Food and Agriculture Organization of the United Nations

World Health Organization

JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES
Sixty-fourth meeting
Rome, 8-17 February 2005

SUMMARY AND CONCLUSIONS

A meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) was held in Rome, Italy, from 8 to 17 February 2005. The purpose of the meeting was to evaluate certain food contaminants.

Dr John Larsen, Division of Toxicology and Risk Assessment, Danish Institute of Food and Veterinary Research, Søborg, Denmark, served as Chairman and Mrs Inge Meyland, Danish Institute of Food and Veterinary Research, Søborg, Denmark, served as Vice-Chairman.

Dr Monica Olsen, Food Quality and Standards Service, Food and Nutrition Division, Food and Agriculture Organization of the United Nations, and Dr Angelika Tritscher, International Programme on Chemical Safety, World Health Organization, served as joint secretaries.

The present meeting was the sixty-fourth in a series of similar meetings. The tasks before the Committee were (a) to elaborate further principles for evaluating the health risk of food contaminants; (b) to evaluate certain food contaminants.

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 substances, and recommendations for future work.

Monographs or monograph addenda on the substances that were considered, which will include information on analytical and technological aspects, concentrations in food, as well as detailed intake assessments, will be published in a joint FAO/WHO publication under WHO Food Additives Series No. 55/FAO Food and Nutrition Paper 83.

More information on the work of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is available at:

www.fao.org/es/esn/jecfa/index_en.stm

http://www.who.int/ipcs/food/jecfa/en/

An edited and extended version of this electronic summary report will be published as full report of the sixty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in the WHO Technical Report Series.
Main conclusions and evaluations are reproduced here in a shorter version so that the information is disseminated quickly.
This draft is subject to further technical editing.

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1. General Considerations

1.1 The formulation of advice on compounds that are both genotoxic and carcinogenic

The Committee has established procedures for determining health-based guidance values, such as the acceptable daily intake (ADI) or provisional tolerable weekly intake (PTWI), for chemicals that produce adverse effects that are thought to show a threshold in their dose–response relationships. Compounds that are both genotoxic and carcinogenic may show non-linear dose–response relationships, but the no-observed-effect level (NOEL) in a study of carcinogenicity represents the limit of detection in that bioassay, rather than an estimate of a possible threshold. Therefore the Committee does not establish health-based guidance values for compounds that are genotoxic and carcinogenic using the NOEL and safety/uncertainty factors. In the absence of evidence on the influence of non-linearity on the incidence of cancer at low levels of exposure, the advice given previously by the Committee on compounds that are both genotoxic and carcinogenic has been that intakes should be reduced to as low as reasonably achievable (ALARA). Such advice is of limited value, because it does not take into account either human exposure or carcinogenic potency, and has not allowed risk managers to prioritize different contaminants or to target risk management actions. In addition, ever-increasing analytical sensitivity means that the numbers of chemicals with both genotoxic and carcinogenic potential detected in food will increase.

The Committee at its present meeting considered a number of compounds for which genotoxicity and carcinogenicity were important issues. The Committee was aware of a number of recent developments relevant to the risk assessment of such compounds, including a WHO Workshop that developed a strategy for dose–response assessment and the formulation of advice (IPCS Workshop 2004), discussions within the European Food Safety Authority about a margin of exposure (MOE) that would indicate the level of priority for risk management action, and Australian recommendations for genotoxic and carcinogenic soil contaminants regarding a guideline dose that would be protective of human health based on a modified benchmark dose and the application of uncertainty factors to allow for interspecies differences, intraspecies variability, quality of the database and the seriousness of the carcinogenic response (Australia, 1999).

The Committee discussed approaches to the formulation of advice on contaminants that are both genotoxic and carcinogenic that would inform risk managers about the possible magnitude of health concerns at different levels of intake in humans.

Hazard identification would normally be based on data from studies on genotoxicity and from cancer bioassays. Some chemicals increase the incidence of cancer in experimental animals by non-genotoxic mechanisms, and establishing a health-based guidance value, such as a PTWI,

would be appropriate. The present guidance relates to chemicals that are both genotoxic[*] and carcinogenic.

Hazard characterization (dose–response assessment) would be based on the available dose–response data for cancer, which would mostly be derived from studies in rodents given daily doses many orders of magnitude greater than the estimated intakes in humans. Dose–response data from studies of epidemiology may also be used for hazard characterization, and would avoid interspecies comparisons and extrapolation over many orders of magnitude. When based on data from a bioassay for cancer in animals, the recent WHO workshop recommended the use of the benchmark dose lower confidence limit (BMDL) as a starting point for hazard characterization when the data are suitable for dose–response modelling. The BMDL is the lower one-sided confidence limit of the benchmark dose (BMD) for a predetermined level of response, called the benchmark response (BMR), such as a 5 or 10% incidence. The BMD in most cases shows less variation than the BMDL for different mathematical models and may be more suitable for ranking different compounds in terms of their potency, while the BMDL may be more appropriate for risk characterization purposes because it reflects the quality of the data. The derivation and interpretation of a BMDL requires considerable statistical and biological expertise.

A number of aspects of the database need to be considered in dose–response modelling, including data selection, model selection, statistical linkage, parameter estimation, implementation and evaluation (IPCS Workshop 2004). The dose-metric used for modelling could be a biomarker, providing that it was critically related to the process by which cancer arises and had been validated in relation to the external dose or intake. For carcinogenesis, selection of the dose–response data for modelling will need to consider both site-specific incidences of tumours, especially for the site showing the greatest sensitivity, as well as combined data (e.g. numbers of tumour-bearing animals) for compounds that do not show clear organ specificity. Analyses based on the numbers of tumour-bearing animals may also be appropriate under other circumstances, for example in the assessment of complex mixtures of compounds that are both genotoxic and carcinogenic. Dose–response characterization should aim to define the BMDL for the carcinogenic response(s) of relevance to human health, at the lowest level of response (the BMR) that reliably defines the bottom end of the observed experimental dose–response relationship. A BMR of a 10% incidence is likely to be the most appropriate for modelling of data from cancer bioassays, because the values for different mathematical models show wider divergence at incidences below 10%. The consistent use of the same benchmark response, i.e. 10%, will facilitate comparisons of the risks associated with different compounds that are both genotoxic and carcinogenic. Non-cancer effects produced by compounds that are both genotoxic and carcinogenic may be analysed using the same approach, and comparison of the derived BMDL values and their associated slopes can help to identify the adverse effect that is critical to risk assessment of the compound.

Exposure (intake) assessment for a compound that is both genotoxic and carcinogenic is no different to that for other types of contaminants.

Risk characterization involves comparison of the estimated exposure with the identified BMDL. In principle, this can take different forms.

- *Calculation of the margin of exposure* (MOE: the ratio of [the BMDL] / [the estimated intake in humans]). The MOE can be used to prioritize different contaminants, providing that a consistent approach has been adopted. The acceptability of an MOE depends on its magnitude and is ultimately a risk management decision (IPCS Workshop 2004). To aid that decision, the risk assessor should provide information on the nature and magnitude of uncertainties in both the toxicological and exposure data. Although the risk assessor should

[*] The present guidance does not address the situation where a compound shows genotoxicity, or has structural alerts for genotoxicity, but where a cancer bioassay has not been performed. The Committee is aware of developments, such as the threshold of toxicological concern (TTC) for compounds with structural alerts for genotoxicity, which may allow the formulation of limited advice to risk managers, and would welcome a critical evaluation of such approaches.

not provide an assessment of the acceptability of the MOE, guidance should be given on its adequacy taking into account the inherent uncertainties and variability.

- *Dose–response analysis outside the observed dose range.* Quantitative dose–response analysis could be used to calculate the incidence of cancer that is theoretically associated with the estimated exposure for humans, or the exposure associated with a predetermined incidence (e.g., 1 in 10^6). In order to provide realistic estimates of the possible carcinogenic effect at the estimated exposure for humans, mathematical modelling would need to take into account the shape of the dose–response relationship between the high doses used in the cancer bioassay and much lower intakes by humans. Such information cannot be derived from the available data on cancer incidence in studies in animals. In the future, it may be possible to incorporate data on dose–response or concentration–response relationships for the critical biological activities involved in the generation of cancer, such as metabolic bioactivation and detoxication processes, DNA binding, DNA repair, rates of cell proliferation and apoptosis, into a biologically-based dose–response model for cancer that would also incorporate data on species differences in these processes. However, such data are not currently available. At present, any estimate of the possible incidence of cancer in experimental animals at intakes equal to those for humans has to be based on empirical mathematical equations that may not reflect the complexity of the underlying biology. A number of mathematical equations have been proposed for low-dose extrapolation. The resulting risk estimates are dependent on the mathematical model used; the divergence increases as the dose decreases and the output by different equations can differ by orders of magnitude at very low incidences.
- *Linear extrapolation from a point of departure.* Because the estimated risks at low doses are model-dependent, linear extrapolation from the BMDL, which is conservative and simple to apply, has been used as a matter of policy by some agencies in order to calculate levels of exposure associated with different theoretical incidences of cancer. The incidence used is regarded as an upper-bound estimate for lifetime risk of cancer and the actual risk may lie anywhere between zero and the calculated upper-bound estimate. Calculation of the intake associated with an incidence of 1 in 10^6 from the BMDL for a 10% incidence using linear extrapolation is simply equivalent to dividing the BMDL by 100 000, and this approach is therefore no more informative than calculation of a MOE.

Of the three options given above, the MOE and linear extrapolation from a point of departure are the most pragmatic and usable at the present time. Linear extrapolation from a point of departure offers no advantages over an MOE and the results are open to misinterpretation because the numerical estimates may be regarded as quantification of the actual risk.

The Committee at its present meeting decided that advice on compounds that are both genotoxic and carcinogenic should be based on estimated MOEs. The strengths and weaknesses inherent in the data used to calculate the MOE should be given as part of the advice to risk managers, together with advice on its interpretation.

1.2 Establishing an acute reference dose

The Committee routinely considers the toxicity of chemicals in food and establishes acceptable or tolerable levels of intake, usually on the basis of data on chronic toxicity. Certain substances, however, e.g. some metals, mycotoxins or veterinary drug residues, could present an acute risk, i.e. could raise concern regarding acute health effects in relation to short periods of intake at levels greater than the ADI or TDI.

The FAO/WHO Joint Meeting on Pesticide Residues (JMPR) evaluates the acute and chronic effects of pesticide residues in food, and has developed guidance on the setting of acute reference doses (ARfDs) for pesticides. The Committee at its present meeting was made aware that JMPR had recently developed a detailed guidance document on the setting of ARfDs. This guidance

document can also serve as basis for considerations for chemicals in food, other than pesticide residues.

The JMPR defined the ARfD as follows:

> "The ARfD of a chemical 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 the known facts at the time of the evaluation".

The Committee agreed with this definition.

Building on the experience of and the guidance developed by JMPR, the Committee may consider setting ARfDs, where relevant, in the future. The need to establish an ARfD should be considered on a case-by-case basis, and only if the substance, on the basis of its toxicological profile and considering the pattern of its occurrence and intake, is likely to present an acute health risk resulting from exposure in a period of 24 h or less.

When considering substances such as inorganic tin having a local irritant or caustic effect, however, there is no need to establish an ARfD because, at reasonable portion sizes, it is the concentration of the substance in the food that causes the effect and not the dose.

1.3 Short-term dietary intakes of contaminants

There is no internationally agreed methodology for estimating short-term intakes of contaminants from food (i.e. in a period of 24 h or less). International Estimated Short-Term Intakes (IESTI) for pesticide residues have been routinely calculated for a number of years. The Joint FAO/WHO Meeting on Pesticide Residues (JMPR) uses specific methodologies and equations to calculate short-term dietary intakes of pesticide residues based on the highest reported 97.5th percentile daily food consumption amount for consumers only and usually matched with the 97.5th percentile residue level in a single commodity unit. IESTIs are calculated for the general population as well as for children aged ≤ 6 years.

The Committee noted the need to develop methodologies to assess the short-term dietary intakes of contaminants. The methodologies should be specified on a case-by-case basis as a function of both the distribution of the contaminant of concern and its toxicological properties. Some of the basic principles of the methodology used to estimate short-term intakes for pesticide residues could be used for contaminants. However, guidelines are required on the data sets and equations that should be used, and the population groups which should be assessed.

1.4 Data on levels of contaminants in food and on food consumption

In advance of a meeting of the Committee, FAO and WHO issue calls for data on levels of contaminants in food and the total diet. The quality and quantity of those data are essential for intake assessment.

The Committee welcomed the fact that for its 64th meeting a greater than usual number of countries had provided information in response to the call for data, but noted that data from developing countries were sparse or lacking altogether.

The Committee was aware that in many cases data existed, but for various reasons, are not submitted. Often the call for data does not reach those persons holding such data or the mechanism for the submission of data by institutions is not clear. In addition, some of the data which are submitted often lack critical information on how the data were generated. Data on contaminants were also available in the scientific literature. However, these data are often lacking

essential information necessary to permit their use by the Committee. Incomplete information can greatly diminish the usefulness of the data for risk assessment purposes.

Therefore, the Committee recommends that FAO and WHO seek ways to make the calls for data more widely known at both technical and policy levels in developing countries and to directly contact governments and other potential data providers to facilitate the submission of such data to the Committee.

The Committee also recommends that data providers in both developing and industrialized countries enhance their efforts to submit their information to JECFA and to use the electronic GEMS/Food format in order to facilitate the collation and quality control of data. Detailed instructions are provided in the GEMS/Food Electronic Reporting Manual available http://www.who.int/foodsafety/publications/chem/en/gemsmanual.pdf. In order to allow the Committee to independently assess the quality of the data, the submission of data should be accompanied by additional details on the sampling plan and analytical method used to generate the data. These details are described in the questionnaire provided in Appendix 5 of the above mentioned reporting manual.

2. Toxicological and Intake Evaluations and Recommendations on Specific Contaminants

2.1 Acrylamide

Explanation

Acrylamide (CH_2=CHCONH_2, CAS Registry Number 79-0601) is an important industrial chemical used since the mid 1950s as a chemical intermediate in the production of polyacrylamides, which are used as flocculants for clarifying drinking water and other industrial applications. The neurotoxicity of acrylamide in humans is well-known from occupational and accidental exposures. In addition, experimental studies with acrylamide in animals have shown reproductive, genotoxic and carcinogenic properties.

Studies conducted in Sweden in 2002 showed that high levels of acrylamide were formed during the frying or baking of a variety of foods. Due to the concerns about the possible public health risks from dietary exposure to acrylamide, a consultation was held by the FAO/WHO in June 2002 (Health Implications of Acrylamide in Food, Report of the FAO/WHO Consultation, 2002).

JECFA was asked by the 36th Session of Codex Committee on Food Additives and Contaminants (2004, ALINORM 04/27/12):

a) to comment on the extent to which acrylamide is bioavailable in food and on the safety implications;

b) to consider the threshold-based end-points of concern such as neurotoxicity and reproductive toxicity and eventually derive a tolerable dietary intake;

c) to evaluate the degree of uncertainty related to the assessments made;

d) to provide estimates of dietary exposure for various population groups including susceptible groups such as young children and regional populations and identify and quantify as far as possible the major sources of dietary exposure;

e) to provide estimates and margins of exposure, safety, and or exposure for various endpoints of concern (non-cancer and cancer). These estimates should contain comparisons between the levels of acrylamide exposure shown to produce effects in animal studies and the demonstrated no-effect levels vs. estimates of dietary exposure for humans;

f) to provide quantitative estimates of risk for various endpoints including cancer for varying degrees of dietary exposure to acrylamide; and

g) to provide comments on the toxicological significance of the main metabolite, glycidamide, and whether this may be more genotoxic than the parent compound.

The Committee has not previously evaluated acrylamide.

Absorption, distribution, metabolism, and excretion

In experimental animals, AA is rapidly and extensively absorbed from the gastrointestinal tract following oral administration and is widely distributed to the tissues, as well as the foetus. It has also been found in human milk. AA is metabolised to a chemically reactive epoxide, glycidamide (GA), in a reaction catalyzed by CYP2E1. An alternate pathway for metabolism of AA is conjugation with glutathione. AA and its metabolites are rapidly eliminated in urine, primarily as mercapturic acid conjugates of AA and GA. The absolute bioavailablity of AA (i.e., the fraction entering the circulation as parent compound) is in the range of 23-48% in rodents for a dose of 0.1 mg/kg bw administered in the diet over a 30-minute period. Extensive first-pass metabolism of AA to glycidamide leads to much higher relative internal exposures to glycidamide after dietary administration compared to intravenous administration.

GA is much more reactive than AA with DNA and several purine base adducts have been identified in vitro. Studies using knockout and wild-type mice showed that CYP2E1-mediated oxidation is the predominant pathway leading to GA and DNA adduct formation. In rodents dosed with acrylamide, glycidamide-DNA adducts are formed at comparable levels in all tissues examined and accumulate to apparent steady state levels from repetitive dosing regimens. DNA adducts have been found in liver, lung, testis, leukocytes, and kidney of mice and in liver, thyroid, testis, mammary gland, bone marrow, leukocytes, and brain of rats treated with either AA or GA. Formation of DNA adducts in mice shows a monotonic dependence on AA dose, from measurable adduct levels at background exposure, with evidence for saturation of adduct levels at higher doses. Kinetic studies of adduct loss from DNA in vitro and in vivo showed that spontaneous depurination, as opposed to active repair, is operative.

Both AA and GA also bind covalently to amino acids in haemoglobin and adducts with the N-terminal valine residue have been widely used to estimate internal exposures in human biomonitoring studies. Preliminary studies that measured concentrations of AA- and GA-haemoglobin adducts in rodents and humans with background exposure to AA through the diet suggested that there may be species differences in the relative formation of GA with mouse > rat > human. However, the long half-life of haemoglobin means that the measured adduct levels reflect a time-weighted average over the lifetime of the erythrocyte. Thus, similar levels of adducts could arise from the same total exposure over an extended time period as over a short period. This has limited the utility of these biomarkers for dose-response modelling under circumstances where there is variability in the magnitude and frequency of exposure.

Toxicological data

Single oral doses produced acute toxic effects only at doses above 100 mg/kg bw, and reported LD50s are generally above 150 mg/kg bw.

Numerous studies conducted in a number of animal species have shown that the nervous system is a principal site of the toxic actions of AA. Sufficient, repeated exposure to AA causes a degenerative peripheral nerve changes that result from an accumulation of damage at the sites of toxicity (Table 1). For example, the same degree of neurotoxicity was observed in rats given AA at a dose of 50 mg/kg bw IP for 11 d as in rats given drinking water containing AA at a dose of 21 mg/kg bw for 40 d. Continued dosing with AA has been shown to induce nerve terminal degeneration in brain areas critical for learning, memory and other cognitive functions (i.e., cerebral cortex, thalamus, and hippocampus) and these lesions may precede the morphological changes in nerves. In rats exposed to AA in drinking water for 90 d the NOEL for morphological changes in nerves detected using electron microscopy was 0.2 mg/kg bw/d and no exposure-related non-neoplastic lesions were found at other tissues at dose levels below 5 mg/kg bw/d.

In reproduction studies, male rodents showed reduced fertility, dominant lethal effects, and adverse effects on sperm count and morphology at oral doses of AA > 7mg/kg bw/d. In female rodents, no adverse effects on fertility or reproduction have been observed, apart from slight reductions in rat offspring body weight at oral doses of 2.5 mg/kg bw/d (LOEL) and above. In developmental toxicity studies, AA was foetotoxic in mice only at a maternally toxic oral dose of 45 mg/kg bw/day, and was not teratogenic in mice or rats. In a developmental neurotoxicity study, in which AA was dosed orally from gestational day 6 to lactation day 10, the NOEL for developmental neurotoxicity was 10 mg/kg bw/day. The overall NOEL for reproductive and developmental effects was 2 mg/kg bw/d.

Table 1. Noncancer effects in animals repeatedly exposed to acrylamide by the oral route

Species	Exposure conditions (mg/kg-d)	NOEL (mg/kg-d)	LOEL (mg/kg-d)	Effect
Fischer 344 rat, M&F[1]	0, 0.05, 0.2, 1, 5, 20 90 days in DW	0.2 1 5 5 5	1 5 20 20 20	Morphological nerve changes (EM) Degenerative nerve changes (LM) Hindlimb foot splay Decreased BW (8-20%) Atrophy of testes & skeletal muscle
Fischer 344 rat, M&F[2]	0, 0.01, 0.1, 0.5, or 2.0 2 years in DW	0.5 2 0.5 0.5 2	2 ND 2 2 ND	Degenerative nerve changes (LM) Hindlimb foot splay Decreased BW (<5%, M only) Early mortality after 24 weeks Other nonneoplastic lesions
Fischer 344 rat, M&F[3]	0, 0.1, 0.5, 2.0 (M) 0, 1.0, 3.0 (F) 2 years in DW	0.5M 1.0F 2.0M 3.0F 0.5M 1.0F 0.5 2.0M 3.0F	2.0M 3.0F ND ND 2.0M 3.0F 2.0 ND ND	Degenerative nerve changes (LM) Hindlimb foot splay Decreased BW (8-9%) Early mortality after 60 weeks Other non-neoplastic lesions
Fischer 344 rat, M&F[4]	0, 0.5, 2.0, 5.0 2 generations in DW	2.0 2.0 ND 0.5	5.0 5.0M 0.5M 2.0M	MM implantation losses (F_0&F_1) Degenerative nerve changes (LM) Hindlimb foot splay & head tilt (F_0 M only) Decreased BW (4-6%)
CD-1 mouse, M&F[5]	0, 0.8, 3.1, 7.5 2 generations in DW	3.1 7.5 3.1 7.5 3.1F	7.5 ND 7.5 ND 7.5F	MM implantation losses (F0&F1) Degenerative nerve changes (F1,LM) Mild grip strength deficits (F1&F2) Hindlimb foot splay Decreased BW (8%, F1 only)
Sprague-Dawley rat, F[6]	0, 5, 10, 15, 20 GD 6-10 by gavage	10 10 ND 10	15 15 5 15	Decreased maternal weight gain Hindlimb splay, maternal Decreased BW in offspring Increased overall horizontal activity & decreased auditory startle response in offspring

BW = body weight; DW = drinking water; EM = electron microscopy; F = female; GD = gestation days; LM = light microscopy; LOEL = lowest-observed -effect level; M = male; MM = male-mediated; ND = not determined; NOEL = no- observed -effect level; PND = postnatal days
[1]Burek et al., 1980; [2]Johnson et al., 1986; [3]Friedman et al., 1995; [4]Tyl et al., 2000b; [5]Chapin et al., 1995; [6]Wise et al., 1995.

Genotoxicity
Although acrylamide did not show mutagenicity in the Ames Salmonella assay, glycidamide clearly did. Acrylamide is both clastogenic and mutagenic in mammalian cells in vitro and in vivo. In addition, dominant lethality studies have shown AA to be a germ cell mutagen in male rodents. The mutational spectra produced by acrylamide and glycidamide in transgenic mouse cells are consistent with formation of promutagenic purine DNA adducts in vivo.

Metabolism of AA to GA appears to be a prerequisite for the genotoxicity of AA in vitro and in experimental animals. Studies using knockout and wild-type mice showed that CYP2E1-mediated oxidation is the predominant pathway leading to DNA adduct formation. Estimates of internal exposures to GA on the basis of haemoglobin (Hb) adduct measurements following administration of either AA or GA, indicated that GA was the active clastogen responsible for micronuclei induction in mice. Studies using wild-type and CYP2E1 knockout mice have also shown that GA is the active metabolite of AA responsible for germ cell mutations and dominant lethality in male mouse spermatids. GA is the presumed active mutagen because GA dosing produced comparable or greater increases in mutant frequencies at the hprt and cII loci in Big Blue transgenic mice compared with AA dosing.

Carcinogenicity
AA in drinking water has been tested for carcinogenicity in two experiments in Fischer 344 rats. There were increases in tumor incidences at a variety of sites (see Tables 2 and 3). Information about total tumor-bearing animals was not available for either study.

Table 2. Numbers of Fischer 344 rats with tumors after receiving acrylamide in the drinking-water for two years. Data from Johnson et al, 1986 as compiled by Rice, 2004.

Type of tumor	Sex	Dose (mg/kg bw per day)				
		0	0.01	0.1	0.5	2.0
Thyroid gland, follicular adenomas	M	1/60	0/58	2/59	1/59	7/59*
Peritesticular mesotheliomas	M	3/60	0/60	7/60	11/60*	10/60*
Adrenal gland[a], pheochromocytomas	M	3/60	7/59	7/60	5/60	10/60*
Mammary tumors	F	10/60	11/60	9/60	19/58	23/61*
Central nervous system, glial tumors	F	1/60	2/59	1/60	1/60	9/61*
Thyroid gland, follicular adenomas or adenocarcinomas	F	1/58	0/59	1/59	1/58	5/60*
Oral cavity, squamous papillomas	F	0/60	3/60	2/60	1/60	7/61*
Uterus, adenocarcinomas	F	1/60	2/60	1/60	0/59	5/60*
Clitoral gland, adenomas[b]	F	0/2	1/3	3/4	2/4	5/5*
Pituitary adenomas[a]	F	25/59	30/60	32/60	27/60	32/60*

[a]*The historical control incidence of adrenal gland phaeochromocytomas in males was 8.7% (range, 1.2-14.0%); that of pituitary adenomas in females was 38.1% (range, 28.2-46.9%).*

[b]*Only clitoral glands with gross lesions were examined histologically.*

**p = 0.05; pair-wise Mantel-Haenszel comparison with the control group adjusted for mortality*

Table 3. Numbers of Fischer 344 rats with tumors at selected organ sites after receiving acrylamide in the drinking-water for two years. Data from Friedman et al., 1995 as compiled by Rice, 2004

Type of tumor	Sex	Dose (mg/kg bw per day)						
		0	0	0.1	0.5	1.0	2.0	3.0
Peritesticular mesotheliomas	M	4/102	4/102	9/204	8/102	-	13/75*	-
Brain & spinal cord, glial neoplasms [b]	M	1/102 [e]	1/102 [e]	2/204 [c]	1.102 [d]	-	3/75 [e]	-
	F	0/50 [f]	0/50 [f]	-	-	2/100 [f]	-	2/100 [f]
Thyroid gland, follicular adenomas	M	2/100	1/102	9/203	5/101	-	15/75*[g]	-
	F	0/50	0/50	-	-	7/100	-	16/100*[g]
Thyroid gland, follicular cell carcinomas	M	1/100	2/102	3/203	0/101	-	3/75	-
	F	1/50	1/50	-	-	3/100	-	7/100
Total rats with follicular cell neoplasms	M	3/100	3/100	12/203	5/101	-	17/75	-
	F	1/50	1/50	-	-	10/100	-	23/100*
Mammary gland, fibroadenomas and adenocarcinomas	F	7/46	4/50	-	-	21/94*	-	30/95*

**Statistically significant, $p < 0.001$.*

[a] *Certain tumors that occurred at increased incidence in treated rats in the previous study (Johnson et al., 1986) were not reported as occurring at increased incidences in this study. These included papillomas of the oral cavity in females, adenomas of the clitoral gland and uterine adenocarcinomas. Numbers of these neoplasms were not given.*

[b] *Does not include 7 rats with "malignant reticulosis" of the brain, including 5 dosed females, 1 dosed male and 1 control male.*

[c] *Only 98/204 brains and 68/204 spinal cords were examined.*

[d] *Only 50/102 brains and 37/102 spinal cords were examined.*

[e] *All brains of high-dose rats and all control brains (both subgroups) were examined, but only 82/102 and 90/102 control spinal cords and 51-75 high dose spinal cords were examined.*

[f] *All brains were examined, but only 45/50, 44/50, 21/100 and 90/100 spinal cords in control, control, low- and high-dose females respectively were examined.*

[g] *Includes 3 male and 1 female rats with multiple tumors in the highest dose groups.*

AA was evaluated by the International Agency for Research on Cancer in 1994 and classified as "probably carcinogenic to humans (IARC Group 2A)" on the basis of a positive cancer bioassay result, supported by evidence that AA is efficiently biotransformed to a chemically reactive genotoxic metabolite, GA, in both rodents and humans. The endocrine-responsive nature of several tumor sites from the two chronic bioassays of AA in F344 rats has elicited speculation about neuroendocrine-mediated mechanisms. However, no published studies have linked hormonal changes with the carcinogenicity of AA in any tissue nor is there any indication of hormonal effects from reproductive studies. Moreover, the wide body of evidence supporting a genotoxic mechanism is not incompatible with hormonal dysregulation by AA because it is clear that other factors beyond DNA damage are probably required for the observed target tissue specificity of tumorigenesis of AA.

Observations in humans
Epidemiological studies of human industrial and accidental exposures suggest that the nervous system is a principal site for toxicity in humans.

Epidemiological studies have been carried out on workers occupationally exposed to acrylamide. Acrylamide exposure was not associated with overall cancer mortality, nor with any statistically significant dose-related increase in cancer risk at any organ site, except a statistically significant doubling of risk for pancreatic cancer for workers with the highest cumulative exposure. These studies, however, were based on low numbers of cases and measurements of dietary exposure to acrylamide were not made and potential confounders such as tobacco smoking were not considered.

The only information available that considers dietary intake of AA comes from case-control studies originally designed to assess the potential cancer risk of dietary factors other than AA. The available results from epidemiological studies that estimate oral exposure to AA are not suitable for use in risk assessment of AA.

Acrylamide adducts to haemoglobin have been used as biomarkers of AA exposure in humans. Although levels of AA adducts were often higher among exposed workers and smokers, including a positive correlation with the amount smoked, some uncertainties remain precluding its current use as a marker of dietary AA intake. Because analytical methods may vary between laboratories, there is a need for improved and validated analytical methodology. A means to link biomarkers of AA exposure in humans with toxicity measurements in experimental animals is not currently available.

Formation during cooking and heat processing

Acrylamide may be formed when dietary items, typically plant commodities high in carbohydrates and low in protein, are subjected to high temperatures during cooking or other thermal processing. The most important precursor is the free amino acid asparagine which reacts with reducing sugars in the Maillard reactions that also form color and flavor. Alternative mechanisms might be important in some speciality foods.

Although trace amounts of acrylamide can be formed by boiling, significant formation generally requires a processing temperature of 120 ^{o}C or higher. Concentrations are likely to represent a balance of competing complex processes of formation and destruction of acrylamide. Most acrylamide is accumulated during the final stages of baking, grilling or frying processes as the moisture content of the food falls and the surface temperature rises, with the exception of coffee where levels fall considerably at later stages of the roasting process. Acrylamide seems to be stable in the large majority of the affected foods, again with the exception of ground coffee for which levels can decline during storage over months.

Since formation is dependent on the exact conditions of time and temperature used to cook or heat-process a food, there can be large variations between brands of the same product and between batches of the same brand. Large variations are also to be expected during home-

cooking although this aspect has been less well documented. The composition of the food also has an influence, crucially the content of free asparagine and reducing sugars. Varietal, storage and seasonal variations can occur. Within ranges of natural variation, the limiting precursor in cereals is asparagine while fructose and glucose are more important in potatoes. Other important factors are pH and water content. Addition of ammonium bicarbonate, a leavening agent used in some bakery products, significantly increases formation. High concentrations of other amino acids or proteins competing with asparagine in the Maillard reaction or that reacts with formed acrylamide reduces the acrylamide concentration.

Prevention and control

Research on acrylamide formation and mitigation is ongoing and has been the subject of several international scientific meetings and reviews. The European food industry (CIAA) submitted a review on the mitigation achievements made by food producers up to December 2004. An average reduction by 30-40% in potato crisps was stated to have been achieved by introducing several adjustments in the existing production procedures. The detailed data behind this calculation were not reported and it is not known to what extent it has been applied by crisp producers. Significant reduction was also reported from process-optimisation for non-fermented crispbread, while little progress was obtained so far in reducing levels in various other important intake sources, e.g., roasted coffee and breakfast cereals.

Experiments on food models have indicated a number of possible mitigation options. The most efficient reduction has been achieved by using the enzyme asparaginase to selectively remove asparagine prior to heating. Although tested both in cereal and potato models, the use is probably limited to specific food products manufactured from liquidised or slurried materials. Several other means of lowering the precursor levels can be applied at various stages of the food chain, e.g. by variety selection and plant breeding, controlling growth and storage factors affecting sugar concentrations in potatoes, pre-treatment of potato pieces by soaking or blanching, and prolonged yeast fermentation time in breadmaking. Other mitigation possibilities include alteration of the product composition, e.g. addition of competing amino acids or acidic compounds, and alteration of process conditions, e.g. lowering the frying temperature. The feasibility of adapting these methods to large-scale food processing has not been completely studied in most cases. Furthermore, any major changes would need to be checked for consumer acceptability, nutritional quality, and the possible increased formation of other undesirable substances.

Levels and pattern of food contamination

Acrylamide occurrence data for different food items analysed from 2002 to 2004 were provided for the current meeting from 24 countries. The total number of analytical results (single or composite samples) was 6,752 with 67.6% from Europe, 21.9% from North America, 8.9% from Asia and 1.6% from Pacific. No data from Latin America and Africa were submitted. The committee noted that the occurrence data evaluated at this meeting were more comprehensive than those available at the FAO/WHO, 2002 consultation (240 samples).

Mainly the choices of food items analysed for acrylamide concentration were based on what was known since 2002-2003 on the occurrence of acrylamide in foods and beverages and also based on recommendations made at the last consultation, especially concerning other foods and beverages that undergo similar processing which might also contain acrylamide such as meat, milk, rice, cassava, soy products, vegetables and processed fruits.

Data were available from Sweden for 4 archived human milk samples, one for each of the years 1998-2001. Each of the 4 samples was a pooled sample from 10 mothers. A further 15 samples collected from individual mothers in 2000-2004 were also analysed. No information on sampling times or on the food consumption by the mothers was available. One of the 19 samples of milk contained acrylamide at 0.5 μg/kg which was just above the limit of quantification, the other 18 samples were below the limit of quantification, <0.5 μg/kg.

Table 4: Summary of the distribution weighted concentration of acrylamide in several commodities from 2002 to 2004

Commodities	*N° of samples***	*Mean concentration (µg/kg)* ***	*CV*[b] *(%)*	*Reported Maximum (µg/kg)*
Cereals and cereals-based products*	3,304 (12,346)	**343**	156	7,834
Cereals and pasta, raw and boiled	113 (372)	15	71	47
Cereals and pasta processed (toasted, fried, grilled)	200 (634)	123	110	820
Cereal-based processed products, all	2,991 (11,327)	366	151	7,834
Breads and rolls	1,294 (5,145)	446	130	3,436
Pastry and biscuits (US=cookies)	1,270 (4,980)	350	162	7,834
Breakfast cereals	369 (1,130)	96	131	1,346
Pizza	58 (85)	33	270	763
Fish and seafood (including e.g breaded, fried, baked)*	52 (107)	**25**	180	233
Meat and offals (including e.g coated, cooked, fried *	138 (325)	**19**	174	313
Milk and milk products*	62 (147)	**5.8**	119	36
Nuts and oilseeds*	81 (203)	**84**	233	1,925
Pulses*	44 (93)	**51**	137	320
Root and tubers*	2,068 (10,077)	**477**	108	5,312
Potato puree/mashed/boiled	33 (66)	16	92	69
Potato baked	22 (99)	169	150	1,270
Potato crisps (US=Chips)	874 (3,555)	752	73	4,080
Potato chips (US=French fries)	1,097 (6,309)	334	128	5,312
Potato chip, croquettes (frozen, not ready-to-serve)	42 (48)	110	145	750
Stimulants and analogue[a]*	469 (1,455)	**509**	120	7,300
Coffee (brewed), ready-to-drink	93 (101)	13	100	116
Coffee (ground, instant or roasted, not brewed)	205 (709)	288	51	1,291
Coffee extracts	20 (119)	1,100	93	4,948
Coffee decaffeinate	26 (34)	668	169	5,399
Coffee substitutes	73 (368)	845	90	7,300
Cocoa products	23 (23)	220	111	909
Green tea (''roasted'')	29 (101)	306	67	660
Sugars and honey (mainly chocolate)*	58 (133)	**24**	87	112
Vegetables*	84 (193)	**17**	206	202
Raw, boiled and canned	45 (146)	4.2	103	25
Processed (toasted, baked, fried, grilled)	39 (47)	59	109	202
Fruits, fresh	11 (57)	<1	188	10
Fruits, dried, fried, processed	37 (49)	131	125	770
Alcoholic beverages (beer, gin, wine)	66 (99)	6,6	143	46
Condiments and sauces	19 (22)	71	345	1,168
Infant formula	82 (117)	<5	82	15
Baby food (canned, jarred)	96 (226)	22	82	121
Baby food (dry powder)	24 (34)	16	125	73
Baby food (biscuits, rusks, etc..)	32 (58)	181	106	1,217
Dried food	13 (13)	121	266	1,184

** According the correspondence with GEMS/Food commodities, mean contamination of only food groups in bold character have been used to estimate international intake.*

*** Number of analytical results for individual samples and for composite samples. In parenthesis the total numbers of individual samples, allowing for the number of samples blended into composites.*

**** Data below the detection and quantification limits have been assumed to be half of those limits.*

[a] *Concentrations for brewed coffee (coffee as consumed multiplied by 28 to convert concentration in beverage into concentration in coffee powder).*

[b] *CV = coefficient of variation*

Food consumption and dietary intake assessment

National dietary intake data for 17 countries were evaluated at this meeting. All regions were represented except Latin America and Africa where no dietary intakes were available. National intakes were calculated mainly using deterministic modelling by linking national individual consumption data with national mean occurrence data obtained from national surveys, using the actual consumer body weight reported in consumption surveys.

Intake estimates at national level ranged from 0.3 to 2.0 µg/kg bw per day for the average in the general population. For high percentiles consumers (90th to 97.5th) intake estimates ranged from 0.6 to 3.5 µg/kg bw per day, and up to 5.1 µg/kg bw per day for the 99th percentile consumer. Based on the available data, children had intakes of acrylamide that were around two to three times those of adult consumers when expressed on a body weight basis. The Committee noted that these estimates are consistent with the long-term dietary intake assessment performed by the FAO/WHO consultation (2002) which was based on a limited data set of analytical results representing only a fraction of the diet.

In the absence of a health-based guidance value for acrylamide (i.e. tolerable intake levels), the relative contribution of food commodities to the total intake is reported. The relative contribution of each food group may be different between studies depending of the numbers of food categories considered in the intake evaluation.

The major contributing foods to total exposure for most countries were potato chips (US=French fries) (16-30%), potato crisps (US=Chips) (6-46%), coffee (13-39%), pastry and sweet biscuits (US=Cookies) (10-20%) and bread and rolls/toasts (10-30%). Others foods items contributed less than 10% of the total exposure.

International estimates of intake were prepared by combining the international weighted means of contamination levels with the food consumption values reported in the GEMS/Food database. The committee noted that that these estimates are conservative as the foods considered are raw commodities while the acrylamide levels are for specifically processed foods (i.e., the intake of all raw potatoes is being combined with contaminant levels taken from fried or baked potato products). Additionally, in regions with little or no acrylamide concentration data, the use of this broad assumption may result in a mismatch between the foods considered and the acrylamide concentration data employed (e.g., cassava consumption combined with acrylamide levels from processed-potato products).

Considering these points, the range for the international mean intakes was estimated to be 3.0 µg/kg bw per day up to 4.3 µg/kg bw per day for the five GEMS/Food regional diets, assuming a body weight of 60 kg. Cereals and root and tubers are the main contributor to the total exposure calculations for each regional diet. Intakes from cereals are about 1.3 µg/kg bw per day to 2.6 µg/kg bw per day. Intakes from root and tubers are about 0.5 µg/kg bw per day to 2.6 µg/kg bw per day. Others GEMS/Food groups contribute less than 5% to the total exposure calculations.

The committee concluded that based on national estimates, an intake of 1 µg/kg bw per day of acrylamide could be taken to represent the average for the general population and that an intake of 4 µg/kg bw per day could be taken to represent high consumers. In these intake estimates for average to high intake, children are also included.

Dose-response analysis

The NOEL for induction of morphological changes in nerves detected using electron microscopy in rats exposed to AA in drinking water for 90 days was 0.2 mg/kg bw/d. The overall NOEL for reproductive and developmental effects and other non-neoplastic lesions was 2 mg/kg bw/day. The Committee considered that the pivotal effects of AA for risk assessment were its genotoxicity and carcinogenicity. The available epidemiological data, as well as human and animal biomarker data, are inadequate to establish a dose-response relationship and therefore the assessment was performed (Annex 1) on the basis of available animal studies. In the dose-response analysis, eight

different statistical models were fitted to the experimental data considered relevant for further consideration. Those resulting in acceptable fits based on biological and statistical considerations were selected to derive the BMD and BMDL for a 10% extra risk of tumors. This procedure results in a range of BMD and BMDL values for each endpoint considered (Table 4).

The results summarized in Table 5 show that the BMDLs are only moderately lower than the BMDs, indicating that the confidence intervals are quite narrow. The reason for the narrow confidence intervals in this case is that the uncertainty is reduced to a large extent, by imposing the constraint that the slope at zero dose should be finite. An infinite slope at dose zero is biologically implausible. When the constraint is omitted in fitting the models, the resulting BMDLs are extremely low for some of the fitted models, showing that the dose-response data by themselves contained a high degree of uncertainty regarding the shape of the dose-response curve.

The lowest range of BMDLs is found for total mammary tumors, i.e. 0.30 – 0.46 mg/kg bw/d. The Committee decided to use the more conservative lower end of this range of values for the evaluation.

Table 5. Summary of dose-response modelling results for induction of selected tumors in rats administered AA with drinking water.

	Range of BMD (mg/kg bw-d)	Range of BMDL (mg/kg bw-d)	Range of BMD (mg/kg bw-d)	Range of BMDL (mg/kg bw-d)
Tumor Site	Johnson et al		Friedman et al.	
Total Mammary Tumors	0.48-0.57	0.30-0.46	1.4-1.5	0.89-1.1
Peri-testicular mesothelioma	0.97	0.63-0.97		
Thyroid follicular adenoma			0.88-1.2	0.63-0.93
CNS tumors of glial origin	1.9-2.0	1.3-1.6		

BMD, benchmark dose for 10% extra risk of tumors; BMDL, 95% lower confidence limit for the benchmark dose. Extra risk is defined as the additional incidence divided by the tumor-free fraction of the population in the controls.

In order to integrate the results from all the models used for both mammary tumor datasets, a composite analysis was conducted in which the model outputs were combined. This resulted in a BMD of 1.0 mg/kg bw/d and a BMDL of 0.4 mg/kg bw/d, which supports the other analysis.

The lowest range of BMDLs is found for total mammary tumors, i.e. 0.30 – 0.46 mg/kg bw/d. The Committee decided to use the more conservative lower end of this range of values for the evaluation.

Evaluation

Margins of exposure (MOEs) have been calculated at intakes of 0.001 mg acrylamide/kg bw/d, to represent the average intake of the general population based on national estimates, and 0.004 mg acrylamide/kg bw/d to represent the intake by high consumers. Comparison of these intakes with the NOEL of 0.2 mg/kg bw/day for morphological changes in nerves detected in rats by electron microscopy would provide MOEs of 200 and 50, respectively. Comparison of the selected intakes with the NOEL of 2.0 mg/kg bw/d for reproductive, developmental, and other non-neoplastic effects in rodents would provide margins of exposure of 2000 and 500, respectively. Based on these margins of exposure, the Committee concluded that adverse effects based on these endpoints are unlikely at the estimated average intakes, but that morphological changes in nerves cannot be excluded for some individuals with very high intake. Ongoing studies of neurotoxicity and neurodevelopmental effects in rats will more clearly define whether effects may arise from long-term, low doses of acrylamide.

When the value of 0.001 mg acrylamide/kg bw/day taken to represent the average intake of the general population was compared with the BMDL of 0.30 mg/kg bw/day for induction of mammary

tumors in rats, the MOE is 300. For intakes taken to represent high consumers (0.004 mg acrylamide/kg bw/d), the MOE is 75. The Committee considered these MOEs to be low for a compound that is genotoxic and carcinogenic and that they may indicate a human health concern. Therefore, appropriate efforts to reduce acrylamide concentrations in foodstuffs should continue.

Uncertainties in the derivation of the MOE values for acrylamide arise from uncertainties and assumptions associated with the data used to derive the BMDL values and the different intake estimates. The Committee noted that the pathways of metabolism of acrylamide are similar in rats and humans, but quantitative differences such as the extent of bioactivation of acrylamide to glycidamide or detoxication of glycidamide could result in species differences in sensitivity. Confidence in the data used to calculate the MOE for acrylamide might be enhanced by the results of the currently ongoing cancer bioassays in rodents. Incorporation of additional data on the influence of dose on the conversion of acrylamide to glycidamide into a PBPK model may facilitate the extrapolation of the incidence data to humans. The intake estimates are based on an extensive database derived primarily from data from industrialized nations. There are limited data for other countries.

Recommendations

The Committee recommended that:

1. acrylamide be re-evaluated when results of ongoing carcinogenicity and longterm neurotoxicity studies become available.
2. work should be continued on using PBPK modelling to better link human biomarker data with exposure assessments and toxicological effects in experimental animals.
3. appropriate efforts to reduce acrylamide concentrations in food should continue.
4. In addition, the Committee noted that it would be useful to have occurrence data on acrylamide in foods as consumed in developing countries. This information will be useful in conducting intake assessments as well as considering mitigation approaches to reduce human exposure.

2.2 Cadmium - impact assessment of different maximum limits

Explanation

The dietary intake of cadmium was evaluated by the Committee at its fifty-fifth and sixty-first meetings (Annex 1, references *149* and *166*). In each of these assessments, cadmium intakes were calculated from available data on concentrations and food consumption taken from the GEMS/Food regional diets. Total intakes of cadmium estimated by the Committee at its sixty-first meeting ranged from 2.8 to 4.2 µg/kg bw per week, which equate to approximately 40–60% of the current PTWI of 7 µg/kg bw per week. The seven commodity groups that contributed significantly to total intake of cadmium included: rice; wheat; root, tuber, leafy, and other vegetables; and molluscs. These commodities accounted for 40–85% of total intake of cadmium in the five GEMS/Food regions.

Before the sixty-first meeting of the Committee, the Codex Committee on Food Additives and Contaminants requested that the Committee evaluate the impact of different maximum levels (MLs) for cadmium in commodities that contribute significantly to intake, but this work could not be undertaken by the Committee at that time. At its 36th Session, the Codex Committee on Food Additives and Contaminants subsequently requested that this analysis be completed. In doing so, it asked the Committee to carry out the following specific tasks:

- to conduct intake and impact assessments for the seven commodity groups, taking into account three different maximum limits (MLs) - that is, the draft Codex MLs proposed by CCFAC for rice (0.4 mg/kg), wheat (0.2 mg/kg), potatoes (0.1 mg/kg), stem/root vegetables (0.1 mg/kg), leafy vegetables (0.2 mg/kg), other vegetables (0.05 mg/kg), and molluscs

(oysters: 3 mg/kg, other molluscs: 1 mg/kg), and one level lower and one level higher than the proposed MLs; and

- to evaluate the impact of MLs on concentrations and intakes in subcategories of molluscs (i.e. bivalves, scallops and cephalopods) on the basis of the data submitted.

This assessment took into account the potential impact of different MLs on the distribution of concentrations of cadmium in each commodity (i.e. how eliminating samples containing cadmium at concentrations greater than the ML affected the mean value of the resulting distribution, and the proportion of samples containing cadmium at concentrations greater than the ML) and the dietary intakes of cadmium from each individual commodity (i.e. how the mean concentrations of cadmium for each ML affected mean intake of cadmium).

Data on cadmium concentrations

Raw data were submitted by Australia, Canada, Germany, Japan, New Zealand, Norway, and the USA. Some aggregated data were also submitted by the European Union, Spain, Sweden, and Thailand.

Average concentrations of cadmium based on the new data on individual samples were similar to those used in the exposure assessment completed by the Committee at its sixty-first meeting. For rice, average concentrations of cadmium were higher in Japanese samples (0.061 mg/kg) than those in samples from other countries (0.017 mg/kg). The average concentration of cadmium in wheat was 0.054 mg/kg. Average concentrations of cadmium in vegetables ranged from 0.012 to 0.040 mg/kg. For molluscs, average concentrations of cadmium derived from more than 7000 samples were: oysters, 1.38 mg/kg; mussels, 0.43 mg/kg; and other bivalves or cephalopods, 0.20 mg/kg.

Assessment of the impact of MLs on mean concentrations of cadmium

For five of the commodity groups (wheat, potatoes, stem/root vegetables, leafy vegetables, and other vegetables), the data from different countries were sufficiently similar to allow all data to be combined for this assessment. Owing to the substantial difference in concentrations of cadmium in rice (by region) and in molluscs (by subcategory), the potential impact of MLs was evaluated separately for subsets of these data. Two estimates of the impact of MLs on concentrations of cadmium were calculated for rice (low estimates were based on European data only and high estimates were based on all data combined) and for molluscs (low estimates were based on data for oysters and other molluscs separately, and high estimates were based on data for all molluscs combined).

For each commodity group or subgroup, a baseline mean concentration of cadmium was calculated from all data on concentrations. For each of the three MLs (proposed, one level higher, and one level lower), the mean was recalculated after excluding values greater than the ML, and the percentage reduction from the baseline mean was calculated. The number and percentage of total data points exceeding the ML were also calculated for each ML. The greatest impacts of MLs on concentrations of cadmium in individual commodities were seen for stem/root vegetables, other vegetables, and molluscs (41%, 68%, and 42%, respectively, when the lowest MLs were used).

Assessment of the impact of MLs on mean intakes of cadmium

For the intake assessment completed by the Committee at its sixty-first meeting, intakes of cadmium, both by commodity and total, were calculated from the GEMS/ Food regional diets and the regional average concentrations of cadmium derived from aggregated data. Total intakes ranged from 2.8 to 4.2 µg/kg bw per week, which corresponds to approximately 40–60% of the PTWI of 7 µg/kg bw per week.

For the present assessment, intakes of cadmium were recalculated for the seven commodity groups on an individual basis; total intakes of cadmium calculated in the previous exposure assessment were used as benchmarks. Baseline intakes were calculated from food consumption reported in the GEMS/Food regional diets, as in the previous assessment, and values for average

baseline concentrations of cadmium derived from the new raw data. Intakes were recalculated based on the mean concentration of cadmium from each of the MLs. The impact of each ML on intake of cadmium was reported in terms of the reduction from baseline intake.

Baseline intakes for the five GEMS/Food regions, expressed as a percentage of the PTWI, ranged from 1 to 34% for rice, 3 to 29% for wheat, 1 to 15% for potatoes, < 1 to 14% for stem/root vegetables, < 1 to 3% for leafy vegetables, < 1 to 3% for other vegetables, < 1 to 3% for oysters, and < 1 to 5% for other molluscs. The lowest MLs generated reductions in intakes as follows, expressed here as a percentage of the PTWI: rice, 4%; wheat, 6%; potatoes, 6%; stem/root vegetables, 5%; oysters, 1%; and other molluscs, 2%. The proposed ML and one level higher had little or no impact on mean intakes of cadmium.

A probabilistic intake assessment for cadmium in rice using national Japanese data was submitted to the Committee. This intake assessment considered four different MLs and showed similar results. Total mean intake from rice was estimated to be about 1.4 µg/kg bw per week, or 20% of the PTWI, compared with estimates based on the GEMS/Food Diets of 33–34% of the PTWI from rice. The consumption values in the GEMS/Food Diets, which are based on Food Balance Sheet data, are generally assumed to be about 15% higher than values for actual average food consumption. Despite the difference in actual estimates of intake of cadmium from rice, both the probabilistic model and the GEMS/Food estimates demonstrated little or no impact on mean intake of cadmium from rice for the four MLs.

Evaluation

The Committee concluded that the effect of different MLs on overall intake of cadmium would be very small. At the proposed Codex MLs, mean intake of cadmium would be reduced by approximately 1% of the PTWI. The imposition of MLs one level lower would result in potential reductions in intake of cadmium of no more than 6% (wheat grain, potatoes) of the PTWI. At the proposed Codex MLs, no more than 9% of a commodity would be violative (oysters). MLs one level below those proposed would result in approximately 25% of molluscs, potatoes, and other vegetables being violative.

The use of MLs to truncate the tail of the distribution of a contaminant in commodities has little impact on the intake of the contaminant from that commodity, unless a large proportion of the commodity is excluded by the ML. The Committee noted that in its previous assessment (Annex 1, reference *166*), the total intake of cadmium was only 40–60% of the PTWI of 7 µg/kg bw per week; therefore, a variation of 1–6% due to the use of the proposed MLs is of no significance in terms of risk to human health.

2.3 Ethyl carbamate

Explanation

Ethyl carbamate (urethane), the ethyl ester of carbamic acid, has not been evaluated previously by the Committee. Although past industrial, medical and veterinary uses of ethyl carbamate have been reported, present information suggested that the major route of exposure to ethyl carbamate in the human population is through consumption of fermented foods and beverages in which it may be present, e.g. as a consequence of its unintentional formation during the fermentation process or during storage. Ethyl carbamate can be formed in fermented foods and beverages such as spirits, wine, beer, bread, soy sauce and yoghurt.

There is an extensive literature (dating from the 1940s to the present day) on the genotoxicity and carcinogenicity of ethyl carbamate. Major reviews of the available data pertaining to carcinogenicity have been performed, most recently in 1989 by the California Department of Health Services (published as Salmon et al., 1991). Ethyl carbamate was designated as “possibly carcinogenic to humans” (Group 2B) by IARC and is listed as “reasonably anticipated to be a

human carcinogen" in the Report on Carcinogens of the United States National Toxicology Program (NTP 2004).

At its present meeting, the Committee evaluated the results of studies assessing the carcinogenic and genotoxic potential of ethyl carbamate, particularly from those studies that had become available since the review by the California Department of Health Services in 1989, as well as data on metabolism and disposition, short-term toxicity, reproductive and developmental toxicity, perinatal carcinogenicity and immunotoxicity. Analytical methods, occurrence and intake were also considered.

The evaluation of ethyl carbamate was carried out in response to a request from the Codex Committee on Food Additives and Contaminants.

Absorption, distribution, metabolism and excretion

Ethyl carbamate is well absorbed from the gastrointestinal tract (and skin), and is rapidly and evenly distributed throughout the body. Elimination is also rapid, with more than 90% being eliminated as carbon dioxide within 6 h in mice. Metabolic pathways of potential importance include hydrolysis to ethanol and ammonia, and side-chain oxidation to vinyl carbamate. In rats and mice, CYP2E1 activity is responsible for about 95% of the metabolism of ethyl carbamate to carbon dioxide. Ethyl carbamate undergoes CYP2E1-mediated metabolic activation to vinyl carbamate epoxide, which binds covalently to nucleic acids and proteins, resulting in the formation of adducts, including those that have been shown to induce base-pair substitutions in DNA from tumour tissue. It has been hypothesized that co-administration of ethyl carbamate with ethanol would reduce CYP2E1-mediated activation and increase elimination by esterase-mediated hydrolysis. High doses of ethanol (4 ml/kg bw or 5 g/kg bw) given to mice 1 h before, or at the same time as, ethyl carbamate delayed elimination as carbon dioxide; in contrast, pre-treatment with 10% ethanol in drinking-water for 3 weeks had no effect.

Toxicological data

The acute oral toxicity of ethyl carbamate is low, the oral LD50 in rodents being approximately 2000 mg/kg bw. In rodents, single doses of 1000 mg/kg bw cause anaesthesia.
After repeated administration of ethyl carbamate via drinking-water for 13 weeks, there was an increase in mortality among mice and rats receiving doses of about 500–600 mg/kg bw per day. In the same study, mice given ethyl carbamate at doses of ≥ 150 mg/kg bw per day showed reduced body-weight gain and effects on lungs, liver, kidney, heart, spleen, lymph nodes, thymus, bone marrow, and ovaries. No such effects were seen at 50 mg/kg bw per day. The same organs, with the exception of the lungs, were affected in rats given drinking-water containing ethyl carbamate at the same concentrations as those at which these effects were observed in mice. A treatment-related decrease in serum lymphocyte and leukocyte counts was observed in rats; no effects were seen in male rats at a dose of about 10 mg/kg bw per day, the lowest dose tested, while a decrease was observed in the females at this dose. Serum haematological parameters were not assessed in mice, but cellular depletion of the spleen and thymus were noted in assays of immunotoxicity in mice treated intraperitoneally with ethyl carbamate at a higher dose range (100–400 mg/kg bw per day) for 1–2 weeks. Co-administration of 5% ethanol in drinking-water with ethyl carbamate at concentrations spanning the range used in the 13-week study in mice and rats (110–10 000 mg/l) attenuated many of the adverse effects of ethyl carbamate.

Ethyl carbamate has been tested in a large number of studies of genotoxicity in vitro and in vivo. The results of assays for point mutations were uniformly negative for mouse lymphoma cells, while assays in bacterial, yeast and other types of mammalian cells produced variable results. Results of assays in somatic cells in vivo (including tests for induction of chromosomal aberrations, micronucleus formation and sister chromatid exchange) were almost uniformly positive. The assay for micronucleus formation in mice showed the strongest positive response, and co-administration of ethanol delayed rather than prevented the genotoxicity of ethyl carbamate in this assay.
There was no evidence for genotoxicity in mammalian germ cells in vivo, according to the results of assays for dominant lethal mutations or in specific locus tests in mice given ethyl carbamate by

intraperitoneal injection or in drinking-water. Treatment of mice with high doses of ethyl carbamate administered by the subcutaneous or intraperitoneal route before mating resulted in increased incidences of tumours in adult offspring.

In most studies of developmental toxicity in which mice, rats or hamsters were given ethyl carbamate at high doses administered by various routes, very high rates of embryonic/fetal mortality and malformations were revealed. In the only two available studies in which ethyl carbamate was administered orally, dose-related increases in skeletal anomalies were observed in mice given a single dose of ethyl carbamate of ≥ 300 mg/kg bw on day 11 of gestation, and increases in external malformations and skeletal abnormalities were observed in rats given ethyl carbamate at a dose of 1000 mg/kg bw for 1, 2 or 7 days during the period of organogenesis. Oral doses of ethyl carbamate that show no effect have not been established. No multigeneration studies that met currently-accepted standard protocols were available.

Ethyl carbamate is a multisite carcinogen with a short latency period. Single doses or short-term oral dosing at 100–2000 mg/kg bw have been shown to induce tumours in mice, rats and hamsters. The upper range of the doses overlaps with the standard anaesthetic dose (1000 mg/kg bw) and the values for LD50s in rodents. In addition, in non-human primates given ethyl carbamate at a dose of 250 mg/kg bw per day by oral administration for 5 years, a variety of tumour types that were analogous to those observed in rodents (including adenocarcinoma of the lung, heptocellular adenoma and carcinoma and hepatic haemangiosarcoma) were induced over an observation period of up to 22 years. Treatment of female mice with single or multiple doses of ethyl carbamate during gestation or lactation was found to increase the incidence or multiplicity of tumours in the adult offspring compared with untreated controls.

In a newly-available lifetime study of carcinogenicity, male and female B6C3F1 mice were given drinking-water containing ethyl carbamate at a concentration of 0, 10, 30 or 90 mg/l together with ethanol at a concentration of 0, 2.5% or 5%. Results from the animals that did not receive ethanol were used as the basis of the present evaluation. In these animals, intakes of ethyl carbamate were equal to approximately 0, 1, 3 or 9 mg/kg bw per day, respectively. Treatment with ethyl carbamate resulted in dose-dependent increased incidences of alveolar and bronchiolar, hepatocellular and Harderian gland adenoma or carcinoma, hepatic haemangiosarcoma, and mammary gland adenoacanthoma or adenocarcinoma (females only). Smaller, but still statistically significant, increases were observed in the incidence of haemangiosarcoma of the heart (males only) and spleen (females only), squamous cell papilloma or carcinoma of the forestomach and skin (males only) and benign or malignant ovarian granulosa cell tumours. Dose-related increases in non-neoplastic lesions affecting the blood vessels of the liver, heart and uterus as well as eosinophilic foci of the liver were also observed. The most sensitive sites for tumor induction (i.e. those at which a significant increase in tumors was observed at the lowest dose tested) were the lung and Harderian gland. The incidence of combined alveolar and bronchiolar adenoma or carcinoma were 5/48, 18/48, 29/47, 37/48 (males); and 6/48, 8/48, 28/48, 39/47 (females). The incidences of combined Harderian gland adenomas or carcinomas were 3/47, 12/47, 30/47, 38/47 (males); and 3/48, 11/48, 19/48, 30/48 (females).

There was also a treatment-related increase in the combined incidence of any tumour type at any site (males: 33/48, 39/48, 46/47, 47/48; females: 37/48, 35/48, 45/48, 47/48). The co-administration of ethyl carbamate and ethanol resulted in marginal changes in the incidence of some of the neoplasms attributed to ethyl carbamate alone, but overall, co-administration of ethanol had no consistent effect on the carcinogenicity of ethyl carbamate. The absence of a clear interaction between ethyl carbamate and ethanol with regard to tumour incidence is consistent with data on CYP2E1, glutathione and apoptosis in the liver, proliferating cell nuclear antigen (PCNA) labelling in the lung and etheno-adducts in liver and lung reported in a related 4-week study in mice given the same treatment regimens.

Observations in humans

Very few data were available and these were not of a quality that could be used for hazard characterization.

Levels and pattern of food contamination

Data on concentrations of ethyl carbamate in foods and beverages were submitted by the US Food and Drug Administration, the UK Food Standards Agency and the Wine Institute of the USA. The alcoholic beverages considered in these reports originate from many countries throughout the world. For alcoholic beverages, only recent data were included because concentrations have been reduced considerably over time as a result of the application of mitigation measures.

Prevention and control

The key to successful prevention and control for ethyl carbamate has been the identification of the main precursor substances responsible for the formation of ethyl carbamate in food and beverages, together with an understanding of the influence of the main external factors of light, time and temperature. This information has led to a mechanistic understanding from which control measures have been devised. Ethyl carbamate can be formed from various substances derived from food and beverages, including hydrogen cyanide, urea, citrulline, and other *N*-carbamyl compounds. Cyanate is probably the ultimate precursor in most cases, reacting with ethanol to form the carbamate ester. Over the past years, major reductions in concentrations of ethyl carbamate have been achieved using two approaches: first, by reducing the concentration of the main precursor substances in the food or beverage; second, by reducing the tendency for these substances to react to form cyanate, e.g. by the exclusion of light from bottled spirits.

Diethylpyrocarbonate, which is an inhibitor of fermentation, can form ethyl carbamate, and for this reason the previous acceptance of diethylpyrocarbonate was revoked by the Committee at its seventeenth meeting (Annex 1, reference *35*). A second exogenous precursor for ethyl carbamate, azodicarbonamide, which has been used as a blowing agent to make certain sealing gaskets, is not recommended for bottling alcoholic beverages. The use of azodicarbonamide as a dough maturing agent is permitted in some countries; at the maximum usage levels, it can give rise to a slight increase in the formation of ethyl carbamate in bread.

Food consumption and dietary intake assessment

The Committee evaluated four published national estimates of intake (Denmark, South Korea, Switzerland, and the USA) and two estimates submitted to the Committee by Australia and New Zealand. The national estimates of mean intake of ethyl carbamate from both food and alcoholic beverages for the population as a whole ranged from approximately 1 to 4 µg/person per day, equivalent to 15–65 ng/kg bw per day. The more recent national estimates of mean intake from Australia (1.4 µg/person per day), South Korea (0.6 µg/person per day), and New Zealand (1.4 µg/person per day) used concentrations of ethyl carbamate in alcoholic beverages that were much lower than those considered at the time of the assessments in Denmark, Switzerland, and the USA, which were conducted in the early 1990s. The Committee noted that mitigation measures have been effective in reducing residual concentrations of ethyl carbamate in alcoholic beverages and that, consequently, the older data published in the early 1990s and used to make the initial estimates of intake of ethyl carbamate no longer accurately reflect current intake from alcoholic beverages.

The Committee prepared international estimates of intake of ethyl carbamate from foods using the five regional diets of the GEMS/Food database. The relevant foods included in the analyses were bread, fermented milk products (including yoghurt and cheese), and soy sauce; alcoholic beverages (with the exception of wine) are not included in the GEMS/Food database, and consequently were not considered in the analyses. The concentrations of ethyl carbamate used were mean values taken from published summaries. The mean intake of ethyl carbamate from food was estimated to be approximately 1 µg/person per day, equivalent to about 15 ng/kg bw per day, for the five regional diets. This value was consistent with the contribution of ethyl carbamate to intake from food in the national estimates, when alcoholic beverages were excluded.

The intake of ethyl carbamate for a high-percentile consumer of alcoholic beverages was modelled using an average concentration of ethyl carbamate of 4 µg/kg in wines (data from 2001) and a 95th

percentile intake of approximately 750 ml of wine (data from France). It was calculated that an additional 3 µg/person per day could be consumed, that when added to the international and national estimates of intake from food of approximately 1 µg/person per day resulted in a total intake of ethyl carbamate of up to 5 µg/person per day (equivalent to 80 ng/kg bw per day). The Committee was aware that high concentrations of ethyl carbamate can be found in stone-fruit brandies and, therefore, that high consumption of such brandies could lead to higher intakes of ethyl carbamate than those considered here.

Dose–response analysis

The Committee concluded that ethyl carbamate is genotoxic and is a multisite carcinogen in all species tested. The pivotal study for risk assessment was a recent long-term study of carcinogenicity in mice. The increased incidence of alveolar and bronchiolar adenoma or carcinoma in mice was considered to be a critical response, and the associated dose–response data were analysed. The dose–response data for animals with Harderian gland tumours were also analysed. The dose-response data for the total number of tumour-bearing animals were not considered suitable for modelling, since the background incidence was already about 75%. In the dose-response analysis, eight different statistical models (Annex 1) were fitted to the experimental data considered relevant for further consideration. Those resulting in acceptable fits based on biological and statistical considerations were selected to derive the BMD and BMDL for a 10% extra risk of tumors. This procedure results in a range of BMD and BMDL values for each endpoint considered (see table 5). The dose–response relationships appeared not to differ statistically significantly between males and females, and the models were fitted to the combined data for both sexes. For both site-specific tumour types, the dose–response data left relatively little uncertainty about the shape of the dose–response relationship. As a result, the ranges of the BMD and BMDL were quite narrow, while the BMDLs were not much lower than their associated BMDs.

Table 6. Ranges of BMD and BMDL values for tumours associated with administration of ethyl carbamate

Tumour type	Range of BMD values (mg/kg bw per day)	Range of BMDL values (mg/kg bw per day)
Lung adenoma or carcinoma	0.50–0.63	0.26–0.51
Harderian gland adenoma or carcinoma	0.47–0.76	0.28–0.61

BMD, benchmark dose for 10% extra risk of tumors; BMDL, 95% lower confidence limit for the benchmark dose. Extra risk is defined as the additional incidence divided by the tumor-free fraction of the population in the controls.

Choosing lung tumours as the critical end-point, the values for BMDLs ranged from 0.3 to 0.5 mg/kg bw. The Committee decided to use the more conservative lower end of this range of values for the evaluation.

Evaluation

When the estimated intake of ethyl carbamate in foods (15 ng/kg bw per day), is compared with the BMDL value obtained for the incidence of alveolar and bronchiolar neoplasms in male and female mice (0.3 mg/kg bw per day), the resulting MOE is 20,000. With the inclusion of alcoholic beverages in the estimated intake (80 ng/kg bw per day), the resulting MOE is 3,800. On the basis of these considerations, the Committee concluded that intake of ethyl carbamate from foods excluding alcoholic beverages would be of low concern. The MOE for all intakes, food and alcoholic beverages combined, is of concern and therefore mitigation measures to reduce concentrations of ethyl carbamate in some alcoholic beverages should be continued.

2.4 Inorganic tin

Explanation

Inorganic tin is found in food in the +2 and +4 oxidation states; it may occur in a cationic form (stannous and stannic compounds) or as inorganic anions (stannites or stannates). Inorganic tin was evaluated by the Committee at its fourteenth, fifteenth, twenty-second, twenty-sixth, thirty-third and fifty-fifth meetings (Annex 1, references *22, 26, 47, 59*, *83* and *150*). At its thirty-third meeting, the Committee converted the previously established provisional maximum tolerable daily intake (PMTDI) of 2 mg/kg bw to a provisional tolerable weekly intake (PTWI) of 14 mg/kg bw. At these meetings, the Committee reviewed data from short- and long-term dietary studies and noted that inorganic tin compounds generally have low systemic toxicity in animals, because of limited absorption from the gastrointestinal tract, low accumulation in tissues, and rapid passage through the gastrointestinal tract. Insoluble tin compounds are less toxic than soluble tin salts.

At its Thirty-first Session, the Codex Committee on Food Additives and Contaminants asked the Committee to review information on the toxicity of inorganic tin in order to establish an acute reference dose (ARfD). At its fifty-fifth meeting (Annex 1, reference *150*), the Committee (JECFA) considered studies of the acute toxic effects seen after consumption of foods containing high concentrations of inorganic compounds of tin. It concluded that the acute toxicity of inorganic tin in animals and humans, however, results from irritation of the mucosa of the gastrointestinal tract, which may lead to vomiting, diarrhoea, anorexia, depression, ataxia, and muscular weakness. There was no clear dose–response relationship, and the vehicle in which the tin was administered may have affected its toxicity. The Committee concluded that insufficient data were available to establish an ARfD for inorganic tin. At that meeting, the PTWI previously established for compounds containing inorganic tin was not reconsidered and was retained at its current value. The Committee did not consider studies on organic tin compounds, since it had concluded at its twenty-second meeting (Annex 1, reference *47*) that these compounds, which differ considerably from inorganic tin compounds with respect to toxicity, should be considered separately.

At its Thirty-fifth session, the Codex Committee on Food Additives and Contaminants asked the Committee (JECFA) to evaluate current levels of inorganic tin in “canned food other than beverages” and “canned beverages”, and to determine an ARfD, since new data would become available. At its Thirty-sixth session, the Codex Committee on Food Additives and Contaminants asked this Committee, when possible, to take population sensitivity into consideration when considering the new data, and to assess the likelihood of the occurrence of effects at the proposed draft maximum levels (200 mg/kg in canned beverages and 250 mg/kg in canned foods other than beverages).

At its present meeting, the Committee reconsidered studies of the acute toxic effects seen in humans after consumption of foods containing high concentrations of inorganic compounds of tin, and also considered a new study.

Observations in humans

Episodes of human poisoning resulting from consumption of food and drink contaminated with inorganic tin have resulted in abdominal distension and pain, vomiting, diarrhoea, and headache. Symptoms commonly start within 0.5–3 h, recovery occurs within 48 h. The doses of inorganic tin ingested in such episodes of poisoning were not estimated, but the symptoms occurred when canned food or beverages were found to contain tin at concentrations varying from 250 to 2000 mg/kg.

In one study, all five volunteers experienced symptoms when they ingested orange juice containing inorganic tin at a concentration of 1370 mg/kg (equal to a dose of 4.4–6.7 mg/kg bw). Orange juice containing inorganic tin at concentrations of 498, 540 or 730 mg/kg (equal to a dose range of 1.6–3.6 mg/kg bw) did not provoke symptoms in groups of five volunteers. Administration of the same

amount of the same juice (containing tin at 1370 mg/kg) to these individuals 1 month later resulted in symptoms in only one person. Although this was explained by the authors as development of tolerance, another possible explanation might be that the longer storage of the juice led to a different speciation.

A newly available study showed that tomato juice freshly spiked with tin (II) chloride at concentrations of ≥ 161 mg/kg causes gastrointestinal disorders in humans in a concentration-related manner. The concentration–response relationship indicated a threshold for acute effects caused by inorganic tin at a concentration of about 150 mg/kg of juice. In the second part of this study, volunteers receiving 250 ml of a tomato soup contaminated with inorganic tin that had migrated from packaging at concentrations of < 0.5, 201 and 267 mg/kg did not experience an increased incidence of adverse effects compared with controls. The results of distribution studies of tin in the soup and juice consumed supported the view that both complexation and adsorption of tin onto solid matter reduce its irritant effect on the gastrointestinal tract.

Overall, the information available showed that gastrointestinal irritation from inorganic tin in canned foods is more related to the concentration and nature of tin in the product than to the dose of tin ingested on a body-weight basis. No information was available regarding subpopulations such as children or people with gastrointestinal disorders.

Evaluation

The Committee concluded that the data available indicated that it is inappropriate to establish an ARfD for inorganic tin, since whether or not irritation of the gastrointestinal tract occurs after ingestion of a food containing tin depends on the concentration and nature of tin in the product, rather than on the dose ingested on a body-weight basis. Therefore, the Committee concluded that the short-term intake estimates were not particularly relevant for the assessment, as they were estimated likely doses of total inorganic tin. The Committee reiterated its opinion, expressed at its thirty-third and fifty fifth meetings, that the available data for humans indicated that inorganic tin at concentrations of > 150 mg/kg in canned beverages or 250 mg/kg in canned foods may produce acute manifestations of gastric irritation in certain individuals. Therefore ingestion of reasonably-sized portions containing inorganic tin at concentrations equal to the proposed standard for canned beverages (200 mg/kg) may lead to adverse reactions. No information was available as to whether there are subpopulations that are particularly sensitive for such adverse reactions. The Committee reiterated its advice that consumers should not store food and beverages in open tinplated cans.

In addition, the Committee noted that the basis for the PMTDI and PTWI established at its twenty-sixth and thirty-third meetings was unclear and these values may have been derived from intakes associated with acute effects. The Committee concluded that it was desirable to (re)assess the toxicokinetics and effects of inorganic tin after chronic exposure to dietary doses of inorganic tin at concentrations that did not elicit acute effects.

2.5 Polybrominated diphenyl ethers

Explanation

Polybrominated diphenyl ethers (PBDEs) are anthropogenic chemicals that are added to a wide variety of consumer/commercial products (e.g. plastics, polyurethane foam, textiles) in order to improve their fire resistance. PBDEs have been produced primarily as three main commercial products: pentabromodiphenyl oxide or ether (PentaBDE), octabromodiphenyl oxide or ether (OctaBDE) and decabromodiphenyl oxide or ether (DecaBDE). Some variability in composition is known to exist between products from different manufacturers, but each technical product can be approximately described by their congener compositions. Theoretically, as with polychlorinated biphenyls (PCBs), 209 distinct PBDE isomers are possible; however, each commercial mixture usually only contains a limited number of congeners from each homologue group. The worldwide demand for PBDEs in 2001 was estimated to be almost 70 000 tonnes, with DecaBDE accounting for almost 80% of the total market.

Table 7. General composition of commercial PBDE flame retardants and substitution pattern of selected congeners

PBDE	
Mixture	*Congener composition (% of total)*
Penta	24–38% tetraBDEs, 50–60% pentaBDEs, 4–8% hexaBDEs
Octa	10–12% hexaBDEs, 44% heptaBDEs, 31–35% octaBDEs, 10–11% nonaBDEs, < 1% decaBDEs
Deca	< 3% nonaBDEs, 97–98% decaBDE
Individual congeners	*Substitution pattern*
BDE-47	2,2',4,4'-tetraBDE
BDE-99	2,2',4,4',5-pentaBDE
BDE-153	2,2',4,4',5,5'-hexaBDE
BDE-209	2,2',3,3',4,4',5,5',6,6'-decaBDE

PBDEs have not been evaluated previously by the Committee. In 1994, WHO published an Environmental Health Criteria document on brominated diphenyl ethers (WHO 1994), as part of an overview on the possible environmental and human health impacts of flame retardants. Recent analysis of archived samples collected over the last three to four decades demonstrated significant increases in concentrations of PBDEs in samples from the environment and in certain samples from humans in Europe and North America. This has led to both voluntary and formal bans on the production and use of certain formulations of PBDEs. Limited national food surveys have identified diet as one of the possible main sources of human exposure. The present evaluation was undertaken in response to a request from the Codex Committee on Food Additives and Contaminants, most recently at its 35th Session, to evaluate the potential risks associated with the presence of PBDEs in food.

Absorption, distribution, metabolism and excretion

The majority of detailed studies of the absorption, distribution, metabolism and excretion of PBDEs are limited to the individual congeners BDE-47, -99 and -209. The absorption of PBDEs is directly related to the extent of bromination of the parent diphenyl ether; as a general rule, greater substitution of bromine leads to a decrease in bioavailability. Intestinal absorption of decaBDE is limited, with greater than 90% of an orally administered dose being rapidly excreted in the faeces. For congeners with a lower degree of bromination (tetra and penta-substituted), greater than 80% of an orally administered dose is absorbed, with patterns of distribution in tissue being largely determined by content of lipid. The metabolism of PBDEs consists of hydroxylation and methoxylation reactions and, in the case of congeners with a higher degree of bromination, oxidative debromination. Faecal excretion appears to be the predominant route of elimination; however, some differences exist between species. Urinary excretion of BDE-47 is a minor pathway in rats, but is as important as faecal excretion in mice. Limited data were available regarding the half-lives of individual PBDE congeners; however, preliminary values in female rats exposed to a commercial PentaBDE mixture, Bromkal 70-5 DE, ranged from 30 to 90 days for the tetra- to hexa-substituted congeners.

Limited pharmacokinetic data were available for humans. On the basis of the observed increase in concentrations of PBDEs in tissue with time, PBDEs are absorbed and bioaccumulate.

Toxicological data

In the toxicology studies reviewed, PBDEs were administered by the oral (gavage or diet) route of exposure, unless otherwise stated.

The acute toxicity of mixtures of PBDEs is low in rodents. Generally, even at the highest doses (several g/kg bw), there are no observable effects in standard tests for acute toxicity after exposure to DecaBDE and OctaBDE, although certain effects (increased mortality, behavioural symptoms and changes in gross pathology) are seen after exposure to PentaBDE at similar high doses. Induction of enzymes, changes in levels of hormones and neurobehavioural effects are observed after bolus administration of mixtures of PBDEs (PentaBDE and OctaBDE), and of specific congeners at considerably lower doses. In short-term studies of toxicity, the main effects of mixtures of PBDEs were seen in the liver, kidney and thyroid of both sexes. Enlargement of the liver is a common finding, which may be connected to increased activity of microsomal enzymes in the liver. Histological changes occur in liver (enlargement, 'round bodies', vacuolization, necrosis), kidney (hyaline degenerative cytoplasmic changes) and thyroid (hyperplasia). In short-term studies, effects on thyroid hormone, vitamin A homeostasis and microsomal enzymes were observed at doses of 1–10 mg/kg bw per day.

The only long-term study with PBDEs was conducted with the DecaBDE mixture (NTP 1986). In this NTP study of carcinogenicity, DecaBDE (purity, 94–99%; brominated dioxins and furans reported not to be detected), given in the diet at high concentrations (2.5% or 5%) for 111–113 weeks, significantly increased the combined incidence of hepatocellular adenomas and carcinomas in male mice, but not in female mice. In spite of an increase in follicular cell hyperplasia, the incidence of thyroid follicular cell adenoma/carcinoma was not significantly increased. In male and female rats, the incidence of liver adenomas, but not hepatocellular carcinomas, was increased. Other effects, such as liver hypertrophy, granulomas, thrombosis and degeneration, thyroid follicular cell hypertrophy, and lymphoid hyperplasia, were also noted. The Committee concluded that evidence for the carcinogenicity of DecaBDE in experimental animals was limited, and noted that no information was available on the carcinogenic potential of the other PBDE mixtures.
The results of the majority of tests for genotoxicity performed in vitro (point mutations, chromosomal aberrations, unscheduled DNA synthesis, sister chromatid exchange) and limited data from tests in vivo (chromosomal aberration) indicated that PBDE mixtures and single congeners are not genotoxic.

The developmental toxicity of the Deca-, Octa- and PentaBDE mixtures has been studied in rats and rabbits. In rats, preparations of pure DecaBDE (purity, 97–98%) had no effects on developmental parameters, while DecaBDE of lower purity (decaBDE, 77.4%; nonaBDE, 21.8%; octaBDE, 0.8%) caused fetotoxic effects. Exposure to commercial OctaBDE mixtures (Saytex 111 and DE-71) produced developmental toxicity as indicated by increased numbers of late resorptions, reduced fetal weight, severe oedemas, reduced ossification of skull bones and bent rib and limb bones at a dose range of 10–50 mg/kg bw per day; only slight maternal toxicity (decreased body weight) was observed at doses of 25–50 mg/kg bw per day. A PentaBDE mixture (Saytex 115) has only been tested in one study, with no clear adverse effects at a dose of 100 mg/kg bw.

In rabbits given a commercial OctaBDE mixture (Saytex 111) during gestation, no major fetotoxic effects were observed, but an increase in the incidence of delayed ossification of sternebrae was seen at 15 mg/kg bw per day.

The Committee concluded that the embryo and fetus may be more sensitive to PBDEs than maternal animals, and that exposure to OctaPBDE mixtures causes an increase in the incidence of developmental abnormalities.

Special studies

Studies with purified PBDE congeners in vitro have shown a lack of Ah-receptor activation at doses six orders of magnitude higher than the half-maximal effective concentration (EC50) of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), suggesting that some toxicity data may be confounded by the presence of traces of impurities that are potent Ah-receptor agonists.

In studies with the commercial PBDE mixtures, PentaBDEs (Bromkal 70-5 DE and DE-71), OctaDBE (DE-79) and DecaBDE (DE-83R), various strains and both sexes of adult mice and rats have been used and acute or short-term dosing schedules applied to examine effects on thyroid hormone homeostasis. In the majority of studies, concentrations of total thyroxine (T4) and, in some cases, free T4 in the blood were found to be suppressed, with almost no corresponding alteration in thyroid-stimulating hormone (TSH). DE-79 was reported to be more potent than DE-71, while no effects were found after exposure to DE-83R. When pregnant rats were given DE-71 at maternal doses of ≥ 3 mg/kg bw per day, circulating concentrations of T4 in the offspring were found to be reduced until weaning, with recovery of T4 values within 2 weeks thereafter. In juvenile rats given DE-71, reductions in serum concentrations of T4 were similar in both sexes, but concentrations of TSH were elevated and serum concentrations of triiodothyronine (T3) were significantly decreased only in males. Plasma concentrations of total and free T4 were decreased in adult female mice and rats given Bromkal 70-5 DE at a dose of 18 mg/kg bw per day for 2 weeks. At doses at which circulating concentrations of T4 were decreased (> 1 mg/kg bw per day), the activities of UDP-glucuronyltransferase (UDPGT) and ethoxyresorufin-O-deethylase (EROD) were often found to be increased, suggesting that Ah receptor-dependent effects are most likely to be mediated by contamination of commercial PBDE mixtures with dioxin-like compounds. A similar observation was also seen in studies with individual PBDE congeners (BDE-47 and BDE-99).
Of the individual congeners, only BDE-47, BDE-99, and BDE-209 have been studied. With regard to effects on the concentrations and activities of thyroid hormones, the available data indicated that BDE-209 is much less potent than BDE-47 and BDE-99, but lack of data precluded a comparison of the potencies of BDE-47 and BDE-99. In general, the results of studies with individual congeners indicated that their effects on thyroid hormones were similar to those observed with mixtures. The most pronounced effects were reduced concentrations of circulating total and free T4. TSH was not affected in the majority of studies.

Recent studies, available as extended abstracts, showed that the offspring (both males and females) of rats given a single oral dose of BDE-99 (60 µg/kg bw) or BDE-47 (140 µg/kg bw) on day 6 of gestation had altered concentrations of T3 and T4 during the weaning period. Serum concentrations of TSH were also reduced during lactation. These alterations in thyroid hormones recovered during postnatal development. In general, examination of effects on the thyroid after maternal exposure to mixtures of PBDEs or to individual congeners demonstrated that the offspring were more susceptible than the dams.
While competitive inhibition of the binding of T4 to transthyretin (TTR) by hydroxylated metabolites of PBDE is thought to be one of the mechanisms responsible for decreases in circulating concentrations of thyroid hormone in rats, the significance of this for human exposure is questionable. Thyroid-binding globulin (TBG), which is absent in rats, is the main thyroid-hormone transport protein in humans. Metabolites of PBDE have been shown to have limited binding affinity to human TBG. A general observation by the Committee was the apparent lack of consistency in the results of a number of experimental studies measuring thyroid hormone changes; significant decreases in serum concentrations of T4 were observed in the absence of corresponding effects on TSH. At the present time, there is insufficient information about the effects of PBDEs on feedback mechanisms in the hypothalamus and pituitary. In a number of studies in which the effects of PBDE congeners or mixtures on thyroid hormones were measured, induction of hepatic EROD was also observed, which could indicate the presence of dioxin-like contaminants. Alterations in thyroid hormones are also a sensitive response in experimental animals exposed to dioxin-like chemicals. The available data were considered to be insufficient to determine the mechanism for the reported effects on thyroid hormones and the possible role of pure PBDEs in altering delivery of maternal thyroid hormones across the placental barrier to the developing embryo/fetus and into the brain.

Possible effects of PBDEs on steroid hormones and steroid-related end-points have been reported in a limited number of studies (mainly in extended abstracts) with a commercial pentaBDE mixture (DE-71) and two congeners, BDE-47 and BDE-99. In weanling rats treated by oral administration with a commercial pentaBDE mixture (DE-71) for 20 days (female) or 31 days (male), the onset of puberty was delayed in both sexes at doses of 30–60 mg/kg bw per day. After a single oral dose of BDE-47 (700 µg/kg bw) on day 6 of gestation, decreased serum concentrations of follicle-stimulating hormone were seen in male rat offspring. With the same exposure protocol, BDE-99 was recently reported to reduce sperm production at a dose of 60 µg/kg bw. Induction of hepatic EROD was observed in all these experiments, therefore Ah receptor-mediated effects by possible dioxin-like contaminants could not be excluded.

In rats given BDE-99 at doses as low as 1 mg/kg bw per day by subcutaneous administration during days 10–18 of gestation, decreases in the circulating concentrations of sex steroid hormones (estradiol and testosterone) were observed in weanling and adult male offspring. Anogenital distance was reduced in male offspring and reproductive organ weights were altered in both sexes. The onset of puberty was delayed in females and accelerated in males, while there was a marked reduction in the expression of androgen receptor (AR) mRNA in the ventral prostate on postnatal day 120. In the same study, exposure to a technical PCB mixture(Aroclor 1254), known to possess dioxin-like activity, at a dose of 30 mg/kg bw per day did not affect several of these end-points, indicating that contamination of the BDE-99 with dioxin-like compounds was unlikely to account for these observations.

The majority of investigations examining neurotoxicity in vivo involved oral exposure of mice and rats to individual congeners. In almost all experiments in mice, individual congeners (e.g. BDE-47, -99, -153, -183, -203, -206 and -209) given to neonates as a single oral dose on a specific postnatal day, produced changes in activity patterns and habituation, which became more pronounced with ageing. Essentially identical results were observed in the same laboratory with two different strains of mice, in both sexes, and also in rats. In general, the congeners with a lower degree of bromination appeared to be more potent than the congeners with a higher degree of bromination. Most of the neurotoxicological examinations were performed in rats treated with BDE-99 during gestation. Decreases in long-term potentiation in the cortex and hippocampus, as well as influences on sexually dimorphic brain structures, reductions in mating behaviour, and feminization of sweet-preference behaviour were reported at doses of ≥ 1 mg/kg bw per day administered subcutaneously. As some of these end-points were not affected by administration of Aroclor 1254 at higher doses, this would indicate that mechanisms similar to those for dioxins are unlikely to be involved. Impaired hippocampal long-term potentiation and conditioned behaviour were also detected in the offspring of female rats treated with the PentaBDE mixture (DE-71) at oral doses of 30–100 mg/kg bw per day from day 6 of gestation to postnatal day 21. Altered locomotor activity was reported in the offspring of female rats given a single oral dose of BDE-47 (140 or 700 µg/kg bw) or BDE-99 (60 or 300 µg/kg bw) on day 6 of gestation. Because of the preliminary nature of these findings, an interpretation of significance for human health could not be made.

Observations in humans

No clinical observations have been reported in humans after oral ingestion of PBDEs. Although several studies have been conducted in workers exposed occupationally to PBDEs, these subjects were also exposed to other substances, making it difficult to attribute any observed effects solely to PBDEs. Therefore the Committee did not consider these studies to be useful for evaluation of the potential health effects of dietary exposure to PBDEs. In a case–control study, elevated concentrations of BDE-47 were found in adipose tissue for incident cases of non-Hodgkin lymphoma, but the etiological significance of this association is uncertain. In a study of adult male consumers of Baltic fish, plasma concentrations of BDE-47 were inversely related to concentrations of TSH and were not related to the concentrations of any of the thyroid hormones measured, suggesting that exposure to BDE-47 via frequent consumption of fish does not impair thyroid function in adult men.

The Committee concluded that the available studies in humans were not adequate to evaluate whether exposure to PBDEs, at the levels studied, is associated with adverse health effects.
In human milk collected in Sweden between 1972 and 1997, the concentrations of PBDEs increased, doubling every 5 years, resulting in current concentrations in the low ng/g lipid range. Recent investigations with human milk from other European countries showed similar levels of contamination.

Analysis of a limited number of samples of human serum, collected between 1985 and 1999 in the USA, also showed an increase in concentrations of PBDEs over time.

Analysis of a limited number of recently collected human samples (blood, milk, adipose) from North America has indicated that average concentrations of PBDEs are 10 to 20 times higher than those in samples collected in European countries. The reason for the higher values found for North America was not thought to be solely related to dietary exposure. The significance of pathways other than food, such as indoor air and indoor dust, are currently under investigation.
Generally, lipid-based concentrations are similar in different human samples, such as milk, blood and adipose tissue.

The typical pattern of congeners found in humans is normally dominated by BDE-47, followed by BDE-99 and the hexa-brominated congener, BDE-153. Preliminary results indicated that congener BDE-153 is becoming more prominent in European samples.

Analytical methods

Gas chromatography coupled with high-resolution mass spectrometry (GC/HRMS) using the isotope dilution technique (^{13}C-labelled standards) has been found to be the most reliable method for the determination of PBDE congeners in food and environmental samples, as well as in samples of human tissues. Only a limited number of congeners has been measured in recent years. Typical limits of detection for Tetra/PentaBDEs range from 0.005 to 0.05 ng/g, depending on lipid content and sample size.
The Committee noted that as DecaBDE was the only commercial formulation currently marketed in Europe and North America, analytical methods should include the determination of this fully brominated congener.

Levels and pattern of food contamination

The Committee reviewed data available on concentrations of PBDEs in foods. Some of the data were from total diet studies conducted either at the national level (Finland, Netherlands, and Sweden), or at the regional level within a given country (e.g. Vancouver and Whitehorse in Canada, and Catalonia in Spain), while others were from more limited, market basket surveys targeting special foods, e.g. foods of animal origin or fish and seafood, or were from grab samples collected from local markets (Canada, Germany, Japan, UK, USA). The data from the Canadian total diet study and Special Fish and Seafood Survey and the total diet studies from the Netherlands and Sweden were available in reports published by the respective national agencies, while the data from the other studies were available in published scientific journals or were submitted by local governments. Concentration data were available for individual congeners or their sum. The patterns of congeners detected were not uniform across the various foods tested and were different from those present in any one commercial mixture.

In general, the available data on concentrations of PBDEs in food for the various countries have not covered the entire diets in these countries or are based on a small number of samples. Thus, the currently available data do not allow a comprehensive assessment of contamination in all foods. Differences in concentrations were detected in similar food samples collected from various geographical areas.

Food consumption and dietary intake assessment

Preliminary estimates of mean intake of PBDEs, based on limited samples from Canada, Japan, the USA, and some European countries, as reported in published studies and reports, range from 13 to113 ng/day. Fish and shellfish were the main contributors to total intakes of PBDEs in the European countries and Japan, while meats, poultry and products of these foods were the major contributors to the total intakes of PBDEs in Canada and the USA.

Estimates of regional intakes for the European and North American region were estimated using the GEMS/Food regional diets and the available concentration data. Although the North American diet is included under the European diet in GEMS/Food, intake estimates for the North American and European regions were derived separately in light of the potential differences between concentrations of PBDEs detected in foods in Europe and North America. The estimated mean intakes of PBDEs for the European and North American regions were 2.2 and 3.6 ng/kg bw per day, respectively. Consumption of fish contributed most to European intake estimates, while meats and poultry contributed most to the North American intake estimates. No data on concentrations of PBDEs were available for countries in the following GEMS/Food regions: Africa, the Middle East, or Latin America, and limited data were available for the Far East. The Committee derived estimates of international intake for these regions using the GEMS/Food regional diets and assuming that concentrations of PBDEs in food in these regions were equal to the average levels of contamination derived from European and North American data. Estimated intakes for Africa, the Middle East, Latin America and the Far East were 1.5, 1.3, 2.1 and 1.2 ng/kg bw per day, respectively. Fish and shellfish contributed most to estimated intakes in the Africa, Latin American and Far Eastern regions, while fats and oils contributed most to the estimates for the Middle East. It should be noted that these estimates were only rough approximations since they were based on concentration data from other regions.

A regional difference was apparent when considering intake by breastfeeding infants. Based on a median concentration of PBDEs of approximately 23 ng/g of lipid in human milk (n = 145), intake by a breastfeeding infant in North America was estimated at 120 ng/kg bw per day (average fat content of milk, 3.0%; 750 ml of milk per day; 5.0 kg body weight during nursing). In comparison, based on a median concentration of PDBEs of 1.8 ng/g of lipid in samples of human milk, estimated intake for a breastfeeding infant in Germany would be approximately 10 ng/kg bw per day.

The Committee recognized the preliminary nature of the data on concentrations of PBDEs in food and human milk, which adds considerable uncertainty to the intake estimates.

Dose–response analysis

Only the commercial DecaBDE mixture has been tested in a long-term toxicity study; the lowest concentration tested (2.5% in the diet) produced adverse effects. Limited toxicological information was available for commercial PBDE mixtures (PentaBDE and OctaBDE) whose congener patterns resemble that of residues found in food and human tissues. Only short-term feeding studies (up to 13 weeks) have been conducted in rats, with liver, kidney and thyroid being identified as target organs. Dose-related increases in relative liver weights and microscopic liver changes (hepatocellular enlargement with vacuolation) were noted in a study with a commercial OctaBDE mixture (DE-79) at a concentration of 100 mg/kg of diet (approximately 8 mg/kg bw per day). Similar effects were seen in a short-term feeding study with a commercial PentaBDE mixture (DE-71); dose-related increases in liver weights and histological changes (hypertrophy, slight degeneration and necrosis) were noted at the lowest dose, 2 mg/kg bw per day. The effects were still partially evident in females at the lowest dose after a 24-week recovery period. At higher doses (≥ 10 mg/kg bw per day), decreases in concentrations of circulating thyroid hormones (T4) were observed. This latter observation was supported by the results of a study of developmental toxicity in rats given the commercial PentaBDE (DE-71); decreases in serum concentrations of T4 were seen in both fetuses and newborn pups at a maternal dose of 10 mg/kg bw per day administered on day 6 of gestation to post-natal day 21.

The Committee also reviewed a number of preliminary studies of acute toxicity involving dosing with mainly commercial PentaBDE mixtures, BDE-47 or BDE-99 on a single day during gestation or lactation. In mice and rats, there were a variety of effects involving neurological development (behaviour, memory and activity), thyroid hormone perturbation and sexual maturation at doses as low as 60 µg/kg bw. Owing to a lack of mechanistic information and adequate data on dose–response relationships, a clear interpretation of the significance to human health could not be made at the present time.

Evaluation

For non-genotoxic substances, the Committee would normally allocate a PMTDI or PTWI based on the NOEL for the most sensitive adverse effect; however, the available data on PBDEs were not adequate for such an approach because:

- PBDEs represent a complex group of related chemicals and the pattern of PDBE congeners in food is not clearly defined by a single commercial mixture;
- data are inadequate to establish a common mechanism of action that would allow a single congener to be used as a surrogate for total exposure or, alternatively, as the basis for establishing toxic equivalence factors;
- there is no systematic database on toxicity including long-term studies on the main congeners present in the diet, using standardized testing protocols that could be used to define a NOEL for individual PBDEs of importance;
- several of the reported effects are biological outcomes for which the toxicological significance remains unclear;
- studies with purified PBDE congeners in vitro have shown a lack of Ah receptor activation; however, many of the adverse effects reported are similar to those found with dioxin-like contaminants, suggesting that some toxicity data may be confounded by the presence of traces of impurities that are potent Ah receptor agonists

DecaBDE was the only brominated diphenyl ether for which a long-term study of toxicity was available. A complete hazard characterization for this PBDE will become increasingly important as it is currently the primary commercial mixture in use worldwide.

The limited toxicity data suggest that for the more toxic PBDE congeners adverse effects would be unlikely to occur in rodents at doses of less than approximately 100 µg/kg bw per day. The current estimates of dietary intake were approximately 0.004 µg/kg bw per day, while intake by breastfeeding infants could be up to 0.1 µg/kg bw per day for the sum of all measured PBDE congeners, including the less toxic ones. In consequence, there appeared to be a large MOE for a non-genotoxic compound which, despite the inadequacy of the data on toxicity and intake, gave reassurance that intakes of PBDEs are not likely to be a significant health concern. The Committee noted that, as with related bioaccumulative persistent contaminants (PCBs, dioxins), a more appropriate dose-metric for interspecies comparison of risk would be a measure of the internal dose. For the majority of PBDEs studied, however, the data from experimental animals or on concentrations in human tissue were insufficient to allow a comparison with external dose.

The Committee considered that continuing studies of PBDEs in samples from humans, including human milk, would be useful in assessing the overall exposure to PBDEs in foods and other possible sources.

2.6 Polycyclic aromatic hydrocarbons

Explanation

Polycyclic aromatic hydrocarbons (PAHs) constitute a large class of organic compounds containing two or more fused aromatic rings. Hundreds of individual PAHs may be formed during incomplete

combustion or pyrolysis of organic matter, during industrial processes and cooking and food processing.

At its thirty-seventh meeting, the Committee evaluated benzo[a]pyrene (Annex 1, references *94*, *95*), and recognized that it was one member of a family of PAHs that should be considered as a class. The Committee concluded that, for the purpose of the evaluation, the most significant toxicological effect of benzo[a]pyrene was carcinogenicity. The Committee noted that the estimated average daily intake of benzo[a]pyrene by humans was about four orders of magnitude lower than that reported to be without effect on the incidence of tumours in rats given diets containing benzo[a]pyrene. However, at that time the Committee was unable to establish a tolerable intake for benzo[a]pyrene, based on the available data. The Committee noted that the large differences between the estimated intakes in humans and the doses producing tumours in animals suggested that any effects on human health were likely to be small. Despite this, the Committee concluded that the considerable uncertainties in the estimation required that efforts should be made to minimize human exposure to benzo[a]pyrene as far as was practicable.

At its present meeting, in response to a request from CCFAC at its Thirty-fifth session, the Committee reviewed all information relevant to the toxicology, epidemiology, intake assessment, analytical methodology, formation, fate and occurrence of PAHs in food. Two documents were particularly important in this evaluation: the opinion of the European Union Scientific Committee on Food (SCF) (EC 2002) on the risks to human health posed by PAHs, and the International Programme on Chemical Safety (IPCS) Environmental Health Criteria document (WHO 1998) on selected non-heterocyclic PAHs. The present Committee used these assessments as the starting point for its evaluation and also took into account newer studies that were considered to be informative for the evaluation.
The 33 compounds considered in the present evaluation are listed in Table 8. These are the 33 PAHs selected for consideration in the IPCS and SCF documents on the basis of available information on their occurrence and toxic effects.

Table 8. PAHs considered in the present evaluation

Common name	CAS name	CAS registry No.
Acenaphthene	Acenaphthylene	83-32-9
Acenaphthylene	Acenaphthylene, 1,2-dihydro	208-96-8
Anthanthrene	Dibenzo[*def,mno*]chrysene	191-26-4
Anthracene	Anthracene	120-12-7
Benz[*a*]anthracene	Benz[*a*]anthracene	56-55-3
Benzo[*a*]fluorene	11 *H*-benzo[*a*]fluorene	238-84-6
Benzo[*b*]fluorene	11 *H*-benzo[*b*fluorene	243-17-4
Benzo[*b*]fluoranthene	Benz[*e*]acephenanthrylene	205-99-2
Benzo[*ghi*]fluoranthene	Benzo[*ghi*]fluoranthene	203-12-3
Benzo[*j*]fluoranthene	Benzo[*j*]fluoranthene	205-82-3
Benzo[*k*]fluoranthene	Benzo[*k*]fluoranthene	207-08-9
Benzo[*ghi*]perylene	Benzo[*ghi*]perylene	191-24-2
Benzo[*c*]phenanthrene	Benzo[*c*]phenanthrene	195-19-7
Benzo[*a*]pyrene	Benzo[*a*]pyrene	50-32-8
Benzo[*e*]pyrene	Benzo[*e*]pyrene	192-91-2
Chrysene	Chrysene	218-01-9
Coronene	Coronene	191-07-1
Cyclopenta[*cd*]pyrene	Cyclopenta[*cd*]pyrene	27208-37-3
Dibenz[*a,h*]anthracene	Dibenz[*a,h*]anthracene	53-70-3
Dibenzo[*a,e*]pyrene	Naphtho[1,2,3,4-*def*]chrysene	192-65-4
Dibenzo[*a,h*]pyrene	Dibenzo[*b,def*]chrysene	189-64-0
Dibenzo[*a,i*]pyrene	Benzo[*rst*]pentaphene	189-55-9
Dibenzo[*a,l*]pyrene	Dibenzo[*def,p*]chrysene	191-30-0
Fluoranthene	Fluoranthene	206-44-0
Fluorene	9*H*-fluorene	86-73-7

Indeno[1,2,3-*cd*]pyrene	Indeno[1,2,3-*cd*]pyrene	193-39-5
5-Methylchrysene	Chrysene, 5-methyl-	3697-24-3
1-methylphenanthrene	Phenanthrene, 1-methyl	932-69-9
Naphthalene	Naphthalene	91-20-3
Perylene	Perylene	198-55-0
Phenanthrene	Phenanthrene	85-01-8
Pyrene	Pyrene	129-00-0
Triphenylene	Triphenylene	217-59-4

CAS, Chemical Abstract Service

Absorption, distribution, metabolism and excretion

Absorption of dietary PAH is determined by the size and lipophilicity of the molecule and the lipid content of the food. PAH are metabolized by oxidation of the aromatic rings, primarily by enzymes of the CYP1, 2 and 3 families, followed by formation of glutathione, glucuronide and sulfate conjugates. Oxidation can generate electrophilic metabolites that bind covalently to nucleic acids and proteins. Some PAH and some PAH metabolites also bind to the aryl hydrocarbon (Ah) receptor, resulting in up-regulation of several of the enzymes involved in PAH metabolism. This may lead to complex and potentially non-linear dose–response relationships for mixtures of PAH.

Toxicological Data

On the basis of the available information, the Committee concluded that fifteen individual PAHs are clearly genotoxic *in vitro* and *in vivo*. These genotoxic PAHs are benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[ghi]perylene, benzo[j]fluoranthene, benzo[k]fluoranthene, chrysene, cyclopenta[cd]pyrene, dibenz[a,h]anthracene, dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, dibenzo[a,l]pyrene, indeno[1,2,3-cd]pyrene and 5-methylchrysene. The Committee considered that four individual PAHs (anthracene, benzo[a]fluorene, naphthalene, pyrene) were not genotoxic.

An important observation was the binding of the active metabolites of PAHs to DNA, predominantly to amino groups of guanine and adenine. The major stable adduct is formed at the N2 position of desoxyguanosine. The formation of DNA adducts by electrophilic metabolites is generally regarded as one of the earliest steps in carcinogenicity of the mutagenic PAHs. However, there is a poor quantitative relationship between levels of tissue adduct and tumour formation. This indicates that other factors additional to DNA adduct formation are apparently critical for the development of tumours caused by benzo[a]pyrene and some other PAHs, and that genotoxic end-points alone may not adequately predict the site or frequency of tumour development.

With respect to the assessment of risk of cancer, the Committee noted that the levels of benzo[c]fluorene-derived adducts were much higher than those of benzo[a]pyrene-derived adducts in the lungs of rats fed with coal tar. Although this might indicate that benzo[c]fluorene may contribute to the formation of lung tumours after oral exposure to coal tar, the Committee found no data on its occurrence in food.

Overall, the Committee concluded that the following PAHs were clearly carcinogenic and genotoxic: benz[a]anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, chrysene, dibenz[a,h]anthracene, dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, dibenzo[a,l]pyrene, indeno[1,2,3-cd]pyrene and 5-methylchrysene.

There is limited or no evidence on the reproductive toxicity of individual PAHs, other than benzo[a]pyrene, in animals. There was no effect on reproductive capacity in a one-generation study in mice receiving diets containing benzo[a]pyrene at doses of up to 133 mg/kg bw per day. Impaired fertility was seen in the offspring of female mice given benzo[a]pyrene at doses of > 10 mg/kg bw per day by gavage. Developmental toxicity has been reported after oral administration of benz[a]anthracene, benzo[a]pyrene, dibenz[a,h]anthracene or naphthalene. A NOEL for reproductive toxicity of the latter PAHs administered by the oral route has not been established.

The immunosuppressive effects of PAHs have mainly been investigated in studies using parenteral administration. It has been suggested that PAHs exert immune effects via the Ah receptor. Observations in CYP1A1 knock-out mice have indicated that CYP1A1 may protect against immunotoxic effects by benzo[a]pyrene. In a study on immunosuppressive effects in rats treated orally, the NOEL for benzo[a]pyrene was 3 mg/kg bw per day.

Observations in humans

Most data, both on the effects of PAHs in human populations and on biomarkers of exposure to PAHs, mainly refer to occupational and environmental exposure. The available evidence regarding oral exposure to PAHs was indirect and did not include data on quantitative exposure, and thus is not suitable for use in the risk assessment for PAHs.

Analytical Methods

High-performance liquid chromatography (HPLC) with fluorescence detection has been widely used for the determination of PAH in several food matrices. Use of a UV/diode-array detector in conjunction with a fluorescence detector improved the detection limits for compounds with a low fluorescence intensity. GC-FID and GC-MS methods are also employed for their determination.

The Committee noted that most analytical methods developed include the 16 priority pollutant PAH listed by the USA Environmental Protection Agency and do not include most dibenzopyrenes and 5-methylchrysene. The Committee recommended that analytical methods be developed to include these compounds.

Sources and occurrence in foods

There are two main routes of entry of PAHs into the food chain. Foods can be contaminated by environmental PAHs that are present in air (by deposition), soil (by transfer) and water (by deposition and transfer). The PAHs that are airborne (either in the vapour phase or adhered to the particulate matter) become deposited on crops, especially crops with broad leaves. Contamination of fish and marine invertebrates occurs due to the deposition and transfer of PAHs. High concentrations of PAHs have been reported in bivalves (mussels and oysters) that feed by filtering large quantities of water. PAHs also form directly during processing (drying and smoking) or cooking (grilling, roasting, frying etc.) of foods. High values are reported in grilled and barbecued foods. Direct smoking, especially using traditional methods, results in contamination with PAHs. Additional minor routes of contamination may include use of contaminated smoke flavouring additives and migration from contaminated packaging materials.

Grilling of foods has been reported to be responsible for contamination with PAHs. Although not precisely known, it is likely that PAHs are formed from melted fat that undergoes pyrolysis when dripping onto the heat source. Higher concentrations of PAHs have been reported in foods that are cooked using horizontal grilling techniques where fat directly falls on the hot coal than in foods cooked using the vertical technique. Contamination of vegetable oils (including olive residue oils) with PAHs usually occurs during technological processes like direct fire drying, where combustion products may come into contact with the oil seeds or oil.

The Committee concluded that concentrations of PAHs in foods can be reduced by avoiding contact of foods with flames from barbecuing and cooking at a lower temperature for a longer time. Broiling (heat source above) leads to lower concentrations of PAHs than does barbecuing. Fat should not drip down onto an open flame sending up a column of smoke that coats the food with PAHs. The use of medium to low heat, and placing of the meat further from the heat source, can greatly reduce contamination with PAHs. Direct contact of oil seeds or cereals with combustion products during drying processes results in contamination with PAHs. The Committee concluded that contamination of smoked foods with PAHs can be significantly reduced by replacing direct smoking (with smoke developed in the smoking chamber, traditionally in smokehouses) with indirect smoking. Washing or peeling fruit and vegetables before consumption would help to remove surface contaminants.

Levels and pattern of food contamination

The Committee did not receive any data on occurrence in the GEMS/Food format. However, the European Union SCOOP task force 3.2.12 has provided comprehensive data on occurrence in the European Union. Data available from the IPCS and SCF reports, and from the literature were reviewed by the Committee. The major foods containing higher concentrations of PAHs are meat and fish products, particularly grilled and barbecued products, oils and fats, cereals and dry foods. In some cases, lack of information on quality control of the analytical data made it difficult for the Committee to judge the quality of the data on occurrence. It was also observed that some determinations had been carried out following episodes of contamination of a given food, or incidents of environmental pollution.

For some PAHs identified by the Committee as genotoxic and carcinogenic, there were few or no data on concentrations in the major food groups. The Committee recommended that efforts be made to collect data for these PAHs.

Food consumption and dietary intake assessment

The Committee reviewed estimates of intake for a range of PAHs from 18 countries, including data submitted by Australia, Brazil, the United Kingdom and New Zealand. The SCF review and the EU SCOOP report submitted to the Committee also included intake estimates from a number of countries. Other intake estimates were obtained from the literature. In the studies reviewed, intakes of individual PAHs were presented, as well as intakes for 'summed' PAHs and 'carcinogenic' PAHs (which differed according to the authors' classification as 'carcinogenic', and which may have differed from that of the Committee). For the assessment conducted by the Committee, the only intake estimates reviewed were those for the 13 PAHs that the Committee considered to be carcinogenic and genotoxic.

There were no estimates of intake for three of the 13 PAHs assessed, namely dibenzo[a,h]pyrene, dibenzo[a,i]pyrene and dibenzo[a,l]pyrene. Estimated intakes of benzo[a]pyrene ranged from < 1 to 2.0 µg/day, and from 0.0001 to 0.006 µg/kg bw per day. For the other nine PAHs, intakes ranged from < 1 to about 12 µg/day, and from 0.0001 to 0.015 µg/kg bw per day.

In order to provide a likely intake of benzo[a]pyrene covering the main food groups in the whole diet for the purposes of risk characterization, a separate determination of the range of intakes was conducted using only those studies that included foods from the range of major food groups. These studies included foods that were 'ready to eat' (e.g. meat was cooked), and therefore included the likely concentrations of PAHs that arise due to cooking of food. From this analysis, mean intakes of benzo[a]pyrene ranged from 0.0014 to 0.42 µg/day, and from 0.0002 to 0.005 µg/kg bw per day. From this range, the Committee selected the value of 0.004 µg/kg bw per day as being representative of a mean intake for use in the present evaluation. The highest reported intake of benzo[a]pyrene from any study in µg/day was 0.77, and in µg/kg bw per day was 0.0062 (from different studies). If the intake of 0.77 µg/day were divided by an assumed body weight of 60 kg, this would result in a higher estimate on a body-weight basis of 0.013 µg/kg bw per day. On the basis of these data, the Committee identified a high-level intake of 0.01 µg/kg bw per day for use in the present evaluation.

Children generally had intakes of PAHs that were about 2 – 2.5 times higher than those of adults when expressed on a body-weight basis.

The major contributors to intakes of PAHs were cereals and cereal products (owing to high consumption in the diets of many countries) and vegetable fats and oils (owing to higher concentrations of PAHs in this food group). Food is the major contributor to total intake of PAHs in the general population, with smaller contributions from water and inhalation. Smokers and people exposed occupationally will have additional exposures to PAHs. In developing countries, the release of PAHs during residential heating and cooking is an important cause of contamination when biomass is burnt in relatively simple stoves.

No data on concentrations of PAHs for individual samples were either submitted to the Committee or available in the literature. Therefore, no distributions of concentrations of PAHs or mean concentrations of PAHs in foods were available in the required format to be used in calculating intakes of PAHs at the regional level. Should PAHs be reassessed by the Committee in the future, the Committee recommended that raw data from individual samples be submitted to allow estimates of the regional intakes to be made.

Overall, the Committee concluded that there was considerable variation in the intake assessments, but that a representative mean intake of 4 ng benzo[a]pyrene/kg bw per day and a high-level intake of 10 ng benzo[a]pyrene/kg bw per day could be used in the present evaluation. However, some population groups may have higher intakes of PAHs, for example those with regular high consumption of food cooked over open fires or barbecues, or people habitually consuming foods from areas of higher PAH contamination.

Dose–response analysis

For the risk assessment for PAHs, modelling of the dose–response relationship was applied to data from two studies on the incidence of tumours in mice or rats treated by oral administration. Groups of mice and rats were given purified benzo[a]pyrene, while additional groups of mice were also given one of two mixtures of coal tar, using content of benzo[a]pyrene as a comparator. In the dose–response analysis for the study in mice, the results for animals receiving the two higher doses of coal tar mixture I were omitted, owing to the premature deaths of all animals at these doses.

In the dose–response analysis, eight different statistical models (Annex 1) were fitted to the experimental data. Those resulting in acceptable fits based on biological and statistical considerations were selected to derive the BMD and BMDL for a 10% extra risk of tumours. This procedure resulted in a range of BMD and BMDL values for each end-point considered.

Taking into account the fact that mixtures of PAHs are present in food, and the possibility that different PAHs may act by different mechanisms, the Committee concluded that the data on the total number of tumour-bearing mice treated with coal tar mixtures provided the most appropriate basis for the present evaluation. For this endpoint, the values for the BMDL ranged from 0.10 to 0.23 mg benzo[a]pyrene/kg bw per day. The Committee decided to use the more conservative lower end of this range for its evaluation. Thus a BMDL equivalent to 0.1 mg benzo[a]pyrene/kg bw per day was derived for mixtures of PAHs in food.

Approaches for mixtures of PAHs

As a variety of PAHs are found together it is necessary to evaluate the combined toxicity of PAHs. There are two general approaches to this problem. The first technique is based on the assumption of dose-additivity, where the effective dose of the mixture is assumed to be equal to the sum of the effective doses of each individual compound. Because different compounds differ in their ability to produce a toxic effect, adjustment factors (toxic equivalency factors, TEFs) are used to scale the effect of each compound relative to that of a standard compound, which is typically chosen because it has a high relative potency and/or has been best characterized with respect to its effects and dose–response relationship. The Committee noted that the TEFs that have been proposed for PAHs were derived from studies involving parenteral administration or in-vitro approaches and that no data on oral administration were available that were suitable for this purpose.

The second option is the use of the surrogate approach. This method uses a single component as the measure of concentration in relation to the response of the whole mixture. For PAHs, benzo[a]pyrene is used as a marker of exposure and of the effects of the mixture.

The Committee compared the PAHs profiles in the coal tar mixtures used in the study of carcinogenicity in mice with those profiles typically reported in food. The concentrations of the

genotoxic and carcinogenic PAHs relative to that of benzo[a]pyrene were generally within a factor of two, but some of the non-genotoxic PAHs of lower relative molecular mass (e.g. phenanthrene, pyrene, fluoranthene) were present at much higher concentrations relative to benzo[a]pyrene in food than in the coal tar mixtures. The Committee concluded that a surrogate approach should be used in the present evaluation, with benzo[a]pyrene being used as a marker of exposure to the genotoxic and carcinogenic PAHs, because this approach is based on data from a study of carcinogenicity with a relevant mixture of PAHs administered by the oral route. Furthermore, the surrogate approach is much simpler to apply and is generally as accurate as the TEF approach for most purposes.

Since benzo[a]pyrene is not a good marker for some of the PAHs of lower relative molecular mass, and because some of these PAHs are tumour promoters when administered by the dermal route, further information was needed to establish whether these substances may act as promoters after administration by the oral route. However, because tumour promotion is more likely to occur at higher doses than carcinogenicity arising from genotoxic effects, a margin-of-exposure (MOE) approach for genotoxic and carcinogenic substances is also likely to adequately allow for the effects of PAHs of lower relative molecular mass.

Evaluation

The Committee concluded that the critical effect of PAHs is carcinogenicity. As some PAHs are genotoxic, it is not possible to assume a threshold mechanism and a PTWI could not be established. The present evaluation focused on 13 PAHs that the Committee identified as being genotoxic and carcinogenic: benz[a]anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, chrysene, dibenz[a,h]anthracene, dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, dibenzo[a,l]pyrene, indeno[1,2,3-cd]pyrene and 5-methylchrysene.

The Committee decided to apply a surrogate approach to the evaluation, in which benzo[a]pyrene was used as a marker of exposure to, and effect of, the 13 genotoxic and carcinogenic PAHs. A BMDL equivalent to 100 µg benzo[a]pyrene/kg bw per day was derived for PAHs in food on the basis of a study of carcinogenicity in mice involving oral administration of mixtures of PAHs representative of the genotoxic and carcinogenic PAHs present in food.

A wide range of estimates of intake were available for benzo[a]pyrene and, to a lesser extent, for nine of the other genotoxic and carcinogenic PAHs. While these may not completely reflect levels of PAHs generated during cooking of food over barbecues and open fires, the Committee concluded that a representative mean intake of 4 ng benzo[a]pyrene/kg bw per day and an estimated high-level intake of 10 ng benzo[a]pyrene/kg bw per day could be used in the present evaluation as a marker for PAHs in food. Comparison of these mean and high-level intakes with the BMDL indicates MOEs of 25 000 and 10 000, respectively. Based on these MOEs, the Committee concluded that the estimated intakes of PAHs were of low concern for human health.

Measures to reduce intake of PAHs could include avoiding contact of foods with flames, and cooking with the heat source above rather than below the food. Efforts should be made to reduce contamination with PAHs during drying and smoking processes, e.g. by replacing direct smoking (with smoke developed in the smoking chamber, traditionally in smokehouses) with indirect smoking. Washing or peeling fruit and vegetables before consumption would help to remove surface contaminants.

Recommendations

The Committee recommended that future monitoring should include, but not be restricted to, analysis of the 13 PAHs identified as being genotoxic and carcinogenic, i.e benz[a]anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, chrysene, dibenz[a,h]anthracene, dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, dibenzo[a,l]pyrene, indeno[1,2,3-cd]pyrene and 5-methylchrysene. In addition, analysis of benzo[c]fluorene in food may help to inform future evaluations.

Annex 1: Modeling approach

At the present meeting cancer dose-response data were analysed by dose-response modelling, in accordance with the IPCS document "Principles for modeling dose-response analysis for the risk assessment of chemicals" (IPCS 2004) The statistical methods of dose-response modeling as applied at this meeting are briefly described below.

For each tumor endpoint considered relevant, the following quantal dose-response models were fitted to the dose-incidence data:

Model Name	Model Equation	Constraints
One-stage	$R = a + (1-a)(1-\exp(-x/b))$	$0 \le a \le 1$,
Two-stage	$R = a + (1-a)(1-\exp(-(x/b)-c(x/b)^2))$	$0 \le a \le 1$
Log-logistic	$R = a+(1-a)/(1+\exp(c \log10(b/x)))$	$0 \le a \le 1$, $c \ge \ln(10)$
Log-probit	$R = a+(1-a)\ \Phi(c \log10(x/b))$	$0 \le a \le 1$
Weibull	$R = a + (1-a)(1-\exp(-(x/b)^c))$	$0 \le a \le 1$, $c>1$
Proast M2	$y = \exp(bx)$, *th1*	
Proast M3	$y = \exp(b\ x^d)$, *th1*	$d \ge 1$
Proast M4	$y = c - (c-1)\exp(-bx)$, *th1*	

Φ denotes the (cumulative) standard normal distribution function.

The first five of these models directly relate the incidence (R, expressed as a fraction) to the dose (x). In these models, the parameter a (also expressed as a fraction) reflects the incidence in the controls, the parameter b denotes the slope, and parameter c can be considered as a shape parameter. The last three models (Proast M2-M4) are a specific family of models that assume an underlying continuous response (indicated by y), which is translated into a binary response (incidence) by incorporating a cut-off point (*th1*) in the normal distribution around y, below which an animal does not, and above which it does respond.

Some of the models are nested members of a larger family of models. Two models are nested, when the one model can be seen as an extension of the other (simpler) model, by incorporating one or more parameters. For instance, the two-stage model is an extension of the one-stage model by including parameter c. Also, the Proast models are a nested family of models. Nested models can be formally compared to each other as follows. Inclusion of an extra model parameter should result in a higher log-likelihood value, and if this increase is larger than 1.92 inclusion of the parameter has resulted in a significantly better fit (log-likelihood ratio test). If the increase is less than 1.92, the fit is not significantly better and the parameter is omitted.

When dose-response data are available from more than one study, or for both sexes, these models are fitted simultaneously to both such subgroups. This was done by either assuming all parameters in the model being the same for all subgroups, or by assuming only the background response parameter (a) being different, or only the slope (b). When all parameters are assumed the same, a single curve results, otherwise different curves for the subgroups will result. A model in which a parameter is assumed to be different represents a model that is nested to the same model with the parameter assumed the same for the subgroups. Hence, the log-likelihood ratio test can be used for testing if an additional background or slope parameter results in a significantly better fit.

Selection of models

In general, those models that do not result in a significantly worse fit than the saturated model (one parameter per data point) are considered acceptable. For instance, when the saturated model has 8 parameters (i.e. 8 observed incidences available) a fitted dose-response model with 3 parameters should result in a log-likelihood that is no more than 5.54 lower than the log-likelihood associated with the saturated model. Table 8 summarizes the critical differences in log-likelihood

for various numbers of degrees of freedom (= difference in number of parameters between the models to be compared).

Table 8. Critical values of log-likelihoods making an increase by a number of parameters (= number of degrees of freedom) to result in a significantly better fit.

Number of degrees of freedom	Critical difference in log-likelihood (α = 0.05)
1	1.92
2	3.00
3	3.91
4	4.74
5	5.54
6	6.30
7	7.03
8	7.75

For those models that were considered acceptable according to the criteria just mentioned, the BMD values, as well as the BMDL values were calculated. All BMD and BMDL values were calculated for a 10% extra risk, defined as:

$$extra\,risk = \frac{R(BMD) - R(0)}{1 - R(0)} ,$$

which represents the additional response fraction divided by the tumor free fraction in the controls. The BMD and BMDL values were estimated by the bootstrap method, usually performing 500 bootstrap runs. These values therefore contain some random error, but usually no more than around 10% for the BMDL.

The calculations were performed using the dose-response software package PROAST, version V07, developed at RIVM, which is freely available.

Annex 2: Participants

Sixty-fourth meeting of the
Joint FAO/WHO Expert Committee on Food Additives (JECFA)
Rome, 8-17 February 2005

Members

Dr L. Barraj, Senior Managing Scientist, Exponent, Washington, DC, USA

Professor D.C. Bellinger, Professor of Neurology, Harvard Medical School and Professor in the Department of Environmental Health, Harvard School of Public Health, Children's Hospital Boston, Boston, MD, USA

Dr M. Bolger, Division of Risk Assessment, Food and Drug Administration, College Park, MD, USA

Dr L. Castle, Principal Scientist, Central Science Laboratory, Sand Hutton, York, United Kingdom

Professor J. Chen, Institute of Nutrition and Food Safety, Chinese Center for Disease Control and Prevention, Beijing, China

Professor M.C. de Figueiredo Toledo, Professor of Food Toxicology, State University of Campinas, Faculty of Food Engineering — Unicamp, Campinas São Paolo, Brazil (*Rapporteur*)

Mrs T. Hambridge, Team Manager — Dietary Modelling, Modelling, Evaluation and Surveillance Section, Food Standards Australia, New Zealand, Canberra BC ACT, Australia

Dr J.C. Larsen, Senior Consultant, Division of Toxicology and Risk Assessment, Danish Institute of Food and Veterinary Research, Søborg, Denmark (*Chairman*)

Mrs I. Meyland, Senior Scientific Adviser, Danish Institute of Food and Veterinary Research, Søborg, Denmark (*Vice-Chairman*)

Dr L.V. Moreno, Centro de Investigación en Alimentación y Desarrollo, Sonora CP, Mexico (*Unable to attend*)

Professor A.G. Renwick, Emeritus Professor, University of Southampton, School of Medicine, Southampton, United Kingdom

Dr J. Schlatter, Head of Food Toxicology Section, Swiss Federal Office of Public Health, Zurich, Switzerland

Professor C. Tohyama, Division of Environmental Health, Center for Disease Biology and Integrative Medicine, Graduate School of Medicine, University of Tokyo, Bunkyo-ku, Tokyo, Japan

Dr M.V. Rao, Director, Central Laboratories Unit, United Arab Emirates University, Al Ain, United Arab Emirates

Dr P. Verger, Directeur de l'Unité INRA 1204, Unité méthodologie d'analyse du risque alimentaire (mét@risk)/Food risk analysis methodologies, Institut Nationale de la Recherche Agronomique/Institut Nationale Agronomique Paris-Grignon, Paris, France

Professor R. Walker, Emeritus Professor of Food Science, University of Surrey, Gilford, United Kingdom

Secretariat

Dr A. Agudo, Senior Epidemiologist, Catalan Institute of Oncology (ICO), Unit of Epidemiology and Cancer Registration (SERC), L'Hospitalet de Llobregat, Spain (*WHO Temporary Adviser*)

Dr S. Barlow, Toxicologist, Brighton, East Sussex, United Kingdom (*WHO Temporary Adviser*)

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

Dr C. Carrington, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA (*WHO Temporary Adviser*)

Ms R. Charrondiere, Nutrition Officer, Nutrition Planning, Assessment and Evaluation Service, Food and Nutrition Division, FAO, Rome, Italy (*FAO Staff Member*)

Ms M.L. Costarrica, Senior Officer, Food Quality and Standards Service, Food and Nutrition Division, FAO, Rome, Italy (*FAO Staff Member*)

Dr P.O. Darnerud, Senior Toxicologist, Toxicology Division, National Food Administration, Uppsala, Sweden (*WHO Temporary Adviser*)

Ms A. de Veer, Deputy Director of the Department of Food and Veterinary Affairs, Chairman of the Codex Committee on Food Additives and Contaminants, Ministry of Agriculture, Nature and Food Quality, The Hague, Netherlands (*WHO Temporary Adviser*)

Dr M. DiNovi, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, Food and Drug, College Park, MD, USA (*WHO Temporary Adviser*)

Dr D.R. Doerge, National Center for Toxicological Research, Food and Drug Administration, Jefferson, AZ, USA (*WHO Temporary Adviser*)

Ms S.K. Egan, Division of Risk Assessment, Center for Food Safety & Applied Nutrition, Food and Drug Administration, College Park, MD, USA (*WHO Temporary Adviser; Unable to attend*)

Ms B. Engeli, Swiss Federal Office of Public Health, Food Toxicology Section, Zurich, Switzerland (*WHO Temporary Adviser*)

Mr M. Feeley, Bureau of Chemical Safety, Food Directorate, Health Canada, Tunney's Pasture, Ottawa, Ontario, Canada (*WHO Temporary Adviser*)

Dr S. Hale Henry, Office of Plant and Dairy Foods and Beverages, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA (*WHO Temporary Adviser*)

Dr K.-E. Hellenäs, Senior Scientist, National Food Administration, Chemistry Division 1, Uppsala, Sweden (*FAO Consultant*)

Dr A. Hirose, Senior Researcher, Division of Risk Assessment, National Institute of Health Sciences, Setagaya-ku, Tokyo, Japan (*WHO Temporary Adviser*)

Mr J. Howlett, Consultant, Wembley Park, Middlesex, United Kingdom (*FAO Consultant*)

Professor F. Kayama, Division of Environmental Medicine, Center for Community Medicine, Jichi Medical School, Tochigi, Japan (*WHO Temporary Adviser*)

Professor R. Kroes, Institute for Risk Assessment Sciences, Utrecht University, Soest, Netherlands (*WHO Temporary Adviser*)

Dr J.-C. Leblanc, Physiologist, Unité méthodologie d'analyse du risque alimentaire (mét@risk)/Food risk analysis methodologies, Institut Nationale de la Recherche Agronomique/Institut Nationale Agronomique Paris-Grignon, Paris, France (*FAO Consultant*)

Dr H. Lilienthal, Senior Research Scientist, Berufsgenossenschaftliches Forschungsinstitut für Arbeitsmedizin (BGFA), Klinische Arbeitsmedizin, Bochum, Germany (*WHO Temporary Adviser*)

Dr G. Moy, Food Safety Department, WHO, Geneva, Switzerland (*WHO Staff Member)*

Dr M. Olsen, Food and Nutrition Division, FAO, Rome, Italy (*FAO Joint Secretary*)

Dr S. Page, International Programme on Chemical Safety, WHO, Geneva, Switzerland (*WHO Staff Member)*

Mr O. Päpke, Senior Scientist**,** Ergo Research, Hamburg, Germany (*FAO Consultant; Rapporteur*)

Professor W. Slob, National Institute of Public Health and the Environment (RIVM), Bilthoven, Netherlands (*WHO Temporary Adviser*)

Dr A. Tritscher, International Programme on Chemical Safety, WHO, Geneva, Switzerland (*WHO Joint Secretary*)

Dr F.X.R. van Leeuwen, Toxicologist, National Institute of Public Health and the Environment (RIVM), Bilthoven, Netherlands (*WHO Temporary Adviser*)

Ms E. Vavasour, Section Head, Pre-Market Toxicology Assessment Section, Food Directorate, Health Canada, Ottawa, Ontario, Canada (*WHO Temporary Adviser*)

Annex 3: Key References

1. Principles for modelling low-dose response for risk assessment of chemicals, IPCS workshop (2004), http://www.who.int/ipcs/food/risk_assessment/en/
2. National Health and Medical Research Council (1999): Toxicity Assessment for carcinogenic soil contaminants. Commonwealth of Australia.Canberra
3. FAO/WHO Joint Meeting on Pesticide Residues (JMPR): Pesticide Residues in Food, Report 2004 http://www.fao.org/ag/agp/agpp/Pesticid/Default.htm
4. FAO/WHO (2002) Health implications of acrylamide in food. Report of a Joint FAO/WHO Consultation, WHO Headquarters, Geneva, Switzerland, 25-27 June 2002. http://www.who.int/foodsafety/publications/chem/acrylamide_june2002/en/
5. Joint FAO/WHO Food Standards Programme (2004) Codex Alimentarius Commission, Report of the 36th session of the Codex Committee on Food Additives and Contaminants, ALINORM 04/27/12.
6. International Agency for Research on Cancer (1994) Acrylamide, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Some Industrial Chemicals, vol. 60, IARC, Lyon, pp. 389-433.
7. Salmon, A.G., Painter, P., Dunn, A.J., Wu-Williams, A., Monserrat, L and Zeise, L. (1991) Carcinogenic effects. In: Salmon, A.G. & Zeise, L., eds. Risks of carcinogenesis from urethane exposure. Boca Raton, FL, CRC Press, pp 48-77
8. NTP (National Toxicology Program) (2004) NTP Technical Report on the toxicology and carcinogenesis studies of urethane, ethanol, and urethane/ethanol (urethane, CAS no. 51-79-6; ethanol, CAS no. 64-17-5) in B6C3F1 mice (drinking water studies). NIH Publication No. 04-4444. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.
9. WHO (Food Safety Group) (1998) GEMS/Food Regional Diets. Regional Per Capita Consumption of Raw and semi-processed agricultural commodities. Geneva: WHO.
10. IARC (1974) Monographs on the evaluation of the carcinogenic risk of chemicals to man: Some antithyroid and related substances, Nitrofurans and industrial chemicals. Volume 7, Urethane. International Agency for Research on Cancer, Lyon, pp. 111-140.
11. Joint FAO/WHO Food Standards Programme (2003) Codex Alimentarius Commission, Report of the 35th session of the Codex Committee on Food Additives and Contamainants, ALINORM 03/12A.
12. WHO (1994). Brominated Diphenyl Ethers. (Environmental Health Criteria 162). Geneva.
13. National Toxicology Program. 1986. Toxicology and Carcinogenesis Studies of Decabromodiphenyl Oxide (CAS No 1163-19-5) in R344/N rats and B6C3F1 mice (feed studies). NTP Technical Report Series no 309. Research Triangle Park, NC:National Toxicology Program.
14. WHO (1998) International Program on Chemical Safety (IPCS). Environment Health Criteria 202. Selected non-heterocyclic Polycyclic aromatic hydrocarbons. WHO, Geneva.
15. EC (2002) Opinion of the Scientific Committee on Food on the risks to human health of Polycyclic Aromatic Hydrocarbons in food. Brussels, European Commission, Scientific Committee on Foods (SCF/CS/CNTM/PAH/29). http://europa.eu.int/comm./food/fs/sc/scf.
16. EC (2004) Reports on tasks for scientific cooperation (SCOOP). Collection of occurrence data on polycyclic aromatic hydrocarbons in food. Report of experts participating in Task 3.2.12. October 2004. http://europa.eu.int/comm/food/food/chemicalsafety/contaminants/index_en.htm

Annex 4 : Summary of Toxicological Evaluations

JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES
Sixty-fourth meeting
Rome, 8-17 February 2005

1. Acrylamide

Intake estimates:
mean 0.001 mg/kg bw/day high 0.004 mg/kg bw/day

Effect	NOEL/BMDL mg/kg bw/d	Margin of Exposure (MOE) *at*		Conclusion/Comment
		Mean Intake	*High Intake*	
Morphological changes in nerves	NOEL 0.2	200	50	The Committee concluded that adverse effects based on these endpoints are unlikely at the estimated average intakes, but that morphological changes in nerves cannot be excluded for some individuals with very high intake.
Reproductive, developmental and other non-neoplastic effects	NOEL 2	2000	500	
Cancer	BMDL 0.3	300	75	The Committee considered these MOEs to be low for a compound that is genotoxic and carcinogenic and that they may indicate a human health concern. Therefore, appropriate efforts to reduce acrylamide concentrations in foodstuffs should continue.

2. Cadmium - impact assessment of different maximum limits

The Committee concluded that the effect of different MLs on overall intake of cadmium would be very small. At the proposed Codex MLs, mean intake of cadmium would be reduced by approximately 1% of the PTWI. The imposition of MLs one level lower would result in potential reductions in intake of cadmium of no more than 6% (wheat grain, potatoes) of the PTWI. At the proposed Codex MLs, no more than 9% of a commodity would be violative (oysters). MLs one level below those proposed would result in approximately 25% of molluscs, potatoes, and other vegetables being violative.

3. Ethylcarbamate

Intake estimates:
from food (=mean) 15 ng/kg bw/day; from food and alcoholic beverages (=high) 80 ng/kg bw/day

Effect	BMDL mg/kg bw/d	Margin of Exposure (MOE) *at*		Conclusion/Comment
		Mean Intake	*High Intake*	
Cancer	0.3	20'000	3'800	The Committee concluded that intake of ethyl carbamate from foods excluding alcoholic beverages would be of low concern. The MOE for all intakes, food and alcoholic beverages combined, is of concern and therefore mitigation measures to reduce concentrations of ethyl carbamate in some alcoholic beverages should be continued.

4. Inorganic Tin

The Committee concluded that the data available indicated that it is inappropriate to establish an ARfD for inorganic tin, since whether or not irritation of the gastrointestinal tract occurs after ingestion of a food containing tin depends on the concentration and nature of tin in the product, rather than on the dose ingested on a body-weight basis. The Committee reiterated its opinion, expressed at its thirty-third and fifty fifth meetings, that the available data for humans indicated that inorganic tin at concentrations of > 150 mg/kg in canned beverages or 250 mg/kg in canned foods may produce acute manifestations of gastric irritation in certain individuals. Therefore ingestion of reasonably-sized portions containing inorganic tin at concentrations equal to the proposed standard for canned beverages (200 mg/kg) may lead to adverse reactions.

5. Polybrominated diphenyl ethers (PBDEs)

Intake estimates:
mean approximately 4 ng/kg bw/day
The Committee recognized the preliminary nature of the data on concentrations of PBDEs in food and human milk, which adds considerable uncertainty to the intake estimates.

PBDEs are non-genotoxic substances, however, the available data on PBDEs were not adequate to allocate a PMTDI or PTWI because:

- PBDEs represent a complex group of related chemicals and the pattern of PDBE congeners in food is not clearly defined by a single commercial mixture;
- data are inadequate to establish a common mechanism of action that would allow a single congener to be used as a surrogate for total exposure or, alternatively, as the basis for establishing toxic equivalence factors;
- there is no systematic database on toxicity including long-term studies on the main congeners present in the diet, using standardized testing protocols that could be used to define a NOEL for individual PBDEs of importance;

- several of the reported effects are biological outcomes for which the toxicological significance remains unclear;
- studies with purified PBDE congeners in vitro have shown a lack of Ah receptor activation; however, many of the adverse effects reported are similar to those found with dioxin-like contaminants, suggesting that some toxicity data may be confounded by the presence of traces of impurities that are potent Ah receptor agonists

Based on limited toxicity data, the Committee concluded that there appeared to be a large MOE for a non-genotoxic compound which, despite the inadequacy of the data on toxicity and intake, gave reassurance that intakes of PBDEs are not likely to be a significant health concern.

6. Polycyclic aromatic hydrocarbons (PAHs)

Intake estimates for benzo[a]pyrene as marker for PAHs:
mean 4 ng/kg bw/day high 10 ng/kg bw/day

Effect	**BMDL[2] ng/kg bw/d**	**Margin of Exposure (MOE)** *at*		**Conclusion/Comment**
		Mean Intake	*High Intake*	
Cancer	100'000	25'000	10'000	The Committee applied a surrogate approach to the evaluation, in which benzo[a]pyrene was used as a marker of exposure to, and effect of, the 13 genotoxic and carcinogenic PAHs. Based on the derived MOEs, the Committee concluded that the estimated intakes of PAHs were of low concern for human health. Measures to reduce intake of PAHs could include avoiding contact of foods with flames, and cooking with the heat source above rather than below the food. Efforts should be made to reduce contamination with PAHs during drying and smoking processes.

[2] BMDL for benzo[a]pyrene as marker for mixtures of PAHs.