the highest range of estimates is 200 times higher than the PTWI of 2 mg/kg bw.

Therefore, the Committee recommended that provisions for food additives containing aluminium included in the GSFA should be compatible with the

revised PTWI for aluminium compounds of 2 mg/kg bw as aluminium from all sources.

There is a need for convincing data to demonstrate that aluminium is not bioavailable from potassium aluminium silicate–based pearlescent pigments.

No data were available to identify the forms of aluminium present in soya-based formula and their bioavailability. Such studies were requested at the sixty-seventh meeting and are still required.

An addendum to the toxicological monograph was prepared.

The Committee received no data on the use of aluminium lactate as a food additive or on the manufacture, assay, impurities or use levels in food. The Committee decided that it was not appropriate to develop specifications for aluminium lactate.

The Committee prepared new tentative specifications for pearlescent pigments containing potassium aluminium silicate at the request of CCFA at its Forty-second Session (*6*). Limited data were received on potassium aluminium silicate itself as well as for the three general types of pearlescent pigments made using potassium aluminium silicate with titanium dioxide, iron oxide or both titanium dioxide and iron oxide. Based on the data received, the Committee decided to prepare specifications for potassium aluminium silicate itself, as well as a combined specification for the three general types of potassium aluminium silicate–based pearlescent pigments manufactured using potassium aluminium silicate combined with titanium dioxide, iron oxide or both titanium dioxide and iron oxide.

In the case of potassium aluminium silicate, information is required on preparation and purification methods, particle size distribution, methods of identification for silicate and aluminium, data on the levels of the inorganic impurities, the suitability of an inductively coupled plasma atomic emission spectroscopy (ICP-AES) method for the determination of inorganic impurities, and the suitability of a proposed method based on alkali fusion followed by ICP-AES for the assay for potassium aluminium silicate based on the determination of aluminium.

In the case of potassium aluminium silicate–based pearlescent pigments, information is required on their manufacture, stability in food, particle size distribution, pH range, methods for the identification of iron, titanium and aluminium, data on the levels of the inorganic impurities, the suitability of an ICP-AES method for the determination of inorganic impurities, a filtration method appropriate for the small particle sizes associated with the pigments, and the suitability of a proposed method based on alkali fusion followed by ICP-AES for the assay for titanium, iron and aluminium.

New specifications for potassium aluminium silicate and potassium aluminium silicate–based pearlescent pigments were prepared and made tentative. The requested information should be made available by the end of 2012.

3.1.2 *Benzoe Tonkinensis*

Explanation

Benzoe Tonkinensis was placed on the agenda of the current meeting at the request of CCFA during its Forty-second Session (*6*). The Committee has not previously evaluated Benzoe Tonkinensis.

Benzoe Tonkinensis is a balsamic resin from the *Styrax tonkinensis* (Pierre) Craib ex Hartwich tree, which belongs to the Styracaceae family. It is variously referred to as Siam benzoin gum, Siam benzoin and benzoin Laos or in a generic way as "benzoin gum".

Two varieties of benzoin gums occur: Benzoe Tonkinensis and Benzoe Sumatranus. These two resins differ in their botanical source, geographical origin and chemical composition. The term "benzoin gum" can include resins from one or the other of the two sources or their mixtures.

The Committee previously considered benzoin gum at its twenty-first and fifty-fifth meetings (Annex 1, references *44* and *149*) but did not evaluate it owing to the lack of analytical and toxicological data. At its twenty-first meeting, the Committee prepared a tentative specification covering the two forms of benzoin gum. However, no ADI was established, and no monograph was prepared. At its fifty-fifth meeting, the Committee withdrew the tentative specification for benzoin gum, as the relevant information was not provided.

Benzoe Tonkinensis is intended to be used as a flavouring agent in foods and beverages.

Chemical and technical considerations

Benzoe Tonkinensis has an opaque appearance and consists of grainy, ovoid, flattened almond-like splits, sometimes agglomerated by a brown-red transparent resin. The product has a strong vanilla flavour and is insoluble in water and soluble in ethanol.

The resin is composed mainly of coniferyl benzoate (15–60%) and benzoic acid (15–45%), with lesser amounts of vanillin (<5%), benzyl benzoate (<2%), 2-hydroxy-1-phenylethanone and 1-(4-hydroxy-3-methoxyphenyl)-2-propanone.

The Committee noted that there are large variations between samples of Benzoe Tonkinensis in the amounts of the four main components (coniferyl

benzoate, benzoic acid, vanillin and benzyl benzoate) determined in the ethanolic extract. Further, the resin contains a significant amount of unidentified compounds in the ethanolic extract.

Toxicological data

The Committee considered the previous evaluations of three of the components of Benzoe Tonkinensis—namely, benzoic acid, vanillin and benzyl benzoate. At its forty-sixth meeting, the Committee confirmed the group ADI of benzyl derivatives, including benzoic acid and benzyl benzoate, of 0–5 mg/kg bw as benzoic acid equivalents (Annex 1, reference *122*), which was maintained at its fifty-seventh meeting (Annex 1, reference *154*). At its eleventh meeting, the Committee assigned vanillin an ADI of 0–10 mg/kg bw (Annex 1, reference *14*), which was maintained at the fifty-seventh meeting.

At the current meeting, the Committee evaluated toxicological studies with Benzoe Tonkinensis, including a 90-day oral toxicity study and genotoxicity assays.

In a 90-day oral toxicity study, rats were administered Benzoe Tonkinensis in corn oil by gavage at dose levels of 0, 500, 1000 or 2000 mg/kg bw per day; the study included two satellite groups of vehicle control and a high-dose group (2000 mg/kg bw per day) kept for an additional 28 days without any treatment. The Committee noted that the test material was inadequately characterized. There were no treatment-related effects on body weight, feed consumption, functional observational battery parameters, ophthalmology, haematology or gross pathology. Statistically significant increases in relative weights of the liver in female rats and of the liver, brain, heart and kidneys in male rats were observed in the high-dose group. Clinical biochemistry parameters showed dose-dependent alterations in alanine aminotransferase (ALT) activity and total cholesterol level, reaching statistical significance at the highest dose. After the treatment was stopped, ALT activity and total cholesterol level returned to normal values at day 119, indicating reversibility of these effects. Histopathological findings of bile duct hyperplasia and multinucleated hepatocytes in the liver as well as lacteal ectasia (prominent white villi) in the jejunum were treatment related in both sexes in the intermediate-dose, high-dose and satellite high-dose groups. These findings correlated with the observed organ weight changes in liver. The Committee concluded that these effects in liver would not progress to neoplastic alterations. The NOAEL was considered to be 500 mg/kg bw per day.

The Committee concluded that Benzoe Tonkinensis was not mutagenic or clastogenic based on the results of the genotoxicity assays (reverse mutation tests in bacteria, chromosomal aberration assay and a gene mutation assay in mammalian cells). However, the Committee noted that there was cytotoxic-

ity in bacteria, mouse lymphoma cells and human lymphocytes at relatively low concentrations. The Committee also noted from published literature that some of the triterpenoids that were isolated from Benzoe Tonkinensis resin were cytotoxic to human leukaemic HL-60 cells. Benzoe Tonkinensis has not been tested in a carcinogenicity study.

Assessment of dietary exposure

The Committee received one submission for Benzoe Tonkinensis that contained information concerning food uses and estimated dietary exposure. Benzoe Tonkinensis can be used as a flavouring agent in solid foods and beverages. The sponsor stated that it has been used in foods in Europe. Benzoe Tonkinensis has a number of technical effects in food beyond use as a flavouring agent.

Benzoe Tonkinensis also finds use in pharmaceuticals and cosmetics. These uses were not considered by the Committee in the dietary exposure assessment, as cosmetic uses are not relevant to dietary exposure and pharmaceutical uses would not likely contribute significantly to overall chronic dietary exposure.

The Committee was requested to evaluate the use of Benzoe Tonkinensis as a flavouring agent in solid foods at levels up to 10 mg/kg and in beverages at levels up to 5 mg/kg. Using the budget method, assuming these use levels in all foods and beverages, the sponsor calculated a dietary exposure of 0.75 mg/kg bw per day for a 60 kg individual.

Benzoe Tonkinensis is manufactured predominantly in the Lao People's Democratic Republic and exported for food use throughout the world. The sponsor reported a total disappearance volume for the Lao People's Democratic Republic for the years 2000–2008 of 70 tonnes. Conservatively assuming that all of that material is sold in Europe for food use and applying the maximized survey-derived intake method, the Committee estimated a dietary exposure for Europe of 0.1 mg/kg bw per day for a 60 kg individual.

The Committee reviewed an assessment of dietary exposure to Benzoe Tonkinensis that was prepared using individual food consumption data from France and use levels submitted by the sponsor. This estimate included all uses in foods. For 60 kg adults, the mean exposure was 0.05 mg/kg bw per day, with a 95th percentile estimate of 0.1 mg/kg bw per day. The mean dietary exposure for children was 0.1 mg/kg bw per day for a 25 kg child, with a 95th percentile exposure of 0.2 mg/kg bw per day.

The Committee concluded that chronic dietary exposure to Benzoe Tonkinensis is expected to be below 0.2 mg/kg bw per day for children, the highest estimate including all uses in foods. As the levels of the individual components of Benzoe Tonkinensis are variable, the Committee did not enumerate

exposure to each individual component, but the dietary exposure to benzoic acid, vanillin and benzyl benzoate would all be below 0.2 mg/kg bw per day, even if each comprised 100% of Benzoe Tonkinensis.

Evaluation

The Committee noted that exposure to benzoic acid and benzyl benzoate from the use of Benzoe Tonkinensis is well below the upper limit of the group ADI (0–5 mg/kg bw) for benzyl derivatives, and exposure to vanillin is also well below the upper limit of its ADI (0–10 mg/kg bw). The Committee further noted that benzoic acid, one of the major components of Benzoe Tonkinensis, is used as a preservative, but that Benzoe Tonkinensis has not been assessed for this application. Comparing the conservative dietary exposure estimate for Benzoe Tonkinensis of 0.2 mg/kg bw per day with the NOAEL of 500 mg/kg bw per day identified in a 90-day oral toxicity study in rats, the margin of exposure is at least 2500. Because of the variability in composition of Benzoe Tonkinensis and the inadequate characterization of the material tested, the Committee concluded that the available data were inadequate to establish an ADI.

Considering the margin of exposure of 2500 for Benzoe Tonkinensis when used in food, the nature of the hepatic effects observed at doses above the NOAEL and the negative genotoxicity results, the Committee concluded that Benzoe Tonkinensis would not pose a health concern at current estimated dietary exposures, provided that it complies with the tentative specifications prepared at the current meeting, when used as a flavouring agent and in accordance with good manufacturing practice.

The Committee prepared new tentative specifications for Benzoe Tonkinensis, requesting additional information regarding the complete composition of the ethanolic extract, data on microbiological contaminants and data on inorganic contaminants (lead, arsenic, antimony, chromium, mercury and cadmium). The Committee also requested an analytical method to distinguish between Benzoe Tonkinensis and Benzoe Sumatranus.

A toxicological monograph and a Chemical and Technical Assessment were prepared.

3.1.3 *Glycerol ester of gum rosin*

Explanation

At its seventy-first meeting, the Committee evaluated 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 (Annex 1, reference *196*). The Committee established a group ADI of 0–25 mg/kg bw

for GEGR and glycerol ester of wood rosin (GEWR). GEGR was evaluated based on the toxicity data of 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, in view of the limited toxicity data available for GEGR and the submission of only the summarized results of two 90-day oral toxicity studies, the Committee concluded that the full reports of the two 90-day oral toxicity studies with GEGR in rats are needed to confirm the validity of the comparison of GEGR with GEWR.

Further, at the seventy-first meeting, new specifications for GEGR were made tentative pending submission of additional data regarding the identity and compositional analysis of GEGR to establish the extent of the chemical similarity between GEGR and GEWR.

Chemical and technical considerations

GEGR is a complex mixture of triglycerol and diglycerol esters of resin acids from gum rosin, with a residual fraction of monoglycerol esters. Gum rosin is an exudate of living pine trees.

Sourcing gum rosin from many different pine species and geographical locations may lead to a high variability in the gum rosin composition. The Committee was informed that the variability in the resin acid composition of GEGR has been reduced by using raw materials exclusively from *Pinus oocarpa* Schiede and by the use of countercurrent steam distillation for purification of the raw material.

Although the submitted analytical data confirmed that GEGR and GEWR have a similar resin acid composition, 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.

No description of the methods used to generate the data on the GEGR composition was provided.

Toxicological data

At the current meeting, the Committee was not provided with the requested full reports of the two unpublished 90-day toxicity studies with GEGR in rats necessary to validate the comparison between GEGR and GEWR. The sponsor informed the Committee that the study reports would not be provided because the sponsor considered them confidential business information belonging to a third party. Therefore, the Committee could not evaluate these studies at the current meeting. At the seventy-first meeting, the Committee evaluated the toxicological studies with gum rosin, which included acute toxicity, 90-day toxicity and 2-year toxicity/carcinogenicity studies as well as a

summary statement by the sponsor regarding the results of two unpublished 90-day toxicity studies with GEGR.

Assessment of dietary exposure

The Committee received no new information on uses for GEGR. At the seventy-first meeting, the Committee considered a maximum use level of 100 mg/kg for GEGR, combined with the total consumption of soft drinks for different worldwide regions, to derive mean and 90th percentile dietary exposure estimates of 0.57 mg/kg bw per day and 1.65 mg/kg bw per day, respectively, for a 60 kg adult.

An evaluation of GEGR by EFSA that was published after the seventy-first meeting reported a mean dietary exposure to GEGR of 1.7 mg/kg bw per day and a 97.5th percentile exposure of 5.8 mg/kg bw per day for children. The Committee noted that both its and EFSA's estimates were conservative, as GEGR is not intended to be added to all non-alcoholic flavoured drinks but to citrus fruit–based drinks only. The Committee concluded that estimates for the purpose of safety assessments range from 1 mg/kg bw per day for adults up to 6 mg/kg bw per day for children at the 97.5th percentile.

Evaluation

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 failure of the sponsor to provide the requested data leaves the Committee's concerns unanswered.

The Committee withdrew the group ADI for GEGR and GEWR at the current meeting (see section 3.1.5), and a temporary group ADI for GEGR and GEWR of 0–12.5 mg/kg bw was established, pending the availability of additional compositional information on the GEWR from *Pinus elliottii*. 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.

Although the tested physical and chemical properties of GEGR and GEWR indicate that these substances are similar, incomplete or limited information was available on the identity and quantity of individual components of the glycerol esters of resin acids and "neutrals" (non-acidic saponifiable and unsaponifiable substances) as a basis for comparison. Furthermore, as variations in the composition of GEGR may arise as a result of differences in the rosin source and processing conditions, the Committee decided to set limits

for monoglycerol esters and neutrals. Additionally, the Committee found it appropriate to include an assay for triglycerol and diglycerol esters.

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.

The specifications were revised to include the name of the *Pinus* species from which the gum is sourced and a representative infrared spectrum of GEGR. The specifications were made tentative pending the submission of the above information by the end of 2012.

An addendum to the toxicological monograph was not prepared.

3.1.4 *Glycerol ester of tall oil rosin*

Explanation

Glycerol ester of tall oil rosin (GETOR) was evaluated by the Committee at its seventy-first meeting for proposed use as an emulsifier/density adjustment agent for flavouring agents in non-alcoholic beverages (Annex 1, reference *196*). The Committee concluded in principle that the data for GEWR, which has been evaluated previously, could be used in the evaluation of GETOR. Because information on the composition of GETOR was inadequate, the Committee could not complete the evaluation and requested additional compositional data on the product in commerce, in order to clarify the extent and significance of any differences relative to other glycerol esters of rosins. Tentative specifications were established at the seventy-first meeting, and additional information was requested.

Chemical and technical considerations

GETOR is a complex mixture of triglycerol and diglycerol esters of resin acids from tall oil rosin (TOR), with a residual fraction of monoglycerol esters. TOR is a by-product of kraft (paper) pulp processing.

Limited data on the composition of GETOR were provided. Although the tested physical and chemical properties of GETOR and GEWR indicate that these substances are similar, inconsistent information was available on the identity and quantity of individual components of the glycerol esters of resin acids and “neutrals” (non-acidic saponifiable and unsaponifiable substances) as a basis for comparison.

The Committee was informed that the sponsor does not analyse GETOR for its composition because this information either is available for the source material TOR or could be theoretically calculated from the TOR data. The Committee noted that although data on TOR are available, they cannot be considered representative for GETOR as a result of the various chemical side reactions that may occur during its manufacture, including isomerization, dehydrogenation, oxidation and dimerization of resin acids. It was also noted that the data provided to the Committee on the ester distribution of GEWR for use as a basis for comparison with GETOR indicated that the analysed GEWR did not meet the specifications for GEWR previously evaluated by the Committee. Therefore, the similarities claimed between GETOR and GEWR could not be confirmed. No description of the methods used to generate these data was provided.

Evaluation

The information received by the Committee was inadequate to conclude that GETOR is sufficiently similar to GEWR to allow the use of the toxicological data from GEWR for the evaluation of GETOR. Furthermore, as variations in the composition of GETOR may arise as a result of differences in the rosin source and processing conditions, the Committee decided to set limits for monoglycerol esters and neutrals. Additionally, the Committee found it appropriate to include an assay for triglycerol and diglycerol esters.

To complete the evaluation of GETOR, additional data are required to characterize the GETOR in commerce in relation to the composition of 1) the refined tall oil rosin used as the source rosin, 2) the glycerol ester of tall oil 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.

The specifications were revised to include a representative infrared spectrum of GETOR and made tentative pending the submission of the above information by the end of 2012.

3.1.5 *Glycerol ester of wood rosin*

Explanation

GEWR was previously considered by the Committee at its eighteenth, twentieth, thirty-third, thirty-seventh, forty-fourth and forty-sixth meetings (Annex 1, references *35*, *41*, *83*, *94*, *116* and *122*). At its forty-sixth meeting, the Committee established an ADI of 0–25 mg/kg bw for GEWR.

At its seventy-first meeting, the Committee extended this ADI to a group ADI of 0–25 mg/kg bw for GEGR and GEWR (Annex 1, reference *196*).

Chemical and technical considerations

GEWR is a complex mixture of triglycerol and diglycerol esters of resin acids from wood rosin, with a residual fraction of monoglycerol esters. Wood rosin is obtained by solvent extraction of aged pine stumps.

The GEWR originally evaluated by the Committee was obtained from just one *Pinus* species (*Pinus palustris*). However, information received by the Committee indicates that the product now in commerce is also produced from the species *Pinus elliottii.* Considering that the rosin source may have an impact on the composition of the final glycerol ester, the Committee decided that the specifications should include the *Pinus* species from which the wood rosin is obtained.

The proposal to use resin acid ratios to distinguish between GEWR and GEGR was evaluated based on the submitted information on the resin acid composition of the two glycerol esters. As wide and overlapping ranges of levels were identified for individual resin acids in GEWR and GEGR, the Committee concluded that it was not possible to differentiate between these esters of rosins based on their resin acid composition alone. It was noted that GEWR can be distinguished from GETOR based on the presence of sulfur compounds in GETOR.

The Committee also noted that the information submitted on the ester distribution and the "neutrals" (non-acidic saponifiable and unsaponifiable substances) of GEWR was incomplete.

Evaluation

Because new information on GEWR raises questions about the identity of the product in commerce, the Committee decided to withdraw the current group ADI of 0–25 mg/kg bw for GEGR and GEWR and establish a new temporary ADI of 0–12.5 mg/kg bw by applying an additional uncertainty factor of 2. The Committee noted that it was not rounding the temporary ADI to one significant figure, as this would allow the re-establishment of the original group ADI once the required information had been submitted. The Committee noted that the temporary group ADI will be withdrawn if both additional compositional information on the GEWR from *Pinus elliottii* currently used as a food additive to assess its similarity with the GEWR from *Pinus palustris* previously evaluated as well as the full reports of the 90-day toxicity studies on GEGR (see section 3.1.3) are not submitted by the end of 2012.

As variations in the composition of GEWR may arise as a result of differences in the rosin source and processing conditions, the Committee decided to set limits for monoglycerol esters and for neutrals. Additionally, the Committee found it appropriate to include an assay for triglycerol and diglycerol esters.

To complete the evaluation of GEWR, additional data are required to characterize the GEWR in commerce in relation to the composition of 1) the refined wood rosin currently used as the source rosin for the production of GEWR, 2) the glycerol ester of wood 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.

The specifications were revised to include the name of the *Pinus* species from which the wood rosin is sourced and a representative infrared spectrum of GEWR. The specifications were made tentative pending the submission of the above information by the end of 2012.

3.1.6 *Octenyl succinic acid modified gum arabic*

At its seventy-first meeting (Annex 1, reference *196*), the Committee decided to establish a temporary ADI "not specified" for OSA modified gum arabic, pending submission of data by the end of 2011 showing hydrolysis of OSA modified gum arabic in the gastrointestinal tract, to confirm the validity of using toxicological data on gum arabic in the evaluation of OSA modified gum arabic. The Committee at its seventy-first meeting prepared new specifications for OSA modified gum arabic.

At the present meeting, the Committee evaluated new data on the hydrolysis of OSA modified gum arabic and reviewed the specifications. The Committee concluded that the results from the experiments on the hydrolysis of OSA modified gum arabic do not demonstrate that OSA modified gum arabic hydrolyses completely in the stomach into gum arabic and OSA. Furthermore, the hydrolysis experiments raised questions regarding the previously reported stability of OSA modified gum arabic in food. Therefore, the Committee deferred further evaluation of OSA modified gum arabic and requested that data resolving the concern about the stability of OSA modified gum arabic in food as well as data on the extent to which OSA modified gum arabic is hydrolysed in the gastrointestinal tract be provided by the end of 2013.

The existing temporary ADI was retained. The specifications were revised, with changes in the test methods for the degree of esterification and for residual OSA content.

3.1.7 *Polydimethyl siloxane*

Explanation

Polydimethylsiloxane (PDMS) (synonyms: dimethylpolysiloxane, dimethicone; Chemical Abstracts Service [CAS] No. 9006-65-9) is widely used in foods as an antifoaming and anticaking agent. At its eighteenth meeting in

1974 (Annex 1, reference *35*), the Committee established an ADI of 0–1.5 mg/kg bw for PDMS. When reviewing this ADI at its twenty-third meeting (Annex 1, reference *50*), the Committee stated that the ADI applied to PDMS with 200–300 subunits of $[(CH_3)_2SiO]$ (weight-average molecular weight range, 15 000–22 000) because of concern that the material of lower molecular weight might be more readily absorbed. At its thirty-seventh meeting (Annex 1, reference *94*), the Committee revised the specifications to a material with a weight-average molecular weight range of 6800–30 000 (90–410 subunits) and a viscosity range of 100–1500 cSt (mm^2/s). However, the toxicological properties of this material were not re-evaluated. As a consequence, material with an average weight at the lower end of this range was outside the limits covered by the previously established ADI.

At its sixty-ninth meeting in 2008 (Annex 1, reference *190*), the Committee considered the applicability of the ADI of 0–1.5 mg/kg bw to the material currently in commerce by evaluating new studies on the absorption and toxicity of two PDMS products: 1) a material with a viscosity of 10 cSt and a number-average molecular weight of 1000 and 2) a material with a viscosity of 350 cSt and a number-average molecular weight of 10 000. These studies did not reveal any significant differences between the two materials: neither product was absorbed to any significant extent, and the main finding for both materials in the short- and long-term studies of toxicity was a dose-dependent increase in the incidence and severity of ocular lesions after oral dosing both in the diet and by gavage. Although the reports of the toxicological studies on the basis of which the ADI was established at the eighteenth meeting did not refer to any ocular effects, ophthalmological examination was not performed. The Committee at its sixty-ninth meeting concluded that the mechanism by which the ocular lesions arose is unclear, although the lack of absorption of PDMS indicated that it is unlikely to be a direct systemic effect. Consequently, the relevance of the ocular lesions for food use of PDMS could not be determined. The Committee withdrew the previously established ADI of 0–1.5 mg/kg bw and, using an additional uncertainty factor of 2, established a temporary ADI of 0–0.8 mg/kg bw for PDMS, pending submission of the results of studies to elucidate the mechanism and relevance of the ocular toxicity. The Committee stated that the temporary ADI applied to PDMS that meets the revised specifications prepared at the sixty-ninth meeting. These specifications refer to a material with a weight-average molecular weight range of 6800–30 000 (90–410 subunits) and a viscosity range of 100–1500 cSt.

Additional data have now been provided, and the Committee at its present meeting considered the following new information: a short-term study of toxicity not previously available to the Committee, in which rats were fed

diets containing PDMS at one of three viscosities; a review of the results on ocular toxicity in all toxicological studies previously evaluated by the Committee in 2008 and 1974; some additional data on the ocular effects of PDMS; and more general information on corneal degeneration in rats. Data on actual use levels in foods were not provided.

Toxicological data

In a 90-day study of toxicity not previously evaluated by the Committee, groups of 20 male and 20 female rats received a diet containing PDMS fluids at viscosities of 35, 350 or 1000 cSt. Each of the three PDMS fluids was mixed in the diet at 1%, 5% or 10%. Two control groups (each consisting of 20 animals of each sex) received basal diet. The animals were checked during workdays for mortality and clinical signs. Body weights and feed consumption were measured weekly. Ophthalmological examination was performed prior to initiation and prior to termination of the study. At termination, all animals were necropsied, and blood and urine samples were collected for haematology and clinical chemistry (10 animals of each sex per group) and urinalysis (5 animals of each sex per group). Liver, kidneys, adrenals, testes and brains were weighed. An extensive range of organs and tissues was histologically examined.

Slight anal leakage, covering up to a 2 cm radius of the anal region, was observed in some rats fed diets containing 5% low-viscosity PDMS. Slight to marked anal leakage of fluid, covering the entire anal area and dorsal surface and extending over the top of the tail and rump, was observed in animals fed each of the diets containing 10% PDMS. The amount of anal leakage decreased with increasing viscosity. No other treatment-related clinical signs, mortality or effects on haematology, clinical chemistry or urinalysis were observed. In general, body weights were comparable between control and PDMS-treated rats. An increased feed consumption in rats treated with diets containing 5% or 10% PDMS was considered to reflect compensation for the lower nutritional value of the food. Necropsy revealed increased incidences of opacities and neovascularization of the cornea in all treatment groups. Histologically, treatment-related changes were observed only in the eyes of the rats. Most animals with corneal lesions showed minimal to mild chronic inflammation of the cornea, and mineralization was also present in some animals. The corneal lesions were present in a non-dose-related pattern, and the study authors speculated that these lesions were due to direct ocular irritation from PDMS fluid in the feed (*9*).

The findings in the 90-day study are consistent with the findings in the various short- and long-term studies of toxicity in rodents evaluated by the Committee at its sixty-ninth meeting. In these studies, oral administration of PDMS

fluids of various viscosities in the diet or by gavage consistently caused an increase in corneal lesions, but did not result in any other toxicologically relevant effect at doses up to and including 100 000 mg/kg diet (equivalent to 5000 mg/kg bw per day), the highest dose tested, in 28-day and 90-day studies and 1000 mg/kg bw per day, the highest dose tested, in a 12/24-month study. In the 12/24-month study, inflammation of the nasolacrimal duct was observed with slightly increased incidence and severity in males at 1000 mg/kg bw per day. Anal leakage of fluids and matting of the fur were also reported in some of these studies (Annex 1, reference *191*). It is noted that in those studies in which ophthalmological examination was performed, corneal opacities (crystals) were already present in high incidences before treatment, and the incidences increased further with time in all animals, including controls, and were different from controls only in the first few weeks of treatment when taking severity into account.

A few studies on the corneal effects of external application of PDMS fluids to the eyes were available. In a study with anaesthetized pigmented rabbits, 0.7–1.0 ml of oxygenated generic PDMS (500, 1000 and 12 500 cSt) or medical-grade PDMS 360 (1000 cSt) was placed for 3–6 hours in an eye cup, formed by hanging sutures in the lid. The untreated eye served as control. The PDMS treatment induced increased epithelial and whole corneal thickness, which persisted for several days in most cases. There was a marked irregularity in the thickness of the epithelium and cornea in most treated eyes, especially from 3 days after treatment. Light microscopic evaluation of the eyes immediately after treatment showed intracellular oedema of the epithelium and some vacuolization of the superficial cells. The most common findings 3–7 days after treatment were irregular thickening of the epithelium and a general disorganization of the normal epithelial architecture. Transmission electron microscopic examination 5 days after treatment with medical-grade PDMS showed mild intracellular epithelial oedema, particularly in the basal cell layers and in the middle layers of the cornea (*10*).

In an eye irritation study in New Zealand White rabbits, in which 0.1 ml of PDMS fluids of 2, 100, 500, 1000 or 12 500 cSt was instilled in the conjunctival sac of the right eye, all PDMS fluids caused mild (grade 1), transitory conjunctival redness. Higher-viscosity grades of PDMS showed more persistent irritation (up to 2 days after instillation). No effects on the cornea or iris were observed. No histological examination of the eye was performed (*11*).

In humans, PDMS fluids of various viscosities (generally called silicone oils) are often used for intra-ocular treatment following vitrectomy as a tamponade to repair retinal detachment. Retinal and corneal lesions (keratopathy and degeneration of the corneal endothelium) have been reported as

a complication of this use of PDMS. Examination of corneal tissue of two patients by light microscopy showed increased cellularity and irregularity of collagen fibres of the stroma layer, decreases in the number of endothelial cells and endothelial cell degeneration. Electron microscopy demonstrated a decrease in endothelial cell population density, accompanied by flattening and thinning of the remaining cells and attenuation of cell borders (*12*). Foulks and co-workers (*13*) described corneal oedema, corneal hypaesthesia, endothelial opacification, band keratopathy and peripheral corneal vascularization upon clinical examination of patients. Histologically, retrocorneal membranes and different degrees of stromal hypercellularity, superficial stroma calcification and vascularization were noted in these patients.

The corneal toxicity observed in humans following intra-ocular treatment with PDMS has also been demonstrated in animal studies. Injection of PDMS in the anterior chamber of the eyes of rabbits and cats induced a 40% reduction in endothelial density in the area of the PDMS bubble. In one study in rabbits, corneal progressive stromal thinning, with gradual development of a retrocorneal membrane at the junction of PDMS–endothelial contact, was observed (*14*). A second study in rabbits showed damage to the corneal endothelial layer, ranging from loose intercellular connections to severe damage of the plasma membranes where the cells were degenerating (*15*). In cats, persistent stromal oedema, peripheral vascularization, irregular plaques on the endothelium and eventual epithelial ulceration and corneal thinning occurred (*14*).

These data indicate that following intra-ocular treatment, PDMS can damage the cornea in humans and animals, although no effects on the cornea were observed in a traditional eye irritation study with rabbits.

Assessment of dietary exposure

A new assessment of dietary exposure was not made at the present meeting. Based on use levels in foods, the comprehensive review of dietary exposure made by the Committee at its sixty-ninth meeting gave conservative estimates of dietary exposure that ranged from 0.1–0.4 mg/kg bw per day in consumers with average consumption up to 1.1 mg/kg bw per day in consumers with high-percentile consumption.

Evaluation

In most of the more recent short- and long-term studies of toxicity with PDMS, dose-dependent increases in the incidence and severity of ocular lesions were observed at all dose levels after oral dosing both in the diet and by gavage.

The weight of evidence indicates that the corneal effects are caused by external exposure of the eyes to PDMS. Support for this can be found in the fact that topical administration of PDMS for 3–6 hours in the rabbit also induced damage to the cornea, although in a classical eye irritation study in rabbits, no corneal effects were noted. In addition, in a 2-year study, inflammation of the nasolacrimal duct was observed with slightly increased incidence and severity in males at 1000 mg/kg bw per day, which is also indicative of a local effect of PDMS. Furthermore, absorption studies evaluated at the sixty-ninth meeting of the Committee indicate that PDMS fluids of various viscosities are not absorbed into the systemic circulation from the gastrointestinal tract to any significant extent.

Topical exposure in animal studies could result from the presence of PDMS in the feed or in faeces. Where the substance was administered in the diet, it is possible that the eyes were directly exposed to PDMS from dusty feed particles. More indirect exposure of the eyes can occur when the substance, after administration via diet or by gavage, has passed through the body and is excreted in the faeces. Anal leakage of fluids, staining of the anogenital area and matting of the fur were frequently reported in the available studies with PDMS.

The Committee concluded that the ocular lesions are caused by local toxicity when the eyes of laboratory animals are exposed to PDMS through contact with PDMS in feed or faeces or through grooming of contaminated fur. As none of the routes leading to local toxicity are likely to occur following use of PDMS as a food additive, the Committee concluded that the ocular effects observed in the short- and long-term toxicity studies evaluated at the sixty-ninth and present meetings are not relevant in establishing an ADI for the dietary route of exposure following use of PDMS as a food additive.

The Committee withdrew the temporary ADI of 0–0.8 mg/kg bw per day and re-established the ADI of 0–1.5 mg/kg bw, originally established at the eighteenth meeting.

The conservatively estimated dietary exposure at the sixty-ninth meeting does not exceed the ADI established at the current meeting.

A toxicological monograph was not prepared.

The existing specifications were maintained.

3.1.8 *Ponceau 4R*

Explanation

Ponceau 4R (CAS No. 2611-82-7), also known as Cochineal Red and New Coccine, is a synthetic food colour. Ponceau 4R consists essentially of

trisodium 2-hydroxy-1-(4-sulfonato-1-naphthylazo)-6,8-naphthalenedisulfonate and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.

Ponceau 4R was evaluated by the Committee at its present meeting at the request of CCFA at its Forty-second Session (*6*). Ponceau 4R was previously evaluated by the Committee at its eighth, thirteenth, eighteenth, twenty-second, twenty-fifth and twenty-seventh meetings (Annex 1, references *8*, *19*, *35*, *47*, *56* and *62*). At its eighth meeting, the Committee did not establish an ADI for Ponceau 4R because of inadequate toxicological data but recognized that some long-term feeding studies were available. At its thirteenth meeting, the Committee reviewed these data and established a temporary ADI of 0–0.75 mg/kg bw based on a NOEL[1] of 150 mg/kg bw per day in a long-term feeding study in rats. The ADI was made temporary because the Committee noted the absence of suitable information on the metabolism and kinetics of Ponceau 4R and a long-term feeding study in a second mammalian species. At its eighteenth meeting, the Committee considered an additional long-term feeding study in mice that had become available and revised the temporary ADI to 0–0.125 mg/kg bw based on a NOEL of 25 mg/kg bw per day (this NOEL assumed 500 mg/kg in the diet to be equivalent to 25 mg/kg bw per day). The current method of calculating administered dose from a concentration of test material present in the feed would yield an equivalent dose of 75 mg/kg bw per day (*4*). At that meeting, the Committee reiterated the need to review more studies on metabolism and reproduction and a long-term feeding study in a non-rodent species.

At the twenty-second and twenty-fifth meetings, the Committee extended the temporary ADI on the understanding that the data requested at the eighteenth meeting would become available for review. At the twenty-seventh meeting, the Committee reviewed new data on metabolism, a long-term study in rats that had been exposed in utero and through lactation, a multigeneration feeding study and a teratogenicity study. The Committee noted that the long-term study in rats showed no adverse effects in the kidneys and had a NOEL of 500 mg/kg bw per day based on reduced body weight gain at higher doses. The results of this study in rats and a reconsideration of the severity of the renal effects observed in the long-term study in mice led the Committee to establish an ADI of 0–4 mg/kg bw. The ADI was derived by applying a 100-fold uncertainty factor to the higher NOEL from the mouse dietary study, which was equivalent to 375 mg/kg bw per day.

[1] At its sixty-eighth meeting (Annex 1, reference *187*), the Committee decided to differentiate between NOEL and NOAEL. This NOEL would now be considered a NOAEL.

At its present meeting, the Committee based its evaluation on data previously reviewed together with a limited number of published studies that had become available since the twenty-seventh meeting. The new data included a reproduction study in mice that measured several neurological end-points, studies on genotoxicity and biochemical enzyme activity, and studies on additive intolerance. The Committee took note of the content of a recently completed review of Ponceau 4R by EFSA.

Toxicological data

This summary of the available toxicological data combines the studies previously reviewed (Annex 1, references *8*, *19*, *35*, *47*, *56* and *62*) with recently published data.

The absorption of ingested Ponceau 4R is limited. After Ponceau 4R is anaerobically reduced by microflora in the gastrointestinal tract, small amounts of its metabolites, in the form of the free sulfonated aromatic amines, naphthionic acid and 7-hydroxy-8-amino-naphthalene-1,3-disulfonic acid, reach the systemic circulation. Ponceau 4R does not accumulate in tissues. Almost all of an orally administered dose is excreted in urine and faeces within 72 hours, with the majority (90%) being present in faeces.

Repeated-dose feeding studies of short and long duration revealed no adverse findings. In 90-day studies, NOAELs of 500 mg/kg bw per day in rats and 300 mg/kg bw per day in pigs were reported. For long-term daily exposure, the NOAELs were 375 mg/kg bw per day in mice and 500 mg/kg bw per day in rats.

There was no evidence of carcinogenicity in long-term feeding studies in rats at doses up to 1500 mg/kg bw per day and in mice at doses up to 1875 mg/kg bw per day. Despite a recent report of a comet assay showing evidence of deoxyribonucleic acid (DNA) damage in the colon and bladder at 10 mg/kg bw and in the stomach at 100 mg/kg bw, there was no evidence of any neoplasia in the stomach, bladder or colon of mice in the carcinogenicity studies. The authors of the comet assay study noted that a histopathological examination did not reveal any treatment-related effects in the colon, bladder or glandular stomach. No mutagenic or cytotoxic effects were found when Ponceau 4R was tested in a range of in vitro experiments.

Reproduction studies revealed no adverse effects of Ponceau 4R in the feed at doses equivalent to 1250 mg/kg bw per day in the rat and up to 205 mg/kg bw per day in a neurobehavioural study in mice. Adverse neurobehavioural findings among mouse pups were inconsistent. Teratogenicity studies in mice at oral gavage doses up to 100 mg/kg bw per day (the highest dose tested) and in rats at 4000 mg/kg bw per day did not reveal any adverse effects.

Urticarial and vasculitic reactions have been reported in humans following exposure to Ponceau 4R. However, most of these reports are characterized by poorly controlled challenge procedures. Although recent studies performed with better control conditions are available, no conclusion on idiosyncratic responses to Ponceau 4R could be drawn from the available evidence.

The administration of six different food colours and a preservative, sodium benzoate, and the presence of multiple methodological deficiencies limited the value of a recent study that investigated a possible relationship between hyperactivity in children and the consumption of beverages containing food colours. The use of mixtures in dosing studies does not permit any observed effects to be ascribed to individual components.

Assessment of dietary exposure

Estimates of dietary exposure to Ponceau 4R prepared and published by EFSA and Food Standards Australia New Zealand (FSANZ) were available to the Committee. The estimates of dietary exposure to Ponceau 4R calculated by EFSA were much higher than those of FSANZ (0.02 mg/kg bw per day at the 90th percentile for children). The Committee concluded that this was due to the use of maximum reported use levels by EFSA, as opposed to the use of the mean analysed levels for all foods by FSANZ. The latter approach is considered to be more realistic for estimating lifetime dietary exposure. Because of the conservative assumptions used by EFSA in making the exposure estimates, the Committee concluded that the 97.5th percentile estimate of 6 mg/kg bw per day for children should be considered in the safety assessment for Ponceau 4R in addition to the more realistic FSANZ estimate.

Evaluation

The Committee noted that the data do not indicate a need to revise the existing ADI of 0–4 mg/kg bw for Ponceau 4R. The Committee noted that EFSA's conservative 97.5th percentile dietary exposure for children was above the ADI, whereas the 90th percentile dietary exposure for children, estimated by the more realistic FSANZ approach, was 0.5% of the upper limit of the ADI. In consequence, the Committee concluded that the dietary exposure of children to Ponceau 4R does not present a health concern.

An addendum to the toxicological monograph was prepared.

The Committee recognized that the specifications for Ponceau 4R in the Compendium of Food Additive Specifications (Annex 1, reference *103*) and the Combined Compendium of Food Additive Specifications (Annex 1, reference *180*) were inconsistent with the original specifications published in FAO Food and Nutrition Paper, No. 31/1 (Annex 1, reference *68*). The Committee revised the chemical formula, the formula weight, the

assay limit of total colouring matters and the titration factor in the method of assay to be consistent with the specifications in FAO Food and Nutrition Paper, No. 31/1 (Annex 1, reference *68*). The Committee replaced the paper chromatographic method for subsidiary colouring matters with a high-performance liquid chromatographic method. The Committee also revised the method for the determination of organic compounds other than colouring matters.

3.1.9 *Pullulan*

Explanation

Pullulan is a naturally occurring fungal polysaccharide. The Committee previously evaluated pullulan at its sixty-fifth meeting (Annex 1, reference *178*).

Pullulan has a linear structure consisting mainly of repeating maltotriose units, which are made up of three α-1,4-linked glucose molecules, linked by α-1,6-glucosidic bonds. The maltotriose units are interspersed with about 6% maltotetraose units consisting of four α-1,4-linked glucose molecules; rarely, branch points occur, at which polymaltotriosyl side-chains are attached to the main chain by a 1,3-glucosidic bond. The product contains not less than 90% glucan on a dried basis. The main impurities are monosaccharides, disaccharides and oligosaccharides that are carried over from the starting material. The average relative molecular mass of pullulan varies considerably, depending on culture conditions. Pullulan as specified at the sixty-fifth meeting has an average relative molecular mass of 200 000 daltons (Da).

Pullulan is used as a glazing agent, as a film-forming agent, as a thickener or as a carrier in the production of capsules for dietary supplements as a substitute for gelatine, in coatings for coated tablets containing dietary supplements, for production of edible flavoured films used as breath fresheners, and in the production of jams and jellies, confectionery and some meat and fruit products. It is also used as a texturizer in chewing gum and as a foaming agent in milk-based desserts.

At its sixty-fifth meeting, the Committee concluded that the current uses of pullulan as a food additive and the studies on its safety provided sufficient information to establish an ADI "not specified" (Annex 1, reference *178*). CCFA at its Forty-second Session (*6*) requested that the Committee at its present meeting review the safety of pullulan when used as a low digestible carbohydrate/dietary fibre in various types of food.

It is stressed that the Committee at the present meeting evaluated only the safety of the estimated intake of pullulan resulting from the proposed use levels and did not assess the efficacy of pullulan used as a dietary fibre.

Toxicological data

A number of in vitro and in vivo studies on the digestibility of pullulan were described in the data submitted to the Committee at its sixty-fifth meeting. However, most of these studies used types of pullulan with lower relative molecular masses than that of pullulan as specified. Data on the digestibility of pullulan as specified were available only from an in vitro digestibility study (*16*) and a pilot study in five human volunteers (*17*). In this latter study, pullulan exhibited a glycaemic index of less than 20% on average in comparison with maltose as the reference carbohydrate. Low digestibility of pullulan was also indicated by the occurrence of caecal enlargement in rats fed diets containing 5% and 10% pullulan for 13 weeks (*18*).

For the present evaluation, new data on the digestibility of pullulan and its effects on glycaemic and insulinaemic responses, bone calcium content and faecal characteristics were available.

Studies in humans and laboratory animals in which the glycaemic and insulinaemic responses to oral administration of pullulan were measured indicate that the higher the relative molecular mass of the pullulan, the lower its digestibility by mammalian intestinal enzymes (*19–24*).

Pullulan is degraded by a large variety of microbial species (*25*). A study in dogs showed that daily consumption of 2 or 4 g of pullulan (relative molecular mass, 100 000 Da) for 14 days increased the ileal bifidobacteria and lactobacilli concentration and the faecal lactobacilli concentration (*26*). No effects of pullulan on nutrient intake or faecal characteristics were noted. Pullulans with low relative molecular masses appear to be more readily fermented than pullulans with high relative molecular masses (*27*).

Pullulan (relative molecular mass not reported) administered to rats in the diet at a concentration of 10% for 2 weeks (equivalent to 5000 mg/kg bw per day) and subsequently at a concentration of 5% for 10 weeks (equivalent to 2500 mg/kg bw per day) caused increased mass of caecal contents and increased levels of short-chain fatty acids, indicating that unabsorbed pullulan is fermented in the lower gastrointestinal tract. No effects of pullulan on food intake, body composition, calcium retention or bone calcium content were observed (*28*).

In a study in humans, in which a single oral dose of 25 g of pullulan conforming to the existing specification was given to 15 subjects, the results of a questionnaire revealed no statistically significant treatment-related differences in intensity or frequency of gastrointestinal symptoms, such as nausea, abdominal cramping, abdominal distension and flatulence (*24*).

Similarly, in a human study with 35 subjects, a dose of 15 g pullulan did not evoke gastrointestinal symptoms (*29*). In comparison with maltodextrin,

pullulan of high relative molecular mass (486 000 Da), consumed by humans at a dose of 6 g twice per day for a period of 14 days, induced small increases in gastrointestinal symptoms, such as bloating, cramping, flatulence and borborygmi (*30*). In this study, no differences were found between pullulan and maltodextrose in fasting plasma levels of triglycerides, cholesterol, glucose, insulin, C-reactive protein, ghrelin or laxation.

Colonic fermentation of pullulan leads to the formation of hydrogen, which is exhaled. In human studies, an increase of the breath hydrogen expiration was observed after consumption of 15–25 g of pullulan, particularly long-chain pullulans with a relative molecular mass of 100 000–200 000 Da (*23*, *29*). The metabolism of pullulan in the gastrointestinal tract is essentially complete, as no pullulan could be detected in faeces of humans who had consumed 10 g pullulan (relative molecular mass, 50 000 Da) per day over a period of 2 weeks (*31*).

Assessment of dietary exposure

The Committee considered dietary exposures to pullulan at its sixty-fifth meeting. Exposure was 1.65 g/day from its use as a gelatine substitute; 2.5 g/day from its use in candies; and 6 g/day from its uses in various foods in Japan. These exposures should not be added, as they were prepared using assumptions that yield conservatively high estimates.

At the present meeting, the Committee received and evaluated one submission from an ingredient manufacturer on the dietary exposure to pullulan from new projected uses in food as a source of dietary fibre (*32*). The dietary exposure from these uses has been calculated using food consumption data from the USA. Although use as a component of chewing gum is included in the proposed uses, the dietary exposure to pullulan from this use was not considered in this analysis because it is not included as a food category in the survey used to provide the food consumption data. A separate analysis of dietary exposure from chewing gum use was prepared and reviewed by the Committee.

Mean and 90th percentile estimates of dietary exposure to pullulan (expressed in grams per day and grams per kilogram body weight per day) were calculated for individual food categories in which pullulan may be used as a dietary fibre and for all these food categories combined. For users 2 years of age and older, the estimates were 11.4 and 19.8 g/day at the mean and 90th percentile, respectively, corresponding to 0.21 and 0.43 g/kg bw per day. The group with the highest dietary exposure was 13- to 19-year-olds, at 21.4 g/day, corresponding to 0.36 g/kg bw per day. On a body weight basis, the highest estimate was 0.98 g/kg bw per day for 2- to 5-year-olds. The overall results are shown in Table 2.

Table 2
Estimated 2-day average exposure to pullulan from its proposed uses as a dietary fibre

Population group	Exposure (g/day)		Exposure (g/kg bw per day)	
	Mean	90th percentile	Mean	90th percentile
Children 2–5 years old	10.2	16.2	0.61	0.98
Children 6–12 years old	11.8	18.7	0.38	0.65
Adolescents 13–19 years old	12.4	21.4	0.20	0.36
Adults 20 years and older	11.3	20.2	0.16	0.28
Population 2 years and older	11.4	19.8	0.21	0.43

The estimation of pullulan exposure from its use in chewing gum was based on a survey of chewing gum consumers in the USA. The survey revealed that the average number of pieces of gum consumed per day varies between 2.1 for preschoolers and 3.8 for teenagers (3.0 pieces of gum per day for the total population). The weight of a piece of gum varies between 2 and 3 g; hence, the chewing gum consumption varies between 6 and 9 g/day. As pullulan may be present in chewing gum at levels of up to 10%, the pullulan intake from chewing gum (3 pieces per day) is estimated to be about 0.6–0.9 g/day for the average consumer.

Consumption of large amounts of poorly digested polysaccharides has been associated with gastrointestinal effects. Therefore, the Committee considered submitted information concerning exposures to pullulan on single eating occasions from its proposed fibre uses. The same survey used for the overall dietary exposure calculations was used, with the exception that individual meals and snack eating occasions were used in place of 2-day average food consumption. The highest eating occasion exposure to pullulan resulted from consumption of soups (by 13- to 19-year-olds), at 14 g per eating occasion.

The Committee concluded that the highest dietary exposures, 1 g/kg bw per day for children 2–5 years of age and 0.4 g/kg bw per day for the general population (2 years of age and older), are appropriate for conducting a safety assessment.

Evaluation

At the sixty-fifth meeting, the Committee evaluated a 90-day oral toxicity study in the rat with pullulan (relative molecular mass, 200 000 Da) in which the NOEL was 7900 mg/kg bw per day (the highest dose tested). The new digestibility and tolerability studies are consistent with the conclusion that pullulan is a substance of low toxicity.

A conservative assessment of the dietary exposure to pullulan as a dietary fibre revealed that exposure could reach 1 g/kg bw per day for 2- to 5-year-old children and 0.4 g/kg bw per day for the general population (2 years of age and older). These exposure estimates are 8 and 20 times lower, respectively, than the NOEL observed in the 90-day study in the rat. The Committee noted that following administration of a single oral dose of 25 g of pullulan to adult humans, no significant gastrointestinal effects were reported, whereas pullulan consumed by adult humans at a dose of 6 g twice per day for a period of 14 days induced small increases in gastrointestinal symptoms. These observed effects of pullulan should be taken into account when considering appropriate levels of use.

The previously established ADI "not specified" for the previously evaluated food additive uses was retained.

A toxicological monograph was not prepared.

The Committee noted an inappropriate term in the existing specifications for pullulan (Annex 1, reference *180*) and agreed to change the description of the production organism from "a non-toxin-producing strain of *Aureobasidium pullulans*" to "a non-toxigenic strain of *Aureobasidium pullulans*". Other minor editorial revisions were also made in the specifications.

3.1.10 *Pullulanase from* Bacillus deramificans *expressed in* Bacillus licheniformis

Explanation

At the request of CCFA at its Forty-second Session (*6*), the Committee evaluated the enzyme pullulanase (pullulan 6-α-glucanohydrolase; Enzyme Commission No. 3.2.1.41) derived from a genetically modified strain of *Bacillus licheniformis*. The Committee had previously evaluated pullulanase from *Klebsiella aerogenes* at its twenty-fifth meeting (Annex 1, reference *56*). Pullulanase catalyses the hydrolysis of the (1,6-α-D) glucosidic linkages in liquefied starch to produce linear oligosaccharides. It is used in the manufacture of starch hydrolysates (maltodextrins, maltose and glucose) and high-fructose corn syrup and in the production of beer and potable alcohol.

Genetic modification

Pullulanase is manufactured by pure culture fermentation of a genetically modified strain of *Bacillus licheniformis* containing the pullulanase gene from *Bacillus deramificans*. *Bacillus licheniformis* is a Gram-positive bacterium that is widely distributed in nature and is considered to be non-pathogenic and non-toxigenic. *Bacillus licheniformis* has a long history of use in the production of enzymes used in food processing, including enzymes from

genetically engineered strains of the organism. *Bacillus licheniformis* has been granted a Qualified Presumption of Safety status by EFSA.

Prior to the introduction of the pullulanase gene, the *B. licheniformis* host strain was genetically modified through deletion of its sporulation capability, amylase activity and chloramphenicol acetyltransferase activity. The modified host strain was then transformed with an amplifiable DNA cassette containing the pullulanase gene from *B. deramificans* and the chloramphenicol acetyltransferase (*cat*) gene from *B. licheniformis*. The *cat* gene was used as a selectable marker in the transformation of the host strain. The transformed host strain was further modified by deletion of genes that encode two major endogenous proteases. The strain was subsequently subjected to a gene amplification procedure to increase the number of pullulanase gene copies. The final production strain was designated as BMP139. The strain is genetically stable and does not contain the plasmid DNA used in the transformation of the host strain.

Chemical and technical considerations

Pullulanase is produced by submerged, aerobic, pure culture fermentation of the genetically modified *B. licheniformis* production strain. The enzyme is secreted into the fermentation broth and is subsequently purified and concentrated. The enzyme concentrate is formulated with dextrose, sodium benzoate and potassium sorbate to produce a final product with the desired pullulanase activity and stability. Total organic solids (TOS) comprise several per cent of the final pullulanase preparation. The pullulanase enzyme preparation conforms to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing (*33*).

The pullulanase enzyme preparation is used as a processing aid in the manufacture of corn syrups, including high-fructose corn syrup, potable alcohol and beer. In the production of sweeteners from starch, pullulanase is used in conjunction with glucoamylase to saccharify starch after its gelatinization with α-amylase. The action of these enzymes allows nearly complete (>95.5%) starch hydrolysis to monomeric glucose. The pullulanase enzyme preparation is typically used at levels ranging between 0.1 and 0.5 litre per tonne of starch on a dry weight basis.

In a typical brewing application, the recommended dosage of the pullulanase enzyme preparation is 0.5–2.0 kg per tonne of grist and up to 2 g/100 litres during fermentation. In the production of potable alcohol, the pullulanase enzyme preparation is used at a rate of 0.2–0.3 kg per tonne of grist during saccharification and at a rate of 0.15 kg per tonne of grist during fermentation.

Pullulanase would be inactivated and/or removed during processing or purification of the reaction products, and its carryover to food is expected to be negligible.

Assessment of potential allergenicity

Pullulanase has been evaluated for potential allergenicity using bioinformatics criteria recommended by the Codex Alimentarius Commission in its Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (*34*).

The amino acid sequence homology search between pullulanase and known allergens included in the Structural Database of Allergenic Proteins revealed no matches greater than 35% sequence identity within a sliding window of 80 amino acids. The search for exact matches of short amino acid fragments that could serve as potential linear immunoglobulin E (IgE) binding sites yielded two matches of seven amino acids with non-food allergens from the German cockroach and the mould *Penicillium citrinum*. These matches were further assessed by analysing the amino acid sequence of pullulanase for hydrophilicity. As neither of the matching amino acid sequences is strongly hydrophilic, they are not likely to be located on the surface of the intact pullulanase or to serve as IgE binding sites.

The Committee concluded that pullulanase has no characteristics of any known allergen.

Toxicological data

For the 13-week repeated-dose oral toxicity study and the genotoxicity studies, an ultrafiltrate pullulanase preparation, using a representative batch (Batch No. 3656), was produced according to the procedure used for commercial production, but was more concentrated. The liquid enzyme concentrate had an activity of 4251 acid-stable pullulanase units per gram (where an acid-stable unit is defined as the amount of pullulanase that liberates reducing sugars equivalent to 0.45 μmol of glucose per minute from pullulan at a pH of 5.0 and a temperature of 40 °C) and a TOS value of 9.82% weight per weight. Stability testing indicated that thawed samples, which had a protein content of 69.8 mg/ml and a specific gravity of 1.034 g/ml, were stable at 4 °C for at least 7 days.

In studies of general toxicity in rats, no significant treatment-related effects were seen when the pullulanase enzyme was administered in the diet at doses of up to approximately 5000 mg/kg bw per day (no TOS level reported) for 28 days or 2500 mg/kg bw per day (i.e. 246 mg/kg bw per day as TOS) by gavage for 13 weeks. Therefore, the NOAEL was identified as 246 mg/kg

bw per day as TOS, the highest dose tested in the 13-week study. Pullulanase enzyme was not mutagenic in an assay for mutagenicity in bacteria in vitro and was not clastogenic in an assay for chromosomal aberration in mammalian cells in vitro. Similarly, an assay for micronucleus formation in mice showed no evidence of a clastogenic effect in vivo.

Assessment of dietary exposure

Pullulanase is not expected to remain in food. However, an estimate of the theoretical dietary exposure to pullulanase was made by the Committee based on the level of TOS in the enzyme preparation and its uses in the saccharification of starch (high-fructose corn syrup production) and the production of potable alcohol and beer. The potential mean dietary exposure to pullulanase enzyme preparation based on national food consumption data for the adult population, assuming a body weight of 60 kg and a level of TOS similar to that in the tested material (9.82%), was 0.1 mg/kg bw per day as TOS. This estimate is conservative, as it is made assuming that all high-fructose corn syrup production and potable alcohol and beer and production processes would use this enzyme preparation.

Evaluation

A comparison of the conservative exposure estimates with the NOAEL of 246 mg/kg bw per day as TOS from the 13-week study of oral toxicity provides a margin of exposure of about 2500. The Committee established an ADI "not specified" for pullulanase from *B. deramificans* expressed in *B. licheniformis*, when used in the applications specified and in accordance with good manufacturing practice.

A toxicological monograph was prepared.

A Chemical and Technical Assessment and new specifications were prepared.

3.1.11 *Quinoline Yellow*

Explanation

Quinoline Yellow is a synthetic food colour. It is prepared by sulfonating either 2-(2-quinolyl)-1,3-indandione (unmethylated variety) or a mixture containing about two thirds 2-(2-quinolyl)-1,3-indandione and one third 2-[2-(6-methyl-quinolyl)]1,3-indandione (methylated variety). It consists essentially of sodium salts of a mixture of disulfonates, monosulfonates and trisulfonates of the above compounds and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.

Quinoline Yellow was evaluated by the Committee at its present meeting at the request of CCFA at its Forty-second Session (*6*). Toxicological data related to Quinoline Yellow were previously evaluated by the Committee at its eighth, thirteenth, eighteenth, twenty-second, twenty-fifth and twenty-eighth meetings (Annex 1, references *8*, *19*, *35*, *47*, *56* and *66*). At its eighth meeting, the Committee did not establish an ADI for Quinoline Yellow because of inadequate toxicological data. At its thirteenth meeting, the Committee reviewed the available data and established a temporary ADI of 0–1 mg/kg bw based on a NOEL[2] of 500 mg/kg bw per day in a long-term feeding study in rats. The ADI was made temporary because of data gaps. In particular, the Committee noted the absence of suitable information on the metabolism and kinetics of Quinoline Yellow and a long-term feeding study in a second mammalian species. At its eighteenth meeting, the Committee considered a suitable long-term feeding study in rats. Using the results of that study, the Committee established a temporary ADI of 0–0.5 mg/kg bw based on the absence of any adverse effects at the highest tested dose of 50 mg/kg bw per day. The Committee reiterated its desire to review a three-generation reproduction study that was in progress, more information on metabolism and a long-term feeding study in a non-rodent species.

At its nineteenth meeting in 1975 (Annex 1, reference *38*), the Committee was informed that there were two types of Quinoline Yellow: non-methylated Quinoline Yellow and partially (30%) methylated Quinoline Yellow. The Committee indicated that data generated using either source could be used to define the toxicological hazard associated with Quinoline Yellow. At its twenty-second meeting, the Committee reviewed a three-generation reproduction study in rats but did not amend the temporary ADI. At its twenty-fifth meeting, the Committee was advised that two major studies were nearing completion and decided to extend the temporary ADI that it had established at its eighteenth meeting until the twenty-eighth meeting.

At the twenty-eighth meeting, the Committee reviewed new data on metabolism and a long-term repeated-dose study in mice that had been exposed to Quinoline Yellow in utero and through lactation. The Committee established an ADI of 0–10 mg/kg bw based on a NOEL of 10 000 mg/kg in the diet (equivalent to a range of 1000–1500 mg/kg bw per day) in the long-term study in mice.

At its present meeting, the Committee based its evaluation on data previously reviewed together with published information that had become available since the twenty-eighth meeting. No new unpublished toxicological studies

[2] At its sixty-eighth meeting (Annex 1, reference *187*), the Committee decided to differentiate between NOEL and NOAEL. This NOEL would now be considered a NOAEL.

were submitted following a public call for data. The Committee took note of the content of a recently completed review of Quinoline Yellow by EFSA.

Toxicological data

This summary of the available toxicological data combines the studies previously reviewed (Annex 1, references *8*, *19*, *35*, *47*, *56* and *66*) with recently published data.

The absorption of ingested Quinoline Yellow is between 3% and 4% in rats and dogs, with most being excreted unchanged in faeces. There is evidence that some of the absorbed Quinoline Yellow is excreted in bile. Quinoline Yellow does not accumulate in tissues, and 85–90% of the Quinoline Yellow absorbed from the gastrointestinal tract is excreted unchanged in the urine.

Repeated-dose feeding studies for 90 days in the rat showed an absence of adverse effects at dose levels up to 2500 mg/kg bw per day. Two-year feeding studies confirmed the absence of any treatment-related effects in mice, rats and dogs at doses equivalent to 1500, 500 and 50 mg/kg bw per day, respectively. The long-term feeding studies in rodents also indicated that Quinoline Yellow was not carcinogenic. This was consistent with an absence of any genotoxicity reported previously or in the new studies completed since the Committee last considered Quinoline Yellow.

No adverse effects on reproductive performance in rats over three generations were reported following dietary exposure to Quinoline Yellow at the highest tested dose of 50 mg/kg bw per day. Similarly, a comprehensive two-generation study involving 65 rats of each sex per group on test showed no adverse reproductive effects at Quinoline Yellow concentrations up to 10 000 mg/kg in the diet (equivalent to a dose range of 1000–1500 mg/kg bw per day).

There are reports suggesting that asthma or chronic idiopathic urticaria/angio-oedema in humans may be induced by oral exposure to Quinoline Yellow. However, most of these reports are characterized by poorly controlled challenge procedures. Although recent studies performed with better control conditions are available, no conclusion on idiosyncratic responses to Quinoline Yellow could be drawn from the available evidence.

The administration of six different food colours and a preservative, sodium benzoate, and the presence of multiple methodological deficiencies limited the value of a recent study that investigated a possible relationship between hyperactivity in children and the consumption of beverages containing food colours. The use of mixtures in dosing studies does not permit any observed effects to be ascribed to individual components.

Assessment of dietary exposure

Estimates of dietary exposure to Quinoline Yellow prepared and published by EFSA and FSANZ were available to the Committee. The estimates of dietary exposure to Quinoline Yellow calculated by EFSA were much higher than those of FSANZ. The Committee concluded that this was due to the use of maximum reported use levels by EFSA, as opposed to the use of the mean analysed levels for all foods by FSANZ (0.01 mg/kg bw per day for children at the 90th percentile). The latter approach was considered by the Committee to be more realistic for preparing long-term dietary exposure estimates. Because of the conservative assumptions used by EFSA in making the exposure estimates, the Committee concluded that EFSA's 97.5th percentile estimate of 4 mg/kg bw per day for children should be considered in the safety assessment for Quinoline Yellow in addition to the more realistic FSANZ estimate.

Evaluation

The Committee noted that there were no new data submitted that would provide a suitable basis on which to revise the existing ADI of 0–10 mg/kg bw for Quinoline Yellow. However, the Committee was aware of unpublished long-term studies in mice and rats with in utero exposure to Quinoline Yellow that had been completed by Biodynamics Laboratories in 1980–1981, but had not been submitted for evaluation. One of these studies was used by EFSA to establish its ADI for Quinoline Yellow. As the results of these studies in rodents might affect the existing ADI, the Committee established a temporary ADI of 0–5 mg/kg bw, incorporating an additional 2-fold uncertainty factor, pending submission of the Biodynamics Laboratories studies by the end of 2013. The previously established ADI of 0–10 mg/kg bw was withdrawn. The conservative exposure estimates were below the upper limit of the temporary ADI.

At the twenty-eighth meeting, the specifications were revised and maintained as tentative (Annex 1, reference *66*). At its present meeting, the Committee recognized that the specifications for Quinoline Yellow had been published as full specifications in FAO Food and Nutrition Paper, No. 31/1 (Annex 1, reference *68*) and then republished in FAO Food and Nutrition Paper, No. 52 (Annex 1, reference *103*) and the Combined Compendium of Food Additive Specifications (Annex 1, reference *180*). The Committee also noted, based on the information received, that only the unmethylated variety of Quinoline Yellow is commercially available for use as a food colour and therefore decided to exclude the methylated variety of Quinoline Yellow from the specifications. Revised specifications were prepared and made tentative pending the submission of information regarding the principal components,

maximum wavelengths for absorption, organic impurities, the level of zinc and a method of assay.

An addendum to the toxicological monograph was prepared.

3.1.12 *Sunset Yellow FCF*

Explanation

Sunset Yellow FCF (CAS No. 2783-94-0) is a synthetic food colour. It is also known as Orange Yellow S, CI Food Yellow 3, FD&C Yellow 6 and C.I. 15985. Sunset Yellow FCF consists principally of the disodium salt of 6-hydroxy-5-(4-sulfonatophenylazo)-2-naphthalenesulfonic acid and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.

Sunset Yellow FCF was evaluated by the Committee at its present meeting at the request of CCFA at its Forty-second Session (*6*). The Committee was asked to evaluate all data necessary for the assessment of the safety, dietary exposure and specifications for Sunset Yellow FCF. Sunset Yellow FCF was evaluated by the Committee at its eighth and twenty-sixth meetings (Annex 1, references *8* and *59*). At its eighth meeting, the Committee considered that sufficient toxicological data were available to establish an ADI of 0–5 mg/kg bw for Sunset Yellow FCF. At the twenty-sixth meeting, the Committee considered new studies on long-term and reproductive toxicity and established an ADI of 0–2.5 mg/kg bw.

At its present meeting, the Committee based its evaluation on data previously reviewed together with published information that had become available since Sunset Yellow FCF was last considered by the Committee. There were no new unpublished toxicological studies submitted following a public call for data. However, a comprehensive review of one unpublished long-term feeding study in mice and two in rats was provided by the United States Food and Drug Administration. The Committee also took note of the content of a recently completed review of Sunset Yellow FCF by EFSA.

Toxicological data

This summary of the available toxicological data combines the studies previously reviewed (Annex 1, references *8* and *59*) with recently published data.

Sunset Yellow FCF has a strongly anionic sulfonated moiety on the molecule, which limits its absorption from the gastrointestinal tract and results in excretion of greater than 95% of an orally administered dose in the faeces, with only about 3% absorbed as the parent compound. However, little of the ingested Sunset Yellow FCF present in faeces remains unchanged, with the

extent of bacterial reduction of the azo group being dependent on the administered dose. Sunset Yellow FCF is metabolized by bacteria in the gastrointestinal tract to yield sulfanilic acid and 1-amino-2-naphthol-6-sulfonic acid, which are absorbed and metabolized to various *N*-acetylated forms.

Dietary administration of Sunset Yellow FCF to rats at doses up to 2330 mg/kg bw per day for 96 days was reported to cause diarrhoea and distension of the caecum at doses equal to and above 1500 mg/kg bw per day. Diarrhoea was also observed in dogs after repeated oral exposure to Sunset Yellow FCF at a dose equivalent to 1250 mg/kg bw per day, but no details on the duration of the study or the sex of the animals were available. At 2330 mg/kg bw per day, increased relative weights of the testes were observed in rats, but without any accompanying histopathological lesions.

In contrast, a feeding study in rats completed in 2005 reported degenerative changes in the testes after 90 days of administration of Sunset Yellow FCF at doses equivalent to 250 or 1500 mg/kg bw per day. However, in that study, the purity of the administered Sunset Yellow FCF, which was purchased at a local market in India, was unknown. The presence of impurities in the administered material may explain the lack of concordance with the absence of any testicular lesions in studies of longer duration (80–104 weeks) and at higher doses (up to 2500 mg/kg bw per day) when using Sunset Yellow FCF of known purity. A consistent adverse finding in repeated-dose feeding studies in mice and rats was reduced body weight gain (>10%) at doses in excess of 2250 mg/kg bw per day in adult mice and in excess of 1500 mg/kg bw per day in adult rats. Reduced body weight gain was also observed in dogs after 2–3 months at a dose of 1250 mg/kg bw per day, but not in pigs after 98 days at dietary concentrations equivalent to a dose of 1000 mg/kg bw per day.

Eight long-term studies previously reviewed by the Committee showed no evidence of carcinogenicity at concentrations in the feed equivalent to an oral dose of up to 3000 mg/kg bw per day in mice and up to 2500 mg/kg bw per day in rats. The present review included five additional long-term repeated-dose studies that tested dietary concentrations of Sunset Yellow FCF equivalent to oral doses of 7500 mg/kg bw per day in mice and up to 2500 mg/kg bw per day in rats. The absence of carcinogenicity in the long-term bioassays is consistent with the weight of evidence from a range of in vitro and in vivo genotoxicity tests reviewed at this meeting and at previous meetings, indicating that Sunset Yellow FCF is not genotoxic.

No adverse effects on reproductive performance in mice and rats have been reported following dietary exposure to Sunset Yellow FCF at doses up to 1000 mg/kg bw per day. However, reduced rat pup survival was observed in comprehensive studies at doses of 1500 and 2500 mg/kg bw per day, with

reduced pup body weight at doses of 750 mg/kg bw per day and above. Dam body weight was affected only at the highest tested dose of 2500 mg/kg bw per day. The NOAEL for reduced pup body weight was 375 mg/kg bw per day.

Teratogenicity studies in rats and rabbits at oral gavage doses up to 1000 mg/kg bw per day (highest tested dose) did not reveal any compound-related adverse effects.

There are reports suggesting that asthma or chronic idiopathic urticaria/angio-oedema in humans may be induced by oral exposure to Sunset Yellow FCF. However, most of these reports are characterized by poorly controlled challenge procedures. Although recent studies performed with better control conditions are available, no conclusion on idiosyncratic responses to Sunset Yellow FCF could be drawn from the available evidence.

The administration of six different food colours and a preservative, sodium benzoate, and the presence of multiple methodological deficiencies limited the value of a recent study that investigated a possible relationship between hyperactivity in children and the consumption of beverages containing food colours. The use of mixtures in dosing studies does not permit any observed effects to be ascribed to individual components.

Assessment of dietary exposure

Estimates of dietary exposure to Sunset Yellow FCF prepared and published by EFSA and FSANZ were available to the Committee. The estimates of dietary exposure to Sunset Yellow FCF calculated by EFSA were much higher than those of FSANZ (0.12 mg/kg bw per day for children at the 90th percentile). The Committee concluded that this was due to the use of maximum reported use levels by EFSA, as opposed to the use of the mean analysed levels for all foods by FSANZ. The latter approach is considered to be more realistic for estimating lifetime dietary exposure. Because of the conservative assumptions used by EFSA in making the exposure estimates, the Committee concluded that the 97.5th percentile estimates of 1 mg/kg bw per day for adults and 6 mg/kg bw per day for children should be considered in the safety assessment for Sunset Yellow FCF in addition to the more realistic FSANZ estimate.

Evaluation

The Committee noted that there were five additional long-term repeated-dose feeding studies that tested Sunset Yellow FCF at dietary concentrations equivalent to doses of 7500 mg/kg bw per day in mice and up to 2500 mg/kg bw per day in rats. One of these long-term studies in rats, which included

in utero exposure, had a NOAEL of 375 mg/kg bw per day for reduced body weight among pups. On the basis of this NOAEL and the usual 100-fold uncertainty factor, the Committee established an ADI of 0–4 mg/kg bw (with rounding). The previous ADI of 0–2.5 mg/kg bw was withdrawn. The Committee noted that EFSA's conservative 97.5th percentile dietary exposure for children was above the ADI, whereas the 90th percentile dietary exposure for children, estimated by the more realistic FSANZ approach, was 3% of the upper limit of the ADI. In consequence, the Committee concluded that the dietary exposure of children to Sunset Yellow FCF does not present a health concern.

An addendum to the toxicological monograph was prepared.

The existing specifications were maintained.

3.2 Revision of specifications

3.2.1 *β-Apo-8'-carotenal*

β-Apo-8′-carotenal was on the agenda for revision of the purity test for carotenoids other than β-apo-8′-carotenal. The Committee was informed about an updated high-performance liquid chromatographic method for the determination of subsidiary colouring matters. The Committee revised the existing specifications and replaced the current thin-layer chromatographic (TLC) method for the determination of carotenoids other than β-apo-8′-carotenal with an HPLC method. The Committee also revised the definition of β-apo-8′-carotenal.

3.2.2 *β-Apo-8'-carotenoic acid ethyl ester*

β-Apo-8′-carotenoic acid ethyl ester was on the agenda for revision of the purity test for carotenoids other than β-apo-8′-carotenoic acid ethyl ester. The Committee was informed about an updated HPLC method for the determination of subsidiary colouring matters. The Committee revised the existing specifications and replaced the current TLC method for the determination of carotenoids other than β-apo-8′-carotenoic acid ethyl ester with an HPLC method. The Committee also revised the definition of β-apo-8′-carotenoic acid ethyl ester.

3.2.3 *β-Carotene, synthetic*

β-Carotene, synthetic, was on the agenda for revision of the purity test for carotenoids other than β-carotene. The Committee was informed about an updated HPLC method for the determination of subsidiary colouring matters. The Committee revised the existing specifications and replaced the current TLC method for the determination of carotenoids other than β-carotene

with an HPLC method. The Committee also corrected the structural formula and revised the definition of β-carotene.

3.2.4 *Hydroxypropyl methyl cellulose*

Hydroxypropyl methyl cellulose (HPMC) was placed on the agenda of the present meeting following a request for the revision of the method for the analysis of propylene chlorohydrins described in the specifications. The Committee replaced the method for the determination of propylene chlorohydrins in HPMC and increased the limit to not more than 1 mg/kg for the sum of both isomers of propylene chlorohydrins.

In consideration of the proposed revision of the limit for propylene chlorohydrins, the Committee took into account the extensive available toxicological database, most notably studies conducted by the United States National Toxicology Program (*35*). These data, together with the Committee's previous estimate of dietary intake of HPMC (Annex 1, references *88* and *89*), indicated that levels of propylene chlorohydrins up to 1 mg/kg in HPMC were not of toxicological concern.

3.2.5 *Magnesium silicate, synthetic*

The Committee at its sixty-first meeting revised the specifications for magnesium silicate, synthetic (Annex 1, reference *166*). At the present meeting, the specifications were considered for revision at the request of CCFA at its Forty-second Session (*6*), to consider deleting the maximum limit for loss on drying. The Committee did not receive any data justifying the deletion of the maximum limit for loss on drying.

The specifications were revised, maintaining the maximum limit for loss on drying for the material intended for use as an anticaking agent and not as a filtering aid. The existing gravimetric method for the assay of magnesium oxide and silicon dioxide was replaced by a more accurate method based on alkali fusion followed by analysis using ICP-AES.

3.2.6 *Modified starches*

While revising two test methods contained in the specifications monograph for OSA modified gum arabic, the Committee recognized that the same methods were also used in the specifications monograph for modified starches.

The specifications were revised to include the amended test methods on the degree of substitution of starch sodium octenyl succinate and residual OSA content. The Committee noted that the analytical methods for the determination of adipate groups and propylene chlorohydrin in modified starches are based on outdated GC methods using packed columns. The Committee

recommends revising the specifications for modified starches to replace these methods with suitable modern test methods.

3.2.7 *Nitrous oxide*

The Committee at its seventy-first meeting revised the specifications for nitrous oxide (Annex 1, reference *196*). The specifications were made tentative, as the assay was based on an outdated GC method using packed columns. The Committee at its current meeting received a method based on infrared spectroscopy for the assay of the gas.

The specifications were revised, and the tentative status was removed. The GC method was replaced by the infrared spectroscopic method, and the identification of the gas was based on the infrared absorption band of the sample instead of the retention time in the GC method.

3.2.8 *Sodium carboxymethyl cellulose*

The Committee noted the request received to consider deleting the synonym CMC for sodium carboxymethyl cellulose in the specifications monograph for this substance, because CMC may indicate the cellulose derivative and not the sodium salt.

The Committee decided to delete the synonym CMC from the specifications monograph for sodium carboxymethyl cellulose.

3.2.9 *Sucrose monoesters of lauric, palmitic or stearic acid*

The Committee at its seventy-third meeting (Annex 1, reference *202*) prepared the new tentative specifications for sucrose monoesters of lauric, palmitic or stearic acid, and information was requested on a test method to distinguish this substance from the substance specified under the title "Sucrose esters of fatty acids".

At the present meeting of the Committee, additional information was received. The specifications were revised to include an identification test based on the composition of fatty acids, and the tentative status was removed. A Chemical and Technical Assessment was prepared.

3.3 Revision of methods

3.3.1 *Method for colouring matters content by spectrophotometry*

The Committee was made aware of the absence of information pertaining to the wavelength of maximum absorbance, absorptivity and/or specific absorbance in individual specifications monographs for synthetic colours used as lakes. This information is necessary to calculate the percentage of total

colouring matters using the method described for Colouring Matters Content by Spectrophotometry (Volume 4) as referenced in the General Specifications for Aluminium Lakes of Colouring Matters (Annex 1, reference *180*). To address this problem, a table that collated information received on the wavelength of maximum absorbance, absorptivity and/or specific absorbance was added to the method for Colouring Matters Content by Spectrophotometry. Specifically, data on the wavelength of maximum absorbance, absorptivity and/or specific absorbance are requested for the following colours: Allura Red AC, Amaranth, Azorubine, Brilliant Black PN, Brilliant Blue FCF, Brown HT, Erythrosine, Fast Green FCF, Fast Red E, Green S, Indigotine, Patent Blue V, Ponceau 4R, Quinoline Yellow, Red 2G, Sunset Yellow FCF and Tartrazine. The data to be provided should also indicate the solvents used as well as any standardization for pH in order to allow for the establishment of consensus values for the wavelength of maximum absorbance, absorptivity and/or specific absorbance.

The method for Colouring Matters Content by Spectrophotometry (Volume 4) was revised and made tentative pending submission of additional data.

4. Contaminants

4.1 Cyanogenic glycosides

Explanation

Cyanogenic glycosides were evaluated by the Committee at its thirty-ninth meeting (Annex 1, reference *101*). The Committee noted that the potential toxicity of a food produced from a cyanogenic plant depends on the likelihood that its consumption will produce a concentration of hydrocyanic acid (HCN) that is toxic to exposed humans. Factors important to this potential toxicity include the following: 1) the plant may not be sufficiently detoxified during processing or preparation, and HCN may therefore remain in the food; 2) if the plant is consumed raw or is insufficiently processed, HCN may be released in the body, until the low pH of the stomach deactivates the plant β-glucosidase enzyme; and 3) a portion of the intact cyanogenic glycoside ingested with the food can be hydrolysed by β-glucosidase of the bacteria of the gut flora. The potential toxicity of HCN from ingested cyanogenic glycosides is dependent on a number of nutritional factors that are involved in detoxification mechanisms, including the availability of sulfur-containing amino acids and vitamin B12.

Although there were reports available showing associations between chronic exposure to cyanogenic glycosides and various diseases in humans (spastic paraparesis, tropical ataxic neuropathy and goitre), these associations were confounded by nutritional deficiencies. Therefore, the previous meeting of the Committee concluded that no causal relationship could be definitively established. Experimental animal studies were also assessed, and the Committee concluded that there were insufficient quantitative data on the level of exposure to cyanogenic glycosides or amount of HCN released with which to estimate a safe exposure level. The Committee also concluded that consumption of cassava flour containing total HCN at levels up to 10 mg/kg as specified in the current Codex standard for edible cassava flour (Codex Standard 176-1989) would not be associated with acute toxicity, but recommended that additional guidelines be developed for analytical methods for releasable HCN from cyanogenic glycoside–containing foods other than cassava.

The Third Session of CCCF in 2009 requested that JECFA reconsider the available data on cyanogenic glycosides, advise on the public health implications of cyanogenic glycosides and their derivatives in food and decide whether risk assessment is feasible and appropriate (*36*).

Absorption, distribution, metabolism and excretion

The biotransformation of cyanogenic glycosides involves two main steps: 1) cleavage of the carbohydrate moiety by β-glucosidases and 2) subsequent enzymatic or spontaneous dissociation of the cyanohydrin to the corresponding aldehyde or ketone and HCN.

Following oral administration, a proportion of ingested cyanogenic glycosides is absorbed and excreted intact in the urine. In limited experiments with human volunteers exposed to cyanogenic glycosides—either linamarin in cassava food products or oral doses of purified amygdalin—between 8% and 32% of the cyanogenic glycoside dose was absorbed and excreted unmetabolized in urine. The residual fraction of unabsorbed cyanogenic glycosides can be enzymatically converted by microorganisms in the gastrointestinal tract to cyanohydrins, following cleavage of the carbohydrate moiety, and then eventually to the corresponding aldehyde and/or ketone and HCN. In experimental animals pretreated with antibiotics, a substantial reduction or elimination of the conversion of cyanogenic glycoside to cyanide is observed. In addition, supplementation of cassava-based experimental diets with D,L-methionine overcomes a dietary deficiency of sulfur-containing amino acids and provides an available source of labile sulfur for cyanide detoxification.

HCN is readily absorbed after oral administration and rapidly distributed in the body through the blood. The concentration of cyanide is higher in erythrocytes than in plasma, as it is known to bind with iron in both methaemoglobin and haemoglobin present in erythrocytes. At the physiological pH of the stomach, cyanide will predominantly form HCN, which can rapidly penetrate mucous and cell membranes. However, after oral exposure, HCN is subjected to extensive presystemic metabolism by the liver.

Cyanide binds to and inactivates several enzymes, particularly those containing iron in the ferric (Fe^{3+}) state and cobalt. Examples include cytochrome c oxidase, catalase, peroxidase, xanthine oxidase and succinic dehydrogenase. Cyanide produces histotoxic anoxia by binding to the active site of cytochrome c oxidase, the terminal protein in the electron transport chain located within the mitochondrial membrane.

Cyanide is detoxified in the liver by conversion to thiocyanate by the intramitochondrial enzyme rhodanese (thiosulfate–cyanide sulfur transferase). Rhodanese catalyses the transfer of sulfur from a donor to cyanide to form

thiocyanate. The availability of sulfur-containing donor molecules is the rate-limiting factor in the detoxification of cyanide. Several polymorphisms in rhodanese have been identified in human populations, although only a minimal effect on cyanide detoxification has been detected. Approximately 80% of cyanide is estimated to be detoxified by conversion to thiocyanate, which undergoes renal clearance with a half-life of approximately 2.7 days. Cyanide can also be detoxified by direct chemical combination with sulfur-containing amino acids (e.g. L-cysteine and L-methionine) or by conjugation with hydroxocobalamin to form cyanocobalamin. Detoxification of cyanide is therefore affected by the presence of nutritional factors, such as sulfur-containing amino acids and vitamin B12.

Toxicological data

Acute toxicity and mortality induced by various cyanogenic glycosides in experimental animals are directly related to, and influenced by, factors associated with the release and detoxification of HCN. As with the previous evaluation, the Committee considered it appropriate to evaluate toxicological data related to both cyanogenic glycosides and HCN. Median oral lethal doses (LD_{50}s) of various cyanogenic glycosides (linamarin, prunasin, amygdalin) have been reported to range from 450 to 880 mg/kg bw in experimental animals. Symptoms associated with acute toxicity are largely similar to those observed with cyanide exposure (metabolic acidosis, decreased cytochrome oxidase activities, atrial fibrillation and decreased respiratory rates).

The majority of animal experiments with cyanogenic glycosides involve only limited numbers of test animals consuming diets containing some fraction of cassava or cassava extract. Effects observed include body weight decrease, impaired glucose tolerance and nonspecific histopathological changes in liver, kidney, thyroid and the central nervous system, including cellular vacuolization, degeneration and necrosis. These effects are consistent with a combination of protein deficiency and cyanide toxicity.

Experimental animals given diets consisting of at least 50% fresh cassava (8–10 mg/kg as HCN equivalents) exhibit significant decreases in body weight within 3 months and signs of decreased motor coordination related to protein malnutrition. Exposure of pregnant hamsters to protein-deficient diets supplemented with cassava meal resulted in delayed fetal ossification and an increased percentage of pups per litter with a reduced body weight. Direct gavage dosing of maternal animals at a critical period of organogenesis (gestation day 8) with linamarin or amygdalin produced a dose-dependent increase in fetal skeletal defects and malformations, but no significant change in associated litter parameters, including number of litters with prenatal deaths, number of live fetuses per litter and fetal body weight.

Simultaneous exposure to amygdalin and a cyanide antagonist, thiosulfate, significantly reduced the incidence of congenital malformations observed with amygdalin alone.

Oral LD_{50} values for cyanide in experimental animals average between 2 and 4 mg/kg bw. Signs of acute toxicity generally occur within minutes of dosing. A similar range of oral human lethal doses has also been reported (0.5–3.5 mg/kg bw).

In various experimental animal species (rats, pigs, goats) exposed to HCN, potassium cyanide or sodium cyanide, qualitative histopathological changes of the central nervous system and thyroid have been reported at continual doses of 0.1–30 mg/kg bw per day (exposure via diet or drinking-water). Thyroid effects noted included increased vacuolization and weight changes, sometimes reported in the absence of thyroid hormone alterations, whereas central nervous system effects reported included neuron loss in the hippocampus, loss of cerebellar white matter and delayed maturation and migration of external granular layer neurons in the cerebellum. However, comparisons of these studies are complicated by differences in dosing procedures, use of animals with compromised health or nutritional status and insufficient reporting of incidences, severity and statistical significance of observed effects.

Subchronic repeated administration of sodium cyanide via drinking-water provided evidence of effects on the male reproductive system in both rats and mice, including decreased epididymis and cauda epididymis weights, decreased testicular sperm counts and decreased sperm motility.

Experimental data for multigenerational reproductive toxicity, chronic toxicity and carcinogenicity are not available for cyanide. Overall, in vitro and in vivo genotoxicity assays for cyanide have been negative.

Observations in humans

Accidental poisoning due to cyanide has been reported as a result of ingestion of foods containing cyanogenic glycosides. Whole blood concentrations of HCN in children with non-fatal episodes of poisoning were 0.32 and 0.56 mg/l, whereas the blood levels in two fatal cases were 0.85 and 1.35 mg/l. The cyanide blood level of an adult with acute cyanide toxicity caused by ingestion of apricot kernels was 1.1 mg/l 5 hours after ingestion. The estimated cyanide exposure was 0.73 mg/kg bw. Cancer patients treated with 0.5 g of laetrile (amygdalin) 3 times per day (25 mg/kg bw per day as amygdalin) had widely varying levels of cyanide in blood, with 5th and 95th percentiles of approximately 0.1 and 2.3 mg/l, respectively; two thirds of these patients had blood concentrations below 1 mg/l.

Long-term consumption of cassava containing high levels of cyanogenic glycosides, usually when cassava constitutes the main source of calories, has been associated with neurological diseases, mainly involving endemic spastic paraparesis (konzo) and tropical ataxic neuropathy. In areas with low iodine intake, development of hypothyroidism and goitre, sometimes accompanied by these neurological diseases, has also been linked to cassava consumption.

Epidemic outbreaks of konzo have been reported in the Democratic Republic of the Congo, Mozambique, the United Republic of Tanzania and the Central African Republic, with potential causal links with high consumption of inadequately processed cassava or reliance on cassava as a diet staple. The epidemiological association between cassava consumption and konzo is considered to be consistent; however, definitive interpretation is complicated by underlying protein and nutrient deficiencies in the populations in question.

Tropical ataxic neuropathy is used to describe several neurological syndromes attributed to toxico-nutritional causes. The syndromes grouped as tropical ataxic neuropathy can differ widely in clinical presentation and characteristics. The main clinical features include sore tongue, angular stomatitis, skin desquamations, optical atrophy, neurosensory deafness and sensory gait ataxia. Tropical ataxic neuropathy has occurred mainly in Africa, particularly Nigeria, but it has also been recently reported in India. Although it has been found associated with consumption of a diet dominated by processed cassava foods, the results of recent studies are not consistent. The confounding effect of nutritional deficiencies cannot be ruled out.

Iodine deficiency disorders, which are endemic in certain African countries, comprise various significant adverse health effects, including goitre, hypothyroidism, mental retardation, cretinism, and increased neonatal and infant mortality. High serum levels of thiocyanate, which acts as an iodide transport inhibitor, can occur after exposure to cyanide from cassava consumption. Cassava consumption has been associated with goitre in both children and mothers, but only when urinary iodine levels were below 10 μg/dl, indicating iodine deficiency, or when intake of iodine was consistently below 100 μg/day.

Acute cyanide toxicity may be caused by ingestion of inadequately processed cassava or other foods containing cyanogens; nevertheless, the available studies do not provide a link between a defined intake and the occurrence of adverse effects due to acute cyanide exposure. There is a consistent association between konzo epidemics and diets consisting mainly of inadequately processed cassava, which involves high cyanide exposure. The Committee, however, concluded that the current data from epidemiological

studies were insufficient to form the basis for establishing a health-based guidance value.

Analytical methods

The compounds of health concern found in food derived from cyanogenic plants are 1) the cyanogenic glycosides initially present in the plants used as food, 2) the cyanogenic glycosides and cyanohydrins that remain in food after processing and food preparation and, ultimately, 3) the HCN and/or cyanide that is present in the food or that could be released from cyanogenic glycosides and cyanohydrins remaining in food when it is consumed. A number of colorimetric and instrumental (gas–liquid chromatography [GLC] and HPLC) methods are available for measuring individual cyanogenic glycosides, cyanohydrins, HCN or cyanide in food.

The term "total HCN" was used by the Committee to describe the total HCN content of all cyanogenic glycosides, cyanohydrins and "free" HCN in a food.

Direct measurement of total HCN by, for example, acid hydrolysis of cyanogenic glycosides present in foods and decomposition of the intermediate cyanohydrins to HCN has the advantages of being applicable to all types of samples and of generating a result directly comparable with Codex MLs (expressed as total milligrams of HCN per kilogram). However, this can lead to an overestimate of dietary exposure, as not all potential HCN of cyanogenic glycosides is likely to be available in vivo. Analysis of individual cyanogenic glycosides or cyanohydrins by GLC or HPLC, especially if using MS detection, has the advantage that both chemical characterization and quantification are achieved.

Levels and patterns of contamination in food commodities

Levels of cyanogenic glycosides, measured as total HCN, in plants used as food or for flavouring can vary greatly, depending on both cultivar and environmental factors. The highest levels reported (expressed as HCN) are associated with bamboo shoots (range 70–8000 mg/kg), bitter almonds (300–4700 mg/kg), lima beans (not detected to 3120 mg/kg) and bitter apricot kernels (90–4000 mg/kg). In regard to cassava tubers, two categories are recognized based on Codex definitions: sweet cassava, less than 50 mg/kg as total HCN; and bitter cassava, greater than 50 mg/kg of cyanides, expressed as HCN (fresh weight basis). The range of total HCN concentrations reported, however, in nominally sweet cassava by various authors is 1–1064 mg/kg as HCN. The range of total HCN concentrations reported in bitter cassava is 15–1120 mg/kg. Drought conditions can lead to significantly higher levels of cyanogenic glycosides, even in sweet cassava tubers. Bitter

almonds and bitter apricot kernels are among the nuts and fruits used to make cyanogenic glycoside–containing flavours for use in products such as marzipan and liqueurs. From a flavouring point of view, the most important are those liberating benzaldehyde as a flavouring compound—namely, amygdalin, sambunigrin and prunasin.

Information about the sampling procedures and number of samples for each food was not provided in detail for many of the papers evaluated.

Prevention and control

The Codex Alimentarius Commission has developed and published standards for sweet cassava, bitter cassava, edible cassava flour and gari. The key aspects of these standards are as follows: sweet cassava, less than 50 mg/kg of "hydrogen cyanide"; bitter cassava, greater than 50 mg/kg of "hydrogen cyanide"; edible cassava flour, "total hydrocyanic acid" must not exceed 10 mg/kg; and gari, "total hydrocyanic acid" must not exceed 2 mg/kg as "free" HCN.

Selection of low-cyanogen cultivars and transgenic methods of reducing cyanogenic glycosides in foods such as cassava have been reported in the literature.

Effects of processing

If the initial level of cyanogenic glycosides in the food is high, commonly used food processing methods may not ensure reduction in the level of total HCN to below Codex MLs. For example, using the least efficient method for making cassava flour from peeled tubers (sun drying), up to 30% of the total HCN present in the cassava has been shown to remain in the flour. The total HCN level in the cassava used must therefore be less than 30 mg/kg to ensure achieving the ML of 10 mg/kg as HCN in cassava flour.

When the most efficient processing method (crushing and sun drying) is used, approximately 3% of the original total HCN remains in the flour. This means that processing cassava containing greater than 330 mg/kg as total HCN is unlikely to achieve the Codex ML of 10 mg/kg as total HCN in cassava flour. This can be the case, for example, when very bitter rather than sweet cassava is used in the preparation of flour or during periods of drought, when higher than normal levels of cyanogenic glycosides are found in cassava tubers. Thus, as well as using the most effective methods of food preparation, the selection and use of cultivars low in cyanogenic glycosides are particularly important if Codex MLs are to be met.

Food processing as a means of detoxification also depends on the availability of endogenous glycosidase enzymes in the food being processed. It has

been shown, in the case of bamboo shoots, for example, that HCN removal is less effective at temperatures above 100 °C, where the glycosidase enzyme is denatured. In relation to cassava, higher degrees of maceration and high levels of moisture result in improvements in HCN removal.

Another aspect to be considered relates to the stability of cyanohydrins at low pH. Gari, a product obtained from processing cassava tubers, has a pH of 4.1, and the cyanogens remaining in gari comprise up to 85% acetone cyanohydrin and only 15% linamarin, whereas cassava flour, a product with a pH of 6.5, contains mainly linamarin and very little acetone cyanohydrin because, at the higher pH of the flour, the acetone cyanohydrin generated readily decomposes to HCN and acetone. This has an impact on the relative potential for gari and cassava flour of equal total HCN content to produce HCN in vivo. The acetone cyanohydrin, which is the predominant cyanogen in gari, releases all its HCN in weakly alkaline environments (e.g. in the intestines), whereas less than 50% of linamarin, the predominant cyanogen in cassava flour, is estimated to be converted to cyanide in the body. Thus, the same total HCN values recorded on analysis of gari and cassava flour do not reflect equal toxicities; as a consequence, gari has a Codex ML of 2 mg/kg as HCN, reflected by "free" HCN, whereas the Codex ML for cassava flour is 10 mg/kg as total HCN.

Assessment of dietary exposure

The dietary exposure estimates evaluated included those submitted to the Committee for Australia and New Zealand and other information found in the literature (primarily for Africa and Europe). Both acute and chronic estimates of dietary exposure were considered. The estimated dietary exposures were generally expressed as exposure to total HCN, as this was the form recorded in most of the occurrence or analytical data. However, dietary exposure can be to cyanogenic glycosides, cyanohydrins or HCN, depending on the processing of the food. The use of total HCN for the dietary exposure estimates represents the maximum exposure to cyanide coming from substances derived from cyanogenic glycosides in foods.

National estimates of acute dietary exposure

Acute dietary exposures to total HCN were estimated using mean, maximum or high-percentile (e.g. 95th percentile) concentrations of total HCN from analytical data. Consumption data used for the estimates were amounts per day of individual foods for consumers with maximum or high consumption at the 97.5th, 95th or 90th percentile, where available.

Estimated acute dietary exposures to total HCN for a range of foods from the small number of countries for which information was available ranged

Table 3
Summary of national estimates of chronic dietary exposure to total HCN

Country/region	Mean dietary exposures (μg/kg bw per day)		High-percentile dietary exposures (μg/kg bw per day)	
	Children	Adults	Children	Adults
Australia[a]	18–27	8–10	50–71	27–33
Democratic Republic of the Congo[b]	20–60	10–25	50–150	25–63
Europe[c]	—	1.6	—	1.7
New Zealand[a]	32–36	15–16	78–86	42–50
Norway[d]	—	1.4	—	5.4
United Kingdom[d]	—	0.8	—	3.6

[a] Includes a range of foods. High percentile is the 90th. Range of results is from lower bound to upper bound.
[b] Included only cassava cossettes. Range is based on analytical data from two different markets. High percentile is the 95th.
[c] Exposures from proposed MLs in foods as flavouring substances. High percentile is the 95th.
[d] Exposures from proposed MLs in foods as flavouring substances. High percentile is the 97.5th.

between 1 and 1044 μg/kg bw per day, depending on the food and the population groups assessed. Foods leading to the highest estimates were bitter apricot kernels (estimates up to 440 μg/kg bw per day in the United Kingdom), cassava (300 μg/kg bw per day for adults in New Zealand), ready-to-eat cassava chips (up to about 1000 μg/kg bw per day for children and up to 370 μg/kg bw per day for adults in Australia and New Zealand) and apple juice (100–110 μg/kg bw per day for children in Australia and New Zealand).

National estimates of chronic dietary exposure

National estimates of chronic dietary exposure to total HCN were available for Australia, the Democratic Republic of the Congo, Europe, New Zealand, Norway and the United Kingdom. These estimates are based on different food sources, including raw and processed foods and foods in which cyanogenic glycosides occur as a result of flavouring uses.

Estimated chronic dietary exposures to total HCN from the countries for which information was available were between less than 1 and 60 μg/kg bw per day for consumers with average exposure and between 2 and 150 μg/kg bw per day for consumers with high exposure. The estimates are summarized in Table 3.

International estimates of dietary exposure

The available occurrence data for cyanogenic glycosides were deemed not to be appropriate for use in determining international estimates of dietary

exposure to total HCN in combination with the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) consumption cluster diets for chronic dietary exposure or with WHO 97.5th percentile large portion data for acute dietary exposure. There were insufficient occurrence data for some foods or no concentration data for prepared or processed foods, which are more reflective of the concentrations in foods as consumed. In addition, cyanogenic glycosides occur in many processed foods, and consumption data for many of these foods are not included in the consumption cluster diets or large portion data. Therefore, no international estimates of chronic or acute dietary exposure were prepared.

Evaluation of existing MLs in relation to dietary exposure to cyanogenic glycosides

MLs for total HCN have been established by Codex and in a number of countries for foods including sweet cassava, cassava flour, gari and ready-to-eat cassava chips/crisps and for many foods containing flavouring agents. Estimates of chronic and acute dietary exposure to total HCN were calculated for Australia and New Zealand, for which analytical survey data for ready-to-eat cassava chips (collected before the FSANZ ML was established) were substituted with the ML of 10 mg/kg. This resulted in mean chronic dietary exposures of 10 µg/kg bw per day for children and 2–11 µg/kg bw per day for adults and 90th percentile exposures of 10–40 µg/kg bw per day for children and 10–12 µg/kg bw per day for adults. These chronic exposure estimates are about 2–5 times lower than estimated dietary exposures based on mean survey values for cassava chips. If all cassava chips were at the ML of 10 mg/kg, the estimated acute dietary exposures to total HCN would be up to a maximum of 100 µg/kg bw per day for children and 25 µg/kg bw per day for adults. These acute estimates are about 4–14 times lower than estimated dietary exposures based on mean survey values for cassava chips.

Acute dietary exposures based on WHO large portion consumption data for sweet cassava using a HCN concentration of 50 mg/kg were 150 µg/kg bw per day for the general population and 330 µg/kg bw per day for children. There was no consumption value for cassava flour in the large portion data set, but if it was assumed that the consumption of cassava flour is equivalent to that of cassava, estimated exposure to HCN based on the Codex ML of 10 mg/kg would be 30 µg/kg bw per day for the general population and 70 µg/kg bw per day for children.

Chronic dietary exposures to HCN from sweet cassava and cassava flour were estimated based on consumption amounts from the GEMS/Food consumption cluster diets and MLs. For sweet cassava at a maximum HCN level of 50 mg/kg (Codex ML for sweet cassava), estimated dietary exposures

ranged between 1 and 235 μg/kg bw per day for the clusters assessed. For cassava flour, based on the Codex ML of 10 mg/kg as total HCN, exposures ranged between less than 0.1 and 14 μg/kg bw per day.

Biomarkers of dietary exposure

Biomarkers of exposure were evaluated as they relate to dietary patterns such as the frequency of consumption of cassava and cassava products. No studies were found that quantitatively link measured levels of biomarkers to numerical estimates of dietary exposure. The information evaluated indicated that consumers of cassava have higher urinary thiocyanate levels than those who never consume cassava; weekly consumers had levels between those of daily consumers and non-consumers. Frequent (e.g. twice per day) or high levels of consumption of cassava can result in low levels of urinary thiocyanate (<100 μmol/l) when effective processing reduces the levels of cyanogenic glycosides. Consumption of varieties of cassava with low levels of HCN results in lower levels of urinary thiocyanate and linamarin. Levels of urinary inorganic sulfate are higher for those who consume a broader range of foods in the diet (other than cassava), which include other amino acids (including sulfur-containing amino acids). Biomarker levels tend to be seasonal, with peaks occurring during times of cassava harvest when cassava consumption is higher. Results from a study in human volunteers suggest that individual differences in liberation of cyanide from ingested linamarin are more important for the internal cyanide exposure.

Dose–response analysis

The Committee recognized that human exposure to HCN from cyanogenic glycosides in food commodities would be from a combination of intact glycoside, partially degraded glycoside (cyanohydrin moiety) and totally degraded glycoside (residual HCN). The ratio of contributors to total food HCN equivalents will vary depending on the commodity and extent of processing.

Following a review of both the experimental animal and human data specific to cyanogenic glycosides, the Committee concluded that, although there were relevant studies in experimental animals for establishing an acute reference dose (ARfD[3]) for oral exposure, there were no appropriate studies on which to base a long-term health-based guidance value. However, as the potential toxicity of ingested cyanogenic glycosides is directly related to the in situ generation of HCN, the Committee concluded that experimental animal

[3] An ARfD by definition is an estimate of the "amount of a substance in food and/or drinking water, normally expressed on a body weight basis, that can be ingested in a period of 24 h or less without appreciable health risk to the consumer on the basis of all known facts at the time of the evaluation" (*37*).

studies with cyanide compounds could serve as the basis for establishing a provisional maximum tolerable daily intake (PMTDI).

The Frakes, Sharma & Willhite (*38*) developmental toxicity study in hamsters was selected for dose–response assessment, as the exposure to linamarin was by the oral route and occurred during a sensitive life stage. Similar developmental effects have also been reported in the same animal model with higher doses of related cyanogenic glycosides (amygdalin and prunasin). The most prominent dose–response effect of linamarin in this developmental toxicity study in hamsters was an increased number of fetal skeletal defects. Data were not reported by the study authors for individual litters; therefore, quantal data on a fetal basis were considered. In the same study, the total number of litters, litters with prenatal deaths, number of live fetuses per litter and fetal body weight were not significantly different between the treated groups and control.

A single linamarin dose of 0, 70, 100, 120 or 140 mg/kg bw was administered by oral gavage to pregnant hamsters on day 8 of gestation, with the fetuses removed on gestation day 15 and examined for internal and external malformations. BMD modelling for dichotomous data was conducted with the United States Environmental Protection Agency's (USEPA) BMD software (BMDS version 2.1.2) using all dichotomous models available within the software with a default benchmark response (BMR) of 10% extra risk. All default constraints as set by the program were accepted. Models that passed the goodness-of-fit test ($P > 0.05$) were considered to be acceptable. As the Akaike's information criteria and BMDs estimated by the acceptable models were all similar, the lowest BMDL was selected as a point of departure. The estimated ranges of BMD_{10}s and $BMDL_{10}$s (the BMD and BMDL for a 10% response) for skeletal defects are 100–111 and 85–98 mg/kg bw per day for linamarin, respectively.

Reproduction-related effects observed in male rats exposed to cyanide doses ranging from 1.4 to 12.5 mg/kg bw per day over 13 weeks in drinking-water included decreased absolute and relative cauda epididymis weight as well as decreased testis weight and testicular sperm count at the highest dose tested. Although additional measurements directly related to cauda epididymis perturbation, such as sperm maturation, fertilization capacity and cauda epididymis sperm count, were not evaluated in this study, a slight reduction in sperm motility (statistically significant) was also noted at all doses, although it was not dose dependent. In addition, related effects associated with the male reproductive tract, such as reduced epididymal and testis weights and reduced testicular sperm counts, were observed at the highest sodium cyanide dose tested (12.5 mg/kg bw per day as cyanide). Taken in combination, these data are indicative of a toxic effect on the male reproductive system in rats.

All available continuous models in the BMDS (version 2.1.2) were fitted to the reproductive parameters (cauda epididymis weight, testicular spermatid count, epididymis weight, testes weight) reported for male rats in the United States National Toxicology Program (NTP) study (*39*). As the biological significance of the observed degree of change in this end-point was unclear, the BMR of one standard deviation (1SD) change in the control mean was considered to be the most appropriate. None of the continuous models for relative cauda epididymis weight and testicular spermatid count passed the goodness-of-fit test for acceptance ($P > 0.05$). Absolute reproductive organ weights were selected for modelling instead of relative organ weights, as body weight decreases were considered minimal (6% in the high-dose group), and there is evidence that absolute weights of testes, cauda epididymis and whole epididymis are not altered in rodents in spite of body weight reductions of up to 30% (*40*).

The ranges of BMD_{1SD} and $BMDL_{1SD}$ values estimated based on the decrease in absolute cauda epididymis weight for rats are 3.5–8.4 and 1.9–5.6 mg/kg bw per day as cyanide, respectively. The lower end of the $BMDL_{1SD}$ range was selected as a point of departure in the evaluation.

Evaluation

Reports of acute human poisoning associated with the consumption of foods containing cyanogenic glycosides were reviewed. The Committee therefore considered it appropriate to establish an ARfD for cyanogenic glycosides, expressed as cyanide equivalents. In addition, as there are a number of human diseases, specifically konzo, tropical ataxic neuropathy and iodine deficiency disorders, associated with the chronic consumption of underprocessed cassava as a staple food, it was recognized that the derivation of a chronic health-based guidance value would also be relevant.

Derivation of the ARfD

Following review of a developmental toxicity study with linamarin (*38*), the Committee considered this study as suitable for establishing an ARfD. BMD modelling of the data from this study provided a $BMDL_{10}$ for linamarin of 85 mg/kg bw for increased skeletal defects in developing hamster fetuses following acute exposure of maternal animals. Although the study did not use dietary exposure, gavage dosing was considered relevant for establishing the ARfD.

Following application of a 100-fold uncertainty factor, the Committee established an ARfD for linamarin of 0.9 mg/kg bw (equivalent to 0.09 mg/kg bw as cyanide). This value was considered, when compared on a cyanide molar basis, to be applicable also to other cyanogenic glycosides. Therefore,

the Committee recommended conversion of the ARfD for linamarin to a cyanide-equivalent dose of 0.09 mg/kg bw. This cyanide-equivalent ARfD applies only to foods containing cyanogenic glycosides as the main source of cyanide.

Derivation of the PMTDI

In a 13-week NTP study (*39*) not previously evaluated by the Committee, in which exposure to sodium cyanide was continuous via drinking-water, a variety of effects related to male reproductive organs were observed—namely, decreased cauda epididymis weights, decreased testis weights and decreased testicular spermatid concentration. Dose–response analysis of continuous data on absolute cauda epididymis weights generated the lowest $BMDL_{1SD}$ of 1.9 mg/kg bw per day. On the basis of this $BMDL_{1SD}$, the Committee established a PMTDI of 20 μg/kg bw by applying a 100-fold uncertainty factor. The Committee decided that it was not necessary to apply an additional uncertainty factor to account for the absence of a long-term study, considering the generally acute nature of cyanide toxicity and the sensitivity of the effect (i.e. the reduction of absolute cauda epididymis weight).

Comparison of estimated dietary exposures with health-based guidance values and the impact of MLs on dietary exposure

Estimated dietary exposures to total available HCN were converted to cyanide equivalents and compared with the health-based guidance values established by the Committee at this meeting.

From the national acute dietary exposure estimates available to the Committee for review, the ARfD of 90 μg/kg bw as cyanide equivalents was exceeded 3-fold for cassava for adults (based on raw samples), less than 2-fold for apple juice for children, between 2- and 5-fold for bitter apricot kernels and up to 10-fold for ready-to-eat cassava chips/crisps, depending on the population group. If ready-to-eat cassava chips contained a level equivalent to the recently established ML in Australia and New Zealand of 10 mg/kg as HCN, there was only a marginal exceedance of the ARfD for children. These results are based on dietary exposure to total HCN, which represents the maximum possible exposure for foods containing cyanogenic glycosides.

Based on national estimates of chronic dietary exposure to total HCN, there is also the potential to exceed the PMTDI of 20 μg/kg bw as cyanide for populations reliant on cassava as a staple food: between 1- and 3-fold in children and between 1- and 2-fold in adults. There is also a potential for those populations not reliant on cassava to exceed the PMTDI: between 1- and 5-fold for children and between 1- and 3-fold for adults. For Australia and New Zealand, ready-to-eat cassava chips were the major contributor to

Table 4
Maximum amount of cassava or cassava food products that could be consumed based on different MLs so that the health-based guidance values are not exceeded[a]

Health-based guidance value[b]	ML (mg/kg as HCN)	Maximum amount of cassava or cassava products that can be consumed (g/day)
ARfD	50	110
	10	560
	2	2800
PMTDI	50	25
	10	125
	2	620

[a] Based on a body weight of 60 kg.
[b] ARfD of 90 µg/kg bw per day as cyanide equivalents; PMTDI of 20 µg/kg bw as cyanide.

dietary exposure to HCN (84–93%). When the cassava chips contain a level equivalent to the ML of 10 mg/kg as HCN, all mean dietary exposures were below the PMTDI. High-percentile exposures for children were between 1- and 2-fold above the PMTDI. All chronic dietary exposure estimates based on exposures from flavouring agents did not exceed the PMTDI. These results are based on dietary exposure to total HCN, which is a worst-case scenario.

Application of the ML of 50 mg/kg as HCN for sweet cassava could result in dietary exposures that exceed the ARfD by less than 2-fold for the general population and up to 4-fold for children and exceed the PMTDI by between 2- and 10-fold, depending on the population group assessed. These estimates do not take into consideration any reduction in concentration of total HCN as a result of food preparation or processing. For the ML of 10 mg/kg as HCN for cassava flour, there are no estimates of dietary exposure available that exceed the ARfD or PMTDI. This is supported by the maximum amount of food that can be consumed based on existing Codex MLs before the health-based guidance values would be exceeded (Table 4), which is as low as 25 g/day for cassava for chronic exposure. More detailed estimates of cassava and cassava flour consumption and concentrations in food for cassava-eating communities would help in supporting the conclusion that dietary exposures to total HCN could exceed health-based guidance values.

The ML for sweet cassava is for the raw product. If the starting level of HCN in the raw sweet cassava were 50 mg/kg as HCN, the minimum effective processing would result in a concentration of 15 mg/kg as HCN, and the most effective processing would give a HCN concentration of 2 mg/kg.

Research needs

Further research is needed to more accurately quantify how nutritional factors ultimately contribute to the human diseases observed in populations whose diets consist mainly of improperly processed cassava, which involves high cyanide exposure.

There is a need for more extensive occurrence data for cyanogenic glycosides. These include data showing the ratio of cyanogenic glycosides to cyanohydrins to HCN in raw and processed versions of a range of foods containing cyanogenic glycosides. More occurrence data for foods other than cassava are needed, as are occurrence data for all foods from a broader range of countries around the world. Concentrations in foods as ready to consume would enable more accurate estimates of dietary exposure to be undertaken. Individual data points from analytical surveys would be of use to evaluate distributions of cyanogenic glycosides in foods and to define adequate sampling protocols. Distributions of occurrence data could then be used for probabilistic dietary exposure assessments.

More consumption data for cassava and cassava products from a broader range of countries would enable more detailed estimates of dietary exposure to be conducted or refined. More acute and chronic dietary exposure assessments from a broader range of countries, particularly African countries, would enable a better estimation of the global risk of dietary exposure to cyanogenic glycosides.

4.2 Fumonisins

Explanation

Fumonisins were previously evaluated by the fifty-sixth meeting of the Committee (Annex 1, reference *152*). The Committee established a group PMTDI for fumonisins B_1 (FB_1), B_2 (FB_2) and B_3 (FB_3), alone or in combination, of 2 μg/kg bw on the basis of the NOAEL of 0.2 mg/kg bw per day for kidney toxicity and application of an uncertainty factor of 100. All of the estimates of exposure to FB_1 based on the available data on national consumption were well below the group PMTDI, even when exposure estimates for FB_1 were increased by 40% to account for the presence of FB_2 and FB_3. For the current evaluation, the Committee reviewed all relevant studies performed on fumonisins since 2001.

The B series of the fumonisins, including FB_1 (CAS No. 116355-83-0), FB_2 (CAS No. 116355-84-1), FB_3 (CAS No. 136379-59-4) and FB_4 (CAS No. 136379-60-7), the major forms found in food, was described previously by the Committee. Since then, the number of known fumonisin analogues has greatly increased. The analogues can be classified into four main groups,

A, B, C and P, which contain two tricarballylic acid moieties. Members of the series FBX are different from these, because they are esterified by other carboxylic acids, such as *cis*-aconitic acid, oxalylsuccinic acid and oxalylfumaric acid. New fumonisins recently described have their 19- or 20-carbon aminopolyhydroxyalkyl chain esterified by fatty acids, such as palmitic acid, linoleic acid and oleic acid (estimated as 0.1% of the FB_1 concentration).

The FB_1 toxin has 10 chiral centres; theoretically, therefore, 1024 stereoisomers can be produced. From culture material, currently 28 FB_1 isomers have been isolated and characterized (2.8% of the possible fumonisins). The identification and absolute configuration of the stereoisomers 3-*epi*-FB_3 and 3-*epi*-FB_4 have been elucidated since the previous evaluation. The hydrolysis of the tricarballylic esters at C-14 and C-15 gives rise to partially hydrolysed fumonisin B (PHFB) or totally hydrolysed fumonisin B (HFB) in food.

Fusarium verticillioides and *F. proliferatum* are the main sources of fumonisins in maize, and both can produce series B and C analogues. *Fusarium proliferatum* can also produce series P analogues.

Recent occurrence data have shown that *Aspergillus niger*, a common fungus growing on grapes, green coffee beans, onions, mango, corn and other cereals, peanuts and dried fruits, is able to produce FB_1, FB_2, FB_4 and FB_6. Some strains of *Fusarium oxysporum*, a worldwide fungal contaminant, also produce fumonisin C analogues.

The term "hidden fumonisin" is used only for non-covalently bound derivatives, which are formed through an interaction between fumonisins and matrix macroconstituents or physical entrapment. "Bound fumonisin" refers only to those compounds that involve covalent linkages between the analyte and the matrix constituents. The linkages may involve the fumonisin free amino group or the carboxylic moieties. A few studies conducted with a limited number of samples show that substantial amounts of hidden or bound fumonisins may be present in raw maize, and commonly used analytical methods are not able to detect their occurrence.

Absorption, distribution, metabolism and excretion

In the previous evaluation, it was concluded that FB_1 and FB_2 were poorly absorbed from the digestive tract and mostly eliminated unchanged in the faeces. The small amount that was absorbed (<4%) was rapidly distributed and eliminated. Liver and kidney contained the highest levels of the absorbed material. It was also concluded that FB_1 does not cross the placenta, and, even after dosing of high levels to cows, no FB_1 was detected in the milk.

Since then, studies show that the bioavailability of FB_1 may be much greater than that of FB_2 and FB_3. Also, recent studies performed in pregnant mice

indicate that FB_1 can cross the placental barrier. In other studies, it was shown that FB_1 did not cross the blood–brain barrier. Bioavailabilities of hidden or bound fumonisins have not been studied.

In the previous evaluation, there was no evidence that FB_1 was metabolized in vitro or in vivo by animal tissues, although hydrolysis by intestinal flora has been demonstrated. Since then, HFB_1 and $PHFB_1$ have been reported in low concentrations in liver (<1 μg/kg) and many other tissues in pigs, but their origin is unclear. It was also shown that FB_1, FB_2 and FB_3 do not carry over in significant amounts from feed to animal products, either as parent compounds or as their hydrolysis products.

The previously described putative mode of action of fumonisins leading to toxicity—i.e. disruption of lipid metabolism—is supported by many recent studies. A new group of sphingoid bases has been discovered and shown to accumulate in livers of FB_1-treated mice. As indicated in the previous evaluation, animals exposed to FB_1 accumulate high levels of sphingoid bases and sphingoid base 1-phosphates in blood and tissues, often at levels of exposure that do not cause overt toxicity. In experimental animal studies, the extent of disruption of sphingolipid metabolism correlates well with toxicity and has been used successfully to demonstrate structure–activity relationships, tissue specificity, strain/sex susceptibility and the efficacy of intervention strategies in poultry, fish, rats, mice, pigs and horses. Hence, the elevation in levels of the sphingoid bases sphinganine (Sa) and sphingosine (So) and the ratio of Sa to So in serum and urine has been validated in numerous animal studies as biomarkers for fumonisin disruption of sphingolipid metabolism. However, the level of accumulation of bioactive lipid metabolites or depletion of lipids that constitutes a quantitative measure of adverse physiological effects is not known. Therefore, the Committee concluded that although disruption of lipid metabolism is an initial response to fumonisin exposure, it could not be used as a toxicological end-point for the final evaluation.

In humans, levels of the sphingoid bases Sa and So and their ratio Sa:So in serum, urine and buccal cells have been studied as potential biomarkers. The conflicting results of several studies on sphingoid bases indicate that levels of these bases and their ratio are not valid biomarkers of human fumonisin exposure. Other suggested biomarkers of exposure in humans were fumonisin levels in hair or urine. Fumonisin levels in hair were measured in one human study, but they were not compared with actual dietary fumonisin exposure, so currently this is also not a validated biomarker of fumonisin exposure. Urinary FB_1 level has emerged as a candidate for a human biomarker of fumonisin exposure, for several reasons. In pilot studies conducted in different parts of the world, urinary FB_1 levels correlated with imputed and actual dietary fumonisin levels. When an intervention was applied in

rural South Africa to reduce fumonisins in maize porridge, levels of urinary FB_1 decreased in a study population whose baseline and post-intervention biomarker levels were measured.

Toxicological data

At the time of the previous evaluation, only a few acute studies using FB_1 were available. From these, the Committee concluded that fumonisins were not acutely toxic. Since then, a study in male rats indicated that the single oral lethal dose of FB_1 would be greater than 46.4 mg/kg bw. Other studies showed that acute effects of FB_1 in rats are similar to those of longer exposures; that is, disruption of sphingolipid metabolism is an early response. Also, increased apoptosis of centrilobular hepatocytes and tubular epithelia in the outer medulla in kidney is an early indicator of FB_1 toxicity. In pigs, early signs of pulmonary porcine oedema were induced with a single oral administration of 5 mg/kg bw of pure FB_1. One gavage study in male rats indicated that the administration of a single dose of pure FB_1 at 0.005 mg/kg bw caused an increase in apoptotic cells in the liver. This dose is orders of magnitude lower than the doses found to increase apoptosis in studies of longer duration (e.g. >0.67 mg/kg bw per day in a 2-year study; *41*). This, together with a lack of clarity in other aspects of the study, led the Committee to consider it inappropriate to use this value in the evaluation without further studies supporting this result.

Since the last evaluation, there have been only two feeding studies examining dose–response relationships in mice using pure FB_1. The first study examined hepatotoxicity and found increased apoptosis and hypertrophy in centrilobular hepatocytes and other microscopic changes indicative of liver toxicity at 11.5 mg/kg bw per day in a 28-day study in female mice. In the second study, male mice (10 mice per group), heterozygous (p53+/−) or wild type (p53+/+), were fed diets containing pure FB_1 at 0, 5, 50 or 150 mg/kg diet, reported to be equal to 0, 0.4, 4 and 12 mg/kg bw per day, for 6 months. Light microscopic evaluation of the livers revealed that increased incidence of megalocytic hepatocytes was a dose-dependent effect observed at all dose levels greater than or equal to 0.4 mg/kg bw per day in both the wild-type and transgenic mice. Multifocal necrosis and increased apoptosis in centrilobular hepatocytes were treatment-related effects in both the medium- and high-dose (≥4 mg/kg bw per day) wild-type and transgenic treatment groups. The incidences of megalocytic hepatocytes, multifocal necrosis and increased apoptosis in centrilobular hepatocytes were not significantly different between the wild-type and transgenic treatment groups. The Committee concluded that both studies were suitable to be used in this evaluation.

Since the last evaluation, there have been three feeding studies using pure FB_1 in male rats. The Committee concluded that these studies were not suitable to be used in this evaluation, as one was conducted with a single high dose (>15 mg/kg bw), and effects were seen only at high doses (>10 mg/kg bw per day) in the other studies. Also, the measured end-points (altered lipid metabolism and reduced body weight gain) were not considered critical to the evaluation.

Two studies conducted in mice using diets prepared with *F. verticillioides* culture material were evaluated by the Committee, and it was concluded that they were not suitable to be used in this evaluation, as one was conducted with a single high dose (>45 mg/kg bw), and effects (altered lipid metabolism) were seen only at high doses (>7.5 mg/kg bw per day) in the other study.

There have been several recent studies conducted in male rats consuming diets prepared with *F. verticillioides* culture material. In two of these studies, renal toxicity was observed (primary effect increased tubular apoptosis in the outer medulla) at FB_1 doses lower than for pure FB_1, as seen in the previous evaluation. The Committee concluded that these studies were suitable for comparing the renal toxicity of FB_1 derived using *F. verticillioides* culture material diets with that of pure FB_1 in the diet. There were also a few studies conducted in rabbits and pigs using culture material in the diet. One study in rabbits showed dose–response effects on kidney and liver parameters (necrosis and other lesions). As the fumonisin content of the diets was uncertain, the Committee concluded that this precluded the use of the rabbit and pig studies for this evaluation.

There have been no new long-term feeding studies reported since the previous evaluation.

Previously, the Committee concluded that "neither fumonisin B_1 nor any other fumonisin was shown unequivocally to be genotoxic", based on a small number of studies in vitro and a single study in vivo. In studies reviewed for the present evaluation, no direct adducts of fumonisin with DNA have been found. The new data that are available generally support the previous conclusion that FB_1 is not mutagenic. The formation of 8-hydroxy-2′-deoxyguanosine adducts observed in new data that are available is indicative of oxidative damage caused by FB_1. This indicates that FB_1 induces effects, such as the formation of reactive oxygen species, that can lead to DNA damage.

In the previous evaluation, it was concluded that reproductive effects (embryotoxicity, skeletal and soft tissue malformations) were secondary to maternal toxicity and that ^{14}C-labelled FB_1 did not cross the placenta when

administered orally. Since then, a mouse model has been used to demonstrate that FB_1 can induce neural tube defects (NTDs) in vivo when administered (gestation days 7.5 and 8.5) either by gavage or by intraperitoneal exposure (dose range around 20 mg/kg bw per day). ^{14}C-labelled FB_1 was shown to enter the embryo when administered intraperitoneally early in organogenesis (gestation day 10.5), and this was confirmed by elevation of Sa levels in embryos. Maternal liver toxicity was not assessed; however, the gavage dose of FB_1 used in this study was similar to that known to induce maternal toxicity in CD-1 mice (25 mg/kg bw per day). Moreover, NTDs were induced in one feeding study conducted in mice, but a follow-up study including higher doses was unable to confirm these results. Studies in rabbits and pigs have shown significant effects on reproductive performance in animals consuming FB_1 in the diet at doses as low as 0.12 mg/kg bw per day; however, the Committee concluded that the uncertainty in the analysis of the diets prepared using *F. verticillioides* culture material precluded these studies from being used in this evaluation.

Two independent studies showed that pure HFB_1 did not cause maternal liver or kidney toxicity or embryotoxicity or induce NTDs in rats or mice at doses much higher than those at which FB_1 induced embryotoxicity in rats and NTDs in mice. This indicates that HFB_1 is not a risk factor for either embryotoxicity or NTDs.

Depression of specific and nonspecific immune responses and altered cytokine profiles were seen after exposure to either pure FB_1 or *F. verticillioides* culture in a variety of studies, mostly performed in pigs. In a pig study using *F. verticillioides* culture material in the diet, males were more sensitive than females to the immunomodulatory effects of FB_1; after subcutaneous exposure of mice to FB_1, females appeared more sensitive. In the last study, an immunostimulatory effect was found. There was one oral repeated-dose pig study performed with culture material derived from *F. moniliforme* (now named *F. verticillioides*), in which the authors concluded that there were no effects on immune response at doses up to 4 mg/kg bw per day; however, not all data were reported, and this study was therefore considered not suitable for the evaluation. Overall, the Committee considered the effects on the immune system to be relevant, as they were observed at low oral doses (i.e. 0.3 mg/kg bw per day for FB_1 as marker for culture material); however, they could not be used in the evaluation, as the relevant studies were performed using single doses.

Intracerebroventricular administration of FB_1 in mice caused severe neurodegenerative and behavioural changes that were not seen when mice were dosed subcutaneously. Dosing by subcutaneous administration caused slight elevation in sphinganine in the brain, but no neurodegenerative changes,

at doses of FB_1 causing large elevations in ALT and aspartate aminotransferase activities and other signs of marked toxicity. The Committee concluded that these sphinganine changes could not be definitively attributed to FB_1 crossing the blood–brain barrier. In pigs fed diets containing pure FB_1, changes in acetylcholinesterase activity in several brain regions were observed. The Committee concluded that, owing to the poorly defined dietary doses, discrepancies in the reported doses and lack of a clear dose–response relationship, these effects on acetylcholinesterase activity could not be attributed to FB_1 exposure. Overall, based on these studies, the Committee concluded that it was unlikely that fumonisins could cross the blood–brain barrier and induce neurotoxic effects in the brain.

Co-exposure to fumonisins and other mycotoxins

In a large number of in vitro and in vivo studies, the combined effects of fumonisins and other mycotoxins have been investigated. The studies show inconclusive and sometimes contradictory results. The effects of simultaneous exposure tend to be at most additive. In some in vitro and in vivo studies, the authors suggested that synergism or antagonism may occur, but often only single doses of each individual mycotoxin were used, and the Committee concluded that these study designs were inadequate to detect synergism. Overall, the available toxicity data on co-exposure were inadequate for their use by the Committee in its evaluation. The Committee concluded that because the fumonisins known to date do not share a similar mode of action with any other mycotoxins, it was unlikely that simple additive effects based on this mode of action would occur, although it recognized that other forms of interaction may occur. The Committee noted that co-exposure to aflatoxin B_1, a compound with known genotoxic properties, and fumonisins, which have the potential to induce regenerative cell proliferation, would be of concern.

In the previous evaluation, it was concluded that *F. verticillioides* culture material containing FB_1 and pure FB_1 were nephrotoxic in male rats. However, in the present evaluation, the nephrotoxicity of culture material was much greater than the nephrotoxicity of pure FB_1. For example, the dose of purified FB_1 that was required to cause renal tubular apoptosis in male rats was about 8 times higher than the dose of culture material containing FB_1 causing the same effect. Analysis of the culture material diets found that in addition to FB_1, FB_2, FB_3 and fumonisin–fructose adducts were present. Other fumonisins (e.g. B series, C series, A series) and unknown metabolites produced by *F. verticillioides* were also likely present in the culture material diet. Thermal processing of culture material used in diets produced fumonisin degradation products and promoted FB_1 binding to protein and carbohydrates. Thus, the Committee concluded that although FB_1 does not completely account for the

total toxic potential of fumonisins and other metabolites of *Fusarium* in the diet, it is a suitable marker for dietary exposure to mycotoxins in food and feed contaminated by *Fusarium*.

Observations in humans

All of the epidemiological studies conducted since the previous evaluation that have investigated the link between fumonisin exposure and oesophageal cancer are ecological studies: whole populations were characterized (e.g. a population with high oesophageal cancer incidence) rather than individuals, as in cohort or case–control studies, and maize samples consumed by those populations were tested for fumonisin levels. Nonetheless, the weight of evidence from these studies and the studies evaluated by the fifty-sixth meeting of the Committee is suggestive of an association between fumonisin exposure and oesophageal cancer. The association has been observed in multiple populations worldwide—the studies evaluated in this report were from China, the Islamic Republic of Iran and South Africa—although these populations have differing levels of other risk factors for oesophageal cancer (e.g. tobacco smoking and alcohol consumption). A dose–response relationship has not yet been established, nor has a toxicological mechanism been elucidated. To date, no study has shown that co-occurrence of fumonisin and aflatoxin exposures resulted in increased oesophageal cancer risk compared with fumonisin exposure alone.

Since the previous evaluation, there has been one epidemiological study on potential associations between fumonisin exposure and human immunodeficiency virus (HIV)–related mortality, one study on childhood stunting and one study on NTD incidence in human babies. As the HIV study did not include measurements of fumonisin levels in food or fumonisin exposure in humans, the Committee concluded that this study alone was insufficient to support an association between fumonisin exposure and HIV-related mortality.

The study investigating the link between fumonisin exposure and childhood stunting in the United Republic of Tanzania indicated that infants whose estimated fumonisin exposure exceeded the PMTDI of 2 μg/kg bw established at the fifty-sixth meeting of the Committee were significantly shorter and weighed less than those whose exposure was below this PMTDI. These results are congruent with toxicological studies in animals in which fumonisin exposure was associated with reduced weight gain and feed conversion efficiency.

The study of NTD incidence among Mexican Americans on the Texas/Mexico border, combined with toxicological and earlier epidemiological studies, indicates that fumonisin exposure in pregnant women may be a contributing factor to increased NTD risk in their babies.

Analytical methods

The Committee reviewed the screening and quantitative methods for the determination of FB_1, FB_2 and FB_3 in various foods made available after the fifty-sixth meeting. It was noted that several new immunoassays and antibodies have been developed, and many manufacturers continue to offer test kits based on enzyme-linked immunosorbent assay (ELISA) or immunochromatographic (lateral flow) devices. Immunoassays are inexpensive, rapid, portable and suitable for routine screening of samples, but have limitations in selectivity and reproducibility. Therefore, results obtained with immunoassays need to be confirmed with quantitative reference methods.

For a quantitative assay, the use of liquid chromatography (LC) with fluorescence detection after *o*-phthalaldehyde derivatization remains the method of choice and continues to be extensively used. Recent improvements in this method include automation of the online post-column derivatization with *o*-phthalaldehyde.

Significant advances in fumonisin analysis have been achieved by the application of LC methods coupled to MS detection systems, as well as the use of MS-based methods for the determination of multiple mycotoxins, including fumonisin. LC with tandem mass spectrometry (MS/MS) methods are now available for the determination of some fumonisins in a large number of matrices. Despite the increasing use of LC-MS or LC-MS/MS for multiple mycotoxin analysis, only one interlaboratory validation study is available for FB_1 and FB_2 in food.

Limitations to be considered for all analytical methods are the lack of suitable reference materials for method validation and the unavailability of standards, particularly for the hydrolysed fumonisins (HFB_1, HFB_2, HFB_3) and FB_6, as well as inadequate knowledge about reactions leading to hidden and bound fumonisins.

Most of the currently used analytical methods for fumonisins are unable to detect hidden or bound fumonisins in the matrix. Further studies are required to elaborate more appropriate methodologies for their determination. The most promising methods for hidden and bound fumonisin quantification include enzymatic or acid digestion, followed by alkaline hydrolysis.

Sampling protocols

The fifty-sixth meeting of the Committee noted that the sampling stage of mycotoxin analysis can represent the greatest contribution to the overall variance of the results. Specific sampling protocols, such as the one provided by the European Commission, which is used worldwide for control, does not cover all sampling necessities. Efforts have been devoted to improving the

reliability of the food-grade maize sampling. At the Fourth Session of CCCF in 2010 (*42*), sampling plans for whole (shelled) maize, corn on the cob and maize products such as maize flour, meal, grits and processed maize flour were proposed. For maize products, it was assumed that sampling variance for these commodities was similar to that associated with aflatoxin in comminuted feeds. However, recent data reviewed for this evaluation showed that the fumonisin distribution in foods and feeds is close to normal. The Committee concluded that further investigations of fumonisin distribution in different foods and feeds are needed to improve the sampling protocols for fumonisins.

Effects of processing

The effects of various processing procedures on the levels of FB_1, FB_2 and FB_3 contamination in cereals were reviewed during the fifty-sixth meeting of the Committee. New studies on extraction characteristics of fumonisins provide a better insight into the fate of fumonisins during sorting, cleaning, thermal processing (including extrusion), milling, fermentation and alkaline treatment.

Reduction of fumonisin levels during sorting and cleaning is dependent on the initial contamination level. The reduction of fumonisins in the wet milling process is due in part to the solubility of the toxins in the steep water. Further studies are required to analyse bound fumonisins during this process as well as to determine the fate of fumonisins and their reactions in heated food. Toxin distribution in dry milled products is dependent on the milling strategies in the plants, and the general distribution pattern is similar for different fractions. Alkaline treatment produces hydrolysed forms of fumonisins that could also be involved in binding with different components of the food matrix.

Prevention and control

Fusarium species are predominantly considered to be field fungi; however, it has been reported that fumonisin production can occur post-harvest when storage conditions are inadequate.

Significantly lower fumonisin levels have been demonstrated in transgenic *Bacillus thuringiensis* (Bt) maize, through reduction of insect pest damage and subsequent fungal infection, when compared with non-Bt isolines. In addition, new transgenic crops are being developed for detoxifying mycotoxins in the crops themselves. The use of antagonistic microorganisms has also received attention. Naturally occurring phenolic compounds, such as chlorophorin, vanillic acid, caffeic acid and maakianin, inhibit mould growth and reduce fumonisin production.

There has been increased interest in the use of natural pesticides to prevent damage to crops, such as the essential oils of menthol, oregano, thyme or clove, as well as natural anti-oxidants, to reduce fumonisin production. New uses of microorganisms to bind or degrade fumonisins are also available. Lactic and propionic acid bacteria bind fumonisins under acidic conditions, with non-viable bacteria showing higher binding capacity. Most of these are recent developments, and their commercial or large-scale applicability remains to be seen.

Levels and patterns of contamination in food commodities

Information on the natural occurrence of fumonisins was drawn from data received from a number of countries (Argentina, Australia, Brazil, Canada, China, Ghana, Japan, Republic of Korea, Singapore, United Republic of Tanzania, Uruguay, USA), results submitted by member states of the EU (Austria, Belgium, Cyprus, Czech Republic, Estonia, France, Germany, Hungary, Lithuania, Luxembourg, the Netherlands, Slovakia and Spain) through EFSA, as well as surveys published in the open literature from 47 countries.

In total, data on 15 755 samples analysed for FB_1 in food were collected, and the occurrence levels of total fumonisins were reported or calculated for 17 091 food samples.

All occurrence data were classified according to the food groups used in the GEMS/Food consumption cluster diets. Occurrence levels of fumonisins (32% and 26% of the samples for FB_1 and total fumonisins, respectively) from processed and not sufficiently described foods were categorized separately and not used to estimate chronic dietary exposure. Two scenarios were considered when calculating the mean values; samples in which the concentration was below the limit of quantification (LOQ) or limit of detection (LOD) were assumed to have a value of zero (lower-bound estimates) or the limit itself (upper-bound estimates).

In total, data on 10 354 samples analysed for FB_1 in food were used to estimate chronic dietary exposure (43% from the Americas, 34% from Asia, 12% from Africa, 10% from Europe and 0.1% from Oceania). More than three quarters of the samples considered (83%) referred to maize. Mean FB_1 levels in maize vary widely between and within the GEMS/Food clusters (Table 5). FB_1 was also detected in "figs, dried" and "groundnuts, shelled". Upper-bound estimates for the global total mean for FB_1 in all other commodities and GEMS/Food clusters did not exceed 100 μg/kg.

In total, occurrence levels of total fumonisins for 12 392 food samples (49% from the Americas, 27% from Asia, 13% from Europe, 11% from Africa and 0.1% from Oceania) were used to assess dietary exposure. The majority

Table 5
Levels of FB_1 in food commodities

Commodity	No. of individual samples	% sample < LOD or LOQ	Global total[a] mean (µg/kg)		Mean across GEMS/Food clusters (lower- and upper-bound estimates) (µg/kg)	
			Lower bound	Upper bound	Minimum	Maximum
Barley	175	82	35	44	0	212
Buckwheat	95	100	0	3	0	3
Figs, dried	230	25	238	250	238	250
Groundnuts, shelled	16	81	97	105	97	105
Maize	8569	30	1237	1260	84	4323
Millet	42	86	0	8	0	8
Oats	17	88	1	10	1	10
Rice	242	96	2	31	0	100
Sorghum	17	76	35	74	0	151
Soya bean (dry)	137	53	33	34	0	84
Sweet corn, kernels	740	66	84	94	0	397
Wheat	74	80	38	48	0	221

[a] Global total lower- and upper-bound mean obtained by pooling the data across all GEMS/Food clusters.

of the samples (85%) were for maize. Also in the case of total fumonisins, levels in maize vary widely between and within the GEMS/Food clusters (Table 6). Lower- and upper-bound estimates for the global total mean of total fumonisins in all other commodities and clusters did not exceed 150 and 200 µg/kg, respectively.

Most of the occurrence data were for FB_1, FB_2 or FB_3, with recent studies showing a few samples naturally contaminated with 3-*epi*-FB_3, FB_4 and FB_6.

From the data analysed for foods, the ratios among FB_1, FB_2 and FB_3 are not constant and depend on the fungal species prevalent in different regions of the world. Fumonisin ratios in food also depend on the process to which they are subjected, sometimes showing different ratios between the free fumonisins in corn, and these ratios tend to be normally distributed.

Extractable and hidden fumonisins in three food products showed almost a normal distribution; however, further studies should be done to confirm these results in order to develop improved sampling protocols.

Table 6
Levels of total fumonisins in food commodities

Commodity	No. of individual samples	Global total[a] mean (µg/kg)		Mean across GEMS/Food clusters (lower- and upper-bound estimates) (µg/kg)	
		Lower bound	Upper bound	Minimum	Maximum
Barley	123	51	102	0	340
Buckwheat	96	0	10	0	10
Maize	10 759	1651	1681	174	5921
Millet	42	0	18	0	18
Oats	26	1	23	0	200
Rice	207	1	59	0	104
Soya bean (dry)	106	6	11	0	30
Sweet corn, kernels	939	131	164	0	549
Wheat	94	0	29	0	200

[a] Global total lower- and upper-bound mean obtained by pooling the data across all GEMS/Food clusters.

The Committee was requested by CCCF to evaluate the co-occurrence, in food and feed, of fumonisins with other mycotoxins. However, this evaluation could not be performed by the Committee, because only aggregated data were available. Levels of fumonisins and other mycotoxins must be available at the level of the individual analytical sample for such an assessment to be conducted.

Levels and patterns of contamination in feed

Information on the natural occurrence of fumonisins in feed materials was drawn from data received from a number of countries (Brazil, China, Japan, Norway, South Africa and Uruguay), results submitted by member states of the EU through EFSA (Belgium, Estonia, France, Hungary, Lithuania, the Netherlands and Slovakia), one commercial feed supplier and published surveys.

Data on a total of 19 631 samples of cereals intended for feed production, silage and finished feed were considered. The ratios between levels of each fumonisin to the sum of fumonisins (FB_1 + FB_2 + FB_3) varied for different countries, mostly due to different fungal prevalence.

The log-normal distribution provides an adequate fit for FB_1 and total fumonisins based on the data provided by Japan.

Table 7
Levels of fumonisins (FB_1 + FB_2 + FB_3) in feed

Commodity	No. of individual samples	% sample < LOD or LOQ	Mean concentration (µg/kg)	
			Lower bound	Upper bound
Cereals	384	87	97	313
Corn gluten meal	18	—	3807	3807
Distillers' grains with solubles	78	23	825	883
Dried distillers' grains with solubles	185	14	1077	1110
Finished feed	2353	30	691	765
Maize	1927	24	1565	1625
Other feed	1391	75	339	524
Rice	20	95	38	275
Silage	248	79	184	383
Soya and soya bean products	362	93	52	283
Wheat	88	89	28	250

Data on 7060 samples originating from Africa (4.5%), America (13.3%), Asia (69.8%), Europe (4.5%) and Australia and New Zealand (7%) were selected for evaluation. These data were generated using similar methods of analysis as used for food. The global lower-bound and upper-bound mean values of the sum of FB_1, FB_2 and FB_3 for the more frequently contaminated feed materials were calculated (Table 7). Corn gluten meal, maize and dried grains plus dried soluble matter from distillers were the most contaminated, and the lower-bound mean values were found to be 3807 µg/kg, 1565 µg/kg and 1077 µg/kg, respectively.

Food consumption and dietary exposure assessment

Since the previous evaluation, a number of national evaluations of dietary exposure have been published. The Committee considered evaluations by Brazil, China, the EU (collectively), France, Guatemala, the Islamic Republic of Iran, Italy, the Netherlands, Portugal, the Republic of Korea, South Africa, Spain, the United Republic of Tanzania and the USA (Table 8). Unprocessed maize was often the only source of fumonisins considered, but in some of the studies, other cereals and cereal-based products were also taken into account. Most of these reports included dietary exposure estimates for FB_1 only (six studies), and total fumonisins were expressed as FB_1 + FB_2 and as FB_1 + FB_2 + FB_3 in six and seven evaluations, respectively. Most of the estimates were below 1 µg/kg bw per day for the general population, but

Table 8
National estimates of dietary exposure to fumonisins

Fumonisins	Country	Population groups	Total dietary exposure (μg/kg bw per day)	
			Mean/median	High percentile
FB_1	7 EU member states	Infants, children, adolescents and adults	0.12×10^{-3}–0.52	—
	China	Regional population	7.6[a]	33.3
	China	General population (TDS)	0.13	—
	Islamic Republic of Iran	Regional population	9×10^{-3}–0.712	—
	Netherlands	General population	0.03–0.15[a]	0.10–0.38
	Netherlands	Children aged 2–6 years	0.3[a]	1.0
	Portugal	General population	0.051	—
	USA	Regional pregnant women (case–control study)	0.156[a]–0.173[a]	—
FB_2	China	General population (TDS)	0.02	—
FB_3	China	General population (TDS)	0.01	—
$FB_1 + FB_2$	7 EU member states	Children, adolescents and adults	0.5×10^{-3}–0.35	—
	Brazil	General population	0.5–7.1	—
	Portugal	General population	0.065	—
	Republic of Korea	Adults	0.087×10^{-3}	—
	South Africa	Regional children, adults and elderly	3.03–14.14	5.96–27.9
	Spain	General population	0.0034–0.0041	—
	United Republic of Tanzania	Regional infants 6–8 months of age	0.47[a]	10.77–37
Total fumonisins	China	General population (TDS)	0.16	—
	France	Children, adults and vegetarians (TDS)	0.014–0.046	0.064–0.29[b]
	Guatemala[c]	Regional adults, women	2.2–10.6	10.6–44.8
	Italy	Infants and adults	0.005–0.020	—
	South Africa	Adults	1.1–8.5	12.0[d]

Table 8 (continued)

Fumonisins	Country	Population groups	Total dietary exposure (μg/kg bw per day)	
			Mean/median	High percentile
	Spain	Infants and adults	0.0017–0.72	—
	United Republic of Tanzania	Infants 6–8 months of age	0.48[a]	3.99

TDS, total diet study
[a] Median (all others are means).
[b] 95th percentile among vegetarians.
[c] The original total fumonisin exposure included FB_1, FB_2, FB_3, HFB_1, HFB_2 and HFB_3. The hydrolysed fumonisins accounted for approximately 50% of the total fumonisin exposure on a molar basis.
[d] Traditional home-brewed maize-based beer consumers only.

particularly high exposures to FB_1 (up to 7.6 and 33.3 μg/kg bw per day for consumers with average and high consumption, respectively) and total fumonisins (up to 10.6 and 44.8 μg/kg bw per day for consumers with average and high consumption, respectively) were registered in specific regions and population groups.

At the current meeting, the Committee prepared updated international estimates using the consumption cluster diets from GEMS/Food.

Individual data points on the concentration of the contaminant (for both FB_1 and total fumonisins) in foods from each cluster have been pooled to derive summary representative concentrations for each cluster for use in the dietary exposure calculations (see Tables 5 and 6 above). For each commodity, when concentration data were not available for a cluster, the global total lower- and upper-bound means, obtained by pooling the data across all clusters, were used to assess exposure. A standard body weight of 60 kg was used to assess exposure per kilogram body weight. Exposures estimated are mean exposures expressed in micrograms per kilogram body weight per day and are representative of chronic dietary exposures.

For the upper-bound scenario, the total dietary exposure to FB_1 was estimated to range from 0.3 μg/kg bw per day (cluster L) to 6.2 μg/kg bw per day (cluster A), and total dietary exposure to total fumonisins ranged from 0.4 μg/kg bw per day (clusters F and L) to 8.4 μg/kg bw per day (cluster A). No differences appeared under the lower-bound scenario.

In the previous evaluation, dietary exposure to FB_1 was evaluated by the Committee, assuming that all maize consumed contains FB_1 at the concentration found in 349 unprocessed maize samples from the Netherlands (1.4 mg/kg at the mean). Maize was the only source of FB_1 considered at that time. Using the then-available five regional diets from GEMS/Food, the total

dietary exposure to FB_1 was estimated to range from 0.2 μg/kg bw per day in the European-type diet to 2.4 μg/kg bw per day in the African diet. The Committee noted that the international exposure estimates obtained in the present evaluation were higher than those of the previous evaluation. This is mainly due to the fact that occurrence data from only one country were used at that time. It was therefore not possible to take into account the high within-cluster variability for the levels of FB_1 in maize as shown by the currently available information.

The contribution of maize to the total exposure to FB_1 and to total fumonisins ranged from 14% (cluster E, lower-bound scenario) to 98% (cluster A, lower-bound scenario) and from 18% (cluster E, upper-bound scenario) to 99% (cluster H, lower-bound scenario), respectively. Wheat was the main contributor to FB_1 exposure in clusters E (80% and 78% in lower-bound and upper-bound scenarios, respectively) and B (50% and 52% in lower-bound and upper-bound scenarios, respectively). The Committee noted that overall exposure in these clusters is relatively low (maximum 1.1 μg/kg bw per day in cluster E) and that levels of FB_1 in wheat are based on only 74 samples, 80% of them below the LOD or LOQ. No differences appeared between the upper- and lower-bound scenarios. Only under the upper-bound scenario did wheat contribute to the exposure to total fumonisins, with percentages ranging from 1% (clusters A, H and M) to 44% (cluster D).

The Committee concluded that, based on the national and international estimates, dietary exposure to FB_1 for the general population ranges from 0.12×10^{-3} to 7.6 μg/kg bw per day at the mean, whereas the 95th percentile exposure was estimated to be up to 33.3 μg/kg bw per day. Dietary exposure to total fumonisins for the general population would range, for a consumer with average consumption, from 0.087×10^{-3} to 14.14 μg/kg bw per day, whereas for consumers with high consumption, exposure was estimated to be up to 44.8 μg/kg bw per day. Maize is still the predominant source of exposure to FB_1 and total fumonisins.

Impact assessment of implementation of Codex MLs in maize

CCCF has proposed the establishment of MLs for fumonisins (FB_1 + FB_2) in maize and maize-based products. In order to evaluate the potential effect of these MLs on chronic dietary exposure, all occurrence data on total fumonisins (as reported or calculated) were categorized into the groups for which an ML has been proposed: "corn/maize grain, unprocessed" (ML = 5000 μg/kg), "corn/maize flour/meal" (ML = 2000 μg/kg), "popcorn grain" (ML = 2000 μg/kg), "maize-based baby food" (ML = 500 μg/kg) and "maize-based breakfast cereals, snacks and chips" (ML = 1000 μg/kg). An international dietary exposure assessment for total fumonisins was performed

Table 9
Effect of the implementation of the proposed Codex MLs in the proposed food categories on the rejection of samples per GEMS/Food cluster

Commodity	GEMS/Food cluster	Number of samples	% of rejected samples after the implementation of Codex MLs
Corn/maize grain, unprocessed	Cluster A	280	88
Corn/maize flour/meal	Cluster G	331	57
Corn/maize flour/meal	Cluster K	721	53
Corn/maize grain, unprocessed	Cluster D	172	28
Popcorn grain	Cluster K	70	17
Corn/maize flour/meal	Cluster B	554	16
Corn/maize grain, unprocessed	Cluster K	2296	12
Corn breakfast cereals and snacks	Cluster K	102	11
Corn/maize flour/meal	Cluster M	781	4
Corn/maize grain, unprocessed	Cluster J	337	2
Corn/maize grain, unprocessed	Cluster H	805	2
Corn/maize grain, unprocessed	Cluster M	1047	1

based on these MLs. For this, all samples for which the upper-bound mean concentration of total fumonisins exceeded its ML were excluded from the calculation.

The percentages of rejected samples after implementation of the proposed MLs are presented, by food category and cluster, in Table 9. MLs did not result in rejected samples in the majority of commodities and clusters. Overall, only 11% of the samples were excluded.

Rejection of samples was noted for "corn/maize flour/meal" in four clusters (from 4% to 57% rejected samples and from 39% to 89% reduction for the upper-bound mean), "corn/maize grain, unprocessed" in six clusters (from 1% to 88% rejected samples and from 7% to 70% reduction for the upper-bound mean), "maize-based breakfast cereals, snacks and chips" in one cluster (11% rejected samples and 30% reduction for the upper-bound mean) and "popcorn grain" in one cluster (17% rejected samples and 40% reduction for the upper-bound mean).

The effect of the implementation of the proposed Codex MLs on chronic dietary exposure to total fumonisins was evaluated by means of the GEMS/Food consumption cluster diets (Table 10). For the upper-bound scenario, reduction in exposure from all commodities occurred in nine clusters (from

Table 10
Effect of the implementation of a range of hypothetical MLs and the proposed Codex MLs on the international estimate of chronic dietary exposure to total fumonisins

ML[a] for corn/ maize grain, unprocessed (µg/kg)	ML[a] for corn/maize flour/meal (µg/kg)	Chronic dietary exposure (µg/kg bw per day) by GEMS/Food cluster												
		A	B	C	D	E	F	G	H	I	J	K	L	M
No limits	No limits	8.4	1.9	2.3	1.9	1.2	0.4	2.9	7.3	2.0	1.0	3.0	0.4	2.0
10 000	4000	8.4	1.9	2.3	1.6	1.2	0.4	1.3	7.3	2.0	1.0	2.9	0.4	1.8
10 000	2000	8.4	1.4	2.3	1.6	1.2	0.4	1.2	7.3	2.0	1.0	2.8	0.4	1.8
7000	3000	8.3	1.9	2.3	0.8	1.2	0.4	1.2	7.3	2.0	1.0	2.6	0.4	1.8
5000	2000	2.7	1.4	2.3	0.8	1.2	0.3	1.2	6.9	2.0	0.9	2.3	0.4	1.8
2500	1000	2.7	0.8	2.3	0.4	1.2	0.3	0.8	3.1	2.0	0.7	2.0	0.4	0.9
1000	500	1.4	0.5	1.5	0.3	1.1	0.2	0.6	3.0	1.1	0.5	0.8	0.3	0.9
500	250	0.4	0.4	1.5	0.3	1.1	0.2	0.5	2.2	1.0	0.5	0.5	0.3	0.6
5000	4000	2.7	1.9	2.3	0.8	1.2	0.3	1.3	6.9	2.0	0.9	2.4	0.4	1.8
5000	3000	2.7	1.9	2.3	0.8	1.2	0.3	1.2	6.9	2.0	0.9	2.3	0.4	1.8
5000	1000	2.7	1.4	2.3	0.8	1.2	0.3	1.2	6.9	2.0	0.9	2.3	0.4	1.8
5000	500	2.7	1.3	2.3	0.8	1.2	0.3	1.2	6.9	2.0	0.9	2.3	0.3	1.8
5000	250	2.7	1.3	2.3	0.8	1.2	0.3	1.2	6.9	2.0	0.9	2.4	0.3	2.3
7000	2000	8.3	1.4	2.3	0.8	1.2	0.4	1.2	7.3	2.0	1.0	2.6	0.4	1.8
2500	2000	2.7	0.8	2.3	0.4	1.2	0.3	0.9	3.1	2.0	0.7	2.0	0.4	0.9
1000	2000	1.4	0.8	1.5	0.3	1.1	0.3	0.8	3.1	1.1	0.5	1.3	0.4	0.9
500	2000	0.6	0.8	1.5	0.3	1.1	0.2	0.7	2.2	1.0	0.5	1.3	0.4	0.7

[a] Proposed MLs by CCCF: 5000 µg/kg for "corn/maize grain, unprocessed", 2000 µg/kg for "corn/maize flour/meal", 2000 µg/kg for "popcorn grain", 500 µg/kg for "maize-based baby food" and 1000 µg/kg for "maize-based breakfast cereals, snacks and chips".

Table 11
Effect of the implementation of a range of hypothetical MLs for "corn/maize grain, unprocessed" on the rejection of samples per GEMS/Food cluster

GEMS/Food cluster	Number of samples	% of rejected samples after the implementation of MLs					
		500 µg/kg	1000 µg/kg	2500 µg/kg	5000 µg/kg	7000 µg/kg	10 000 µg/kg
Cluster A	280	100	96	88	88	3	
Cluster B	300	31	31	31	—	—	—
Cluster C	20	100	100	—	—	—	—
Cluster D	172	51	51	40	28	28	6
Cluster E	96	58	58	—	—	—	—
Cluster F	0	—	—	—	—	—	—
Cluster G	431	60	49	27	—	—	—
Cluster H	805	90	31	31	2	—	—
Cluster I	572	29	22	—	—	—	—
Cluster J	337	27	27	9	2	—	—
Cluster K	2296	100	95	32	12	3	—
Cluster L	339	8	8	—	—	—	—
Cluster M	1047	88	33	33	1	1	1

6% to 68%), and the total dietary exposure to total fumonisins ranged from 0.3 µg/kg bw per day (cluster F) to 6.9 µg/kg bw per day (cluster H).

The Committee also evaluated the impact of a range of hypothetical MLs for the categories "corn/maize grain, unprocessed" (10 000, 7000, 5000, 2500, 1000 and 500 µg/kg) and "corn/maize flour/meal" (4000, 3000, 2000, 1000, 500 and 250 µg/kg) on the rejection of samples (Tables 11 and 12) and the chronic dietary exposure to total fumonisins (Table 10). Exposure estimates were obtained by using different combinations of MLs for the two above-mentioned food categories, whereas for the other food categories, the MLs proposed by CCCF were used.

No or little effect was noticed on the international exposure estimates resulting from the implementation of MLs higher than those proposed by CCCF. None of the evaluated MLs, including the one proposed by CCCF for the category "corn/maize flour/meal", produced a relevant reduction in exposure in all clusters. The exposure estimate in cluster M even increased, as a result of the rejection of samples with relatively low fumonisin levels within the GEMS/Food category "maize". MLs of 5000 and 2500 µg/kg for "corn/maize grain, unprocessed" reduced exposure estimates more than 50% in three and four clusters, respectively. The resulting exposure estimates were

Table 12
Effect of the implementation of a range of hypothetical MLs for "corn/maize flour/meal" on the rejection of samples per GEMS/Food cluster

GEMS/Food cluster	Number of samples	% of rejected samples after the implementation of MLs					
		250 µg/kg	500 µg/kg	1000 µg/kg	2000 µg/kg	3000 µg/kg	4000 µg/kg
Cluster A	0	—	—	—	—	—	—
Cluster B	554	63	42	16	16	—	—
Cluster C	0	—	—	—	—	—	—
Cluster D	0	—	—	—	—	—	—
Cluster E	70	53	53	—	—	—	—
Cluster F	0	—	—	—	—	—	—
Cluster G	331	85	85	85	57	57	43
Cluster H	3	100	100	—	—	—	—
Cluster I	101	9	9	—	—	—	—
Cluster J	0	—	—	—	—	—	—
Cluster K	721	87	85	76	53	32	11
Cluster L	878	15	13	4			
Cluster M	781	90	12	4	4	4	2

above 2 µg/kg bw per day in four, one and zero clusters based on hypothetical MLs for "corn/maize grain, unprocessed" at 2500, 1000 and 500 µg/kg, respectively.

Dose–response analysis

Four of the reviewed studies were identified as providing data suitable for BMD estimation. The first was a 28-day study performed with pure FB_1 in female mice, with the incidences of hepatic apoptosis and hypertrophy used as critical end-points (*43*). The second was a recent study (*44*) of the effects of pure FB_1 on male transgenic p53+/− and corresponding wild-type mice. Incidences of two lesions were modelled from this study: megalocytic hepatocytes and apoptosis, where the pathology scores of 1 and 2 were used as cut-off points for the megalocytic hepatocytes and apoptosis, respectively. The results for the transgenic and wild-type mice were pooled for dose–response analysis, as there were no differences in histopathological results between these strains of mice. The third study (*45*) examined the effects of 10-day oral exposure of male rats to *F. verticillioides* culture material in the diet. The results from day 5 and day 10 were pooled, as the results were similar. Renal toxicity was selected as an end-point, and this effect, as well as FB_1 levels in the kidney, reached a maximum for the high dose on day 5. The fourth study

Table 13
Ranges of BMD_{10} and $BMDL_{10}$ values for dietary exposure to purified FB_1

End-point and study	BMD_{10} (μg/kg bw per day)	$BMDL_{10}$ (μg/kg bw per day)
Rat renal toxicity (*41*)	431–602	286–356
Rat renal cell tumours (*41*)	1603–2118	1108–1692
Mouse hepatocyte apoptosis (*43*)	2053–8443	944–2064
Mouse hepatocyte hypertrophy (*43*)	1109–10 260	673–3939
Mouse megalocytic hepatocytes (*44*)	284–1675	165–1178
Mouse hepatocyte apoptosis (*44*)	969–3342	463–1216

Table 14
Ranges of BMD_{10} and $BMDL_{10}$ values for dietary exposure to *Fusarium* culture material using FB_1 as a marker

End-point and study	BMD_{10} (μg/kg bw per day)	$BMDL_{10}$ (μg/kg bw per day)
Rat renal toxicity 5–10 days (*45*)	134–778	62–208
Rat renal toxicity 3 weeks (*46*)	35–139	21–79
Rat renal toxicity 8 weeks (*46*)	47–76	17–47

(*46*) examined the effects of *F. verticillioides* culture material on male rats using FB_1 as a marker. The incidence of renal toxicity as a function of dose was modelled for exposure durations of 3 and 8 weeks. In order to compare the results with those of the previous evaluation, the NTP (*41*) study that served as its basis was also modelled. Two end-points were selected from this data set: renal toxicity and renal cell tumours in male rats.

Modelling was carried out using the USEPA's BMD software (BMDS version 2.1.2). The nine different dichotomous models provided by the program were fit to each of the data sets identified as critical. If exponential shape parameters were present, these were constrained to have values above 1. Those resulting in acceptable fits based on quantitative comparisons of each model were selected to derive BMD_{10} and $BMDL_{10}$ values for a BMR of 10% extra risk (see Tables 13 and 14). The lowest $BMDL_{10}$ for purified fumonisin, which came from the Bondy et al. (*44*) study, was 165 μg/kg bw per day. The lowest calculated $BMDL_{10}$ for FB_1 as a marker came from the Voss et al. (*46*) study and was 17 μg/kg bw per day.

Evaluation

Exposure to fumonisins has been associated with a wide range of effects, which are often species and sex specific. Laboratory studies have identified

the liver as the most sensitive organ in mice and the kidney as the most sensitive organ in rats.

Studies suitable for dose–response analysis have been conducted with rodents employing either purified FB_1 or *F. verticillioides* culture material containing FB_1. The latter studies typically use FB_1 as a marker for dietary exposure to the fumonisins and other metabolites of *Fusarium*. The studies employing purified FB_1 are generally better in experimental design for dose–response analysis. However, the Committee concluded that the studies with culture material were of sufficient quality to clearly indicate that other toxins produced by *F. verticillioides* either add to or potentiate the toxicity of FB_1. Although naturally contaminated corn would probably be more representative of actual human dietary exposure than either purified FB_1 or culture material, no suitable studies were identified that used naturally contaminated corn as a test material. As the implications are somewhat different, the Committee evaluated studies with purified FB_1 and *F. verticillioides* culture material separately.

For pure FB_1, the lowest identified $BMDL_{10}$ was 165 μg/kg bw per day for megalocytic hepatocytes in male mice. Using an uncertainty factor of 100 for intraspecies and interspecies variation, the Committee derived a PMTDI of 2 μg/kg bw. As this was the same value as the previously established group PMTDI, this group PMTDI, for FB_1, FB_2 and FB_3, alone or in combination, was retained.

For culture material, the lowest identified $BMDL_{10}$ using FB_1 as a marker was 17 μg/kg bw per day for renal toxicity in male rats. The Committee chose not to establish a health-based guidance value for culture material because its composition was not well characterized and may not be representative of natural contamination.

The Committee concluded that, based on the national and international estimates, dietary exposure to FB_1 for the general population ranges from 0.12×10^{-3} to 7.6 μg/kg bw per day at the mean, whereas the 95th percentile exposure was estimated to be up to 33.3 μg/kg bw per day. Dietary exposure to total fumonisins for the general population would range, for an average consumer, from 0.087×10^{-3} to 14.14 μg/kg bw per day, whereas for consumers with high consumption, exposure was estimated to be up to 44.8 μg/kg bw per day. Maize is still the predominant source of exposure to FB_1 and total fumonisins.

Comparison of these estimates with the group PMTDI indicates that the group PMTDI is exceeded at the population level in some regions within some countries. The Committee concluded that adverse effects from fumonisin exposure may occur and that reduction of exposure to fumonisin and

other toxins produced by *F. verticillioides* is highly desirable, particularly in areas of the world where maize is a major dietary staple food and where high contamination can occur.

As fumonisins do not carry over from feed to animal products in significant amounts, the occurrence of fumonisins in feed was considered not to be a human health concern.

The Committee concluded that implementation of the MLs proposed by CCCF could significantly reduce exposure (by more than 20%) to total fumonisins in six clusters (A, B, D, F, G, K). The main contribution to reduction was due to the proposed Codex ML for the category "corn/maize grain, unprocessed". The Committee noted that implementation of the proposed MLs would result in rejection of 1–88% of "corn/maize grain, unprocessed" and 4–57% of "corn/maize flour/meal" across the clusters. The Committee also noted that the national estimates of exposure to fumonisins show that the exceedance of the PMTDI occurs only in limited regions presenting high maize consumption levels and highly contaminated maize.

The Committee concluded that no or little effect on the international exposure estimates was noticed as a result of implementing MLs higher than those proposed by CCCF.

Research needs

To be able to fully assess the toxic potential of culture material or naturally contaminated food, characterization and quantification of their mycotoxin content are necessary.

To obtain a realistic representation of the effects of "real life" exposure, and in order to compare its toxic potential with the studies used for the final evaluation, naturally contaminated feed should be tested in dose–response studies in animals.

As hidden and bound fumonisins have been detected in corn and corn products, the Committee recommended that further studies be performed to elaborate more appropriate analytical methods to obtain additional occurrence data and information on the effects of processing.

As dietary exposure to fumonisins may occur together with exposure to other mycotoxins, such as aflatoxins, well-designed laboratory and epidemiological studies are needed to assess interactions.

For evaluation of the co-occurrence, in food and feed, of fumonisins with other mycotoxins, levels of fumonisins and other mycotoxins must be provided at the level of the individual analytical sample (i.e. not aggregate data).

Additional data on fumonisin distribution in corn food products should be collected in order to establish appropriate sampling procedures.

To validate the potential candidate urinary FB1 level for a human biomarker of short-term exposure, large-scale human studies that indicate a well-characterized dose–response relationship between urinary FB1 level and dietary fumonisin exposures are needed. A biomarker for long-term exposure is also needed.

To investigate the association of fumonisin exposure with oesophageal cancer risk, child growth impairment and NTDs in humans, studies on fumonisin exposure and incidence of these conditions in individuals (such as a cohort or case–control study) are needed. These studies should use a validated fumonisin exposure biomarker and control for confounders and for known risk factors.

5. Future work

Methods for analysis of propylene chlorohydrins

The GC-MS method introduced at the present meeting into the specifications monograph for hydroxypropyl methyl cellulose should be validated for use in the specifications monograph for hydroxypropyl cellulose, hydroxypropyl starch and hydroxypropyl distarch phosphate.

Modified starches

The Committee recommends revising the specifications for modified starches to replace the outdated GC methods using packed columns with suitable modern test methods.

6. Recommendations

Aluminium-containing food additives

Provisions for food additives containing aluminium included in the GSFA should be compatible with the revised PTWI for aluminium compounds of 2 mg/kg bw as aluminium from all sources.

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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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Annex 2

Tolerable or acceptable intakes, other toxicological information and information on specifications

Food additives evaluated toxicologically or assessed for dietary exposure

Food additive	Specifications[a]	Acceptable or tolerable daily intakes and other toxicological recommendations
Aluminium-containing food additives (including new food additives potassium aluminium silicate and potassium aluminium silicate–based pearlescent pigments)	N, T[b]	**The Committee established a provisional tolerable weekly intake (PTWI) of 2 mg/kg body weight** based on a no-observed-adverse-effect level (NOAEL) of 30 mg/kg body weight per day and application of an uncertainty factor of 100. The PTWI applies to all aluminium compounds in food, including food additives. **The previous PTWI of 1 mg/kg body weight was withdrawn.** For adults, the estimates of mean dietary exposure to aluminium-containing food additives from consumption of cereals and cereal-based products are up to the PTWI. Estimates of dietary exposure of children to aluminium-containing food additives, including high dietary exposures (e.g. 90th or 95th percentile), can exceed the PTWI by up to 2-fold. For potassium aluminium silicate–based pearlescent pigments at the maximum proposed use levels and using conservative estimates, anticipated dietary exposure at the highest range of estimates is 200 times higher than the PTWI. The Committee emphasized that whereas substances that have long half-lives and accumulate in the body are not generally considered suitable for use as food additives, consumption of aluminium-containing food additives would not be a health concern, provided that total dietary exposure to

Food additive	Specifications[a]	Acceptable or tolerable daily intakes and other toxicological recommendations
		aluminium is below the PTWI. The Committee recommended that provisions for food additives containing aluminium included in the Codex General Standard for Food Additives should be compatible with the revised PTWI for aluminium compounds of 2 mg/kg body weight as aluminium from all sources.
Benzoe Tonkinensis	N, T	**The Committee concluded that the available data were inadequate to establish an acceptable daily intake (ADI)** because of the variability in composition of Benzoe Tonkinensis and the inadequate characterization of the material tested. The margin of exposure between the conservative dietary exposure estimate of 0.2 mg/kg body weight per day and the NOAEL of 500 mg/kg body weight per day identified in a 90-day oral toxicity study in rats is 2500. Given this margin of exposure as well as the nature of the hepatic effects observed at doses above the NOAEL and the negative genotoxicity results, **the Committee concluded that Benzoe Tonkinensis would not pose a health concern at current estimated dietary exposures, provided that it complies with the tentative specifications prepared at the current meeting, when used as a flavouring agent and in accordance with good manufacturing practice.** The Committee also noted that exposure to benzoic acid and benzyl benzoate from the use of Benzoe Tonkinensis is well below the upper limit of the group ADI (0–5 mg/kg body weight) for benzyl derivatives, and exposure to vanillin is also well below the upper limit of its ADI (0–10 mg/kg body weight). The Committee further noted that benzoic acid, one of the major components of Benzoe Tonkinensis, is used as a preservative, but that Benzoe Tonkinensis has not been assessed for this use.
Glycerol ester of gum rosin (GEGR)	R, T	**The Committee withdrew the group ADI for GEGR and glycerol ester of wood rosin (GEWR) and established a temporary group ADI for GEGR and GEWR of 0–12.5 mg/kg body weight,** pending the submission of the full reports of the 90-day toxicity studies on GEGR as well as additional compositional information

Food additive	Specifications[a]	Acceptable or tolerable daily intakes and other toxicological recommendations
		on the GEWR from *Pinus elliottii*. The Committee noted that the temporary group ADI will be withdrawn if the requested information is not submitted by the end of 2012.
Glycerol ester of tall oil rosin (GETOR)	R, T	**The Committee was unable to complete the evaluation of GETOR** because additional data are required to characterize the GETOR in commerce. Validated methods for the determination of the substances considered in the specifications are also required. The above information should be submitted by the end of 2012.
Glycerol ester of wood rosin (GEWR)	R, T	**The Committee withdrew the group ADI for GEGR and GEWR and established a temporary group ADI for GEGR and GEWR of 0–12.5 mg/kg body weight**, applying an additional uncertainty factor of 2, because new information raises questions about the identity and composition of the product in commerce. Additional compositional information on the GEWR from *Pinus elliottii* to assess similarity with the GEWR from *Pinus palustris* is required. The Committee noted that the temporary group ADI will be withdrawn if the requested information is not submitted by the end of 2012.
Octenyl succinic acid (OSA) modified gum arabic	R	**The Committee deferred further evaluation of OSA modified gum arabic** pending the submission of data on its stability in food and on the extent to which it is hydrolysed in the gastrointestinal tract, to be provided by the end of 2013. **The existing temporary ADI "not specified"[c] was retained.**
Polydimethyl siloxane	M	**The Committee withdrew the temporary ADI of 0–0.8 mg/kg body weight and re-established the ADI of 0–1.5 mg/kg body weight,** originally established at the eighteenth meeting.
Ponceau 4R	R	**The Committee concluded that new data do not indicate a need to revise the existing ADI of 0–4 mg/kg body weight** and that dietary exposure to Ponceau 4R does not present a health concern.
Pullulan	R	Dietary exposure to pullulan as a dietary fibre could reach 1 g/kg body weight per day for children (2–5 years old) and 0.4 g/kg body weight per day for the general population (2 years of age

Food additive	Specifications[a]	Acceptable or tolerable daily intakes and other toxicological recommendations
		and older). These estimates are 8 and 20 times lower, respectively, than the no-observed-effect level (NOEL) observed in the 90-day rat study evaluated previously. Gastrointestinal effects observed in humans should be taken into account when considering appropriate use levels. The Committee stressed that it assessed the safety of use and not the efficacy of pullulan used as a dietary fibre. **The Committee maintained the previously established ADI "not specified"[c] for the previously evaluated food additive uses.**
Pullulanase from *Bacillus deramificans* expressed in *Bacillus licheniformis*	N	**The Committee established an ADI "not specified"[c] for pullulanase from *B. deramificans* expressed in *B. licheniformis*** when used in the applications specified and in accordance with good manufacturing practice.
Quinoline Yellow	R, T	**The Committee established a temporary ADI of 0–5 mg/kg body weight,** incorporating an additional 2-fold uncertainty factor, pending submission of requested toxicological studies by the end of 2013. **The previously established ADI of 0–10 mg/kg body weight was withdrawn.** The conservative exposure estimates were within the range of the temporary ADI. Additional information on the composition of the product in commerce is required, in particular relating to the identity and purity of the unmethylated form of Quinoline Yellow.
Sunset Yellow FCF	M	**The Committee established an ADI of 0–4 mg/kg body weight and withdrew the previous ADI of 0–2.5 mg/kg body weight.** The Committee concluded that dietary exposure to Sunset Yellow FCF does not present a health concern.

[a] M, existing specifications maintained; N, new specifications prepared; R, existing specifications revised; T, tentative specifications.

[b] For potassium aluminium silicate and pearlescent pigments containing potassium aluminium silicate.

[c] 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.

Food additives considered for specifications only

Food additive	Specifications[a]
β-Apo-8′-carotenal	R
β-Apo-8′-carotenoic acid ethyl ester	R
β-Carotene, synthetic	R
Hydroxypropyl methyl cellulose	R[b]
Magnesium silicate, synthetic	R
Modified starches	R
Nitrous oxide	R
Sodium carboxymethyl cellulose	R
Sucrose monoesters of lauric, palmitic or stearic acid	R

[a] R, existing specifications revised.
[b] The Committee concluded that levels of propylene chlorohydrins up to the new limit of not more than 1 mg/kg for the sum of both isomers in hydroxypropyl methyl cellulose were not of toxicological concern.

Analytical methods for food additives in the Combined Compendium of Food Additive Specifications, Volume 4 (FAO JECFA Monographs 1, 2006)

Food additive	Method[a]
Colouring matters content by spectrophotometry	R, T

[a] R, existing method revised; T, tentative method.

Contaminants evaluated toxicologically

Cyanogenic glycosides

The Third Session of the Codex Committee on Contaminants in Food (CCCF) in 2009 requested that JECFA reconsider the available data on cyanogenic glycosides, advise on the public health implications of cyanogenic glycosides and their derivatives in food and decide whether risk assessment is feasible and appropriate.

Reports of acute human poisoning associated with the consumption of foods containing cyanogenic glycosides were reviewed. The Committee therefore considered it appropriate to establish an acute reference dose (ARfD) for cyanogenic glycosides, expressed as cyanide equivalents. In addition, as there are a number of human diseases, specifically konzo, tropical ataxic neuropathy and iodine deficiency disorders, associated with the chronic consumption of underprocessed cassava as a staple food, it was recognized that the derivation of a chronic health-based guidance value would also be relevant.

Derivation of the ARfD

Following review of a developmental toxicity study with linamarin, the Committee considered this study as suitable for establishing an ARfD. Benchmark dose (BMD) modelling of the data from this study provided a lower limit on the benchmark dose for a 10% response ($BMDL_{10}$) for linamarin of 85 mg/kg body weight for increased skeletal defects in developing hamster fetuses following acute exposure of maternal animals. Although the study did not use dietary exposure, gavage dosing was considered relevant to establishing the ARfD.

Following application of a 100-fold uncertainty factor, the Committee established an ARfD for linamarin of 0.9 mg/kg body weight (equivalent to 0.09 mg/kg body weight as cyanide). This value was considered, when compared on a cyanide molar basis, to also be applicable to other cyanogenic glycosides. Therefore, the Committee recommended conversion of the ARfD for linamarin to a cyanide-equivalent dose of 0.09 mg/kg body weight. This cyanide-equivalent ARfD applies only to foods containing cyanogenic glycosides as the main source of cyanide.

Derivation of the provisional maximum tolerable daily intake (PMTDI)

In a 13-week United States National Toxicology Program study not previously evaluated by the Committee, in which exposure to sodium cyanide was continuous via drinking-water, a variety of effects related to male reproductive organs were observed—namely, decreased cauda epididymis weights, decreased testis weights and decreased testicular spermatid concentration. Dose–response analysis of continuous data on absolute cauda epididymis weights generated the lowest BMDL for a one standard deviation response ($BMDL_{1SD}$) of 1.9 mg/kg body weight per day. On the basis of this $BMDL_{1SD}$, the Committee established a PMTDI of 0.02 mg/kg body weight by applying a 100-fold uncertainty factor. The Committee decided that it was not necessary to apply an additional uncertainty factor to account for the absence of a long-term study, considering the generally acute nature of cyanide toxicity and the sensitivity of the effect (i.e. the reduction of absolute cauda epididymis weight).

Comparison of estimated dietary exposures with health-based guidance values and the impact of maximum limits (MLs) on dietary exposure

Estimated dietary exposures to total available hydrocyanic acid (HCN) were converted to cyanide equivalents and compared with the health-based guidance values established by the Committee at this meeting.

From the national acute dietary exposure estimates available to the Committee for review, the ARfD of 0.09 mg/kg body weight as cyanide equivalents

was exceeded 3-fold for cassava for adults (based on raw samples), less than 2-fold for apple juice for children, between 2- and 5-fold for bitter apricot kernels and up to 10-fold for ready-to-eat cassava chips/crisps, depending on the population group. If ready-to-eat cassava chips contained a level equivalent to the recently established ML in Australia and New Zealand of 10 mg/kg as HCN, there was only a marginal exceedance of the ARfD for children. These results are based on dietary exposure to total HCN, which represents the maximum possible exposure for foods containing cyanogenic glycosides.

Based on national estimates of chronic dietary exposure to total HCN, there is also the potential to exceed the PMTDI of 0.02 mg/kg body weight as cyanide for populations reliant on cassava as a staple food: between 1- and 3-fold for children and between 1- and 2-fold for adults. There is also a potential for those populations not reliant on cassava to exceed the PMTDI: between 1- and 5-fold for children and between 1- and 3-fold for adults. For Australia and New Zealand, ready-to-eat cassava chips were the major contributor to dietary exposure to HCN (84–93%). When the cassava chips contain a level equivalent to the ML of 10 mg/kg as HCN, all mean dietary exposures were below the PMTDI. High-percentile exposures for children were between 1- and 2-fold above the PMTDI. All chronic dietary exposure estimates based on exposures from flavouring agents did not exceed the PMTDI. These results are based on dietary exposure to total HCN, which is a worst-case scenario.

Application of the ML of 50 mg/kg as HCN for sweet cassava could result in dietary exposures that exceed the ARfD by less than 2-fold for the general population and up to 4-fold for children and exceed the PMTDI by between 2- and 10-fold, depending on the population group assessed. These estimates do not take into consideration any reduction in concentration of total HCN as a result of food preparation or processing. For the ML of 10 mg/kg as HCN for cassava flour, there are no estimates of dietary exposure available that exceed the ARfD or PMTDI. This is supported by the maximum amount of food that can be consumed based on existing Codex MLs before the health-based guidance values would be exceeded, which is as low as 25 g/day for cassava for chronic exposure. More detailed estimates of cassava and cassava flour consumption and concentrations in food for cassava-eating communities would help in supporting the conclusion that dietary exposures to total HCN could exceed health-based guidance values.

The ML for sweet cassava is for the raw product. If the starting level of HCN in the raw sweet cassava were 50 mg/kg as HCN, the minimum effective processing would result in a concentration of 15 mg/kg as HCN, and the most effective processing would give a HCN concentration of 2 mg/kg.

ARfD: 0.09 mg/kg body weight as cyanide (applies only to foods containing cyanogenic glycosides as the main source of cyanide)

PMTDI: 0.02 mg/kg body weight as cyanide

Fumonisins

For the current evaluation of fumonisins, the Committee reviewed all relevant studies performed on fumonisins since 2001.

Exposure to fumonisins has been associated with a wide range of effects, which are often species and sex specific. Laboratory studies have identified the liver as the most sensitive organ in mice and the kidney as the most sensitive organ in rats.

Studies suitable for dose–response analysis have been conducted with rodents either employing purified fumonisin B_1 (FB_1) or using *Fusarium verticillioides* culture material containing FB_1. The latter studies typically use FB_1 as a marker for dietary exposure to the fumonisins and other metabolites of *Fusarium*. The studies employing purified FB_1 are generally better in experimental design for dose–response analysis. However, the Committee concluded that the studies with culture material were of sufficient quality to clearly indicate that other toxins produced by *F. verticillioides* either add to or potentiate the toxicity of FB_1. Although naturally contaminated corn would probably be more representative of actual human dietary exposure than either purified FB_1 or culture material, no suitable studies were identified that used naturally contaminated corn as a test material. As the implications are somewhat different, the Committee evaluated studies with purified FB_1 and *F. verticillioides* culture material separately.

For pure FB_1, the lowest identified $BMDL_{10}$ was 165 μg/kg body weight per day for megalocytic hepatocytes in male mice. Using an uncertainty factor of 100 for intraspecies and interspecies variation, the Committee derived a PMTDI of 2 μg/kg body weight per day. As this was the same value as the previously established group PMTDI for FB_1, FB_2 and FB_3, alone or in combination, this group PMTDI was retained.

For culture material, the lowest identified $BMDL_{10}$ using FB_1 as a marker was 17 μg/kg body weight per day for renal toxicity in male rats. The Committee chose not to establish a health-based guidance value for culture material, because its composition was not well characterized and may not be representative of natural contamination.

The Committee concluded that, based on the national and international estimates, dietary exposure to FB_1 for the general population ranges from 0.12×10^{-3} to 7.6 μg/kg body weight per day at the mean, whereas the 95th

percentile exposure was estimated to be up to 33.3 μg/kg body weight per day. Dietary exposure to total fumonisins for the general population would range, for a consumer with average consumption, from 0.087×10^{-3} to 14.4 μg/kg body weight per day, whereas for consumers with high consumption, exposure would be up to 44.8 μg/kg body weight per day. Maize is still the predominant source of exposure to FB_1 and total fumonisins.

Comparison of these estimates with the group PMTDI indicates that the group PMTDI is exceeded at the population level in some regions within some countries. The Committee concluded that adverse effects from fumonisin exposure may occur and that reduction of exposure to fumonisin and other toxins produced by *F. verticillioides* is highly desirable, particularly in areas of the world where maize is a major dietary staple food and where high contamination can occur.

As fumonisins do not carry over from feed to animal products in significant amounts, the occurrence of fumonisins in feed was considered not to be a human health concern.

The Committee concluded that implementation of the MLs proposed by CCCF could significantly reduce exposure (by more than 20%) to total fumonisins in six Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) consumption clusters (A, B, D, F, G, K). The main contribution to reduction was due to the proposed Codex ML for the category "Corn/maize grain, unprocessed". The Committee noted that implementation of the proposed MLs would result in rejection of 1–88% of "Corn/maize grain, unprocessed" and 4–57% of "Corn/maize flour/meal" across the clusters. The Committee also noted that the national estimates of exposure to fumonisins show that the exceedance of the PMTDI occurs only in limited regions presenting high maize consumption levels and highly contaminated maize.

The Committee concluded that no or little effect was noticed on the international exposure estimates resulting from the implementation of MLs higher than those proposed by CCCF.

Group PMTDI for FB_1, FB_2 and FB_3, alone or in combination, of 2 μg/kg body weight was retained

Annex 3

Further information required or desired

Aluminium-containing food additives

There is a need for convincing data to demonstrate that aluminium is not bioavailable from potassium aluminium silicate–based pearlescent pigments.

No data were available to identify the forms of aluminium present in soya-based formula and their bioavailability. Such studies were requested at the sixty-seventh meeting and are still required.

In the case of potassium aluminium silicate, information is required on preparation and purification methods, particle size distribution, methods of identification for silicate and aluminium, data on the levels of the inorganic impurities, the suitability of an inductively coupled plasma atomic emission spectrometry (ICP-AES) method for the determination of inorganic impurities, and the suitability of a proposed method based on alkali fusion followed by ICP-AES for the assay for potassium aluminium silicate based on the determination of aluminium.

In the case of potassium aluminium silicate–based pearlescent pigments, information is required on their manufacture, stability in food, particle size distribution, pH range, methods for the identification of iron, titanium and aluminium, data on the levels of the inorganic impurities, a filtration method appropriate for the small particle sizes associated with the pigments, and the suitability of a proposed method based on alkali fusion followed by ICP-AES for the assay for titanium, iron and aluminium.

The requested information should be made available by the end of 2012.

Benzoe Tonkinensis

The Committee requested additional information regarding the complete composition of the ethanolic extract, data on microbiological contaminants and data on inorganic contaminants (lead, arsenic, antimony, chromium,

mercury and cadmium). The Committee also requested an analytical method to distinguish between Benzoe Tonkinensis and Benzoe Sumatranus.

Cyanogenic glycosides

Further research is needed to more accurately quantify how nutritional factors ultimately contribute to the human diseases observed in populations whose diets consist mainly of improperly processed cassava, which involves high cyanide exposure.

There is a need for more extensive occurrence data for cyanogenic glycosides. These include data showing the ratio of cyanogenic glycosides to cyanohydrins to HCN in raw and processed versions of a range of foods containing cyanogenic glycosides. More occurrence data for foods other than cassava are needed, as are occurrence data for all foods from a broader range of countries around the world. Concentrations in foods as ready to consume would enable more accurate estimates of dietary exposure to be undertaken. Individual data points from analytical surveys would be of use to evaluate distributions of cyanogenic glycosides in foods and to define adequate sampling protocols. Distributions of occurrence data could then be used for probabilistic dietary exposure assessments.

More consumption data for cassava and cassava products from a broader range of countries would enable more detailed estimates of dietary exposure to be conducted or refined. More estimates of acute and chronic dietary exposures from a broader range of countries, particularly African countries, would enable a better estimation of the global risk of dietary exposure to cyanogenic glycosides.

Fumonisins

To be able to fully assess the toxic potential of culture material or naturally contaminated food, characterization and quantification of its mycotoxin content are necessary

To obtain a realistic representation of the effects of “real life” exposure and in order to compare the toxic potential of naturally contaminated feed with the findings in the studies used for the final evaluation, naturally contaminated feed should be tested in dose–response studies in animals.

As hidden and bound fumonisins have been detected in corn and corn products, further studies should be performed to elaborate more appropriate analytical methods in order to obtain additional occurrence data and information on the effects of processing.

As dietary exposure to fumonisins may occur with other mycotoxins, such as aflatoxins, well-designed laboratory and epidemiological studies are needed to assess interactions.

For the evaluation of co-occurrence, in food and feed, of fumonisins with other mycotoxins, concentrations of fumonisins and other mycotoxins must be provided at the level of the individual analytical sample.

Additional data on fumonisin distribution in corn food products should be collected in order to establish appropriate sampling procedures.

To validate urinary FB_1 as a potential candidate for a human biomarker of short-term exposure, large-scale human studies that indicate a well-characterized dose–response relationship between urinary FB_1 and dietary fumonisin exposures are needed. A biomarker for long-term exposure is also needed.

To investigate the association of fumonisin exposure with oesophageal cancer risk, child growth impairment and neural tube defects in humans, studies on fumonisin exposure and the incidence of these conditions in individuals (such as a cohort or case–control study) are needed. These studies should use a validated fumonisin exposure biomarker and control for confounders and for known risk factors.

Glycerol ester of gum rosin (GEGR)

The requested full reports of the unpublished 90-day oral toxicity studies were not provided, and the validity of evaluating GEGR on the basis of toxicological data on glycerol ester of wood rosin (GEWR) still requires confirmation. To complete the evaluation of GEGR, the unpublished studies are required as well as additional data 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. The information is required by the end of 2012.

Glycerol ester of tall oil rosin (GETOR)

To complete the evaluation of GETOR, additional data are required to characterize the GETOR in commerce in relation to the composition of 1) the refined tall oil rosin used as the source rosin, 2) the glycerol ester of tall oil 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. The above data are required by the end of 2012.

Glycerol ester of wood rosin (GEWR)

To complete the evaluation of GEWR, additional data are required to characterize the GEWR in commerce in relation to the composition of 1) the refined wood rosin used as the source rosin for the production of GEWR, 2) the glycerol ester of wood 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.

Method for colouring matters content by spectroscopy (Volume 4)

Data on the wavelength of maximum absorbance, absorptivity and/or specific absorbance are requested for the following colours: Allura Red AC, Amaranth, Azorubine, Brilliant Black PN, Brilliant Blue FCF, Brown HT, Erythrosine, Fast Green FCF, Fast Red E, Green S, Indigotine, Patent Blue V, Ponceau 4R, Quinoline Yellow, Red 2G, Sunset Yellow FCF and Tartrazine. The data to be provided should also indicate the solvents used as well as any standardization for pH in order to allow for the establishment of consensus values for the wavelength of maximum absorbance, absorptivity and/or specific absorbance.

Octenyl succinic acid (OSA) modified gum arabic

The Committee requested that data resolving the concern about the stability of OSA modified gum arabic in food as well as data on the extent to which OSA modified gum arabic is hydrolysed in the gastrointestinal tract be provided by the end of 2013.

Quinoline Yellow

The Committee is aware of unpublished long-term studies in mice and rats with in utero exposure to Quinoline Yellow that had been completed by Biodynamics Laboratories in 1980–1981 but had not been submitted for evaluation and which might affect the acceptable daily intake (ADI). These studies are requested by the end of 2013. The specifications are tentative pending submission of information regarding the principal components, maximum wavelengths for absorption, organic impurities, the level of zinc and a method of assay.

This report represents the conclusions of a Joint FAO/WHO Expert Committee convened to evaluate the safety of various food additives, with a view to recommending acceptable daily intakes (ADIs) and to preparing specifications for identity and purity. The Committee also evaluated the risk posed by two food contaminants, with the aim of deriving tolerable intakes where appropriate and advising on risk management options for the purpose of public health protection.

The first part of the report contains a general discussion of the principles governing the toxicological evaluation of and assessment of dietary exposure to food additives and contaminants. A summary follows of the Committee's evaluations of technical, toxicological and dietary exposure data for certain food additives (aluminium-containing food additives, Benzoe Tonkinensis, glycerol ester of gum rosin, glycerol ester of tall oil rosin, glycerol ester of wood rosin, octenyl succinic acid modified gum arabic, polydimethyl siloxane, Ponceau 4R, pullulan, pullulanase from *Bacillus deramificans* expressed in *Bacillus licheniformis*, Quinoline Yellow and Sunset Yellow FCF) and two food contaminants (cyanogenic glycosides and fumonisins).

Specifications for the following food additives were revised: aluminium lakes of colouring matters; β-apo-8'-carotenal; β-apo-8'-carotenoic acid ethyl ester; β-carotene, synthetic; hydroxypropyl methyl cellulose; magnesium silicate, synthetic; modified starches; nitrous oxide; sodium carboxymethyl cellulose; and sucrose monoesters of lauric, palmitic or stearic acid.

Annexed to the report are tables summarizing the Committee's recommendations for dietary exposures to and toxicological evaluations of the food additives and contaminants considered.

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