This report represents the conclusions of a Joint FAO/WHO Expert Committee convened to evaluate the safety of various food contaminants, with the aim to advise on risk management options for the purpose of public health protection.

The first part of the report contains a general discussion of the principles governing the toxicological evaluation of contaminants and assessments of intake. A summary follows of the Committee's evaluations of technical, toxicological and intake data for certain food contaminants (acrylamide, ethyl carbamate, inorganic tin, polybrominated diphenyl ethers and polycyclic aromatic hydrocarbons). Cadmium was assessed to determine the impact of different maximum limits on intake. Annexed to the report are tables summarizing the Committee's recommendations for intakes and toxicological evaluations of the food contaminants considered, and a description of the statistical methods for dose–response modelling as applied at this meeting.

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EVALUATION OF CERTAIN FOOD CONTAMINANTS

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

World Health Organization
Geneva

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

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

Safety evaluation of certain contaminants in food. WHO Food Additive Series, No. 55

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

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

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

1. Introduction

A meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) was held at the headquarters of the Food and Agriculture Organization of the United Nations (FAO) in Rome, Italy, from 8 to 17 February 2005. The meeting was opened by Dr E. Boutrif, Chief, Food Quality and Standards Service, Food and Nutrition Division, FAO, on behalf of the Directors-General of FAO and the World Health Organization (WHO).

Dr Boutrif noted that the sixty-fourth meeting was devoted to the evaluation of food contaminants. He stressed that reducing human dietary exposure to undesirable contaminants, such as those that were to be evaluated, was an important element in the effort to improve consumer protection. He stated that the first step in determining how that could be done most effectively was to carry out a structured evaluation of the risks they posed, and that it was in this field that the Committee's expertise played an essential role. Dr Boutrif also noted that the recommendations of the Committee were highly considered by FAO and WHO, not only for direct input into the work of the Codex Alimentarius Commission, but also for use by FAO and WHO Member countries. FAO and WHO had recently engaged in a process to improve the procedure and mechanisms for the provision of scientific advice to Member countries and to the Codex Alimentarius Commission, with a view to ensuring transparency, independence, scientific soundness and inclusiveness. This process, which was expected to conclude at the end of 2005, would produce specific recommendations for consideration by FAO and WHO, and should lead to an improved and harmonized procedure in both organizations. In this regard, FAO and WHO were seeking comments and suggestions from the Committee on how to continue improving their work in the risk assessment of chemicals in food.

2. General considerations

The tasks before the Committee were:

— to elaborate further principles for evaluating the safety of food contaminants (section 2); and
— to undertake toxicological evaluations of certain food contaminants (section 3, and Annex 2).

2.1 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 for 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 number 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 are 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 (*1*);
— discussions within the European Food Safety Authority about a margin of exposure (MOE) that would indicate the level of priority for risk management action (*2*); 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 (*3*).

The Committee discussed approaches to the formulation of advice on contaminants that are both genotoxic* and carcinogenic, which 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. The recent WHO workshop recommended the use of the benchmark dose lower confidence limit (BMDL) as a starting point for hazard characterization based on data from a bioassay for cancer in animals 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 (*1*). 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

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* The present guidance does not address the situation where a compound shows genotoxicity, or has structural alerts for genotoxicity, but where a bioassay for cancer 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, that may allow the formulation of limited advice to risk managers, and would welcome a critical evaluation of such approaches.

modelling will need to consider both site-specific incidences of tumours, especially for the site showing the greatest sensitivity, and 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 lower 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 bioassays for cancer, 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.

The intake (exposure) 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 to 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 (*1*). 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 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 1 million). 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 for the high doses used in the bioassay for cancer and for the much lower intakes by humans. Such information cannot be derived from the available data on cancer incidence from 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 (e.g. 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 extrapolation to low doses. 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 1 million 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.

2.2 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 from long-term studies of toxicity. Certain substances, however, such as certain 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 tolerable daily intake (TDI).

The FAO/WHO Joint Meeting on Pesticide Residues (JMPR) evaluates the acute and chronic effects of pesticide residues in food; the Committee at its present meeting was made aware that the Joint Meeting had recently developed a detailed document giving guidance on the setting of ARfDs for pesticides (*4*). This document could also serve as a basis for considerations of chemicals in food, other than pesticide residues.

The JMPR has 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 the 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) that have a local irritant or caustic effect, however, there is no need to establish an ARfD because it is the concentration[†] of the substance in the food that causes the effect and not the dose.

[†] The IPCS glossary of terms (*5*) defines these terms as follows: concentration = amount of a material or agent dissolved or contained in unit quantity in a given medium or system; dose = total amount of an agent administered to, taken up or adsorbed by an organism, system or (sub) population.

2.3 Short-term dietary intakes of contaminants

There is no internationally agreed methodology for estimating short-term intakes of contaminants in 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 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 for consumers only and usually matched with the residue level in the 97.5th percentile in a single unit of the commodity. 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 that should be assessed.

2.4 Data on levels of contaminants in food and the total diet

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. For the assessment of intake, it is essential that the data provided are adequate in quantity and quality.

The Committee welcomed the fact that for its present meeting more countries than usual 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 in possession of such data, or the mechanism for the submission of data by institutions is not clear. In addition, critical information is often lacking 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 recommended that FAO and WHO seek ways to make the calls for data more widely known in developing

countries at both technical and policy levels, and to directly contact governments and other potential data providers to facilitate the submission of such data to the Committee.

The Committee also recommended that data providers in both developing and developed countries enhance their efforts to submit their information using the electronic format for the Global Environment Monitoring System — Food Contamination Monitoring and Assessment Programme (GEMS/Food) in order to facilitate the collation and quality control of data. Detailed instructions are provided in the GEMS/Food manual *Instructions for electronic submission of data on chemical contaminants in food and the diet* (*6*). 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 manual.

3. The toxicological evaluation of compounds on the agenda

The Committee considered three food contaminants for the first time and re-evaluated three others. Information on the safety evaluations is summarized in Annex 2.

3.1 Acrylamide

Explanation

Acrylamide (CH_2=CHCONH$_2$, CAS Registry Number 79-06-1) is an important industrial chemical that has been used since the mid 1950s as a chemical intermediate in the production of polyacrylamides, which are used as flocculants for clarifying drinking-water and in other industrial applications. It is well established that acrylamide is neurotoxic in humans, as revealed by the consequences of occupational and accidental exposures. In addition, experimental studies in animals have shown that acrylamide has reproductive toxicity, and is genotoxic and carcinogenic.

Studies conducted in Sweden in 2002 showed that high concentrations of acrylamide are formed during the frying or baking of a variety of foods. Owing to concerns about the possible public health risks associated with dietary exposure to acrylamide, a consultation was held by FAO/WHO in June 2002 (*7*). On the basis of the recommendations

arising from this consultation, numerous studies of metabolism, bioavailability, toxicokinetics, DNA adduct formation, and mutagenicity in vitro and in vivo have been performed. Concurrently, a major worldwide effort has produced extensive survey data that can be used to estimate the extent and levels of contamination in food and to estimate national intakes.

At its present meeting, the Committee responded to a request from the Codex Committee on Food Additives and Contaminants (CCFAC) at its Thirty-sixth Session (*8*) to:

- comment on the extent to which acrylamide is bioavailable in food and on the safety implications;
- consider the threshold-based end-points of concern, such as neurotoxicity and reproductive toxicity, and eventually derive a tolerable dietary intake;
- evaluate the degree of uncertainty related to the assessments made;
- provide estimates of dietary intake for various population groups, including susceptible groups such as young children and regional populations, and to identify and quantify as far as possible the major sources of dietary intake;
- provide estimates and MOEs, safety, and intake for various end-points of concern (non-cancer and cancer). These estimates should contain comparisons between the levels of exposure shown to produce effects in animal studies and demonstrated no-effect levels versus estimates of dietary intake for humans;
- provide quantitative estimates of risk for various end-points, including cancer, for varying degrees of dietary exposure to acrylamide; and
- provide comments on the toxicological significance of the main metabolite, glycidamide, and whether this may be more genotoxic than the parent compound.

Acrylamide has not been evaluated previously by the Committee.

Absorption, distribution, metabolism, and excretion

In animals, acrylamide administered orally is rapidly and extensively absorbed from the gastrointestinal tract and is widely distributed to the tissues, as well as the fetus. It has also been found in human milk. Acrylamide is metabolized to a chemically reactive epoxide, glycidamide, in a reaction catalysed by cytochrome P450 2E1 (CYP2E1). An alternate pathway for the metabolism of acrylamide is conjugation with glutathione. Acrylamide and its metabolites are rapidly eliminated in the urine, primarily as mercapturic acid conjugates of acrylamide and glycidamide. The absolute bioavailablity of

acrylamide (i.e. the fraction entering the circulation as parent compound) is in the range of 23% to 48% in rodents for a dose of 0.1 mg/kg of body weight administered in the diet over a period of 30 min. The relative internal exposure to glycidamide is much higher after dietary administration than after intravenous administration, owing to extensive first-pass metabolism of acrylamide to glycidamide.

Glycidamide is much more reactive than acrylamide with DNA, and several purine base adducts have been identified in vitro. Studies in knockout and wild-type mice have shown that CYP2E1-mediated oxidation is the predominant pathway leading to the formation of glycidamide–DNA adducts. In rodents given acrylamide, glycidamide–DNA adducts are formed at comparable levels in all tissues examined and accumulate to apparent steady-state levels after regimens involving repeated dosing. DNA adducts have been found in the liver, lung, testis, leukocytes, and kidney of mice, and in the liver, thyroid, testis, mammary gland, bone marrow, leukocytes, and brain of rats treated with either acrylamide or glycidamide. The formation of DNA adducts in mice shows a monotonic dependence on the dose of acrylamide administered, from measurable levels of adduct at background exposure, to evidence for saturation of levels of adduct 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 acrylamide and glycidamide 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 biomonitoring studies in humans. Preliminary studies measuring concentrations of acrylamide–haemoglobin and glycidamide–haemoglobin adducts in rodents and humans with background exposure to acrylamide in the diet suggested that there may be species differences in the relative formation of glycidamide, (relative formation, mice > rats > humans). However, the long half-life of haemoglobin means that the measured levels of adduct reflect a time-weighted average over the lifetime of the erythrocyte. Thus the same total exposure over an extended period of time or over a short period of time could produce similar levels of adducts. This has limited the usefulness 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 of >100 mg/kg of body weight, and reported median lethal doses (LD_{50}s) are generally >150 mg/kg of body weight.

Numerous studies in a number of animal species have shown that the nervous system is a principal target site for the toxic effects of acrylamide. Sufficient repeated exposure to acrylamide causes 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 acrylamide at a dose of 50 mg/kg of body weight per day by intraperitoneal administration for 11 days and in rats given drinking-water containing acrylamide at a dose of 21 mg/kg of body weight per day for 40 days. Continued dosing with acrylamide has been shown to induce degeneration of nerve terminals in brain areas (i.e. cerebral cortex, thalamus, and hippocampus) critical for learning, memory and other cognitive functions, and these lesions may precede the morphological changes in nerves. In rats given drinking-water containing acrylamide for 90 days, the NOEL was 0.2 mg/kg of body weight per day for morphological changes in nerves, detected by electron microscopy, and no exposure-related non-neoplastic lesions were found in other tissues at doses of <5 mg/kg of body weight per day.

In studies of reproductive toxicity, male rodents given acrylamide showed reduced fertility, dominant lethal effects, and adverse effects on sperm count and morphology at oral doses of ≥7 mg/kg of body weight per day. In female rodents, no adverse effects on fertility or reproduction were observed, apart from slight reductions in the body weight of rat offspring at oral doses of 2.5 mg/kg of body weight per day (the lowest-observed-effect level, LOEL) and above. In studies of developmental toxicity, acrylamide was fetotoxic in mice only at a maternally toxic oral dose of 45 mg/kg of body weight per day, and was not teratogenic in mice or rats. In a study of developmental neurotoxicity, in which rats were given acrylamide orally from day 6 of gestation until day 10 of lactation, the NOEL for developmental neurotoxicity was 10 mg/kg of body weight per day. The overall NOEL for reproductive and developmental effects was 2 mg/kg of body weight per day.

Genotoxicity

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

Table 1
Non-cancer effects reported in animals receiving acrylamide by repeated oral administration

Species; sex (reference)	Dose (mg/kg of body weight per day)	NOEL (mg/kg of body weight per day)	LOEL (mg/kg of body weight per day)	Effect
Fischer 344 rats; males and females (*9*)	0, 0.05, 0.2, 1, 5, or 20; in drinking-water, for 90 days	0.2	1	Morphological changes in nerves (EM)
		1	5	Degenerative changes in nerves (LM)
		5	20	Hindlimb foot splay
		5	20	Decreased body weight (8–20%)
		5	20	Atrophy of testes and skeletal muscle
Fischer 344 rats; males and females (*10*)	0, 0.01, 0.1, 0.5, or 2.0; in drinking-water, for 2 years	0.5	2	Degenerative changes in nerves (LM)
		2	ND	Hindlimb foot splay
		0.5	2	Decreased body weight (<5%, males only)
		0.5	2	Early mortality after 24 weeks
		2	ND	Other non-neoplastic lesions
Fischer 344 rats; males and females (*11*)	0, 0.1, 0.5, or 2.0 (males) 0, 1.0, or 3.0 (females); in drinking-water, for 2 years	0.5 (males), 1.0 (females)	2.0 (males), 3.0 (females)	Degenerative changes in nerves (LM)
		2.0 (males) 3.0 (females)	ND ND	Hindlimb foot splay
		0.5 (males) 1.0 (females)	2.0 (males) 3.0 (females)	Decreased body weight (8–9%) Early mortality after 60 weeks
		0.5	2.0	Other non-neoplastic lesions
		2.0 (males) 3.0 (females)	ND ND	

Fischer 344 rats; males and females (*12*)	0, 0.5, 2.0, or 5.0; in drinking-water, for two generations	2.0 2.0 ND 0.5	5.0 5.0 (males) 0.5 (males) 2.0 (males)	Male-mediated implantation losses (F_0 and F_1) Degenerative changes in nerves (LM) Hindlimb foot splay and head tilt (F_0, males only) Decreased body weight (4–6%)
CD1 mouse; males and females (*13*)	0, 0.8, 3.1, or 7.5; in drinking-water, for two generations	3.1 7.5 3.1 7.5 3.1 (females)	7.5 ND 7.5 ND 7.5 (females)	Male-mediated implantation losses (F_0 and F_1) Degenerative nerve changes (F_1; LM) Mild deficits in grip strength (F_1 and F_2) Hindlimb foot splay Decreased body weight (8%, F_1 only)
Sprague-Dawley rat; females (*14*)	0, 5, 10, 15, or 20; given on days 6–10 of gestation, by gavage	10 10 ND 10	15 15 5 15	Decreased maternal weight gain Hindlimb splay, maternal Decreased body weight in offspring Increased overall horizontal activity and decreased auditory startle response in offspring

EM: electron microscopy; LM: light microscopy; LOEL: lowest-observed-effect level; ND: not determined; NOEL: no-observed-effect level

The metabolism of acrylamide to glycidamide appears to be a prerequisite for the genotoxicity caused by acrylamide in vitro and in experimental animals. Studies using knockout and wild-type mice showed that CYP2E1-mediated oxidation is the predominant pathway leading to the formation of DNA adducts. Estimates of internal exposures to glycidamide, based on measurements of haemoglobin adducts after administration of either acrylamide or glycidamide, indicated that glycidamide was the active clastogen responsible for induction of micronuclei in mice. Studies in wild-type and CYP2E1 knockout mice have also shown that glycidamide is the active metabolite of acrylamide responsible for germ cell mutations and dominant lethality in spermatids of male mice. Glycidamide is the presumed active mutagen because dosing with glycidamide produced increases in the frequency of mutation at the *Hprt* and *cII* loci in Big Blue transgenic mice that were comparable to or greater than those resulting from dosing with acrylamide.

Carcinogenicity

Acrylamide, administered in drinking-water, has been tested for carcinogenicity in two experiments in Fischer 344 rats. There were increases in the incidence of tumours at a variety of sites (see Tables 2 and 3). Information about the total number of tumour-bearing animals was not available for either study.

Acrylamide was evaluated by the International Agency for Research on Cancer (IARC) in 1994 and classified as "probably carcinogenic to humans (IARC Group 2A)" (*16*) on the basis of a positive result in a bioassay for cancer (Table 2), supported by evidence that acrylamide is efficiently biotransformed to a chemically reactive genotoxic metabolite, glycidamide, in both rodents and humans. The endocrine-responsive nature of several tumour sites from the two long-term bioassays with acrylamide in F344 rats has elicited speculation about neuroendocrine-mediated mechanisms. However, no published studies have linked hormonal changes with the carcinogenicity of acrylamide in any tissue, nor is there any indication of hormonal effects in studies of reproductive toxicity. Moreover, the wide body of evidence supporting a genotoxic mechanism is not incompatible with hormonal dysregulation by acrylamide, because it is clear that other factors beyond DNA damage are probably required for the observed target tissue specificity of tumourigenesis caused by acrylamide.

Observations in humans

Epidemiological studies in humans exposed in industry or accidentally suggest that the nervous system is a principal target site for toxicity caused by acrylamide in humans.

Table 2
Number of Fischer 344 rats with tumours at various organ sites after receiving drinking-water containing acrylamide for 2 years (data from Johnson et al., 1986 (*10*))

Type of tumour	Sex	Dose (mg/kg of body weight per day)				
		0	0.01	0.1	0.5	2.0
Thyroid gland, follicular adenomas	Male	1/60	s0/58	2/59	1/59	7/59*
Peritesticular mesotheliomas	Male	3/60	0/60	7/60	11/60*	10/60*
Adrenal gland,[a] phæochromocytomas	Male	3/60	7/59	7/60	5/60	10/60*
Mammary tumours	Female	10/60	11/60	9/60	19/58	23/61*
Central nervous system, glial tumours	Female	1/60	2/59	1/60	1/60	9/61*
Thyroid gland, follicular adenomas or adenocarcinomas	Female	1/58	0/59	1/59	1/58	5/60*
Oral cavity, squamous papillomas	Female	0/60	3/60	2/60	1/60	7/61*
Uterus, adenocarcinomas	Female	1/60	2/60	1/60	0/59	5/60*
Clitoral gland, adenomas[b]	Female	0/2	1/3	3/4	2/4	5/5*
Pituitary adenomas[a]	Female	25/59	30/60	32/60	27/60	32/60*

* $P = 0.05$; pair-wise Mantel-Haenszel comparison with the control group, adjusted for mortality
[a] The incidence of phæochromocytomas of the adrenal gland in males among the historical controls was 8.7% (range, 1.2 to 14.0%); that of pituitary adenomas in females was 38.1% (range, 28.2 to 46.9%).
[b] Only clitoral glands with gross lesions were examined histologically.
Source: reprinted from reference (*15*) with permission from Elsevier

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

The only information available that included dietary intake of acrylamide came from case–control studies originally designed to assess the potential risk of cancer associated with dietary factors other than acrylamide. The available results from epidemiological studies that estimated oral exposure to acrylamide were not suitable for use in risk assessment for acrylamide.

The formation of acrylamide adducts to haemoglobin has been used as a biomarker of exposure in humans. Although levels of acrylamide adducts were often found to be higher among exposed workers and smokers, and there was a positive correlation with the amount of tobacco product smoked, some uncertainties remained that precluded use of this measure as a marker of dietary intake of acrylamide at the

Table 3
Number of Fischer 344 rats with tumours at various organ sites after receiving drinking-water containing acrylamide for 2 years (data from Friedman et al., 1985 (*11*))[a]

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

* Statistically significant, $P < 0.001$.
[a] Certain tumours that occurred at increased incidence in treated rats in the previous study (*10*) 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 seven rats with "malignant reticulosis" of the brain, including five treated females, one treated male and one control male.
[c] Brains of all rats receiving the highest dose and all rats in the control group (both subgroups) were examined, but only 82/102 and 90/102 spinal cords from rats in the control group and 51–75 spinal cords from rats at the highest dose were examined.
[d] Only 98/204 brains and 68/204 spinal cords were examined.
[e] Only 50/102 brains and 37/102 spinal cords were examined.
[f] All brains were examined, but only 45/50, 44/50, 21/100 and 90/100 spinal cords of females in the control and (second) control, low- and high-dose groups respectively were examined.
[g] Includes three male rats and one female rat with multiple tumours in the groups receiving the highest dose.
Source: reprinted from reference (*15*) with permission from Elsevier

present time. Because analytical methods may vary between laboratories, there is a need for improved and validated analytical methodology. At the time of the present meeting, it was not possible to link biomarkers of exposure to acrylamide in humans with measurements of toxicity in experimental animals.

Analytical methods

The analytical methodology used to measure concentrations of acrylamide in food appeared to be adequate, although no methods for rapid screening had yet been developed. Acrylamide is freely soluble in water and studies indicated that the extraction procedures employed gave complete extraction. Most survey data for acrylamide have been obtained using either liquid chromatography–tandem mass spectrometry (LC–MS/MS) or gas chromatography–mass spectrometry (GC–MS). Stable isotope-labelled derivatives of acrylamide are used widely as internal standards. Both LC–MS/MS and GC–MS methods have been found to be accurate in the many schemes for proficiency tests and exercises for checking samples that have been conducted. There are currently no certified reference materials or analytical methods that have been tested collaboratively to internationally recognized standards. There is a need for improvement in analytical precision, but given the large number of data available on acrylamide concentrations in food, this does not affect the current estimates of intake. LC–MS/MS methods can routinely quantify acrylamide at concentrations as low as 10 μg/kg. Similarly, a limit of quantification of 5 μg/kg is well within the reach of the average laboratory equipped with a standard bench-top GC–MS instrument.

Formation of acrylamide 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 colour and flavour. Alternative mechanisms might be important in some speciality foods.

Although trace amounts of acrylamide can be formed by boiling, formation of more significant quantities of acrylamide generally requires a processing temperature of 120 °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 of acrylamide 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, in which concentrations of acrylamide can decline during storage over months.

Since the formation of acrylamide is dependent on the exact conditions of time and temperature used to cook or to heat process a food, there can be large variations between different brands of the same product and between different 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 the 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 acrylamide formation. High concentrations of other amino acids or proteins that compete with asparagine in the Maillard reaction or that react with already-formed acrylamide reduce the concentration of acrylamide.

Prevention and control

Research into the formation and mitigation of acrylamide 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 of food producers up to December 2004 (*17*). An average reduction of acrylamide of 30–40% in potato crisps was stated to have been achieved by introducing several adjustments into the existing production procedures. The detailed data behind this calculation were not reported and it was not known to what extent these adjustments had been applied by producers of crisps. Significant reduction was also reported from process-optimization for non-fermented crispbread, while little progress had been obtained so far in reducing concentrations in various other foods making an important contribution to intake, e.g. roasted coffee and breakfast cereals.

Experiments in food models have indicated a number of possible options for mitigation. The most efficient reduction has been achieved by using the enzyme asparaginase to selectively remove asparagine prior to heating. Although tested in models in cereals and potatoes, use is probably limited to specific food products manufactured from liquidized or slurried materials. Several other means of lowering the

levels of precursor can be applied at various stages of the food chain, e.g. by variety selection and plant breeding, controlling growth and storage factors affecting concentrations of sugar in potatoes, pre-treatment of potato pieces by soaking or blanching, and prolonged time for yeast fermentation in bread-making. Other possibilities for mitigation include alteration of the composition of the product, 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 had not been studied sufficiently 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

At its present meeting, the Committtee reviewed data provided by 24 countries on the occurrence of acrylamide in different food items analysed between 2002 and 2004 (Table 4). The total number of analytical results (single or composite samples) was 6752, with 67.6% coming from Europe, 21.9% from North America, 8.9% from Asia and 1.6% from the Pacific. No data from Latin America or Africa were submitted. The Committee noted that the occurrence data evaluated at its present meeting were more comprehensive than those available at the FAO/WHO consultation in 2002 (*7*) (240 samples).

The choices of food items analysed for concentration of acrylamide were mainly made on the basis of knowledge acquired since 2002–2003 on the occurrence of acrylamide in foods and beverages and also on the basis of recommendations made at the last FAO/WHO consultation, especially concerning other foods and beverages that undergo similar processing and that might also contain acrylamide, such as meat, milk, rice, cassava, soya products, vegetables and processed fruits.

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

Table 4
Summary of the distribution-weighted concentration of acrylamide in several food commodities, 2002–2004

Commodities	No. of samples[a]	Mean concentration (μg/kg)[b]	Coefficient of variation (%)	Reported maximum concentration (μg/kg)
Cereals and cereals-based products[c]	3304 (12346)	**343**	156	7834
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	2991 (11327)	366	151	7834
Breads and rolls	1294 (5145)	446	130	3436
Pastry and biscuits (USA = "cookies")	1270 (4980)	350	162	7834
Breakfast cereals	369 (1130)	96	131	1346
Pizza	58 (85)	33	270	763
Fish and seafood (including breaded, fried, sbaked)[c]	52 (107)	**25**	180	233
Meat and offals (including coated, cooked, fried)[c]	138 (325)	**19**	174	313
Milk and milk products[c]	62 (147)	**5.8**	119	36
Nuts and oilseeds[c]	81 (203)	**84**	233	1925
Pulses[c]	44 (93)	**51**	137	320
Root and tubers[c]	2068 (10077)	**477**	108	5312
Potato puree/mashed/boiled	33 (66)	16	92	69
Potato baked	22 (99)	169	150	1270
Potato crisps (USA = "chips")	874 (3555)	752	73	4080
Potato chips (USA = "french fries")	1097 (6309)	334	128	5312
Potato chip, croquettes (frozen, not ready-to-serve)	42 (48)	110	145	750

Stimulants and analogues[b,c]	469 (1455)	**509**	120	7300
Coffee (brewed), ready-to-drink	93 (101)	13	100	116
Coffee (ground, instant or roasted, not brewed)	205 (709)	288	51	1291
Coffee extracts	20 (119)	1100	93	4948
Coffee decaffeinated	26 (34)	668	169	5399
Coffee substitutes	73 (368)	845	90	7300
Cocoa products	23 (23)	220	111	909
Green tea ("roasted")	29 (101)	306	67	660
Sugars and honey (mainly chocolate)[c]	58 (133)	**24**	87	112
Vegetables[c]	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	1168
Infant formula	82 (117)	<5	82	15
Baby food (canned, in sealed jars)	96 (226)	22	82	121
Baby food (dry powder)	24 (34)	16	125	73
Baby food (biscuits, rusks, etc.)	32 (58)	181	106	1217
Dried food	13 (13)	121	266	1184

[a] Number of analytical results for individual samples and for composite samples. The total numbers of individual samples, allowing for the number of samples blended into composites, are given in parentheses.
[b] Results that were below the limits of detection and quantification were assumed to be half of those limits.
[c] According to the correspondence with GEMS/Food commodities, only the mean concentration of acrylamide in food groups given in bold type were used to estimate international intake.
[d] Concentrations for brewed coffee ([concentration of acrylamide in coffee-as-consumed] × 28, to convert concentration in beverage into concentration in coffee powder).

Food consumption and dietary intake assessment

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

Estimates of average intake at the national level ranged from 0.3 to 2.0 µg/kg of body weight per day for the general population. For consumers in the 90 to 97.5th percentile, estimates of intake ranged from 0.6 to 3.5 µg/kg of body weight per day, while the intake for consumers in the 99th percentile was up to 5.1 µg/kg of body weight per day. Based on the available data, children had intakes of acrylamide that were around two to three times higher than those of adults, when expressed on a body-weight basis. The Committee noted that these estimates were consistent with the long-term dietary intake assessment performed by the FAO/WHO consultation (*7*), 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, the relative contribution of food commodities to the total intake is reported here. The relative contribution of each food group may be different between studies, depending on the numbers of food categories considered in the intake evaluation.

The foods that made the biggest contribution to total exposure in most countries were: potato chips (USA = "french fries"), 16–30%; potato crisps (USA = "chips"), 6–46%; coffee, 13–39%, pastry and sweet biscuits (USA = "cookies"), 10–20%; and bread and rolls/toasts, 10–30%. Other food items contributed <10% of the total exposure.

International estimates of intake were prepared by combining the international weighted means of contamination levels (Table 4) 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 concentrations of acrylamide apply to specifically processed foods (i.e. the intake of all raw potatoes was combined with concentrations of contaminant in fried or baked potato products). Additionally, in regions with little or no data on concentrations of acrylamide, the use of this broad assumption may result in a mismatch between the foods considered and the data employed (e.g. cassava consumption

combined with concentrations of acrylamide in processed potato products).

Taking these points into consideration, the Committee estimated the range for the international mean intakes to be 3.0 to 4.3 μg/kg of body weight per day for the five GEMS/Food regional diets, assuming a body weight of 60 kg. Cereals and root and tubers are the main contributors to the total exposure calculations for each regional diet. Intakes from cereals range from about 1.3 to 2.6 μg/kg of body weight per day. Intakes from root and tubers are about 0.5 to 2.6 μg/kg of body weight per day. Other GEMS/Food groups contribute <5% to the total exposure calculations.

The Committee concluded that based on national estimates, an intake of acrylamide of 1 μg/kg of body weight per day could be taken to represent the average for the general population and that an intake of 4 μg/kg of body weight per day could be taken to represent consumers with a high intake. Children are also included in these estimates for average to high intake.

Dose–response analysis

The NOEL for induction of morphological changes in nerves, detected by electron microscopy, in rats given drinking-water containing acrylamide for 90 days was 0.2 mg/kg of body weight per day. The overall NOEL for reproductive and developmental effects and other non-neoplastic lesions was 2 mg/kg of body weight per day.

The Committee considered that the pivotal effects of acrylamide for the present risk assessment were its genotoxicity and carcinogenicity. The available epidemiological data, as well as data on biomarkers in humans and animals, were inadequate to establish a dose–response relationship and therefore the assessment was performed (Annex 3) on the basis of available studies in animals. In the dose–response analysis, eight different statistical models were fitted to the experimental data that were 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 tumours[‡]. This procedure results in a range of BMD and BMDL values for each end-point considered (Table 5).

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

[‡] Extra risk is defined as the additional incidence divided by the tumour-free fraction of the population in the controls.

Table 5
Summary of the results of dose–response modelling for induction of selected tumours in rats given drinking-water containing acrylamide

Tumour	Study			
	(*10*) Johnson et al. (1986)		(*11*) Friedman et al. (1985)	
	Range of BMD (mg/kg of body weight per day)	Range of BMDL (mg/kg of body weight per day)	Range of BMD (mg/kg of body weight per day)	Range of BMDL (mg/kg of body weight per day)
Total mammary tumours	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	NA	NA
Thyroid follicular adenoma	NA	NA	0.88–1.2	0.63–0.93
Central nervous system tumours of glial origin	1.9–2.0	1.3–1.6	NA	NA

BMD: benchmark dose for 10% extra risk of tumours; BMDL: 95% lower confidence limit for the benchmark dose. Extra risk is defined as the additional incidence divided by the tumour-free fraction of the population in the controls; NA: not applicable

In order to integrate the results from all the models used for both mammary tumour data sets, a composite analysis was conducted in which the model outputs were combined. This resulted in a BMD of 1.0 mg/kg of body weight per day and a BMDL of 0.4 mg/kg of body weight per day, which supports the other analysis.

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 contained a high degree of uncertainty regarding the shape of the dose–response curve.

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

Evaluation

MOEs were calculated at intakes of 0.001 mg/kg of body weight per day, to represent the average intake of acrylamide for the general population, based on national estimates, and 0.004 mg/kg of body weight per day to represent the intake of acrylamide by high consumers. Comparison of these intakes with the NOEL of 0.2 mg/kg of body weight per day for morphological changes in nerves, detected by electron microscopy, in rats would provide MOEs of 200 and 50, respectively. Comparison of the selected intakes with the NOEL of 2.0 mg/kg of body weight per day for reproductive, developmental,

and other non-neoplastic effects in rodents would provide MOEs of 2000 and 500, respectively. Based on these MOEs, the Committee concluded that adverse effects were unlikely at the estimated average intakes, but that morphological changes in nerves could not be excluded for some individuals with a very high intake. Ongoing studies of neurotoxicity and neurodevelopmental effects in rats would more clearly define whether effects may arise in the long-term, at low doses of acrylamide.

When the value of 0.001 mg/kg of body weight per day taken to represent the average intake of acrylamide of the general population is compared with the BMDL of 0.30 mg/kg of body weight per day for induction of mammary tumours in rats, the MOE is 300. For the value taken to represent consumers with a high level of intake, 0.004 mg/kg of body weight per day, the MOE is 75. The Committee considered these MOEs to be low for a compound that is genotoxic and carcinogenic and that this may indicate a human health concern. Therefore, appropriate efforts to reduce concentrations of acrylamide in food and beverage should be continued.

Uncertainties in the derivation of the MOEs for acrylamide arise from uncertainties and assumptions associated with the data used to derive the BMDL values and the different estimates of intake. The Committee noted that the pathways by which acrylamide is metabolized are similar in rats and humans, but that 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 currently ongoing cancer bioassays in rodents. Incorporation of additional data on the influence of dose on the conversion of acrylamide to glycidamide into a physiologically based pharmacokinetic 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.

A toxicological and technical monograph was prepared.

Recommendations

1. The Committee recommended that acrylamide be re-evaluated when the results of planned and ongoing studies of carcinogenicity and long-term studies of neurotoxicity become available.
2. The Committee recommended that work should be continued on the use of physiologically based pharmacokinetic modelling to

better link data on biomarkers in humans with intake assessments and toxicological effects in experimental animals.

3. The Committee recommended that appropriate efforts to reduce concentrations of acrylamide in food should continue.
4. In addition, the Committee noted that it would be useful to have data on the occurrence of acrylamide in foods as consumed in developing countries. This information would be useful in conducting intake assessments, as well as considering mitigation approaches to reduce human exposure.

3.2 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, intakes of cadmium 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 of body weight per week, which equate to 40–60% of the current PTWI of 7 µg/kg of body weight per week. The seven commodity groups that contributed significantly to total intake of cadmium included rice, wheat, root vegetables, tuber vegetables, leafy vegetables, other vegetables, and molluscs. These commodities accounted for 40–85% of the total intake of cadmium in the five GEMS/Food regions.

Before the sixty-first meeting of the Committee, CCFAC at its Thirty-sixth Session (*8*) 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. CCFAC subsequently requested that this analysis be completed. Specifically, the Committee was asked:

- To conduct intake and impact assessments for the seven commodity groups, taking into account three different MLs (i.e. the draft Codex ML proposed by CCFAC and one level lower and one level higher than the proposed ML). The draft Codex MLs were as follows: 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).
- 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). Table 6 summarizes the concentrations of cadmium in each commodity, the highest intake derived in a GEMS/Food region, the MLs assessed, and the impact of each ML on the mean concentration of cadmium, the percentage of the commodity that would contain cadmium at greater than the ML, and intakes of cadmium from consumption of the commodity.

Data on concentrations of cadmium in food

In order to conduct this assessment, information about the distribution of concentrations of cadmium in each commodity group (e.g. analytical results for each sample, or raw data) was required. 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 intake assessment completed by the Committee at its sixty-first meeting. For rice, average concentrations of cadmium were higher in samples from Japan (0.061 mg/kg) than 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 >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

Table 6
Overall summary of impact of different MLs for cadmium

Commodity		Level relative to proposed ML	ML (mg/kg of food)	Impact on mean concentration of cadmium		Impact on mean intakes of cadmium
				% reduction from baseline mean	% of samples >ML of PTWI)[a]	Reduction in highest mean intakes (as %
Rice (all data combined)						
Baseline mean concentration (mg/kg)	0.061	Two levels lower	0.2	12	3	4
Highest baseline intake (% of PTWI):	34	One level lower	0.3	3	1	1
		Proposed	0.4	2	<1	1
		One level higher	0.5	<1	<1	0
Wheat grain						
Baseline mean concentration (mg/kg)	0.054	One level lower	0.1	22	10	6
Highest baseline intake (% of PTWI):	29	Proposed	0.2	6	1	1
		One level higher	0.3	3	<1	1
Potatoes						
Baseline mean concentration (mg/kg)	0.037	One level lower	0.05	39	25	6
Highest baseline intake (% of PTWI):	15	Proposed	0.1	8	2	1
		One level higher	0.2	3	<1	1

Stem and root vegetables (excluding potatoes and celeriac)						
Baseline mean concentration (mg/kg)	0.028	One level lower	0.05	41	15	5
Highest baseline intake (% of PTWI):	14	Proposed	0.1	16	4	2
		One level higher	0.2	3	<1	0
Leafy vegetables						
Baseline mean concentration (mg/kg)	0.040	One level lower	0.1	22	7	Not evaluated
Highest baseline intake (% of PTWI):	<5	Proposed	0.2	7	1	
		One level higher	0.3	2	<1	
Other vegetables (excluding tomatoes and fungi)						
Baseline mean concentration (mg/kg)	0.012	One level lower	0.01	68	27	Not evaluated
Highest baseline intake (% of PTWI):	<5	Proposed	0.05	27	4	
		One level higher	0.1	9	1	
Molluscs						
Oysters						
Baseline mean concentration (mg/kg)	1.384	One level lower	2	39	23	1
Highest baseline intake (% of PTWI):	3	Proposed	3	22	9	1
		One level higher	4	13	4	1
Molluscs excluding oysters						
Baseline mean concentration (mg/kg)	0.391	One level lower	0.5	42	25	2
Highest baseline intake (% of PTWI):	5	Proposed	1	18	6	1
		One level higher	2	3	1	0

ML: maximum level; PTWI: provisional tolerable weekly intake;
[a] This represents the reduction in intake for the GEMS/Food Region with the highest intake from this commodity.

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 of body weight per week, which corresponds to 40–60% of the PTWI of 7 µg/kg of body weight 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 intake 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 data from Japan was submitted to the Committee. This intake assessment considered four different MLs and showed similar results. Total mean intake of cadmium from rice was estimated to be about 1.4 µg/kg of body weight 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 data from food balance sheets, are generally assumed to be about 15% higher than values for actual average food consumption (*18*). 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 of body weight per week; therefore, a variation of 1–6% attributable to the use of the proposed Codex MLs, and one level higher or lower, is of no significance in terms of risk to human health.

An addendum to the toxicological monograph was prepared.

3.3 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 re-

ported, information available to the Committee at its present meeting 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 (*19*). Ethyl carbamate was designated as "possibly carcinogenic to humans" (Group 2B) by IARC (*20*) and is listed as "reasonably anticipated to be a human carcinogen" in the *Report on Carcinogens* of the United States National Toxicology Program (*21*).

At its present meeting, the Committee evaluated the results of studies assessing the genotoxic and carcinogenic potential of ethyl carbamate, particularly from those studies that had become available since the review 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 request for evaluation of ethyl carbamate originated from CCFAC at its Thirty-fifth Session (*22*).

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 >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 reduces CYP2E1-mediated activation and increases elimination by esterase-mediated hydrolysis. High doses of ethanol (4 ml/kg of body weight in

one study and 5 g/kg of body weight in another) 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 LD_{50} in rodents being approximately 2000 mg/kg of body weight. In rodents, single doses of 1000 mg/kg of body weight cause anaesthesia.

In mice and rats given drinking-water containing ethyl carbamate for 13 weeks, there was an increase in mortality at doses of about 500–600 mg/kg of body weight per day. In the same study, mice given ethyl carbamate at doses of ≥150 mg/kg of body weight per day showed reduced body-weight gain and effects on the lungs, liver, kidney, heart, spleen, lymph nodes, thymus, bone marrow, and ovaries. No such effects were seen at 50 mg/kg of body weight 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 of body weight 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 range of doses (100–400 mg/kg of body weight 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 ethyl carbamate as a single dose at ≥300 mg/kg of body weight 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 of body weight 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 of body weight have been shown to induce tumours in mice, rats and hamsters. The upper range of these doses overlaps with the standard anaesthetic dose (1000 mg/kg of body weight) and the values for LD_{50}s in rodents. In addition, in non-human primates given ethyl carbamate at a dose of 250 mg/kg of body weight 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 $B6C3F_1$ 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 of body weight per day, respectively. Treat-

ment 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 tumour induction (i.e. those at which a significant increase in tumours was observed at the lowest dose tested) were the lung and Harderian gland. The incidences 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.

Analytical methods

Over the past 30 years, methods have been developed for the extraction and analysis of ethyl carbamate in all the food and beverage types in which the substance is known to be formed. These methods have been tested in two international collaborative trials, for application to beers and whiskeys and to wine, fortified wine, spirits and soy sauce.

As a small organic ester, ethyl carbamate is suitable for analysis using gas chromatography. Ethyl carbamate labelled with heavy isotopes (deuterium and/or ^{15}N) is available for use as an internal standard during analysis. The use of gas chromatography coupled with mass spectrometry gives confidence in the analytical aspects of correct identification and quantification in food and beverages. The methods are capable of routinely detecting ethyl carbamate at concentrations of 1 µg/kg, and have limits of quantification in the range of 3 to 5 µg/kg in food and beverages. Sensitivity is further improved if extra sample clean-up and concentration steps are performed.

Levels and pattern of food contamination

Data on concentrations of ethyl carbamate in foods and beverages were submitted by the Food and Drug Administration of the USA, the Food Standards Agency of the United Kingdom (UK) and the Wine Institute of the USA. The alcoholic beverages considered in these reports originate from many countries throughout the world. Means and ranges of concentrations of ethyl carbamate in foods and beverages as reported in publications or data submitted to the Committee are presented in Table 7. 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 stone-fruit brandies.

Diethylpyrocarbonate, which is an inhibitor of fermentation, can form ethyl carbamate, and for this reason the previous acceptance of

Table 7
Concentrations of ethyl carbamate in food and beverages[a]

Food and beverages	Country of origin	No. of samples	Mean concentration (μg/kg)	Range of concentrations reported (μg/kg)
Alcoholic beverages				
Wine	Various	228	8	ND–36
	Various	13	10	ND–24
	Various	5189	4	ND–61
Fortified wines	Various	125	41	ND–262
	Various	15	32	14–60
Whiskeys	Various	205	29	ND–239
	Various	30	32	ND–102
Cordials, liqueurs,	Various	14	37[b]	ND–170, 6131
brandies	Various	31	64	ND–243
Sake	Japan	90	73	ND–202
	Japan	2	122	81, 164
Beer	Various	10	—	ND
	Various	52	1	ND–5
Other foods				
Bread	UK	157	—	ND
	Denmark	33	4	0.8–12
	UK	65	2	0.4–4.5
Kimchi	South Korea	20	4	ND–16
Yogurt	UK	4	—	ND
	Various	9	1	ND–1.3
	Denmark	19	0.2	ND–0.3
Cheese	Various	17	—	ND
Soy sauce	Japan	10	—	ND
		20	16	ND–84
		18	16	ND–78

ND: not detected
[a] As reported in publications or data submitted to the Committee.
[b] This mean concentration excludes the single highest value reported, 6131 μg/kg.

diethylpyrocarbonate was revoked by the Committee at its seventeenth meeting (Annex 1, reference *32*). 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 approximately 15 to 65 ng/kg of body weight 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 of body weight 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, which 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 after rounding to one significant figure (equivalent to 80 ng/kg of body weight 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.

Table 8
Ranges of BMD and BMDL values for tumours associated with administration of ethyl carbamate

Tumour type	Range of BMD values (mg/kg of body weight per day)	Range of BMDL values (mg/kg of body weight 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 tumours; BMDL: 95% Lower confidence limit for the benchmark dose. Extra risk is defined as the additional incidence divided by the tumour-free fraction of the population in the controls.

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 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 tumours. This procedure results in a range of BMD and BMDL values for each end-point considered (Table 8). 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 curve. As a result, the ranges of the BMD and BMDL values were quite narrow, while the BMDLs were not much lower than their associated BMDs.

Choosing lung tumours as the critical end-point, the values for BMDLs ranged from 0.3 to 0.5 mg/kg of body weight per day. The Committee decided to use the more conservative lower end of this range of values for the evaluation. The Committee used these BMDLs to estimate MOEs.

Evaluation

When the estimated intake of ethyl carbamate in foods (15 ng/kg of body weight per day), is compared with the lower end of the range of BMDL values obtained for the incidence of alveolar and bronchiolar neoplasms in male and female mice (0.3 mg/kg of body weight per day), the resulting MOE is 20 000. With the inclusion of alcoholic beverages in the estimated intakes (80 ng/kg of body weight per day), the resulting MOE is 3800. On the basis of these considerations, the Committee concluded that exposure to ethyl carbamate in food would be of low concern. The MOE for all intakes, food and beverages, is of concern and therefore mitigation measures to reduce concentrations of ethyl carbamate in some alcoholic beverages should be continued.

A toxicological and technical monograph was prepared.

3.4 Inorganic tin

Explanation

Inorganic tin is found in food in the +2 and +4 oxidation states; it may occur in cationic form (stannous and stannic compounds) or as 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 *149*). At its thirty-third meeting, the Committee converted the previously established provisional maximum tolerable daily intake (PMTDI) of 2 mg/kg of body weight to a provisional tolerable weekly intake (PTWI) of 14 mg/kg of body weight. 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, CCFAC asked the Committee to review information on the toxicity of inorganic tin in order to establish an ARfD (*23*). At its fifty-fifth meeting (Annex 1, reference *149*), the Committee 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, CCFAC (*22*) decided to ask the Committee 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 (*8*), CCFAC asked the 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 MLs (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 and 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 of body weight). 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 of body weight) did not provoke any 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 (*24*) showed that tomato juice freshly spiked with tin (II) chloride at a concentration 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 closely 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.

Prevention and control

The lacquering of tinplated cans prevents the migration of inorganic tin into food and beverages. Food and beverages should not be stored in opened tinplated cans.

Levels and pattern of food contamination

Data on the concentrations of inorganic tin in a range of foods from four countries (Australia, France, Lithuania and the UK) had become available since the last review of inorganic tin by the Committee and were reviewed at this meeting. The Committee noted that the reported concentrations of inorganic tin were in the same range as those previously assessed by the Committee, the new values ranging from "not detected" to 300 mg/kg.

Food consumption and dietary intake assessment

The major dietary source of inorganic tin is food packaged in unlacquered or partially lacquered tinplated cans. The migration of inorganic tin from tinplate into foods is greater in highly acidic foods such as pineapples and tomatoes; with increased time and temperature of food storage; and in foods, such as fruit juice, in opened cans. The inorganic tin content of canned foods is variable, and some foods may have concentrations high enough to cause an acute toxic reaction. Information previously evaluated by the Committee and

additional data from Australia and the UK indicated that the mean long-term dietary intakes of inorganic tin by individuals ranged from <1 to about 14 mg/person per day. Population groups with higher intakes of canned foods may have higher intakes of inorganic tin. A small number of estimates of short-term dietary intake (i.e. in a period of 24 h or less) were assessed by the Committee. Based on limited data, preliminary short-term intakes of inorganic tin were estimated to be between 0.004 and 3.3 mg/kg of body weight per day, depending on the food considered.

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 of food 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 opened 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 long-term exposure to dietary doses of inorganic tin at concentrations that did not elicit acute effects.

An addendum to the toxicological monograph was prepared.

Table 9
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

PBDE: polybrominated diphenyl ethers

3.5 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, given in Table 9. 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.

PBDEs have not been evaluated previously by the Committee. In 1994, WHO published an Environmental Health Criteria document on brominated diphenyl ethers (*25*), 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 has 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 CCFAC, most recently at its Thirty-fifth Session (*22*), 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 with bromine leads to a decrease in bioavailability. Intestinal absorption of decaBDE is limited, with >90% of an orally administered dose being rapidly excreted in the faeces. For congeners with a lower degree of bromination (tetra and penta-substituted), >80% of an orally administered dose is absorbed, with patterns of distribution in tissue being largely determined by lipid content. 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 in mice is as important as faecal excretion. 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 toxicological 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 grams/kg of body weight), 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 of body weight per day.

The only long-term study with PBDEs was conducted with the decaBDE mixture. In this National Toxicology Program study of carcinogenicity (*26*), 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 individual congeners are not genotoxic.

The developmental toxicity of 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-79) 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 of body weight per day; only slight maternal toxicity (decreased body weight) was observed at doses of 25–50 mg/kg of body weight 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 of body weight per day.

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 of body weight 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 lack of activation of the aryl hydrocarbon receptor at doses six orders of magnitude higher than the half-maximal effective concentration (EC_{50}) 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 agonists of the aryl hydrocarbon receptor.

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. 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 of body weight 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 thyroid-stimulating hormone 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 of body weight per day for 2 weeks. At doses at which circulating concentrations of T4 were

decreased (>1 mg/kg of body weight per day), the activities of UDP-glucuronyltransferase (UDPGT) and ethoxyresorufin-*O*-deethylase were often found to be increased, suggesting that aryl hydrocarbon receptor-dependent effects are most likely to be mediated by contamination of commercial PBDE mixtures with dioxin-like compounds. A similar observation was also made 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. Thyroid-stimulating hormone 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 of body weight) or BDE-47 (140 µg/kg of body weight) on day 6 of gestation had altered concentrations of T3 and T4 during the weaning period. Serum concentrations of thyroid-stimulating hormone 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 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, 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 thyroid-binding globulin. 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 thyroid-stimulating hormone. There was 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 ethoxyresorufin-*O*-deethylase 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 of body weight per day. After a single oral dose of BDE-47 (700 µg/kg of body weight) on day 6 of gestation, decreased serum concentrations of follicle-stimulating hormone were seen in male offspring. With the same exposure protocol, BDE-99 was recently reported to reduce sperm production at a dose of 60 µg/kg of body weight. Induction of hepatic ethoxyresorufin-*O*-deethylase was observed in all these experiments, therefore aryl hydrocarbon 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 of body weight 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 messenger RNA (mRNA) in the ventral prostate on postnatal day 120. In the same study, exposure to a technical mixture of PCBs (Aroclor 1254), known to possess dioxin-like activity, at a dose of 30 mg/kg of body weight 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 of body weight 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 of body weight 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 of body weight) or BDE-99 (60 or 300 µg/kg of body weight) on day 6 of gestation. Because of the preliminary nature of these findings, an interpretation of their 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 the adipose tissue of patients with non-Hodgkin lymphoma (incident cases), 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 thyroid-stimulating hormone 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 nanogram/g of 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 intake. The significance of pathways of exposure 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.

The total number of possible PBDE congeners is 209. For reasons of occurrence in food and human samples, and analytical capability, only a limited number of congeners has been measured in recent years. This number ranged between three and nine congeners (BDE-28, -47, -66, -99, -100, -153, -154, -183, -209). With increasing analytical power and availability of standards, the number of individual congeners measured in food and human samples could be increased.

The physical and chemical properties of the BDE-209 congener are such that great demands are made of the analytical method, including

sample preparation, extraction and clean-up, as well as final chromatographic separation. The problems encountered during the analysis of PBDE congeners of high relative molecular mass are associated with thermal instability and sensitivity to light rather than with their high boiling points.

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.

Effects of processing

No data were available on the effect of processing on concentrations of PBDEs in foods.

Prevention and control

As with other lipophilic contaminants, control of PBDE residues in animal feed is likely to have an impact on the concentrations of PBDEs found in meat, poultry, farmed fish, and other animal-derived products.

Levels and pattern of food contamination

The Committee reviewed data available on concentrations of PBDEs in foods (Table 10). 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 coun-

tries or are based on a small number of samples. Thus, the currently available data do not allow a comprehensive assessment to be made of contamination in all foods. Differences in concentrations were detected in samples of similar foods collected from various geographical areas.

Food consumption and dietary intake assessment

Preliminary estimates of mean intake of PBDEs, based on a limited number of samples from Canada, some European countries, Japan, and the USA, as reported in published studies and reports, range from 13 to 113 ng/day (Table 10). 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 concentration data from studies summarized in Table 10. Table 11 summarizes the food consumption data used in this estimation and the estimated mean intakes of total PBDEs for these regions. 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 of body weight 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 of body weight per day, respectively. Fish and

** For the Far Eastern region, limited data were available for some food groups and were used in combination with data from the North American and European regions for the remaining food groups.

Table 10
Summary of available data on concentrations of PBDEs and associated national intakes

Country	Type of study	Foods	PBDEs measured
Canada	Food basket survey	Foods of animal origin	NA[a]
	Total diet study, (1998)	About 50 foods representing the total diet	Total PBDE (28, 47, 99, 100, 153, 154, 183)
	Total Diet Study, Vancouver (2002)	About 50 foods representing the total diet	Total PBDE (28, 47, 99, 100, 153, 154, 183)
	Special Fish & Seafood Survey (2002)	70 samples of farmed and wild fish	Total PBDE (28, 47, 99, 100, 153, 154, 183)
Finland	Market basket survey (1997–1999)	228 foods grouped in 10 market baskets (about 4000 samples)	Total PBDE (47, 99, 100, 153, 154)
	Total diet basket	228 foods	Total (47, 99, 100, 153, 154)
Germany	Samples collected from German markets (2001–2003)	Fish, meats, dairy (607 samples)	Total (28, 47, 99, 100, 153, 154)
	Fish samples collected from German markets (2004)	13 Fish samples	17, 28, 47, 66, 77, 99, 100, 153, 154, 183, 209, & total
Japan	Total diet study	13 food groups (two composites each for fish, meats and eggs, and milk and milk products, one composite for each of the remaining food groups)	47, 49, 66, 99, 100, 119, 153, 154, 183
	Duplicate diet study	Duplicate meals collected from 6 subjects for 2 to 3 days	47, 49, 66, 99, 100, 119, 153, 154, 183
	Market basket survey	Fish, shellfish, meats & vegetables (26 samples)	28, 47, 99, 100, 153, 154
Netherlands	Total diet study (2001–2002)	84 samples (dairy, eggs, meats, animal fats, fish, oil)	28, 47, 99, 100, 153, 154, 71, 77, 190 & 209

Data reported	Concentrations detected (ng/g fresh weight)	Consumption data	Reported mean intake of PBDEs (ng/day)
NA	NA	National estimates	44
Level/food sample	Range of means/food group 0.024 (dairy) to 0.333 (egg — 1 sample)	National estimates	38
Level/food sample	Range of means/food group 0.035 (dairy) to 0.680 (fish)	National estimates	30
N, Mean, SE, SD, Min, Max per species and source	Range of means: 0.2–2.2 (farmed) 0.1–0.6 (wild)	NA	NA
Level/basket	Range of means/food group 0.009 (other) to 0.85 (fish)	1997 Dietary Survey of Finnish Adults	43
Level in total diet basket	0.043	1997 Dietary Survey of Finnish Adults	44
N, Number of detects; Number of non-detects; Mean concentration (positive samples); Range	Range of means/food group 0.030 (dairy) to 1.45 (fish and shellfish)	NA	NA
Data/congener per sample	Mean: 0.66, Range: 0.01–2.87	NA	NA
Total PBDE/food group	Range of levels/food group (for detects) 0.009 (dairy) to 1.26 (fish) (ND = LOD)	National Nutrition Survey	113
Data/congener per person	Mean: 0.29 Range: 0.003–0.081	Total diet of 6 subjects for 2 to 3 days	68
Total PBDE per sample	Range of means/food group 0.030 (meats & poultry) to 0.91 (fish and shellfish)	NA	NA
Data per congener per sample	Range of means/food group ND (eggs) to 6.82 (fish and shellfish)	Data from 6250 individuals in Dutch National Food Consumption Survey	13

Table 10 (*continued*)

Country	Type of study	Foods	PBDEs measured
Spain	Market basket, in Catalonia (2000)	Fish, meats, dairy, vegetables, cereals, fats and oils ($n = 54$)	Tetra-, penta-, hexa-, hepta-, octaBDE
Sweden	Total diet study	Fish, meats, dairy, fats and oils ($n = 17–74$ 20 composite samples)	47, 99, 100, 153, 154
	Market basket	Fish, meat, dairy products, eggs, fats/oils, pastry	Sum of congeners 47, 99, 100, 153, 154
UK	Targeted study	Fish (18 samples)	Total PBDE
	Duplicate diet study	Total diet of 10 individuals	Sum of 47, 99, 100, 153, 154
USA	Market basket survey (2003)	32 food samples (fish, meats, dairy)	17, 28, 47, 66, 77, 85, 99, 100, 138, 153, 154, 183, 209
	Grab samples from local supermarkets (Texas)	15 meat samples	17, 28, 47, 66, 85, 100, 100, 138, 153, 154, 183, 209
	Grab samples from local supermarkets (Texas)	11 dairy samples	17, 28, 47, 66, 85, 99, 100, 138, 153, 154, 183, 209
	Samples from supermarkets in nine cities	48 bacon and meat trimmings	28/33, 47, 85, 99, 100, 153, 154, 183, 209
	Samples from local markets (California)	Nine meat samples	26 congeners
USA and EU	Review paper	Various fish species	47, 99, 100, 153, 154

bw: body weight; EU: European Union; LOD: limit of detection; N: No. of samples; NA: not available or analysis not conducted; ND: not detected and counted as zero unless otherwise stated; Min: minimum; Max: maximum; SD: standard deviation; SE: standard error

Data reported	Concentrations detected (ng/g fresh weight)	Consumption data	Reported mean intake of PBDEs (ng/day)
Total PBDE per food group & congener level/entire diet	Range of means/food group 0.001 (other foods) to 0.46 (fats & oils)	Consumption data for Catalonia	82
Total PBDE level per sample	Range of means/food group: 0.04 (eggs) to 1.884 (fish and shellfish) (ND = LOD/2)	National Consumption Survey (1997–98)	Adults aged years Females: 41 Males: 47 (ND = LOD/2)
NA	NA	Based on production	51 (ND = LOD/2)
Range per fish type & location	Range 12–53 (control location) 59–288 (target location)	Standard portion sizes	Maximum intakes Control: 9 ng/kg bw per day Target: 56 ng/kg bwper day
NA	NA	10 individuals	90
Total PBDE per sample	Range 0.0009 (fats and oils), 1.487 (fish and shellfish)	USDA 1994–96 Continuing Survey of Food Intakes by Individuals	Females: per day Males: 2.0 ng/kg bw per day
Individual congeners and total PBDE per sample	Mean: 0.58 Range: ND–2.86	NA	NA
Individual congeners and total PBDE per sample	Mean: 0.17 Range: 0.03–0.66	NA	NA
Mean and range for each congener and total PBDE per food type	Mean: 0.20 (bacon), 1.07 (meat trimmings)	NA	NA
Individual congeners and total PBDE per sample	Mean: 0.60 Range: 0.16–2.52	NA	NA
N for each study, Mean concentration for each congener and total PBDEs	Range of means (ng/g lipid): European studies: 6.31–515; USA studies: 12.1–7200	NA	NA

Table 11
Estimated intakes of total PBDEs in GEMS/Food regional diets

Food group	Consumption (g/day)	Estimated intake of PBDEs (ng/day)[a]	
		European diet[b]	North American diet[c]
Dairy and products	336	10	24
Eggs	38	2	8
Fats and oils	49	13	47
Fish and shellfish	47	84	40
Meat and poultry	217	17	66
Other foods	826	6	29[d]
Total (ng/day)		131	213
Total (ng/kg of body weight per day)[e]		2.2	3.6

ND: not detected
[a] ND = 0
[b] Concentration data from Finland, Germany, Netherlands, Spain, and Sweden were used in the estimation.
[c] USA and Canada
[d] Limited data were available for this region, therefore the combined data from the other regions were used instead.
[e] Based on a body weight of 60 kg

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 of body weight per day (average fat content of milk, 3.0%; 750 ml of milk per day; 5.0 kg of 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 of body weight 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 study of toxicity; 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 of body weight 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 of body weight 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 of body weight per day), decreases in concentrations of circulating thyroid hormones (T4) were observed. The latter observation was supported by the results of a study of developmental toxicity in rats given the commercial pentaBDE mixture (DE-71); decreases in serum concentrations of T4 were seen in both fetuses and newborn pups at a maternal dose of 10 mg/kg of body weight per day administered on day 6 of gestation to postnatal 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 of body weight. 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 activation of the aryl hydrocarbon receptor; 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 agonists of the aryl hydrocarbon receptor.

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 at the time of the present evaluation it was the primary commercial mixture in use worldwide.

The limited toxicity data suggested 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 of body weight per day. The current estimates of dietary intake were approximately 0.004 µg/kg of body weight per day, while intake by breastfeeding infants could be up to 0.1 µg/kg of body weight 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 dosemetric 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.

A toxicological and technical monograph was prepared.

3.6 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, on basis of 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 (*22*), 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 on the risks to human health posed by PAHs (*27*), and the International Programme on Chemical Safety (IPCS) Environmental Health Criteria document on selected non-heterocyclic PAHs (*28*). 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 12. These are the 33 PAHs selected for consideration by the IPCS and the Scientific Committee on Food on the basis of available information on their occurrence and toxic effects.

Table 12
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 PAHs from the diet is determined by the size and lipophilicity of the molecule and the lipid content of the food. PAHs are metabolized by oxidation of the aromatic rings, primarily by enzymes of the CYP1, CYP2 and CYP3 families, followed by formation of glutathione, glucuronide and sulfate conjugates. Oxidation can generate electrophilic metabolites that bind covalently to nucleic acids and proteins. Some PAHs and some metabolites of PAHs also bind to the aryl hydrocarbon 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 PAHs.

Toxicological data

The relatively few studies of acute toxicity that were available indicated that PAHs have moderate to low acute toxicity. A limited number of short-term studies of toxicity with individual PAHs (acenaphthene, fluoranthene, fluorene, naphthalene, pyrene) administered orally to rats and mice were available. These studies predominantly showed toxicity in the liver and kidney, with NOELs ranging from 53 to 175 mg/kg of body weight per day. The NOEL for benzo[*a*]pyrene was 3 mg/kg of body weight per day on the basis of toxicity in the liver in rats. The Committee considered that these studies were not pivotal for the present risk assessment.

The carcinogenicity of PAHs administered by dermal, subcutaneous, inhalation or oral routes has been assessed in a large number of studies. In most studies, the site of tumour development was related to the route of administration, e.g. gastric tumours after oral administration, skin tumours after dermal application. However, tumours at sites other than the site of application were also observed (e.g. liver tumours after oral exposure to benzo[*a*]pyrene, or lung tumours after oral exposure to coal tar mixtures containing PAHs). On the basis of all the available information, the Committee concluded that 13 PAHs (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, 5-methylchrysene) are clearly carcinogenic in experimental animals. On the basis of observations in bioassays in animals treated parenterally, dibenz[*a,h*]anthracene, dibenzo[*a,h*]pyrene, dibenzo[*a,l*] pyrene, benzo[*a*]pyrene, and benzo[*b*]fluoranthene seem to be the most potent carcinogens.

For the present evaluation, studies of carcinogenicity after oral administration are most relevant. Benzo[*a*]pyrene, when administered by the oral route, has been reported to produce tumours of the gastrointestinal tract, liver, lungs and mammary glands of mice and rats. Of the few other PAHs tested for carcinogenicity by the oral route, dibenz[*a,h*]anthracene and benz[*a*]anthracene have been reported to produce tumours of the gastrointestinal tract, lungs and liver in mice. No increases in incidences of tumours were seen in rats after oral administration of benz[*a*]anthracene, phenanthrene, fluorene or naphthalene. No other PAHs have been tested for

carcinogenicity after oral administration. All these studies, however, had limitations as to their design and scope. The Committee paid particular attention to two new studies using oral administration: a study in mice in which the tumorigenic response to benzo[*a*]pyrene was compared with that to two mixtures of coal tar, and a study in rats given benzo[*a*]pyrene.

In the first of these studies, groups of 48 female B6C3F_1 mice were fed diets containing benzo[*a*]pyrene at a concentration of 0, 5, 25 or 100 mg/kg of diet (equivalent to doses of 0, 0.7, 3.6 or 14 mg/kg of body weight per day) for 2 years. Papillomas and squamous cell carcinomas were observed in the forestomach, with a combined incidence of 1/48, 4/47, 36/47 and 46/47 in each group. The increased incidences at 25 and 100 mg/kg of diet were significant and dose-related. The combined incidences of papillomas and carcinomas were: oesophagus, 0/48, 0/48, 2/45, 27/46; and tongue, 0/48, 0/48, 2/46, 23/48. In the two latter tissues, only animals receiving the highest dose differed significantly from those receiving the solvent only. Groups of 48 female B6C3F_1 mice were also fed diets containing 0, 0.01, 0.03, 0.1, 0.3, 0.6 or 1.0% coal tar mixture I, which contained benzo[*a*]pyrene at a concentration of 2240 mg/kg (equivalent to doses 0.03, 0.09, 0.32, 0.96, 1.92 or 3.2 mg/kg of body weight per day), or 0, 0.03, 0.1 or 0.3% of coal tar mixture II, which contained benzo[*a*]pyrene at a concentration of 3669 mg/kg (equivalent to doses of 0.16, 0.52 or 1.1 mg/kg of body weight per day). A significantly increased incidence of alveolar and bronchiolar adenomas and carcinomas was found at 0.3, 0.6 and 1.0% of mixture I (27/47, 25/47 and 21/45 versus 2/47 in the control group) and at 0.1 and 0.3% of mixture II (10/48 and 23/47 versus 2/47 in the control group). For tumours of the forestomach, a significant increase was observed at 0.3, 0.6% and 1.0% of mixture I (14/46, 15/45 and 6/41 versus 0/47 in the control) and at 0.3% of mixture II (13/44). The total numbers of tumour-bearing animals were 5/48, 12/48, 14/48, 12/48, 40/48, 42/48 and 43/48 at 0, 0.01, 0.03, 0.1, 0.3, 0.6 and 1.0% of mixture I, and 5/48, 17/48, 23/48 and 44/48 at 0, 0.03, 0.1 and 0.30% of mixture II. This study indicated that benzo[*a*]pyrene alone induced only tumours of the alimentary tract, whereas the coal tar mixtures also induced liver and lung tumours.

Administration of oral doses of benzo[*a*]pyrene at 0, 3, 10 or 30 mg/kg of body weight per day by gavage to groups of 104 male and female Wistar rats on 5 days per week for 2 years resulted in a large variety of tumours, most prominent being those in the liver and forestomach. In the forestomach, the combined incidence of papilloma and carcinoma was, respectively, 1/52, 6/51, 30/51, 50/52 for females, and 0/52, 8/52, 43/52, 52/52 for males. The incidences of combined adenoma and

carcinoma in the liver were respectively 0/52, 2/52, 39/52, 51/52 for females, and 0/52, 4/52, 38/52, 49/52 for males. In addition to these tumours, treatment with benzo[*a*]pyrene also induced soft tissue sarcomas (skin, mammary), as well as tumours of the auditory canal, oral cavity, small intestine and the kidney. The total numbers of tumour-bearing animals were 8/52, 20/52, 47/52, 51/52 for females, and 6/52, 16/52, 51/52, 52/52 for males.

On the basis of the available information, the Committee concluded that 15 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 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.

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 of body weight per day. Impaired fertility was seen in the offspring of female mice given benzo[*a*]pyrene at doses of >10 mg/kg of body weight 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 PAH 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 aryl hydrocarbon 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 of body weight 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 was not suitable for use in this risk assessment.

Analytical methods

High-performance liquid chromatography (HPLC) with fluorescence detection has been widely used for the determination of PAHs in several food matrices. Use of an ultraviolet/diode-array detector in conjunction with a fluorescence detector improved the detection limits for compounds with a low fluorescence intensity. Gas chromatography–flame ionization detector (GC–FID) and GC–MS methods are also employed for their determination. Confirmation of compounds present is achieved through the mass spectral data or by analysing on a different column when the GC–FID technique is used. Confirmation of compounds analysed by HPLC methods is achieved through GC–MS analysis in some cases. Both HPLC and GC–MS methods are suitable for routine monitoring.

The extraction and clean-up techniques used depend on the type of food matrix. Fatty foods such as meat, fish and their products are

saponified with methanolic potassium hydroxide, liquid extraction into non-polar solvents, partitioning with dimethylformamide or dimethylsulfoxide-water followed by re-extraction with solvents like cyclohexane. Solid-phase extraction (SPE) clean-up using reversed phase cartridges has been found to give better recoveries for PAHs of high relative molecular mass and to be suitable for their clean-up in different matrices.

The limits of detection for PAHs are matrix-dependent. Limits of detection for both HPLC and GC–MS methods are adequate to detect the concentrations normally encountered in foods. The recovery of PAHs in different matrices varies, but is usually >70% for most compounds. Available certified reference materials cover a few selected PAHs in certain matrices only. Current proficiency testing programmes seem to be inadequate as they cover a few matrices for selected PAHs only. Lack of certified reference materials and proficiency testing programmes covering a wide range of PAHs in high-risk food commodities has been a limitation in the analysis of PAHs in foods. The Committee noted that most analytical methods developed include the 16 priority pollutant PAHs listed by the United States Environmental Protection Agency and do not include most dibenzopyrenes and 5-methylchrysene. The Committee recommended that analytical methods be developed to include these compounds.

Sampling protocols

PAHs are chemically stable and highly lipophilic in nature. However, they are susceptible to photodegradation in the presence of light, and thus all sampling and analytical operations must be performed under subdued light. Little information was available on the reactions and fate of PAHs in foods. Since PAHs are chemically stable compounds, they are assumed to be stable in different food matrices, although PAHs deposited on the surface of crops may undergo photodegradation. Sampling protocols in the European Union are detailed by the Scientific Cooperation on Food (SCOOP) task force 3.2.12 on the occurrence of PAHs (*29*). Sampling protocols are available for total diet studies in the UK. Several researchers followed either routine sampling or sampling based on suspicion (e.g. edible oils, industrial pollution, oil spills).

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 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 (e.g. grilling, roasting, frying) 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.

Effects of processing and cooking of food

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 during which fat falls directly 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, in which combustion products may come into contact with the oil seeds or oil.

Prevention and control of PAHs in foods

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, European Union SCOOP task force 3.2.12 (*29*) has provided comprehensive data on occurrence in the European Union. Data available from the IPCS and Scientific Committee on Food reports, and from the literature were reviewed by the Committee. The range of concentrations of PAHs in the major food groups, from available data, is summarized in Table 13. 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 being genotoxic and carcinogenic, there were few or no data on concentrations in the major food groups (Table 13). 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, New Zealand and the UK. The Scientific Committee on Food review and the European Union 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. The intake estimates were calculated in a variety of ways including differences in the data on food consumption, derivation of concentrations, the range of foods included, the methodologies of intake calculation, the treatment of results given as "not detected" and the reporting of results.

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. The ranges of intakes of the other 10 PAHs assessed from all of the studies reviewed are shown in Table 14, which incorporates data on specific foods as well as the total diet. The intakes were

Table 13
Range of concentrations (μg/kg) of PAHs in major food groups

PAH	Meat and meat products[a]	Fish and seafoods[a]	Vegetables	Fruits and confections[b]	Cereal and cereal products[c]	Beverages	Oils and fats	Dairy products[d]
Acenaphthene	0.05	ND–83	0.01–0.03	0.02	ND–2.3	—	0.02–45	0.01–0.08
Acenaphthylene	ND–500	<0.02–160	0.07–0.19	0.1–0.14	0.89	—	0.10–29	0.16–0.4
Anthanthrene	ND–67	—	—	—	ND–0.13	—	0.03–2.7	—
Anthracene	ND–133	ND–191	ND–17	ND–2.5	ND–9.4	ND	ND–460	ND–0.30
Benz[*a*]anthracene	ND–144	ND–86	0.05–15	ND–2.0	0.03–4.2	0.003–0.61	ND–79	ND–1.7
Benzo[*a*]fluorene[e]	ND–174	0.2–3.0	0.08–2.6	ND–1.5	—	—	—	—
Benzo[*b*]fluorene	ND–72	—	0.11–2.8	ND–1.0	—	—	ND–45	—
Benzo[*b*]fluoranthene[f]	ND–197	ND–134	ND–28.7	ND–3.5	0.03–1.3	ND–0.65	ND–91	ND–0.7
Benzo[*ghi*]fluoranthene	ND	ND	ND	ND–0.9	ND–0.7	ND	ND–4.9	ND
Benzo[*j*]fluoranthene	ND–7	—	—	—	—	—	ND–5.1	—
Benzo[*k*]fluoranthene	ND–172	ND–55	ND–17	ND–0.2	0.02–1.4	ND–0.24	ND–99	ND–0.1
Benzo[*ghi*]perylene	ND–153	ND–31	ND–11	ND–6.0	ND–120	ND–0.03	ND–66	ND–1.6
Benzo[*c*]phenanthrene	ND–1.4	ND–280	ND–9.2	ND–0.5	ND–0.7	ND	ND	ND
Benzo[*a*]pyrene	ND–212	ND–173	ND–25	ND–1.5	ND–5.4	ND–0.60	ND–164	ND–1.3
Benzo[*e*]pyrene	ND–81	ND–50	ND–7.9	ND–1.5	0.06–5.2	ND–0.06	ND–37	ND–0.2
Chrysene[g]	ND–140	ND–49	ND–62	ND–9.0	ND–2.8	ND–0.02	ND–76	ND–1.5
Coronene	—	ND–2.4	—	—	—	—	ND–7.4	—
Cyclopenta[*cd*]pyrene	—	—	—	—	—	—	ND–1.4	—
Dibenz[*a,h*]anthracene[h]	ND–8.8	ND–39	ND–1.0	ND–0.05	ND–3.6	0.002–0.24	ND–43	ND–0.04
Dibenzo[*a,e*]pyrene	ND	ND–0.3	ND	ND	ND	ND	ND–0.04	ND

Dibenzo[*a,h*]pyrene	—	—	ND–0.7	—	—	—	—	—
Dibenzo[*a,i*]pyrene	—	—	ND–0.3	—	—	—	—	—
Dibenzo[*a,l*]pyrene	—	—	—	—	—	—	—	—
Fluoranthene	ND–376	ND–218	ND–117	ND–27	0.10–130	ND–8.4	ND–460	ND–8.0
Fluorene	ND–0.67	ND–252	0.03–0.06	0.03–3.5	ND–5.9	—	ND–264	0.02–0.07
Indeno[1,2,3-*cd*]pyrene	ND–171	ND–42	ND–7.9	ND–1.0	ND–3.2	ND	ND–81	ND–1.2
5-Methylchrysene	ND–3.7	ND–1.1	ND–2.6	ND–1.6	ND–4.9	ND–0.05	ND–3.7	ND–1.6
1-Methylphenanthrene	ND–58	ND–708	0.1–2.1	—	0.3	—	ND–190	—
Naphthalene	0.9–55	ND–156	0.06–0.5	0.18–4.3	2.6	—	ND–57	0.27–0.9
Perylene	ND–28	ND–24	0.05–1.7	—	0.1–0.7	—	ND–36	ND–0.6
Phenanthrene	ND–618	ND–334	ND–12	ND–30	ND–94	ND	ND–170	ND–1.6
Pyrene	1.2–452	ND–217	ND–70	ND–12	ND–48	ND–9.3	ND–330	ND–4.8
Triphenylene	—	—	—	—	—	—	—	—

Note: compounds shown in bold were considered by the Committee to be genotoxic and carcinogenic.
ND: not detected; PAH: polycyclic aromatic hydrocarbon
[a] Includes grilled and smoked foods and smoke flavouring food additives
[b] Includes sweets and sugar
[c] Includes biscuits, bread, bran and breakfast cereals
[d] Includes butter and cheese
[e] Reported including benzo[*b*]fluorene in some publications
[f] Reported as sum with benzo(*j* + *b* + *k*)fluoranthenes in some publications
[g] Reported including triphenylene in some publications
[h] Reported as sum with dibenzo(*a,h* + *a,c*)anthracenes in some publications

Table 14
Summary of estimated intakes of 10 of the 13 PAHs considered by the Committee to be genotoxic and carcinogenic

The range of intakes presented in μg/day may not be from the same study as those expressed in μg/kg of body weight per day and therefore are not directly divisible by an average body weight.

PAH	No. of estimates of intake (No. of countries)	Lowest reported intake, μg/day (μg/kg of body weight per day)	Range of reported means, μg/day (μg/kg of body weight per day)	Range of reported 95th percentiles, μg/day (μg/kg of body weight per day)	Highest reported intake, μg/day (μg/kg of body weight per day)
Benz[*a*]anthracene	12 (9)	0.005 (0.00007)	0.0006–0.47 (0.00007–0.0018)	0.013–0.093 (0.00031–0.00132)	1.7 (0.003)
Benzo[*b*]fluoranthene	11 (9)	0.009 (0.0001)	0.005–0.46 (0.00045–0.0036)	0.075–0.127 (0.0081–0.0036)	1.0 (0.004)
Benzo[*j*]fluoranthene	[b] 1 (1)	<0.030 [a] (<0.0005)	<0.030 [a] (<0.0005)	—	0.9 [a] (0.015)
Benzo[*k*]fluoranthene	11 (9)	0.007 (0.0002)	0.007–0.26 (0.00016–0.0032)	0.023–0.043 (0.00061–0.0015)	0.4 (0.005)
Benzo[*a*]pyrene	32 (18)	0.0006 (0.0001)	0.0006–2.04 (0.0001–0.005)	0.096–0.27 (0.0012–0.0047)	2.04 (0.006)
Chrysene	8 (8)	0.008 (0.0001)	0.008–5.0 (0.0001–0.0035)	0.045–0.113 (0.001–0.0021)	11.6 (0.006)
Dibenz[*a,h*]anthracene	10 (9)	0.005 (0.0001)	0.0046–0.76 (0.00009–0.0012)	0.016–0.028 (0.0004–0.00079)	1.5 (0.002)
Dibenzo[*a,e*]pyrene	1 (1)	0.010 [a] (0.00017)	0.01–0.63 [a] (0.00017–0.011)	—	0.64 [a] (0.011)
Indeno[1,2,3-*cd*]pyrene	9 (8)	0.009 (0.0001)	0.009–0.46 (0.0001–0.0034)	0.034–0.064 (0.00079–0.0017)	0.55 (0.006)
5-Methylchrysene	1 (1)	0.580 [a] (0.0097)	0.58–0.73 [a] (0.0097–0.012)	—	2.6 [a] (0.040)

[a] No estimates of intake reviewed were expressed in μg/kg of body weight per day for this PAH. Therefore, intakes in μg/day were divided by 60 kg to determine a likely intake in μg/kg of body weight per day.
[b] Two additional estimates of intake were presented as benzo[*b* + *j* + *k*]fluoranthene, therefore have not been included in this summary.

summarized from all of the studies reviewed, some of which reported intakes in units/person per day, and others in units/kg of body weight. Therefore, the range of intakes presented in µg/day may not be from the same study as those expressed in µg/kg of body weight per day and therefore are not directly divisible by an average body weight. Estimated intakes of benzo[*a*]pyrene ranged from <1 to 2.0 µg/day, and from 0.0001 to 0.006 µg/kg of body weight 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 of body weight 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 (Table 15). 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 of body weight per day. From this range, the Committee selected the value of 0.004 µg/kg of body weight 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 of body weight 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 of body weight per day. On the basis of these data, the Committee identified a high-level intake of 0.01 µg/kg of body weight per day for use in the present evaluation.

It was also noted by the Committee that there may be some regions of the world that have higher intakes than the "whole of diet" estimates presented in Table 15. In one study from northern China, intakes of PAH were calculated from vegetables only. This area of China uses a lot of coal fuel for cooking, heating and greenhouse warming. Intake of benzo[*a*]pyrene in this study was estimated at about 2 µg/day. It was noted that a consumption of vegetables of about 440 g/day was used to calculate the estimated intake, although data submitted to the Committee from the Chinese National Nutrition and Health Survey indicated that actual consumption of vegetables was about 270 g/day. Therefore, actual intake of benzo[*a*]pyrene from vegetables alone for this part of China is likely to be about 1 µg/day. This is in the same order of magnitude as the intake of 0.77 µg/day reported as being the highest intake from the major foods in the diet.

Table 15
Summary of estimated intakes of benzo[*a*]pyrene from studies covering the range of major food groups in the diet

The range of intakes presented in μg/day may not be from the same study as those expressed in μg/kg of body weight per day and therefore are not directly divisible by an average body weight.

PAH	No. of estimates of intake (No. of countries)	Range of reported means, μg/day (μg/kg of body weight per day)	Range of reported 95th percentiles, μg/day (μg/kg of body weight per day)	Highest reported intake, μg/day (μg/kg of body weight per day)
Benzo[*a*]pyrene	16 (13)[a]	0.0014–0.42 (0.0002–0.005)	0.07–0.27 (0.0012–0.0049)	0.77[b] (0.0062)

[a] Australia, Austria, Belgium, Brazil, Denmark, Finland, France, Italy, Netherlands ($n = 4$), New Zealand, Norway, Spain, UK
[b] Equates to an intake of 0.013 μg/kg of body weight per day, for a 60 kg person.

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.

The Committee usually calculates international estimates of intake of contaminants using the GEMS/Food regional diets, and extrapolating data on concentrations of the contaminant from regions in which data are collected to regions for which no data exist. In the present case, 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 benzo[*a*]pyrene of 0.004 µg/kg of body weight per day and a high-level intake of benzo[*a*]pyrene of 0.01 µg/kg of body weight 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 where the level of contamination with PAHs is higher.

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 rats or mice 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 3) 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 (Table 16).

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 end-point, the values for the BMDL ranged from 0.10 to 0.23 mg (100 to 230 µg) of benzo[*a*]pyrene/kg of body weight per day. The Committee decided to use the more conservative lower end of this range for its evaluation. Thus a BMDL equivalent to 100 µg of benzo[*a*]pyrene/kg of body weight 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 mixtures 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) 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 toxic equivalency factors that have been proposed for PAHs are 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 PAH 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

Table 16

BMD and BMDL values for carcinogenicity of benzo[*a*]pyrene and coal tar mixtures, based on a battery of statistical models

Modelled data	Range of BMD and BMDL values (mg of benzo[*a*]pyrene/kg of body weight per day)					
	Benzo[*a*]pyrene		Coal tar mixture I		Coal tar mixture II	
	BMD	BMDL	BMD	BMDL	BMD	BMDL
Mouse forestomach	0.40–0.921	0.31–0.74	0.21–0.40	0.16–0.31	0.37–0.59	0.27–0.45
Mouse lung[a]	No observed increase in incidence of tumours		0.14–0.26	0.11–0.20	0.14–0.26	0.11–0.20
Tumour-bearing mice[a]	—	—	0.13–0.29	0.10–0.23	0.13–0.29	0.10–0.23
Rat liver	3.4–4.0	2.9–3.4	—	—	—	—
Tumour-bearing rats	1.2–2.0	1.0–1.7	—	—	—	—

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

[a] The two mixtures did not produce significantly different dose–response curves and therefore the data were combined.

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 PAH administered by the oral route. Furthermore, the surrogate approach is much simpler to apply and is generally as accurate as the toxic-equivalency-factor 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, an 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 of benzo[*a*]pyrene/kg of body weight per day was derived for PAHs in food on the basis of a study of carcinogenicity in mice treated orally with 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 the levels of PAHs generated during cooking of food over barbecues and open fires, the Committee concluded that a representative mean intake of benzo[*a*]pyrene of 0.004 µg/kg of body weight per day and an estimated high-level intake of benzo[*a*]pyrene of 0.01 µg/kg of body weight 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 PAH 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.

A toxicological and technical monograph was prepared.

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.
- The Committee recommended that analytical methods be developed to include most dibenzopyrenes and 5-methylchrysene.
- The Committee recommended that efforts be made to collect data on concentrations in the major food groups for PAHs identified by the Committee as being genotoxic and carcinogenic.
- The Committee recommended that, should PAHs be reassessed by the Committee in the future, raw data from individual samples be submitted to allow estimates of the regional intakes to be made.

4. Future work

- The Committee noted the need to develop methodologies to assess the short-term dietary intakes of contaminants. For this, guidelines

need to be developed on the data sets and equations that should be used to calculate short-term dietary intakes, and the population groups that should be assessed.

- The Committee recommended that acrylamide be re-evaluated when the results of planned and ongoing studies of carcinogenicity and long-term studies of neurotoxicity become available.

5. Recommendations

1. The Committee recommended that FAO and WHO seek ways to make the calls for data more widely known in developing countries at both technical and policy levels and to directly contact governments and other potential data providers to facilitate the submission of such data to the Committee.
2. The Committee also recommended that data providers in both developing and industrialized countries enhance their efforts to submit their information to the Committee and to use the electronic format for GEMS/Food in order to facilitate the collation and quality control of data.
3. The Committee recommended that acrylamide be re-evaluated when the results of planned and ongoing studies of carcinogenicity and long-term studies of neurotoxicity become available.
4. The Committee recommended that work should be continued on the use of physiologically based pharmacokinetic modelling fro acrylamide to better link data on biomarkers in humans with intake assessments and toxicological effects in experimental animals.
5. The Committee recommended that appropriate efforts to reduce concentrations of acrylamide in food should continue.
6. The Committee noted that it would be useful to have data on the occurrence of acrylamide in foods as consumed in developing countries. This information would be useful in conducting intake assessments, as well as considering mitigation approaches to reduce human exposure.
7. The Committee recommended that future PAH 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. Moreover, the Committee recommended that analytical methods be developed to include most dibenzopyrenes and 5-methylchrysene.

8. Although no specific recommendations for PBDEs were made by the Committee, it was 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.
9. The Committee also 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.

Acknowledgements

The Committee learned with sadness of the recent passing of Professor Philippe Shubik who had been a member of the Committee for the first time at its fifth meeting in 1961 and had served more or less continuously as a member or chairman until a few years ago. Professor Shubik also played a leading role in the preparation of Environmental Health Criteria No. 70, which is still to date the most comprehensive and leading reference book on the principles of the safety assessment of additives and contaminants in food. His pioneering work on the two-stage model opened the door to major advances in understanding the mechanisms of the processes of carcinogenesis. He will be remembered for his wise counsel, acute critical mind and acerbic wit, which enlivened many meetings. His contributions to food toxicology and safety evaluation will be sadly missed.

Were Professor Shubik alive today, he would probably say "this is the worst piece of prose that I have ever read!".

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

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Annex 2

Summary of toxicological evaluations

1. Acrylamide

Estimated intake: mean intake, 0.001 mg/kg of body weight per day; high intake, 0.004 mg/kg of body weight per day

Effect	NOEL/BMDL (mg/kg of body weight per day)	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 end-points 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, 2.0	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 concentrations of acrylamide in foodstuffs should continue.

BMDL: 95% lower confidence limit for the benchmark dose; MOE: margin of exposure; NOEL: no-observed-effect level

2. Cadmium – impact assessment of different maximum limits

The Committee concluded that the effect of different maximum levels (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 provisional tolerable weekly intake (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. Ethyl carbamate

Estimated intake: mean intake (from food), 15 ng/kg of body weight per day; high intake (from food and alcoholic beverages), 80 ng/kg of body weight per day

Effect	BMDL (mg/kg of body weight per day)	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.

BMDL: 95% lower confidence limit for the benchmark dose; MOE: margin of exposure

4. Inorganic tin

The Committee concluded that the data available indicated that it is inappropriate to establish an acute reference dose (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)

Estimated intake: mean, approximately 4 ng/kg of body weight 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.

PBDEs are non-genotoxic substances, however, the available data on PBDEs were not adequate to allocate a provisional maximum tolerable daily intake (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 activation of the aryl hydrocarbon receptor; 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 agonists of the aryl hydrocarbon receptor

Based on limited toxicity data, the Committee concluded that there appeared to be a large margin of exposure (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)

Estimated intake for benzo[*a*]pyrene as a marker for PAHs:

mean, 4 ng/kg of body weight per day; high, 10 ng/kg of body weight per day

Effect	BMDL[a] (ng/kg of body weight day)	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.

BMDL: 95% lower confidence limit for the benchmark dose; MOE: margin of exposure
[a] BMDL for benzo[*a*]pyrene as a marker for mixtures of PAHs.

Annex 3
Approach to dose–response modelling

At the present meeting, cancer dose–response data were analysed by dose–response modelling, in accordance with the International Programme on Chemical Safety (IPCS) document *Principles for modeling dose–response analysis for the risk assessment of chemicals* (*1*). The statistical methods of dose–response modelling as applied at this meeting are briefly described below.

For each tumour end-point considered relevant, the following quantal dose–response models were fitted to the dose–incidence data:

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 respond, 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 (*2*). Nested models can be formally compared to each other as follows. Inclusion of an extra model

Table 1
Dose–response models used

Model	Model equation	Constraints
One-stage	$R = a + (1 - a)(1 - \exp(-x/b))$	$0 \leq a \leq 1$,
Two-stage	$R = a + (1 - a)(1 - \exp(-(x/b) - c(x/b)\text{^}2))$	$0 \leq a \leq 1$
Log-logistic	$R = a+(1 - a)/(1 + \exp(c\log 10(b/x)))$	$0 \leq a \leq 1$, $c \geq \ln(10)$
Log-probit	$R = a+(1 - a)\ \Phi(c\log 10(x/b))$	$0 \leq a \leq 1$
Weibull	$R = a + (1 - a)(1 - \exp(-(x/b)\text{^}c))$	$0 \leq a \leq 1$, $c > 1$
Proast M2	$y = \exp(bx)$, *th1*	
Proast M3	$y = \exp(b\ x\ \text{^}d)$, *th1*	$d \geq 1$
Proast M4	$y = c - (c - 1)\exp(-bx)$, *th1*	

Φ denotes the (cumulative) standard normal distribution function.

parameter should result in a higher log-likelihood value, and if this increase is >1.92, inclusion of the parameter has resulted in a significantly better fit (log-likelihood ratio test). If the increase is <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 to be 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 to be acceptable. For instance, when the saturated model has eight parameters (i.e. eight observed incidences available) a fitted dose–response model with three 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 2 summarizes the critical differences in log-likelihood values for various numbers of degrees of freedom (= difference in number of parameters between the models to be compared).

For those models that were considered acceptable according to the criteria mentioned, the benchmark dose (BMD) values, as well as the

Table 2
Critical differences in log-likelihood values 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 ($\alpha = 0.05$)
1	1.92
2	3.00
3	3.91
4	4.74
5	5.54
6	6.30
7	7.03
8	7.75

benchmark dose lower confidence limit (BMDL) values were calculated. All BMD and BMDL values were calculated for a 10% extra risk, defined as:

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

This represents the additional-response fraction divided by the tumour-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 about 10% for the BMDL.

The calculations were performed using the dose–response software package PROAST, version V07 (developed at the National Institute of Public Health and the Environment (RIVM), Bilthoven, the Netherlands), which is freely available.

References for Annex 3

1. *Principles for modelling low-dose response for risk assessment of chemicals*, International Programme on Chemical SafetyWorkshop, Geneva, World Health Organization, 2004 (http://www.who.int/ipcs/methods/harmonization/en/draft_document_for_comment.pdf).
2. Slob W. Dose–response modeling of continuous endpoints. *Toxicological Sciences*, 2002, **66**:298–312.

Corrigendum

WHO Technical Report Series 925: Evaluation of certain veterinary drug residues in food, 2004

Doramectin, p71, footnote [a], line 1:

Replace “15 mg/kg” with “15 µg/kg”.