Food and Agriculture Organization of the United Nations

World Health Organization

JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES
Eighty-third meeting
Rome, 8–17 November 2016

SUMMARY AND CONCLUSIONS

Issued 23 November 2016

A meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) was held in Rome, Italy, from 8 to 17 November 2016. The purpose of the meeting was to evaluate certain contaminants in food.

Dr R. Cantrill, American Oil Chemists' Society, United States of America, served as Chairperson, and Dr D. Benford, Food Standards Agency, United Kingdom, served as Vice-Chairperson.

Dr M. Lipp, Agriculture and Consumer Protection Department, Food and Agriculture Organization of the United Nations, and Dr A. Tritscher, Department of Food Safety and Zoonoses, World Health Organization, served as Joint Secretaries.

The present meeting was the eighty-third in a series of similar meetings. The tasks before the Committee were (a) to elaborate principles governing the evaluation of contaminants in food; (b) to undertake toxicological evaluations and dietary exposure assessments for six contaminants or groups of contaminants in food; and (c) to undertake toxicological evaluations and dietary exposure assessments in relation to co-exposure to two groups of contaminants in food.

The report of the meeting will be published in the WHO Technical Report Series. Its presentation will be similar to that of previous reports – namely, general considerations, comments on specific contaminants or groups of contaminants, and future work and recommendations. An annex will include a summary (similar to the summary in this report) of the main conclusions of the Committee in terms of provisional maximum tolerable daily intakes and other toxicological and safety recommendations.

The participants in the meeting are listed in Annex 1. Items of a general nature that the Committee would like to disseminate quickly are included in Annex 2. Future work and recommendations are listed in Annex 3.

Toxicological and dietary exposure monographs on the contaminants or groups of contaminants considered will be published in WHO Food Additives Series No. 74.

More information on the work of JECFA is available at:

http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/en/

and

http://www.who.int/foodsafety/areas_work/chemical-risks/jecfa/en/

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Evaluations of contaminants

Aflatoxins

Aspergillus flavus is a fungus that was first recognized to cause aflatoxicosis in domestic animals and is the most important aflatoxin-producing species in food on a global basis. It produces aflatoxin B_1 (AFB_1) and aflatoxin B_2 (AFB_2) and affects many commodities, but most human exposure comes from contaminated corn (also referred to as maize), peanuts (also referred to as groundnuts) and rice. Another important producer of aflatoxin, *A. parasiticus*, produces AFB_1, AFB_2, aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2) and is primarily associated with peanuts in the Americas, but can also occur on corn, figs and pistachios. Of these four aflatoxins, AFB_1 is most frequently present in contaminated samples; AFB_2, AFG_1 and AFG_2 are generally not reported in the absence of AFB_1. Aflatoxin M_1 (AFM_1) is the hydroxylated metabolite of AFB_1; in areas of high aflatoxin exposure, humans are exposed to AFM_1 more or less exclusively through milk and milk products, including breast milk.

The aflatoxins were previously evaluated by JECFA at its thirty-first, forty-sixth, forty-ninth, fifty-sixth and sixty-eighth meetings. The Committee updated the aflatoxin risk assessment at the current meeting at the request of the Codex Committee on Contaminants in Foods (CCCF).

The Committee reaffirmed the conclusions of the forty-ninth meeting of JECFA that aflatoxins are among the most potent mutagenic and carcinogenic substances known, based on studies in test species and human epidemiological studies, and that hepatitis B virus (HBV) infection is a critical contributor to the potency of aflatoxins in inducing liver cancer. The more recent information about human polymorphisms in metabolizing enzymes (e.g. cytochrome P450s, sulfotransferases) has described population variability in the balance between activation and detoxification processes for aflatoxins. This knowledge has been used in conjunction with biomarkers to evaluate the effectiveness of pharmacological and dietary interventions with the aim of reducing cancer risk.

Increased reporting and identification of acute aflatoxicosis outbreaks, particularly in areas of Africa, led this Committee to consider the available data on acute exposure. Indeed, loss of lives attributed to aflatoxins was most recently reported in the United Republic of Tanzania during the summer of 2016. Ranges of AFB_1 exposures between 20 and 120 µg/kg body weight (bw) per day for a period of 1–3 weeks or consumption of staple food containing concentrations of 1 mg/kg or higher would be suspected to cause acute aflatoxicosis and possibly death. The Committee did not assess acute dietary exposure, but noted that the estimates of chronic dietary exposure are at least 2–5 orders of magnitude lower than the doses associated with acute effects.

Since the forty-ninth meeting of the Committee, epidemiological data have become available to support the hypothesis that aflatoxin exposure in utero and during early life has negative effects on growth; in particular, decreased height is the most frequently associated anthropometric parameter. The available data did not provide evidence for an exposure level at which there is a significant risk for growth faltering.

The Committee considered that the development of analytical technologies based on aptamers may have relevance in remote areas, because of their inherent stability, ease of production and use.

The Committee noted that there were limited contamination data from developing countries, which hindered a more comprehensive and global evaluation of aflatoxin occurrence and may have resulted in an underestimate of dietary exposure in these countries.

Only five food commodities (maize, peanuts, rice, sorghum and wheat) each contribute more than 10% to international dietary exposure estimates for more than one Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) cluster diets, for either total aflatoxins (AFT) or AFB_1. The Committee noted that international dietary exposure estimates (AFT and AFB_1) were generally higher than those reported at the sixty-eighth meeting. This was predominantly due to the availability of concentration data for rice, sorghum and wheat and their inclusion in the international dietary exposure estimates. Although overall concentrations of aflatoxins in rice and wheat are lower than concentrations in maize and groundnuts (a traditional focus for aflatoxin risk management), the high consumption of rice and wheat in some countries means that these cereals may account for up to 80% of dietary aflatoxin exposure for those GEMS/Food cluster diets. Mean AFB_1 concentrations in sorghum from the GEMS/Food contaminants database are higher than those for maize; combined with high consumption levels of sorghum in some GEMS/Food clusters, this cereal contributes 16–59% of dietary exposure in six GEMS/Food clusters. The database on sorghum is considerably more limited than that on maize.

The Committee calculated global aflatoxin-related hepatocellular carcinoma (HCC) risk based on the new central and upper-bound cancer potency estimates from the current dose–response analysis and international dietary exposures estimated at the current meeting. Aflatoxin-related cancer rates were calculated, accounting for prevalence of chronic hepatitis B virus surface antigen (HBsAg) positivity, by GEMS/Food clusters. The low end of the range refers to lower-bound estimates at the mean dietary AFB_1 exposure, minimum HBsAg+ rates for countries in the cluster and the central cancer potency estimate. The high end of the range refers to upper-bound estimates at the 90th percentile of dietary AFB_1 exposure, maximum HBsAg+ rates for countries in the cluster and upper-bound estimates of cancer potency. The lowest cancer risks were estimated for clusters G07 and G08 (European and other developed countries), with cancer risk estimates in the range <0.01–0.10 aflatoxin-induced cancers per year per 100 000 population, with wheat being the major contributing food commodity. For countries within these clusters, HBsAg+ rates were in the range 0.01–1.2%. The highest cancer risks were for cluster G13 (sub-Saharan African countries and Haiti), with cancer risk estimates in the range 0.21–3.94 aflatoxin-induced cancers per year per 100 000 population, with sorghum and maize being the major contributing food commodities. For countries within this cluster, HBsAg+ rates were in the range 5.2–19%. Other clusters with relatively high cancer risks were G03 (sub-Saharan African countries and Paraguay, with maize and sorghum being the major contributing food commodities), G05 (mainly Central and South American countries, with maize, rice, sorghum and wheat being the major contributing food commodities) and G16 (sub-Saharan African countries, with maize and sorghum being the major contributing food commodities). The Committee noted that the aflatoxin-related HCC risk rates calculated here are within the range of aflatoxin-related foodborne disease (HCC) incidence published by WHO.

The Committee notes that a common background cancer rate was used in the cancer potency estimates. A sensitivity analysis showed that changing the background cancer rates has minimal impact on the analysis.

Given the relative cancer potencies and international dietary exposure estimates for AFB_1 and AFM_1, AFM_1 will generally make a negligible (<1%) contribution to aflatoxin-induced cancer risk for the general population.

On request of the CCCF, the Committee performed an impact assessment of different MLs for ready-to-eat peanuts and concluded that enforcing a maximum limit (ML) of 10, 8 or 4 µg/kg for ready-to-eat peanuts would have little further impact on dietary exposure to AFT for the general population, compared with setting an ML of 15 µg/kg. At an ML of 4 µg/kg, the proportion of the world market of ready-to-eat peanuts rejected would be approximately double the proportion rejected at an ML of 15 µg/kg (about 20% versus 10%).

Diacetoxyscirpenol

4,15-Diacetoxyscirpenol (4,15-DAS; (3α,4β)-3-hydroxy-12,13-epoxytricothec-9-ene-4,15-diyl diacetate; Chemical Abstracts Service [CAS] number 2270-40-8; $C_{19}H_{26}O_7$; molecular weight 366.4 Da) or anguidine is a trichothecene mycotoxin. All trichothecenes have the same core 12,13-epoxytrichothec-9-ene structure, and trichothecene analogues have different patterns of substitution around this core structure. 4,15-DAS is a type A trichothecene, with similar structure to T-2 toxin and HT-2 toxin. Both T-2 toxin and HT-2 toxin have an ester function at the C-8 position, whereas HT-2 toxin additionally has a hydroxyl group on the C-4 position.

4,15-DAS has not previously been evaluated by JECFA. The structurally related type A trichothecenes T-2 toxin and HT-2 toxin were evaluated by JECFA at the forty-seventh meeting. The Committee evaluated 4,15-DAS at the present meeting in response to a request from CCCF.

The Committee concluded that there are insufficient toxicological data available to derive a point of departure for the risk assessment of 4,15-DAS alone. There are limitations in the available short-term toxicity studies and no data from chronic exposure and reproductive and developmental toxicity studies.

4,15-DAS and T-2/HT-2 toxin are structurally similar, and there is evidence that they cause similar effects at the biochemical and cellular levels, have similarities in toxic effects in vivo and have an additive dose effect when co-exposure occurs. Therefore, the evidence was considered sufficient by the Committee to support including 4,15-DAS in the group provisional maximum tolerable daily intake (PMTDI) for T-2 and HT-2 toxin established at the forty-seventh JECFA meeting. The PMTDI of 0.06 µg/kg bw for T-2 and HT-2 toxin, alone or in combination, was established based on a lowest-observed-adverse-effect level (LOAEL) of 0.03 mg/kg bw per day associated with changes in white blood cell counts following 3 weeks of dietary exposure in pigs and the application of an uncertainty

factor of 500. The inclusion of 4,15-DAS in the group PMTDI of 0.06 µg/kg bw is considered to be a conservative approach when taking into consideration the observation that T-2 toxin was consistently more potent than 4,15-DAS when comparing similar in vitro and in vivo end-points.

The Committee noted that there is a paucity of occurrence data and what data were available to the Committee frequently were left censored, thereby increasing the uncertainty in the exposure assessment.

The Committee also noted that the very high degree of censorship in the concentration data set and the relatively high limits of quantification (LOQs) for 4,15-DAS have a considerable influence on the results and therefore provide substantial uncertainty in the dietary exposure estimates.

In the 2001 JECFA evaluation, the total dietary exposure to T-2 and HT-2 toxins was estimated only from the GEMS/Food European diet due to the fact that data on these toxins were not available from regions other than Europe. The total lower-bound (LB) mean dietary exposure to T-2 plus HT-2 toxins was estimated to be 16.3 ng/kg bw per day, with wheat, barley and oats being the major dietary sources.

The Committee noted that only LB dietary exposure estimates for Europe were available for the sum of T-2, HT-2 and 4,15-DAS. From these estimates, the sum of the LB dietary exposure estimates for 4,15-DAS of up to 0.0028 µg/kg bw per day and the total dietary exposures estimated for T-2 plus HT-2 of 0.016 µg/kg bw per day results in a LB mean dietary exposure of 0.019 and in a LB high dietary exposure estimated at 0.038 µg/kg bw per day (twice the mean). It was not possible to estimate the upper-bound (UB) dietary co-exposure because of the lack of UB data reported for T-2 and HT-2 toxins in the previous 2001 JECFA evaluation together with the substantial uncertainty that is reported for UB estimates of dietary exposure to 4,15-DAS. The Committee concluded that these LB estimates for Europe do not exceed the group PMTDI for T-2, HT-2 and 4,15-DAS.

Fumonisins

Fumonisins are common contaminants of maize that are produced by *Fusarium verticillioides* (formerly *F. moniliforme*), *F. proliferatum* and *F. fujikuroi*, as well as some less common *Fusarium* species, such as *F. anthophilum*, *F. dlamini*, *F. napiforme* and *F. thapsinum*. Fumonisin B_2 (FB_2) and fumonisin B_4 (FB_4) are also produced by *Aspergillus niger*.

Fumonisins were evaluated by JECFA for the first time at the fifty-sixth meeting and then re-evaluated at the seventy-fourth meeting. At the seventy-fourth meeting, the Committee used a short-term dose–response study of liver toxicity in male transgenic mice fed diets containing purified fumonisin B_1 (FB_1) to derive a group PMTDI for FB_1, FB_2 and fumonisin B_3 (FB_3), alone or in combination, of 2 µg/kg bw on the basis of a lower 95% confidence limit on the benchmark dose for a 10% response ($BMDL_{10}$) of 0.165 mg/kg bw per day and an uncertainty factor of 100. Because the derived PMTDI at the seventy-fourth meeting of JECFA was the same as the group PMTDI established at the fifty-sixth meeting of JECFA, based on renal toxicity in a 90-day rat study, the group PMTDI for fumonisins B_1, B_2 and B_3, alone or in combination, was retained at the seventy-fourth meeting.

Fumonisins were evaluated by the present Committee in response to a request from CCCF for an updated exposure assessment. The Committee also evaluated toxicological and epidemiological studies that had become available since the previous evaluation in 2011.

The Committee reaffirmed the conclusions of the seventy-fourth meeting that fumonisins are associated with a wide range of toxic effects and that the liver and kidney are the most sensitive target organs. The Committee reviewed the studies that have become available since the 2011 evaluation and concluded that the study by Bondy et al. (2010),[1] subsequently published as Bondy et al. (2012),[2] remained the most relevant for the evaluation. The Committee evaluated the updated Bondy et al. (2012) data and concluded that they would not change the overall toxicological assessment performed previously by the Committee. Thus, the previously established group PMTDI of 2 µg/kg bw for FB_1, FB_2 and FB_3, alone or in combination, was retained by the current Committee.

[1] Bondy GS, Mehta R, Caldwell D, Coady L, Armstrong C, Savard M et al. (2010). Effects of long term exposure to FB_1 on p53+/– transgenic mice. Ottawa: Health Canada, Health Products and Food Branch, Food Directorate, Bureau of Chemical Safety, Toxicology Research Division (unpublished).

[2] Bondy GS, Mehta R, Caldwell D, Coady L, Armstrong C, Savard M et al. (2012). Effects of long term exposure to the mycotoxin FB_1 in p53 heterozygous and p53 homozygous transgenic mice. Food Chem Toxicol. 50:3604–13.

The Committee noted the paucity of new data on the occurrence of fumonisins in food submitted to the GEMS/Food contaminants database since 2011 by all WHO regions except for Europe, as opposed to the data used in the previous evaluation (2011). Owing to these differences in the data sets between 2011 and the current evaluation, a direct comparison was not possible.

The Committee noted that there are limited data on the occurrence of bound fumonisins in different cereals, the impact of processing on these bound mycotoxins and their bioavailability after consumption.

LB mean and high (90th percentile) chronic FB_1 exposures in adults were maximally 0.56 and 1.1 µg/kg bw per day, respectively. For total fumonisins, the corresponding exposure estimates were 0.82 and 1.6 µg/kg bw per day. The UB mean and high exposures were estimated to be as high as 1.2 and 2.3 µg/kg bw per day for FB_1, respectively, and as high as 2.1 and 4.3 µg/kg bw per day for total fumonisins, respectively. In children, the LB mean and high chronic FB_1 exposures were maximally 0.8 and 1.6 µg/kg bw per day, respectively, and for total fumonisins, maximally 1.2 and 2.3 µg/kg bw per day, respectively. In this population group, the UB mean and high exposures were estimated to be as high as 1.6 and 3.9 µg/kg bw per day for FB_1, respectively, and as high as 3.2 and 6.4 µg/kg bw per day for total fumonisins, respectively. Maize is the predominant source of LB exposure to FB_1 and total fumonisins in most cluster diets. In the UB scenario, wheat was also an important contributor to the exposure to fumonisins in some clusters.

Comparison of the estimates of exposure to FB_1 and total fumonisins with the group PMTDI indicates no exceedance at the LB mean exposure level in both children and adults. Assuming that all non-detect samples contained fumonisin at the LOQ, the UB mean exposure to total fumonisins in children exceeded the PMTDI in several countries. This was also true for the high (90th percentile) exposure, independent of the fumonisin concentration assigned to the non-detect samples. For adults, only the UB high exposure exceeded the PMTDI. The Committee noted that, due to the high percentage of non-detect samples in the concentration database (around 70%) and the wide range of LOQs reported in the GEMS/Food contaminants database for fumonisins, the UB estimates may be interpreted as a worst-case estimate of exposure based on the data available.

The Committee noted that the international exposure estimates for FB_1 and total fumonisins were lower than those estimated by the Committee at its seventy-fourth meeting in 2011. In the current assessment, a larger part of the occurrence data was from countries belonging to the WHO European Region compared with 2011, resulting in lower overall fumonisin levels in maize. In the current assessment, no information on fumonisin levels in maize was available from countries belonging to the African, Eastern Mediterranean or South-East Asia regions, where higher fumonisin concentrations are typically detected. Given these limitations of the occurrence data used in the exposure assessment and high exposures reported in the literature in some countries, it is likely that the exposures to fumonisins in areas where maize is a staple food and high contamination with fumonisins can occur are higher than those estimated by the Committee at this meeting, as can be seen in the previous evaluation, which was based on a larger and more representative data set.

Glycidyl esters

Glycidyl esters are processing-induced contaminants primarily found in refined fats and oils and foods containing fats and oils. Initial research related to glycidyl esters was largely performed as part of the investigation into 3-monochloro-1,2-propanediol (3-MCPD) esters. During 3-MCPD ester analysis, variable 3-MCPD concentrations were obtained, leading to a proposal that additional compounds were present in edible oils and converted to 3-MCPD during sample analysis. The presence of additional processing-induced contaminants, glycidyl esters, in refined edible oils was later confirmed. Initially it was assumed that 3-MCPD esters and glycidyl esters were formed by similar processes, but it is now known that their mechanisms of formation are different, with glycidyl ester formation directly associated with elevated temperatures (>240 °C) and time at these elevated temperatures. Glycidyl esters are generally formed from diacylglycerols, with no requirement for the presence of chlorinated compounds. Formation of glycidyl esters occurs following intramolecular rearrangement, elimination of a fatty acid and epoxide formation.

Glycidyl esters have not been evaluated previously by the Committee. The present evaluation was conducted in response to a request from CCCF.

Experimental evidence indicates that glycidyl esters are substantially hydrolysed to glycidol in the gastrointestinal tract and elicit toxicity as glycidol. The Committee therefore based its evaluation on the conservative assumption of complete hydrolysis of glycidyl esters to glycidol. Whereas the experimental data supporting substantial hydrolysis are derived from studies with post-weaning

animals, the Committee concluded that the capacity of the neonate to hydrolyse fatty acids in the gut is efficient, and therefore the same assumption of substantial hydrolysis could be extended to this age group.

The Committee concluded that glycidol is a genotoxic compound and considered its carcinogenicity as the most sensitive end-point on which to base a point of departure. The lowest $BMDL_{10}$ was 2.4 mg/kg bw per day for mesotheliomas in the tunica vaginalis/peritoneum in male rats observed in the NTP (1990)[1] carcinogenicity study (doses adjusted for non-continuous dosing; with quantal linear, gamma, Weibull and multistage 2 degree models giving the same result).

The Committee noted that there are no published collaboratively studied methods for the determination of glycidyl esters in complex foods in contrast to the situation with fats and oils; therefore, caution should be applied when interpreting analytical data from complex foods.

The Committee further noted that there was uncertainty in comparing the reported levels in the same foods from different regions because of the lack of interlaboratory comparisons and the absence of data arising from proficiency testing schemes.

As it is not appropriate to establish a health-based guidance value for substances that are both genotoxic and carcinogenic, the margin of exposure approach is chosen.

National estimates of dietary exposure were used for determining the margins of exposure. This was because they were considered to be the most representative of dietary exposure as they are based on consumption data from national dietary surveys. The majority of the surveys used include 2 or more days of data, which better estimate chronic dietary exposure.

The national dietary exposures are considered to be reliable estimates, as they are based on a range of foods in the diet and include the key foods in which glycidol contamination is known to occur – namely, fats and oils. The concentrations in specific foods in the majority of cases have been able to be matched directly with consumption data for the same foods.

The Committee considered that the lower ends of the ranges of the margins of exposure for infants, children and adults (Table 1) were low for a compound that is genotoxic and carcinogenic and that they may indicate a human health concern.

Table 1
Dietary exposures and margins of exposure compared with the $BMDL_{10}$

Population group	Range of estimated dietary exposures to glycidol (µg/kg bw per day)[a]		Margins of exposure[b]	
	Mean	High percentile	Mean	High percentile
Adults	0.1–0.3	0.2–0.8	8 000–24 000	3 000–12 000
Children	0.2–1.0	0.4–2.1	2 400–12 000	1 100–6 000
Infants	0.1–3.6	0.3–4.9	670–24 000	490–8 000

[a] Includes LB and UB estimates from a range of national estimates of dietary exposure.
[b] Compared with a $BMDL_{10}$ of 2.4 mg/kg bw per day. Margins of exposure are expressed as a range; the lower end of the range relates to UB mean and high-percentile exposures, and the higher end of the range relates to LB mean and high-percentile exposures.

3-MCPD esters

3-Monochloro-1,2-propanediol (3-MCPD) esters are processing-induced contaminants found in various refined oils and fats and are formed from acylglycerols in the presence of chlorinated compounds during deodorization at high temperature. “3-MCPD esters” is a general term for 3-MCPD esterified with one (sn1- and sn2-monoesters) or two identical or different fatty acids (diesters). Depending on the fatty acid composition of the oil or fat, a variety of different 3-MCPD esters can be formed during processing. In foods that contain refined vegetable oils or fats, mainly diesters are found. Concentrations of 3-MCPD esters in refined oils increase incrementally in the following order: rapeseed oil < soya bean oil < sunflower oil < safflower oil < walnut oil < palm oil.

[1] NTP (1990). National Toxicology Program (NTP) technical report on the toxicology and carcinogenesis studies of glycidol (CAS no. 556-52-5) in F344/N rats and B6C3F1 mice (gavage studies). Research Triangle Park (NC): National Toxicology Program (NTP Technical Report 374).

3-MCPD esters have not been previously evaluated by the Committee. The present evaluation was conducted in response to a request from CCCF for an evaluation of 3-MCPD esters. 3-MCPD has been evaluated at the forty-first, fifty-seventh and sixty-seventh meetings of JECFA. At the sixty-seventh meeting, the Committee reaffirmed a PMTDI for 3-MCPD of 2 µg/kg bw, based on a lowest-observed-effect level (LOEL) of 1.1 mg/kg bw per day for tubule hyperplasia in the kidney seen in a long-term carcinogenicity study in rats. An uncertainty factor of 500 was applied to allow for the absence of a clear no-observed-effect level (NOEL) and to account for the effects on male fertility and inadequacies in the studies of reproductive toxicity.

Experimental evidence indicates that 3-MCPD esters are substantially hydrolysed to 3-MCPD in the gastrointestinal tract and elicit toxicity as free 3-MCPD. The Committee therefore based its evaluation on the conservative assumption of complete hydrolysis of 3-MCPD esters to 3-MCPD. Whereas the experimental data supporting substantial hydrolysis are derived from studies with post-weaning animals, the Committee concluded that the capacity of the neonate to hydrolyse fatty acids in the gut is efficient, and therefore the same assumption of substantial hydrolysis could be extended to this age group.

The main target organs for 3-MCPD and its esters in rats and for 3-MCPD in mice are the kidneys and the male reproductive organs. 3-MCPD was carcinogenic in two rat strains, but not in mice. No genotoxic potential has been demonstrated in vivo for 3-MCPD. Two long-term carcinogenicity studies with 3-MCPD in rats[1] were identified as pivotal studies, and renal tubular hyperplasia was identified as the most sensitive end-point. The lowest $BMDL_{10}$ (restricted log-logistic model) for renal tubular hyperplasia was calculated to be 0.87 mg/kg bw per day for male rats. After application of a 200-fold uncertainty factor, the Committee established a group PMTDI of 4 µg/kg bw for 3-MCPD and 3-MCPD esters singly or in combination (expressed as 3-MCPD equivalents) (rounded to one significant figure). The overall uncertainty factor of 200 incorporates a factor of 2 related to the inadequacies in the studies of reproductive toxicity.

The previous PMTDI of 2 µg/kg bw for 3-MCPD, established at the fifty-seventh meeting and retained at the sixty-seventh meeting, was withdrawn.

The Committee noted that there are no published collaboratively studied methods for the determination of 3-MCPD esters in complex foods in contrast to the situation with fats and oils; therefore, caution should be applied when interpreting analytical data from complex foods.

The Committee further noted that there was uncertainty in comparing the reported levels in the same foods from different regions because of the lack of interlaboratory comparisons and the absence of data arising from proficiency testing schemes.

The Committee noted that estimated dietary exposures to 3-MCPD for the general population, even for high consumers (up to 3.8 µg/kg bw per day), did not exceed the new PMTDI. Estimates of mean dietary exposure to 3-MCPD for formula-fed infants, however, could exceed the PMTDI by up to 2.5-fold for certain countries (e.g. 10 µg/kg bw per day in the first month of life).

While the current evaluation was specific to the request for an evaluation of 3-MCPD esters, the Committee was aware that 2-MCPD esters can be detected in some of the same foods as 3-MCPD esters. There are, however, currently limited food occurrence data for 2-MCPD and 2-MCPD esters available in the GEMS/Food contaminants database, and the toxicological database is currently insufficient to allow a hazard characterization.

Sterigmatocystin

Sterigmatocystin is a toxic fungal secondary metabolite (mycotoxin) that has been reliably reported to be produced by many phylogenetically and phenotypically different fungal genera, including more than two dozen species each of *Aspergillus* and *Emercella* and one or more species of *Bipolaris*, *Botryotrichum*, *Chaetomium* (*Botryotrichum*, *Humicola*), *Moelleriella*, *Monocillium*, *Moelleriella* (*Aschersonia*), *Podospora* and a unique species of *Penicillium*, *P. inflatum*, closely related to *A.*

[1] Sunahara G, Perrin I, Marchesini M (1993). Carcinogenicity study on 3-monochloropropane-1,2-diol (3-MCPD) administered in drinking water to Fischer 344 rats. Unpublished report no. RE-SR93003 submitted to WHO by Nestec Ltd, Research & Development, Switzerland.

Cho WS, Han BS, Nam KT, Park K, Choi M, Kim SH et al. (2008). Carcinogenicity study of 3-monochloropropane-1,2-diol in Sprague-Dawley rats. Food Chem Toxicol. 46:3172–7.

tardus. The anamorphic names in parentheses are no longer in use. Sterigmatocystin is a polyketide-derived mycotoxin with CAS No. 10048-13-2 and International Union of Pure and Applied Chemistry (IUPAC) name (3a*R*,12c*S*)-8-hydroxy-6-methoxy-3a,12c-dihydro-7*H*-furo[3′,2′:4,5]furo[2,3-*c*]xanthen-7-one.

Sterigmatocystin has not previously been reviewed by JECFA. The Committee evaluated sterigmatocystin at the present meeting at the request of CCCF.

Taking account of the available information on genotoxicity, carcinogenicity and DNA adduct formation, the Committee concluded that sterigmatocystin is genotoxic and carcinogenic, and the critical effect was determined to be carcinogenicity. The Committee selected the $BMDL_{10}$ of 0.16 mg/kg bw per day for hepatic haemangiosarcoma in male rats in a study by Maekawa et al. (1979)[1] from the restricted log-logistic model as the point of departure for use in the risk assessment.

As it is not appropriate to establish a health-based guidance value for substances that are genotoxic carcinogens, the Committee used a margin of exposure approach. The Committee noted that there is a paucity of occurrence data and what data were available to the Committee frequently were left censored, thereby increasing the uncertainty in the exposure assessment.

The Committee calculated margins of exposure for mean and high estimates of dietary exposure to sterigmatocystin. The margins of exposure for adults range from 9400 to more than 530 000 for mean estimates based on UB and LB assumptions. For high estimates, margins of exposure for adults range from 4700 to 270 000. The lowest margins of exposure are observed for the African Region (from 4700 to 5000 for the high exposure UB–LB range, and from 9400 to 10 000 for the mean exposure UB–LB range). The Committee noted that these estimates, which are based only on adult populations and for which only one food commodity (sorghum) was considered, may indicate a human health concern. Margins of exposure were not calculated for Europe or Japan, as sterigmatocystin was not detected in any samples. For all other regions, the Committee considered that the margins of exposure were not of human health concern even at the high UB exposure.

Overall, the Committee concluded that the data used for calculating the margins of exposure have considerable limitations, both for the dietary exposure estimate and for the toxicological point of departure. Limited data on occurrence in food were available, and analytical detection limits were high in some countries. The only long-term carcinogenicity study suitable for dose–response modelling used an uncommon strain of rat (ACI/N) and, in view of the low incidence of liver tumours in this animal model, it may not be the most appropriate for human risk assessment. Consequently, the derived margins of exposure should be considered only as crude estimates.

The Committee also noted that sterigmatocystin and AFB_1 have the same main target organ (the liver). The comparative animal data on carcinogenicity are very limited, but indicate that sterigmatocystin is less potent than AFB_1.

Co-exposure of fumonisins with aflatoxins

Fumonisins and aflatoxins are mycotoxins produced by fungi of *Fusarium* and *Aspergillus* species. Aflatoxins were previously evaluated by JECFA at its thirty-first, forty-sixth, forty-ninth, fifty-sixth and sixty-eighth meetings. At the thirty-first meeting, the Committee considered aflatoxins to be a potential human carcinogen and urged that dietary exposure to aflatoxins be reduced to the lowest practicable levels (no PMTDI was established). At the subsequent meetings, the Committee evaluated the potency of AFB_1 for liver cancer and analysed the human cancer risk with certain hypothetical MLs for maize, groundnuts, milk, tree nuts and dried figs.

Fumonisins were first evaluated by JECFA at the fifty-sixth meeting and then re-evaluated at the seventy-fourth meeting. At the fifty-sixth meeting, the Committee derived a group PMTDI for FB_1, FB_2 and FB_3, alone or in combination, of 2 µg/kg bw. At the seventy-fourth meeting of JECFA, the group PMTDI for the same fumonisins, alone or in combination, was retained.

Considering that fumonisins and aflatoxins are both frequent contaminants in cereal (especially maize, rice, sorghum and wheat) and cereal-based foods and that aflatoxins are common contaminants in groundnuts and tree nuts, co-exposure to both mycotoxins is likely in areas where these foods are consumed as part of the routine diet.

As part of the evaluation of fumonisins at the seventy-fourth meeting, the Committee evaluated the available data on the concurrent exposure to fumonisins and other mycotoxins. There

[1] Maekawa A, Kajiwara T, Odashima S, Kurata H (1979). Hepatic changes in male ACI/N rats on low dietary levels of sterigmatocystin. Gann. 70:777–81.

were no human studies available showing co-exposure. There were co-exposure toxicological studies available using animal models. None of the co-exposure studies in animal models was considered adequate for use in the Committee's evaluation for fumonisins; the Committee noted that the interaction between AFB_1, a compound with known genotoxic and hepatocarcinogenic properties, and fumonisins, which have the potential to induce regenerative cell proliferation in the liver, would be of concern. The Committee has not performed a full evaluation for the co-exposure of fumonisins and aflatoxins previously.

At the current meeting, the Committee evaluated updated toxicological and exposure data for fumonisins and aflatoxins separately (see above). At the request of CCCF, the Committee also evaluated co-exposure to aflatoxins and fumonisins.

From the international estimates of dietary exposure, two GEMS/Food clusters (G05 and G13) have high dietary exposure to both AFB_1 and FB_1. The countries (Guatemala and the United Republic of Tanzania) where co-exposure has been confirmed using urinary or plasma exposure biomarkers of FB_1 and AFB_1 belong to these two clusters.

Although evidence in laboratory animals from the previous and the present evaluations has suggested an additive or synergistic effect of fumonisin and aflatoxin co-exposure in the development of preneoplastic lesions or hepatocellular carcinoma, currently no data are available on such effects in humans.

Two prospective epidemiological studies do not support the hypothesis of an interaction between aflatoxins and fumonisins in childhood stunting.

The Committee concluded that there are few data available to support co-exposure as a contributing factor in human disease. However, the interaction between AFB_1, a compound with known genotoxic properties, and fumonisins, which have the potential to induce regenerative cell proliferation (particularly at exposures above the PMTDI), remains a concern. This is due to the fact that the incidences of chronic liver disease and stunting are high in the areas of the world where the exposures to both mycotoxins are high and the co-exposure has been confirmed with biomarkers.

Annex 1

Eighty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives[1]

Rome, 8–17 November 2016

Members

Professor J. Alexander, Norwegian Institute of Public Health, Oslo, Norway
Dr S. Barlow, Brighton, East Sussex, United Kingdom
Dr D. Benford, Risk Assessment Unit, Food Standards Agency, London, United Kingdom (*Vice-Chairperson*)
Dr M. Bolger, Annapolis, MD, USA
Dr R. Cantrill, American Oil Chemists' Society, Urbana, IL, USA (*Chairperson*)
Mr P. Cressey, Institute of Environmental Science and Research Ltd (ESR), Christchurch, New Zealand
Dr M. De Nijs, RIKILT Wageningen University & Research, Wageningen, the Netherlands (*Co-Rapporteur*)
Professor S. Edwards, Harper Adams University, Newport, Shropshire, United Kingdom
Mr M. Feeley, Bureau of Chemical Safety, Food Directorate, Health Canada, Ottawa, Ontario, Canada
Dr U. Mueller, Food Standards Australia New Zealand, Canberra, ACT, Australia (*Co-Rapporteur*)
Dr G.S. Shephard, Institute of Biomedical and Microbial Biotechnology, Cape Peninsula University of Technology, Bellville, South Africa

Secretariat

Professor G.O. Adegoke, Department of Food Technology, University of Ibadan, Ibadan, Nigeria (*FAO Expert*)
Professor K.E. Aidoo, Department of Life Sciences, Glasgow Caledonian University, Glasgow, United Kingdom (*FAO Expert*)
Dr N. Arnich, French Agency for Food, Environmental and Occupational Health and Safety (Anses), Maisons-Alfort, France (*WHO Expert*)
Dr D. Bhatnagar, Agricultural Research Service, United States Department of Agriculture, New Orleans, Louisiana, USA (*FAO Expert*)
Dr P. Boon, Centre for Nutrition, Prevention and Health Services, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands (*FAO Expert*)
Dr G. Brisco,* Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy (*Codex Secretariat*)
Dr C. Carrington, Gaithersburg, Maryland, USA (*WHO Expert*)
Dr D.R. Doerge, National Center for Toxicological Research, United States Food and Drug Administration, Jefferson, Arkansas, USA (*WHO Expert*)
Dr L. Edler, German Cancer Research Center, Heidelberg, Germany (*WHO Expert*)
Ms B. Engeli, Federal Food Safety and Veterinary Office (FSVO), Bern, Switzerland (*WHO Expert*)
Dr V. Fattori, Agriculture and Consumer Protection Department, Food and Agriculture Organization of the United Nations, Rome, Italy (*FAO Secretariat*)
Ms Z. Gillespie, Bureau of Chemical Safety, Food Directorate, Health Canada, Ottawa, Ontario, Canada (*WHO Expert*)
Ms T. Hambridge, Food Data Analysis Section, Food Standards Australia New Zealand, Barton, ACT, Australia (*FAO Expert*)
Dr J.C. Leblanc, Food Safety and Quality Unit, Agriculture and Consumer Protection Department, Food and Agriculture Organization of the United Nations, Rome, Italy (*FAO Secretariat*)
Professor P. Li, Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Wuchang, Wuhan, Hubei Province, China (*FAO Expert*)
Dr M. Lipp, Agriculture and Consumer Protection Department, Food and Agriculture Organization of the United Nations, Rome, Italy (*FAO Joint Secretary*)

[1] Participants marked with an asterisk (*) did not attend the entire meeting.

Professor H.A. Makun, Federal University of Technology, Minna, Nigeria (*FAO Expert*)
Dr D. Miller,* Department of Chemistry, Carleton University, Ottawa, Ontario, Canada (*WHO Expert*)
Dr N.J. Mitchell, Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan, USA (*WHO Expert*)
Dr T. Rawn, Food Research Division, Health Canada, Ottawa, Ontario, Canada (*FAO Expert*)
Dr R.T. Riley, Athens, Georgia, USA (*WHO Expert*)
Dr A.-C. Roudot, Université de Bretagne Occidentale, Brest, France (*WHO Expert*)
Ms M. Sheffer, Orleans, Ontario, Canada (*WHO Technical Editor*)
Ms J.H. Spungen, Office of Analytics and Outreach, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, College Park, Maryland, USA (*FAO Expert*)
Dr A. Tritscher, Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland (*WHO Joint Secretary*)
Dr T. Umemura, Division of Pathology, Biological Safety Research Center, National Institute of Health Sciences, Tokyo, Japan (*WHO Expert*)
Dr M. Wheeler, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Cincinnati, Ohio, USA (*WHO Expert*)
Dr T. Yoshinari, Division of Microbiology, National Institute of Health Sciences, Tokyo, Japan (*WHO Expert*)
Dr Y. Zhang, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, College Park, Maryland, USA (*WHO Expert*)

Annex 2

General considerations

An edited version of this section will appear in the report of the eighty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). It is reproduced here so that the information can be disseminated quickly.

Considerations for dose–response modelling

Introduction

The present meeting used dose–response modelling to evaluate exposure-related effects and to derive a point of departure to establish a health-based guidance value or a margin of exposure for risk assessment, referring to previous guidance and practices of JECFA (e.g. Environmental Health Criteria [EHC] 239 and EHC 240 as well as the report of the seventy-second meeting of JECFA). During the meeting, the Committee recognized several issues concerning the selection of models to be included in the set of models fitted to the dose–response data identified as pivotal for risk assessment.

Theoretical considerations

Dose–response models are mathematical models that approximate a biological process in a range of observed data. When extrapolating below the lowest dose of the experimental data, it should not be assumed that any one model is an accurate representation of the true underlying dose–response. There are often several different models that describe the data adequately, and there is often considerable uncertainty in the form of the approximation of the dose–response relationship.

Benchmark dose methodology ideally avoids this problem by confining the modelling process to doses at which the relationship between dose and response is highly constrained by empirical data, so that the differences between the estimates generated by alternative models are slight. For example, when considering quantal data, a dose that results in a 10% increase in excess risk is typically used, because this is a size of effect that is typically bracketed by standard testing methodologies using experimental animals. However, the data often do not conform to that ideal. Laboratory studies may be limited by the number of animals per dose or employ doses that are far apart from the dose at which the critical adverse health effects become evident for risk assessment. Epidemiological studies have a different set of theoretical problems (e.g. dose misclassification).

Therefore, the Committee concluded that model estimates cannot rely solely on empirical guidance on performing dose–response analyses and stressed the need to use toxicological knowledge, weight of evidence and other information. Curve fitting, such as benchmark dose modelling, fulfils one key aspect of such an evaluation – it ensures that the dose is "associated" with an effect. As all models are approximations, fitting the data does not necessarily make the model's estimate plausible. The curve-fitting process must be scrutinized with other criteria based upon biological considerations. These considerations come under the headings of plausibility and analogy:

- *Plausibility*. Quantitative dose–response analysis is rooted in biochemistry. Although absorption, distribution, metabolism and excretion make toxicological interactions more complicated than biochemical interactions in vitro, the combination of such interactions in a living organism should still bear some resemblance to the first- and second-order kinetics suggested by biochemistry. As first-order interactions are approximately linear at low doses and second-order interactions are sublinear at low doses, it is reasonable to suppose that toxicological effects may exhibit dose–response relationships that are linear, highly sublinear (i.e. threshold-like) or anywhere in between. Mathematical models that demonstrate supralinearity at low doses are not toxicologically plausible and should be used with caution.

- *Analogy*. Even if the shape of a dose–response relationship is not well characterized, experience should inform the modelling decisions. In particular, a reasonable approach would assume that it would be rare to observe a completely different dose–response relationship

than previously observed, and caution should be taken when extrapolating risk from such models. This reasoning is by analogy. One uses past experience analogically to guide the decisions in a similar situation.

Supralinearity in benchmark dose estimation

When dose–response curves are fitted to data, the benchmark dose (BMD) as well as the corresponding lower bound (BMDL) are computed from these curves, which are based upon a prespecified excess risk value – the benchmark response (BMR). In many situations, the dose–response curve appears supralinear at the doses tested, and models that support supralinearity may describe the data better than models that do not support supralinear dose–response data. One reason is that the set of models available on modelling software allows for both sublinearity and supralinearity. The Committee agrees that these models should not be dismissed for statistical reasons but should be evaluated based upon biological plausibility, and, in many situations, these models can be used to estimate the BMD. For illustrative purposes, Fig. 1 describes such a situation. The fitted dose–response curve (solid line) and corresponding BMD appear reasonable; however, the dose–response curve that is used to calculate the BMDL (dashed line) is clearly unreasonable, as it is essentially vertical at doses corresponding to risk around the BMR (i.e. the slope is infinite at zero). In such a situation, the model should not be used.

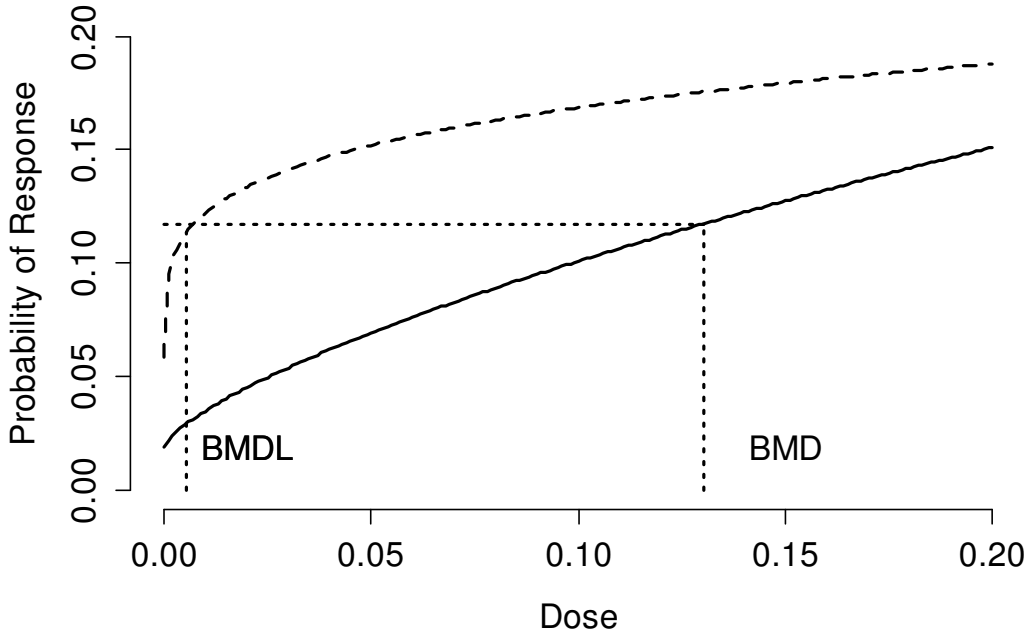

Fig. 1: Plot of a hypothetical dose–response curve (solid line) and its corresponding 95% UB, dashed line. The vertical lines represent the BMD and BMDL. Here, the fitted dose–response curve appears reasonable, but the UB curve, which defines the BMDL, is biologically unreasonable.

It is sometimes the case that the estimate of the BMDL is unreasonable given other considerations; for example, the BMDL may imply that exposure to only a few molecules of a chemical could increase risk by 10%. A check for supralinearity is to estimate the BMD and the BMDL at BMRs above and below the BMR chosen a priori. If the resultant BMDs and BMDLs are approximately located in the linear or sublinear range along the levels tested, the values can be used without objection. If there is a strong pattern of supralinearity, the model may be dismissed as not biologically appropriate. Fig. 2 shows such a plot, where the left pane describes three BMDLs computed at BMRs of 5%, 10% and 20%, and the estimates appear to be on a line. The right pane describes the same circumstance, but there is a large deviation above the line, which indicates supralinearity. In this case, toxicological evidence for that estimate should be investigated, and it should be dismissed if it is found to be biologically implausible.

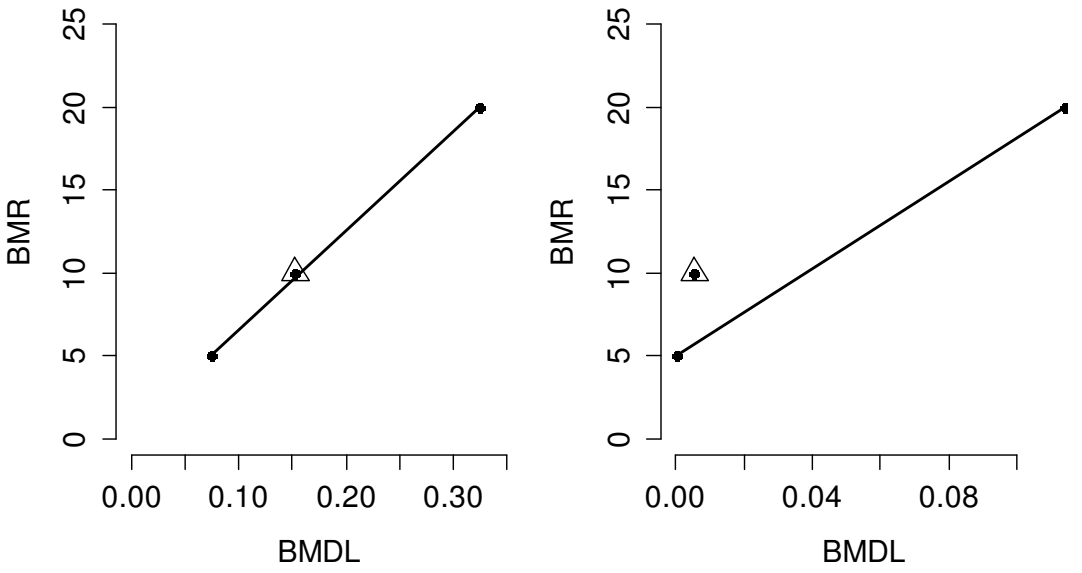

Fig. 2: Comparison of the BMDL computed across different BMRs for a model that is linear (left pane) and supralinear (right pane).

General approaches for identifying a BMDL

Restricted models only. This technique uses models with the default parameter constraints provided with the United States Environmental Protection Agency's (USEPA) benchmark dose modelling software (BMDS). The lowest resulting BMDL is then typically selected as the point of departure. This is the methodology used for past JECFA evaluations for acrylamide, arsenic, fumonisins and cyanogenic glycosides. This method avoids supralinearity, but can result in significantly poorer model fits for some data sets. Additionally, the statistical coverage of this method may be anti-conservative – that is, the BMDL is higher than the true BMD at a rate greater than the confidence limit specified (type I error).

Unrestricted models only. This technique uses models without constraints and also selects the lowest resulting BMDL for identifying a point of departure. This methodology was recently used by JECFA for deoxynivalenol and by the European Food Safety Authority in 2016 for 3-MCPD and other compounds. Although this methodology may avoid the statistical pitfalls of constrained models, as it allows supralinear models, implausible BMDLs may result from using this method.

Model averaging. Model averaging is a method that averages constituent dose–response models. As shown by various authors, it often avoids all of the problems listed above. Such estimates are often less sensitive to supralinear effects and result in estimates that are more reliable statistically. Although there is no current JECFA guidance to using model averaging, it is a useful adjunct to the other methods when computing the BMDL.

Approach taken at current meeting. The Committee used the restricted models to identify the point of departure and also applied the other two methods for comparative purposes.

The current Committee recommends that the JECFA Secretariat establish an expert working group to develop detailed guidance for the application of the methods most suitable to the work of the Committee.

Handling non-detected or non-quantified analytical results for food chemicals

At the current meeting, the Committee discussed two general issues in relation to non-detected or non-quantified analytical results: 1) the handling of a high percentage of left-censored occurrence data (i.e. those analytical results less than the limit of detection [LOD] or LOQ), and 2) dealing with different LODs or LOQs in the same data set for individual chemicals or for a group of chemicals (e.g. aflatoxins or fumonisins). The number of uncensored contaminant data points also needs to be considered. Combination of these parameters can lead to very different results, both in the mean occurrence values derived and in the estimates of dietary exposure. These results will then affect the assessment of risk in relation to the health-based guidance value (e.g. PMTDI) or point of departure (e.g. BMDL). Therefore, how to deal with all of these issues needs careful consideration and consistent approaches for risk assessment purposes, and updating of EHC 240 as needed.

The issue of a high proportion of left-censored data was discussed at the meeting during the evaluations of two mycotoxins, 4,15-DAS and sterigmatocystin, for which the percentages of left-censored data were over 90%. These discussions raised the need to review the current practices used by the Committee on handling left-censored data and to provide the Committee with clear recommendations on how to deal with such situations in its evaluations.

The Committee discussed a proposal but, due to the importance of this topic, decided that further considerations were required. These discussions will be continued after the meeting through a working group.

Annex 3

Future work and recommendations

Considerations for dose–response modelling

Reiterating the recommendations of the seventy-second meeting of JECFA, the current Committee recommends that the JECFA Secretariat establish an expert working group to develop detailed guidance for the application of the methods most suitable to the work of the Committee. The working group should, inter alia, address the following aspects:

- the use of constraints when fitting models that allow for restrictions on the slope and/or power parameters modelling (i.e. the use of restricted versus unrestricted models);
- models to be used from the standard BMDS suite;
- the use of model averaging, including selection of weights;
- the use of non-parametric methods as an alternative for dose–response risk assessment;
- the use of biological information for the selection and specification of models for dose–response;
- transparent presentation of modelling outcomes in JECFA publications;
- review of developments in the USEPA BMDS software.

Handling non-detected or non-quantified analytical results for food chemicals

The Committee discussed a proposal regarding guidance on how to handle left-censored data in its evaluations. However, due to the importance of this topic, the Committee decided that further considerations were required. These discussions will be continued after the meeting through a working group.