Food and Agriculture Organization of the United Nations

World Health Organization

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Joint FAO/WHO Expert Committee on Food Additives
Fifty-seventh meeting
Rome, 5-14 June 2001

SUMMARY AND CONCLUSIONS

A meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) was held in Rome, Italy, from 5 to 14 June 2001. The purpose of the meeting was to evaluate certain food additives and contaminants.

Mrs. I. Meyland, Senior Scientific Adviser, Danish Veterinary and Food Administration, Søborg, Denmark, served as chairman and Professor R. Walker, Emeritus Professor of Food Science, School of Biological Sciences, University of Surrey, Guildford, Surrey, United Kingdom, served as vice-chairman.

Dr J.L. Herrman, International Programme on Chemical Safety, World Health Organization and Dr. Manfred Luetzow, Food Quality and Standards Service, Food and Nutrition Division, Food and Agriculture Organization of the United Nations, served as joint secretaries.

The present meeting was the fifty-seventh in a series of similar meetings. The tasks before the Committee were (a) to elaborate further principles for evaluating the safety of food additives and contaminants; (b) to assess certain food additives, flavouring agents, and contaminants; and (c) review and prepare specifications for selected food additives.

The report of the meeting will appear in the WHO Technical Report Series. Its presentation will be similar to that of previous reports, namely, general considerations, comments on specific substances, and recommendations for future work. An annex will include detailed tables (similar to the tables in this report) summarizing the main conclusions of the Committee in terms of acceptable daily intakes (ADIs) and other toxicological recommendations. Information on specifications for the identity and purity of certain food additives examined by the Committee will also be included.

The participants in the meeting are listed in Annex 1. Further information required or desired is listed in Annex 2. Items of a general nature that contain information that the Committee would like to disseminate quickly are included in Annex 3. Draft report items on the contaminants that were evaluated are included in Annex 4.

Toxicological monographs or monograph addenda on most of the substances that were considered will be published in WHO Food Additives Series No. 48.

Specifications for the identity and purity of the compounds listed in Annex 2 marked as N; N,T; R; or R,T will be published in FAO Food and Nutrition Paper Series 52, Addendum 9. Specifications for substances marked as S and S,T have been published previously in that series. However, if these specifications have not been adopted as Codex Advisory Specifications, they will be re-published in FAO Food and Nutrition Paper Series No. 52, Addendum 9.

Corrected version (corrections are on pages 6 and 16)

Acceptable daily intakes (ADIs), other recommendations, and information on specifications

1. Food additives evaluated toxicologically

Food additive	Specifications[a]	Acceptable daily intake (ADI) and other toxicological recommendations
Emulsifiers		
Diacetyltartaric and fatty acid esters of glycerol	R	0-50 mg/kg bw (temporary)[b]
Tartaric, acetic and fatty acid esters of glycerol, mixed	W[c]	ADI withdrawn[c]
Quillaia extracts	R, T[b]	0-5 mg/kg bw (temporary)[b]
Enzyme preparation		
Invertase from *Saccharomyces cerevisiae*	N	Acceptable[d]
Food colours		
ß-Carotene from *Blakeslea trispora*	N,T[b]	0-5 mg/kg bw (group ADI with synthetic ß-carotene)
Curcumin	R	0-1 mg/kg bw (temporary)[b]
Food salts		
Calcium dihydrogen diphosphate	N	} Included in the maximum tolerable daily intake of 70 mg/kg bw for phosphates, diphosphates, and polyphosphates
Monomagnesium phosphate	N,T[b]	
Sodium calcium polyphosphate	N	
Trisodium diphosphate	N,T[b]	
Glazing agent		
Hydrogenated poly-1-decene	R	0-6 mg/kg bw
Preservative		
Natamycin (pimaricin)	N,T[b]	0-0.3 mg/kg bw
Sweetening agent		
D-Tagatose	S	0-80 mg/kg bw
Thickening agents		
Carrageenan	R	} ADI "not specified"[e] (group ADI for carrageenan and processed *Eucheuma* seaweed)
Processed *Eucheuma* seaweed	R	
Curdlan	R	ADI "not specified"[e]
Miscellaneous substances		
Acetylated oxidized starch	N, R[f]	ADI "not specified"[e]
a-Cyclodextrin	N	ADI "not specified"[e]
Sodium sulfate	S	ADI "not specified"[e]

[a]N, new specifications prepared; R, existing specifications revised; S, specifications exist, revision not considered or required; T, the existing, new, or revised specifications are tentative and new information is needed; W, existing specifications withdrawn.

[b]See Annex 2.

[c]The ADI was withdrawn because the specifications for tartaric, acetic and fatty acid esters of glycerol, mixed, were combined with those of diacetyltartaric and fatty acid esters of glycerol under the latter name at the fifty-first meeting (WHO Technical Report Series, No. 891, 2000).

[d]Invertase from *Saccharomyces cerevisiae* that meets the specifications developed at the present meeting was considered to be acceptable because *S. cerevisiae* is commonly used in the preparation of food. Its use should be limited by Good Manufacturing Practice.

[e]ADI "not specified" is used to refer to a food substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effects and from its acceptable background levels in food, does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for the reasons stated in the individual evaluations, the establishment of

an ADI expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice, i.e. it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal food of inferior quality or adulterated food, and it should not create a nutritional imbalance.

[f] The new specifications for Acetylated Oxidized Starch were integrated into the revised specifications for Modified Starches.

2. Food additives considered for specifications only

Food Additive	Specification[a]	Food Additive	Specification[a]
Acesulfame K (potassium salt)	R	Pectins	R
Blackcurrant extract	R	Smoked flavourings	R
Oxystearin	W	Tagetes extract	R
DL-Malic Acid	R[b]		

[a] N, new specifications prepared; R, existing specifications revised; S, specifications exist, revision not considered or required; T, the existing, new, or revised specifications are tentative and new information is needed; W, existing specifications withdrawn.

[b] The "call for data" asked for information on L-malic acid. However, no information about the uses of L-malic acid, other than its well-established use as a flavouring agent was received. As DL- and L-malic acid are different compounds made by different manufacturing processes, the specifications for DL-malic acid were corrected, and the reference to the specifications for L-malic acid were removed.

3. Revision of heavy metals limits for food additives

At its fifty-fifth meeting, the Committee began its implementation of a systematic five-year programme to replace the outdated test for heavy metals (as lead) in all existing food additive specifications with appropriate limits for individual metals of concern. Proposed lead and arsenic limits for 43 emulsifiers were established. As no alternative proposals were received by the deadline for submission of data for the present meeting, the new proposed limits were adopted, replacing those published in FAO Food and Nutrition Paper 52 and its addenda 1 to 7.

The second group of substances, considered at the present meeting, included 10 anticaking agents, 17 flavour enhancers, 10 sweetening agents, and 13 thickening agents. In response to the call for data, proposed limits and supporting data were received for sodium ferrocyanide.

The proposed changes to the current limits were as follows

- Limits for arsenic were deleted except for ferrocyanides of calcium, potassium and sodium, for which a limit of 3 mg/kg was proposed.
- Proposed limits for lead for the thickening agents and magnesium oxide were 2 mg/kg, for flavour enhancers and sweeteners 1 mg/kg, for phosphates 4 mg/kg, and for silicate anticaking agents 5 mg/kg.
- No limits were proposed for cadmium or mercury, as there were not concerns for their presence in any of the substances under review.
- Limits for heavy metals (as lead) were deleted.

Comments on the Committee's new proposed limits are invited. If alternative values and supporting data are not received by the deadline for submission of data for the fifty-ninth meeting, the proposed metal limits will be adopted and supersede the existing limits, replacing those published in FAO Food and Nutrition Paper 52 and its addenda 1 to 8.

Category	Food additive	INS	Sub	As	Pb	Hg	Cd	Other elements
Anticaking agent	Aluminium silicate	0559		-	5	-	-	
Anticaking agent	Calcium aluminium silicate	0556		-	5	-	-	F<50
Anticaking agent	Calcium silicate	0552		-	5	-	-	F<50
Anticaking agent	Ferrocyanides of Ca, K & Na	0538		3	5	-	-	Cu<10, Zn<25
Anticaking agent	Magnesium oxide	0530		-	2	-	-	
Anticaking agent	Magnesium silicates (synthetic)	0553	a	-	5	-	-	F<10
Anticaking agent	Silicon dioxide (amorphous)	0551		-	5	-	-	
Anticaking agent	Sodium aluminosilicate	0554		-	5	-	-	
Anticaking agent	Tricalcium phosphate	0341	iii	-	4	-	-	F<50
Anticaking agent	Trimagnesium phosphate	0342	iii	-	4	-	-	F<5
Flavour enhancer	Calcium-5’-guanylate	0629		-	1			
Flavour enhancer	Calcium 5’-inosinate	0633		-	1			
Flavour enhancer	Calcium 5'-ribonucleotides	0634		-	1	-	-	
Flavour enhancer	Calcium di-L-glutamate	0623		-	1	-	-	
Flavour enhancer	Dipotassium-5’-guanylate	0628		-	1	-	-	
Flavour enhancer	Dipotassium-5’-inosinate	0632		-	1	-	-	
Flavour enhancer	Disodium-5’-guanylate	0627		-	1	-	-	
Flavour enhancer	Disodium-5’-inosinate	0631		-	1	-	-	
Flavour enhancer	Disodium-5'-ribonucleotides	0635		-	1	-	-	
Flavour enhancer	Ethyl maltol	0637		-	1	-	-	
Flavour enhancer	L-Glutamic acid	0620		-	1	-	-	
Flavour enhancer	5’-Guanylic acid	0626		-	1	-	-	
Flavour enhancer	5’-Inosinic acid	0630		-	1	-	-	
Flavour enhancer	Magnesium di-L-glutamate	0625		-	1	-	-	
Flavour enhancer	Monoammonium L-glutamate	0624		-	1	-	-	
Flavour enhancer	Monopotassium L-glutamate	0622		-	1	-	-	
Flavour enhancer	Monosodium L-glutamate	0621		-	1	-	-	
Sweetening agent	Alitame	0956		-	1	-	-	
Sweetening agent	Aspartame	0951		-	1	-	-	
Sweetening agent	Cyclohexylsulfamic acid	0952		-	1	-	-	Se<30
Sweetening agent	Isomalt	0953		-	1	-	-	Ni<2
Sweetening agent	Lactitol	0966		-	1	-	-	Ni<2
Sweetening agent	Mannitol	0421		-	1	-	-	Ni<2
Sweetening agent	Saccharin and its Na, K and Ca salts	0954		-	1	-	-	Se<30
Sweetening agent	Sorbitol/ sorbitol syrup	0420		-	1	-	-	Ni<2
Sweetening agent	Sucralose	0955		-	1	-	-	
Sweetening agent	Xylitol	0967		-	1	-	-	Ni<2
Thickening agent	Ammonium alginate	0403		-	2	-	-	
Thickening agent	Ethyl cellulose	0462		-	2	-	-	
Thickening agent	Gum ghatti	0419		-	2	-	-	
Thickening agent	Hydroxypropyl cellulose	0463		-	2	-	-	
Thickening agent	Hydroxypropylmethyl cellulose	0464		-	2	-	-	
Thickening agent	Karaya gum	0416		-	2	-	-	
Thickening agent	Konjac flour	0425		-	2	-	-	
Thickening agent	Methylethyl cellulose	0465		-	2	-	-	
Thickening agent	Methyl cellulose	0461		-	2	-	-	
Thickening agent	Polyvinylpyrrollidone	1201		-	2	-	-	
Thickening agent	Powdered cellulose	0460	(ii)	-	2	-	-	
Thickening agent	Tara gum	0417		-	2	-	-	
Thickening agent	Tragacanth gum	0413		-	2	-	-	

4. Flavouring agents evaluated using the Procedure for the Safety Evaluation of Flavouring Agents

A. Pyrazine derivatives

Flavouring agent	No.	Specifi-cations[a]	Conclusions based on current intake
2-Methylpyrazine	761	N	= No safety concern
2-Ethylpyrazine	762	N	
Propylpyrazine	763	N	
Isopropylpyrazine	764	N	
2,3-Dimethylpyrazine	765	N	
2,5-Dimethylpyrazine	766	N	
2,6-Dimethylpyrazine	767	N	
2-Ethyl-3-methylpyrazine	768	N	
2-Ethyl-6-methylpyrazine	769	N	
2-Ethyl-5-methylpyrazine	770	N	
2,3-Diethylpyrazine	771	N	
2-Methyl-5-isopropylpyrazine	772	N	
2-Isobutyl-3-methylpyrazine	773	N	
2,3,5-Trimethylpyrazine	774	N	
2-Ethyl-3,(5 or 6)-dimethylpyrazine	775	N	
3-Ethyl-2,6-dimethylpyrazine	776	N	
2,3-Diethyl-5-methylpyrazine	777	N	
2,5-Diethyl-3-methylpyrazine	778	N	
3,5-Diethyl-2-methylpyrazine	779	N	
2,3,5,6-Tetramethylpyrazine	780	N	
5-Methyl-6,7-dihydro-5H-cyclopentapyrazine	781	N	
6,7-Dihydro-2,3-dimethyl-5*H*-cyclopentapyrazine	782	N	
2-Isobutyl-3-methoxypyrazine	792	N	
Acetylpyrazine	784	N	
2-Acetyl-3-methylpyrazine	950	N	
2-Acetyl-3-ethylpyrazine	785	N	
2-Acetyl-3,(5 or 6)-dimethylpyrazine	786	N	
Methoxypyrazine	787	N	
(2,5 or 6)-Methoxy-3-methylpyrazine	788	N	
2-Ethyl(or methyl)-(3,5 or 6)-methoxypyrazine	789	N	
2-Methoxy-(3,5 or 6)-isopropylpyrazine	790	N	
2-Methoxy-3-(1-methylpropyl)-pyrazine	791	N	
(Cyclohexylmethyl)pyrazine	783	N	
2-Methyl-3,5 or 6-ethoxypyrazine	793	N	
2-(Mercaptomethyl)pyrazine	794	N	
2-Pyrazinylethanethiol	795	N	
Pyrazinylmethyl methyl sulfide	796	N	
(3,5 or 6)-(Methylthio)-2-methylpyrazine	797	N	
5-Methylquinoxaline	798	N	
Pyrazine	951	N	
5,6,7,8-Tetrahydroquinoxaline	952	N	

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B. Aromatic substituted secondary alcohols, ketones and related esters

Flavouring agent	No.	Specifi-cations[a]	Conclusions based on current intake
α-Methylbenzyl alcohol	799	N	No safety concern
α-Methylbenzyl formate	800	N	
α-Methylbenzyl acetate	801	N	
α-Methylbenzyl propionate	802	N	
α-Methylbenzyl butyrate	803	N	
α-Methylbenzyl isobutyrate	804	N	
p, α-Dimethylbenzyl alcohol	805	N	
Acetophenone	806	N	
4-Methylacetophenone	807	N	
p-Isopropylacetophenone	808	N	
2,4-Dimethylacetophenone	809	N	
Acetanisole	810	N	
1-(*p*-Methoxyphenyl)-2-propanone	813	N	
α-Methylphenethyl butyrate	814	N	
4-Phenyl-2-butanol	815	N	
4-Phenyl-2-butyl acetate	816	N	
4-(*p*-Tolyl)-2-butanone	817	N, T	
4-(*p*-Methoxyphenyl)-2-butanone	818	N	
4-Phenyl-3-buten-2-ol	819	N	
4-Phenyl-3-buten-2-one	820	N	
3-Methyl-4-phenyl-3-buten-2-one	821	N	
1-Phenyl-1-propanol	822	N	
α-Ethylbenzyl butyrate	823	N	
Propiophenone	824	N	
α-Propylphenethyl alcohol	825	N	
1-(*p*-Methoxyphenyl)-1-penten-3-one	826	N	
Ethyl benzoylacetate	834	N	
Ethyl 2-acetyl-3-phenylpropionate	835	N	
4-Acetyl-6-*t*-butyl-1,1-dimethylindan	812	N	Additional data required*
α-Isobutylphenethyl alcohol	827	N	No safety concern
4-Methyl-1-phenyl-2-pentanone	828	N	
1-(4-Methoxyphenyl)-4-methyl-1-penten-3-one	829	N	
3-Benzyl-4-heptanone	830	N	
1-Phenyl-1,2-propanedione	833	N	
Methyl β-naphthyl ketone	811	N	
Benzophenone	831	N	
1,3-Diphenyl-2-propanone	832	N	
Benzoin	836	N	

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*Corrected from the earlier version, where this was given as “no safety concern”.

C. Benzyl derivatives

Flavouring agent	No.	Specifi-cations[a]	Conclusions based on current intake
Benzyl alcohol	025	R	= No safety concern
Benzyl formate	841	N	
Benzyl acetate	023	R	
Benzyl propionate	842	N	
Benzyl butyrate	843	N	
Benzyl isobutyrate	844	N	
Benzyl isovalerate	845	N	
Benzyl *trans*-2-methyl-2-butenoate	846	N	
Benzyl 2,3-dimethylcrotonate	847	N, T	
Benzyl acetoacetate	848	N	
Benzyl benzoate	024	R	
Benzyl phenylacetate	849	N	
Benzaldehyde	022	R	
Benzaldehyde dimethyl acetal	837	N	
Benzaldehyde glyceryl acetal	838	N	
Benzaldehyde propylene glycol acetal	839	N	
Benzoic acid	850	N	Evaluation not finalized[b]
Methyl benzoate	851	N	= No safety concern
Ethyl benzoate	852	N	
Propyl benzoate	853	N	
Hexyl benzoate	854	N	
Isopropyl benzoate	855	N	
Isobutyl benzoate	856	N	
Isoamyl benzoate	857	N	
cis-3-Hexenyl benzoate	858	N	
Linalyl benzoate	859	N	
Geranyl benzoate	860	N	
Glyceryl tribenzoate	861	N, T	Evaluations not finalized[b]
Propylene glycol dibenzoate	862	N, T	
Methylbenzyl acetate (mixed *o,m,p*)	863	N	= No safety concern
p-Isopropylbenzyl alcohol	864	N	
4-Ethylbenzaldehyde	865	N	
Tolualdehydes (mixed *o,m,p*)	866	N, T	
Tolualdehyde glyceryl acetal	867	N	
Cuminaldehyde	868	N	
2,4-Dimethylbenzaldehyde	869	N	
Benzyl 2-methoxyethyl acetal	840	N	

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[b]Further information is required to determine whether this substance is in current use as a flavouring agent.

D. Hydroxy- and alkoxy-substituted benzyl derivatives

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
4-Hydroxybenzyl alcohol	955	-[b]	⎫
4-Hydroxybenzaldehyde	956	-[b]	⎪
4-Hydroxybenzoic acid	957	-[b]	= No safety concern
2-Hydroxybenzoic acid	958	-[b]	⎭
Butyl-*p*-hydroxybenzoate	870	N, T	Evaluation not finalized[c]
Anisyl alcohol	871	N	⎫
Anisyl formate	872	N, T	⎪
Anisyl acetate	873	N	⎪
Anisyl propionate	874	N	⎪
Anisyl butyrate	875	N	⎪
Anisyl phenylacetate	876	N	⎪
Veratraldehyde	877	N	⎪
p-Methoxybenzaldehyde	878	N	⎪
p-Ethoxybenzaldehyde	879	N	⎪
Methyl *o*-methoxybenzoate	880	N	⎪
2-Methoxybenzoic acid	881	N	⎪
3-Methoxybenzoic acid	882	N	⎪
4-Methoxybenzoic acid	883	N	⎪
Methyl anisate	884	N	⎪
Ethyl *p*-anisate	885	N	⎪
Vanillyl alcohol	886	N	⎪
Vanillin	889	N	⎪
4-Hydroxy3-methoxybenzoic acid	959	-[b]	⎪
Vanillin acetate	890	N	⎪
Vanillin isobutyrate	891	N	⎪
Salicylaldehyde	897	N	⎪
2-Hydroxy-4-methylbenzaldehyde	898	N	= No safety concern
Methyl salicylate	899	N	⎪
Ethyl salicylate	900	N	⎪
Butyl salicylate	901	N	⎪
Isobutyl salicylate	902	N	⎪
Isoamyl salicylate	903	N	⎪
Benzyl salicylate	904	N	⎪
Phenethyl salicylate	905	N	⎪
o-Tolyl salicylate	907	N	⎪
2,4-Dihydroxybenzoic acid	908	N	⎪
Vanillyl ethyl ether	887	N	⎪
Vanillyl butyl ether	888	N	⎪
Ethyl vanillin	893	N	⎪
Vanillin *erythro*- & *threo*-butan-2,3-diol acetal	960	-[b]	⎪
Ethyl vanillin isobutyrate	953	N	⎪
Ethyl vanillin propylene glycol acetal	954	N, T	⎪
Piperonyl acetate	894	N	⎪
Piperonyl isobutyrate	895	N	⎪
Piperonal	896	N	⎪
Ethyl vanillin β-d-glucopyranoside	892	N	⎭

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[b]Specifications will be considered at the fifty-ninth meeting of the Committee.

[c]Further information is required to determine whether this substance is in current use as a flavouring agent

E. Aliphatic acyclic diols, triols, and related agents

Flavouring agent	No.	Specifi-cations[a]	Conclusions based on current intake
Glycerol	909	N, T	Evaluation not finalized[b]
3-Oxohexanoic acid glyceride	910	N, T	
3-Oxooctanoic acid glyceride	911	N, T	
Heptanal glyceryl acetal (mixed 1,2 and 1,3 acetals)	912	N	= No safety concern
1,2,3-tris[(1'-Ethoxy)ethoxy]propane	913	N	
3-Oxodecanoic acid glyceride	914	N, T	
3-Oxododecanoic acid glyceride	915	N, T	
3-Oxotetradecanoic acid glyceride	916	N, T	
3-Oxohexadecanoic acid glyceride	917	N, T	
Glycerol monostearate	918	N, T	
Glyceryl monooleate	919	N, T	
Triacetin	920	N, T	
Glyceryl tripropionate	921	N, T	= Evaluations not finalized[b]
Tributyrin	922	N, T	
Glycerol 5-hydroxydecanoate	923	N, T	
Glycerol 5-hydroxydodecanoate	924	N, T	
Propylene glycol	925	N, T	
Propylene glycol stearate	926	N, T	
1,2-di[(1-Ethoxy)ethoxy]propane	927	N	
4-Methyl-2-pentyl-1,3-dioxolane	928	N	
2,2,4-Trimethyl-1,3-oxacyclopentane	929	N	
Lactic acid	930	N	
Ethyl lactate	931	N	
Butyl lactate	932	N	
Potassium 2-(1'-ethoxy)ethoxypropanoate	933	N	= No safety concern
cis-3-Hexenyl lactate	934	N	
Butyl butyryllactate	935	N	
Pyruvic acid	936	N	
Pyruvaldehyde	937	N, T	
Ethyl pyruvate	938	N	
Isoamyl pyruvate	939	N	

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[b]Further information is required to determine whether this substance is in current use as a flavouring agent.

F. Aliphatic acyclic acetals

Flavouring agent	No.	Specifi-cations[a]	Conclusions based on current intake
1,1-Dimethoxyethane	940	N	⎫
Acetal	941	N	⎪
Heptanal dimethyl acetal	947	N	⎪
4-Heptenal diethyl acetal	949	N	⎪
Octanal dimethyl acetal	942	N	⎪
2,6-Nonadienal diethyl acetal	946	N	= No safety concern
Decanal dimethyl acetal	945	N	⎪
Citral dimethyl acetal	944	N	⎪
Citral diethyl acetal	948	N	⎪
Acetaldehyde ethyl *cis*-3-hexenyl acetal	943	N, T	⎭

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G. Flavouring agents considered for specifications only

No.	Flavouring agent	Specifi-cations[a]	No.	Flavouring agent	Specifi-cations[a]
10	Allyl tiglate	R	461	3-(Methylthio)propanol	R
12	Allyl cyclohexane acetate	R	478	3-(Methylthio)propyl acetate	R
14	Allyl cyclohexane butyrate	R	490	Allyl thiopropionate	R,T
15	Allyl cyclohexane valerate	R	510	2-Propanethiol	R
16	Allyl cyclohexane hexanoate	R	531	2-Naphthalenethiol	R
51	Isoamyl 2-methylbutyrate	R	543	Trithioacetone	R
58	Geranyl acetate	R	562	2,5-Dimethyl-2,5-dihydroxy-1,4-dithiane	R
64	Rhodinyl propionate	R	580	2-Methyl-2-(methyldithio)propanal	R
70	Geranyl hexanoate	R	581	Ethyl 2-(methyldithio)propionate	R
72	Geranyl isobutyrate	R	591	Methyl 2-oxo-3-methylpentanoate	R
74	Rhodinyl isobutyrate	R	599	Geranyl acetoacetate	R
77	Rhodinyl isovalerate	R	609	1,4-Nonanediol diacetate	R,T
78	3,7-Dimethyl-2,6-octadien-1-yl 2-ethylbutanoate	R	627	Aconitic acid	R,T
111	Lauric acid	R,T	642	3-Phenylpropyl hexanoate	R,T
113	Myristic acid	R,T	645	3-Phenylpropionaldehyde	R
115	Palmitic acid	R,T	648	Cinnamaldehyde ethylene glycol acetal	R
116	Stearic acid	R,T	652	Cinnamyl butyrate	R
172	Isobutyl heptanoate	R	656	Cinnamaldehyde	R
178	Nonyl octanoate	R	660	Propyl cinnamate	R
182	Isoamyl laurate	R,T	663	Butyl cinnamate	R
184	Butyl stearate	R	666	Heptyl cinnamate	R
191	trans-3-Heptenyl 2-methyl propanoate	R	671	Phenethyl cinnamate	R
240	omega-6-Hexadecenlactone	S	672	3-Phenylpropyl cinnamate	R
249	cis-4-Hydroxy-6-dodecenoic acid lactone	R	673	Cinnamyl cinnamate	R
260	2-Methylpentanal	R	676	alpha-Amylcinnamyl formate	R
270	2-Methyloctanal	R	677	alpha-Amylcinnamyl acetate	R
273	2,6-Dimethyloctanal	R	678	alpha-Amylcinnamyl isovalerate	R,T

No.	Flavouring agent	Specifi-cations[a]	No.	Flavouring agent	Specifi-cations[a]
304	Isopropyl formate	R	681	alpha-Amylcinnamaldehyde dimethyl acetal	R
306	Isopropyl propionate	R	698	o-Tolyl acetate	R
308	Isopropyl hexanoate	R	711	p-Vinylphenol	R
334	Methyl 3-hexenoate	R	719	Guaiacyl phenylacetate	R
344	Butyl 10-undecenoate	R	720	Hydroquinone monoethyl ether	R
347	2-Methyl-3-pentenoic acid	R	723	4-Ethyl-2,6-dimethoxyphenol	R
348	2,6-Dimethyl-6-hepten-1-ol	R	724	4-Propyl-2,6-dimethoxyphenol	R
350	Ethyl 2-methyl-3-pentenoate	R	726	4-Allyl-2,6-dimethoxyphenol	R
352	Hexyl 2-methyl-3&4-pentenoate (mixture)	R	729	Dihydroxyacetophenone	R,T
367	Terpinyl formate	R	732	Vanillylidene acetone	R
370	Terpinyl butyrate	R	740	Furfuryl propionate	R
372	Terpinyl isovalerate	R	741	Furfuryl pentanoate	R
374	p-Menth-8-en-1-ol	R	742	Furfuryl octanoate	R
390	gamma-Ionone	R,T	743	Furfuryl 3-methylbutanoate	R
416	5-Hydroxy-4-octanone	R	748	Amyl 2-furoate	R
424	2-Hydroxy-2-cyclohexen-1-one	R	749	Hexyl 2-furoate	R
428	d-Neo-Menthol	R	750	Octyl 2-furoate	R
434	p-Menth-1-en-3-ol	R	752	2-Phenyl-3-carbethoxyfuran	R,T
440	2-Ethyl-1,3,3-trimethyl-2-norbornanol	R	759	Furfuryl butyrate	R
442	Methyl 1-acetoxycyclohexyl ketone	R	760	Cinnamyl benzoate	R
457	(1-Buten-1-yl) methyl sulfide	R			

[a]R, existing specifications revised; S, specifications exist, revision not required; T, the existing, new, or revised specifications are tentative and new information is required.

4. Contaminants

Contaminant	Tolerable intake and other toxicological recommendations
3-Chloro-1,2-propanediol	PMTDI (provisional maximum tolerable daily intake): 2 µg/kg bw[a]
1,3-Dichloro-2-propanol	Establishment of a tolerable intake was considered to be inappropriate because of the nature of toxicity (tumorogenic in various organs in rats and the contaminant can interact with chromosomes and/or DNA); The Committee noted that the dose that caused tumours in rats (19 mg/kg bw per day) was about 20 000 times the highest estimated intake of 1,3-dichloro-2-propanol by consumers of soya sauce (1 µg/kg bw per day).[a]
Polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (PCBs)	PTMI (provisional tolerable monthly intake): 70 pg/kg bw[a]

[a]See Annex 4 for detailed information on the evaluation.

Annex 1

Fifty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives Rome, 5-14 June 2001

Members

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Annex 2

Further information required or desired

b-*Carotene from Blakeslea trispora*

Information is required on the method of analysis for residual solvents (ethyl acetate and isobutyl acetate). This information is required for evaluation in 2003.

Curcumin

The results of a reproductive toxicity study on a substance complying with the specifications for curcumin, known to be in progress, is required for evaluation in 2003.

Diacetyltartaric and fatty acid esters of glycerol

The following information relating to the two-year toxicity study in rats is required for evaluation in 2003:

- To assess whether some of the adverse effects that were observed were treatment-related, the groups treated with diacetyltartaric and fatty acid esters of glycerol should be compared with both untreated and monoglyceride-treated controls and the control groups should be compared with one another.
- Additional information on the incidence of mycocardial fibrosis and adrenal medullary hyperplasia in animals at the low and intermediate doses should be provided.

Monomagnesium phosphate, trisodium diphosphate

Information is required on the loss on drying, loss on ignition, test method for loss on ignition and assay method for the hydrates. This information is required for evaluation in 2003.

Natamycin

Information is required on the level and determination of water content, lead limit, specific rotation, assay value and method of assay for the commercial product. Comments on other aspects of the monograph are invited. This information is required for evaluation in 2003.

Quillaia extracts

The existing specifications for quillaia extracts were revised in order to clarify the differences between unpurified and semi-purified extracts Additional information on composition (minimum and maximum percentages of saponins unpurified and semi-purified extracts) is necessary, so the specifications were designated as tentative. Once the requested information has been received, the Committee will consider whether separate specifications for unpurified and semi-purified extracts are required. This information is required for evaluation in 2003. The ADI was made temporary pending clarification of the specifications. The temporary ADI is applicable only to the unpurified extract.

Annex 3

General considerations

An edited version of this section will appear in the report of the fifty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). It is reproduced here so that the information is disseminated quickly. This draft is subject to extensive editing.

1. Modification of the agenda

The following food additives were removed from the agenda (as announced in the *call for data*):

Annatto extracts	Scheduled for a future meeting, so that the Committee may consider toxicological studies that were being performed.
Amyloglucosidase from *Aspergillus oryzae*, var.	Included in the call for data by mistake
Sodium ethyl *para*-hydroxybenzoate	These food additives had been removed from the draft Codex General Standard for Food Additives and were referred to the Committee for evaluation. There was no indication that they are used as food additives and consequently little information was provided that would permit the establishment of ADIs or specifications.
Sodium propyl *para*-hydroxybenzoate	
Sodium methyl *para*-hydroxybenzoate	
calcium sulfite	
Sodium formate	
Calcium formate	
Synthetic ?-tocopherol	
Synthetic d-tocopherol	
Calcium tartrate	
Sorbitan trioleate	
Dipotassium diphosphate	
Dimagnesium diphosphate	
Phenyl salicylate (No. 906)	Had been evaluated previously at the fifty-fifth meeting (no. 736)*

*Corrected from the earlier version, where it was stated that no data were available.

2. Principles for the assessment of chemicals in food

The committee was informed that FAO and WHO are initiating a project to update and consolidate principles and methods for the assessment of chemicals in food, including food additives, contaminants, residues of veterinary drugs in food, and pesticide residues in food. This project is being undertaken on the basis of a recommendation of the *Conference on International Good Trade Beyond 2000: Science-based decisions, harmonization, equivalence, and mutual recognition* that was held in October 1999 and in view of the tremendous scientific advances and changes in the procedures and complexity of assessments of chemicals in food that have taken place since the publication of *Principles for the safety assessment of food additives and contaminants in food* (Environmental Health Criteria No. 70) and *Principles for the toxicological assessment of pesticide residues in food* (Environmental Health Criteria No. 104). It will be a comprehensive project that will include consideration of all those aspects of the assessment of chemicals in food that are considered by the Committee and the Joint FAO/WHO Meeting on Pesticide Residues.

The Committee recognized the importance of this initiative and recommended that it be undertaken as soon as possible.

3. Flavouring agents evaluated by the Procedure for the Safety Assessment of Flavouring Agents

The Committee questioned whether some of the substances included in the lists of flavouring agents that it had been requested to evaluate at its present meeting were in fact flavouring agents; some of these substances are used extensively in food processing as solvents, emulsifiers, or preservatives.

The Committee stressed that the Procedure for the Safety Evaluation of Flavouring Agents is intended for application to flavouring agents used to impart flavour to foods and not to non-flavour uses or to other chemicals that may be used in flavouring formulations. Consequently, the Committee was unable to finalize the evaluations of certain substances listed on the agenda[1], pending confirmation of their uses and intake as flavouring agents.

A clear definition of 'flavouring agent' has not been elaborated by the Committee. Although *Principles for the safety assessment of food additives and contaminants in food* provides some guidance, the Committee recommended that this issue be addressed at a future meeting.

4. Minimum assay values for flavouring agents

At its fifty-third meeting, the Committee established the criteria required for specifications for flavouring agents. The Committee noted that three criteria – chemical formula and relative molecular mass, identity test, and minimum assay value – constituted the core information required to establish acceptable specifications. At that time, the Committee expressed its view that a minimum assay value for individual flavouring agents of 95% applied to the content of the named flavouring agent or the named agent plus its known secondary components. About 90% of the flavouring agents evaluated to date meet or exceed the 95% minimum assay value for the named flavouring agent itself. For the others, the Committee received information on the nature of the secondary components. The Committee noted that 95% is not a fixed criterion for judging the acceptability of specifications for flavouring agents and that flexibility can be applied in establishing an acceptable level of secondary components, taking into account the likely levels of intake and other considerations.

Many secondary components are structurally related to the named flavouring agents and typically include small amounts of starting materials, isomers, and other flavouring agents. As these secondary components share many of the properties of the named flavouring agent, and in some cases are metabolites, they would not be expected to present a safety concern, or their safety can be determined from appropriate data on metabolism and toxicity.

The Committee noted that, in applying the Procedure for the Safety Evaluation of Flavouring Agents, information on secondary components included in the specification should be considered with data on intake and the potential toxicity of the flavouring agent and its structural analogues. The Committee therefore recommended that data on specifications be submitted before or at the same time as all other information necessary for evaluating safety.

5. Requests for data relating to intake assessments

The Committee recognized that it is not necessary to request data for intake assessments for all substances on its agenda, as it had done recently. Therefore, it developed criteria for determining when it is necessary to request such information. Calls for data should specify the information required for each substance on its agenda, as different data are required for the evaluation of food additives and contaminants.

[1] The substances in question are benzoic acid (No. 850), glyceryl tribenzoate (No. 861), propylene glycol dibenzoate (No. 862), butyl-p-hydroxybenzoate (No. 870), glycerol (No. 909), 3-oxodecanoic acid glyceride (No. 914), 3-oxododecanoic acid glyceride (No. 915), 3-oxotetradecanoic acid glyceride (No. 916), 3-oxohexadecanoic acid glyceride (No. 917), glycerol monostearate (No. 918), glyceryl monooleate (No. 919), (tri)-Acetin (No. 920), glyceryl tripropionate (no. 921), (tri)-Butyrin (No. 922), glycerol 5-hydroxydecanoate (No. 923), glycerol 5-hydroxydodecanoate (No. 924), propylene glycol (No. 925), and propylene glycol stearate (No. 926).

Food additives

Data should be requested for the assessment of intake when food additives are evaluated for the first time or when they are re-evaluated, except for food additives:

- for which only specifications are to be considered and
- on which the committee has recently deferred an evaluation pending the provision of a specific toxicological study or specific information on specifications, provided the Committee has evaluated intake during the preceding 3-5 years.

For food additives included in the draft Codex General Standard for Food Additives (GSFA), information on proposed maximum levels should be provided in the call for data so that national intake assessments based on the maximum levels in the GSFA, national maximum levels, and/or actual levels of use can be submitted. The Committee has formulated data sheets for submission of national intake assessments, which are included in the guidelines for the preparation of working papers on the intake of food additives that are available from the Secretariat.

Contaminants

For contaminants, an intake assessment is required in all cases. The call for data should request data on:

- occurrence and concentration (both individual and summary data) from all available sources, preferably submitted in the GEMS/Food format, with information on sampling and analytical techniques, data quality and reliability, reporting conventions, and appropriate processing factors and
- national intake of the contaminant based on national surveys of food consumption and concentrations.

6. Inclusion of raw materials and manufacturing methods in specifications

With increasing volumes of food additives in international trade, it is becoming increasingly important that specifications include raw materials and methods of manufacture in order to provide a full account of the product that was evaluated. Without this information, a product could be produced from different materials by different methods; consequently, impurities might have arisen that were not considered during the toxicological evaluation of the substance.

Principles for the safety assessment of food additives and contaminants in food states that 'To establish the chemical identities of additives, it is necessary to know the nature of the raw materials, methods of manufacture and impurities. This information is used to assess the completeness of analytical data on the composition of additives, and to assess the similarity of materials used in biological testing with those commercially produced.'

Therefore, the specifications other than those for flavouring agents will include brief details of raw materials and methods of manufacture, excluding proprietary details. The level of detail should be similar to that already used in many specifications published by the committee for additives made by fermentation or from plant materials.

7. General specifications and considerations for enzyme preparations used in food processing

The Committee has, on many occasions, addressed issues related to specifications for enzyme preparations used in food processing. The General Specifications in use today for enzymes were first elaborated by the Committee at its twenty-sixth meeting. Several revisions have been made, including:

(1) an addendum to address issues related to enzymes from genetically modified microorganisms;
(2) addition of an appendix to describe the method for determining antibiotic activity;
(3) an amendment to address microbial strain numbers in the specifications for enzyme preparations; and
(4) addition of the general requirement that source microorganisms be non-pathogenic and non-toxigenic.

At its fifty-fifth meeting, the Committee requested that the General Specifications for enzymes be reviewed and revised. Special consideration was to be given to updating the specifications in light of recent technological advances and to ensure consistency and coherence.

The revised General Specifications require that all new enzyme preparations undergo a general safety assessment. Many of the requirements previously outlined for enzyme preparations from genetically modified microorganisms are appropriate for all preparations, regardless of source, and the present Committee revised the General Specifications to reflect those requirements. For enzymes from genetically modified sources, focus is now placed on the final microbial strain used as the source organism and the genetic material introduced into and remaining in the final microbial production strain.

At its fifty-fifth meeting, the Committee noted that the list of mycotoxins contained in the existing General Specifications was not relevant to all food enzyme preparations from fungal sources. It further agreed that an attempt to list all known mycotoxins of potential concern was impractical and unwarranted. At its present meeting, the Committee agreed that enzyme preparations derived from fungal sources be evaluated for those mycotoxins that are known to be produced by strains of the species used in the production of the enzyme preparation or related species.

With regard to limits on heavy metals, the Committee agreed that the specification for lead contained in the existing General Specifications should be lowered from 10 mg/kg to 5 mg/kg. The Committee recognized that arsenic is not a concern in enzyme preparations, and the limit for this metal was deleted. Moreover, as there is no traceable source of cadmium or mercury in enzyme preparations, the Committee saw no need to establish limits for those metals. Such changes are consistent with the Committee's current policy on heavy metals.

In considering microbiological contamination of enzyme preparations, the Committee agreed that the existing microbiological criteria (for *Salmonella* spp., *Escherichia coli*, and total coliforms) and the requirement that use of preparations not increase the total microbial count in treated food over the level considered to be acceptable for the respective food are sufficient to ensure microbial safety and were thus retained. The Committee noted that the specification for a total viable count of 5×10^4/g contained in the existing General Specifications is arbitrary and is not an indication of the safety of an enzyme preparation. Therefore, it was eliminated.

In considering allergenic potential, the Committee emphasized that when the source organism of an enzyme preparation is a genetically modified microorganism the need for an evaluation for allergenic potential of the gene products encoded by the inserted DNA should be assessed. The Committee agreed that when the DNA sequence of an enzyme from a genetically modified production microorganism is comparable to that coding for an enzyme already known to have a history of safe use in food, there would be no need to assess the allergic potential of that enzyme further.

Finally, the Committee recognized that the revised Specifications include many criteria for safety evaluation that would be more appropriately listed elsewhere. The Committee strongly recommended that *Principles for the safety assessment of food additives and contaminants in food* be revised to include the safety assessment of enzymes intended for use in food and subsequent removal of such guidelines from the General Specifications.

Annex 4

Contaminants

An edited version of this section will appear in the report of the fifty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). It is reproduced here so that the information is disseminated quickly. This draft is subject to extensive editing.

1. 3-Chloro-1,2-propanediol

Certain chlorinated propanols occur as contaminants in hydrolysed vegetable proteins. Processing of defatted vegetable proteins by traditional hydrochloric acid hydrolysis leads to the formation of 3-chloro-1,2-propanediol and 1,3-dichloro-2-propanol. These two compounds were evaluated by the Committee at its forty-first meeting, when it concluded that 3-chloro-1,2-propanediol is an undesirable contaminant in food and considered that its concentration in hydrolysed proteins should be reduced to the lowest level technically achievable. Since that time, new data have become available, and the Codex Committee on Food Additives and Contaminants asked the Expert Committee to re-evaluate 3-chloro-1,2-propanediol.

Absorption, distribution, metabolism, and excretion

3-Chloro-1,2-propanediol crosses the blood-testis barrier and the blood-brain barrier and is widely distributed in body fluids. The parent compound is partly detoxified by conjugation with glutathione, resulting in excretion of the corresponding mercapturic acid, and is partly oxidized to ß-chlorolactic acid and further to oxalic acid. Approximately 30% is broken down to and exhaled as CO_2. In these studies, however, much of the administered dose was not accounted for. Intermediate formation of an epoxide has been postulated but not proven. There is some indication that microbial enzymes can dehalogenate haloalcohols to produce glycidol (a known genotoxin in vitro and in vivo).

Toxicological studies

The oral LD_{50} of 3-chloro-1,2-propanediol in rats is 150 mg/kg bw. In several studies in which the compound was given to rats at repeated doses in excess of 1 mg/kg bw per day, it decreased sperm motility and impaired male fertility. At doses of 10-20 mg/kg bw per day or more, alterations in sperm morphology and epididymal lesions (spermatocoele) were found in rats. 3-Chloro-1,2-propanediol reduced fertility in males of several other mammalian species at slightly higher doses than in the rat.

In rats and mice, 3-chloro-1,2-propanediol at doses of 25 mg/kg bw per day and above was associated with the development of dose-related central nervous system lesions, particularly in the brain stem.

In several short-term studies in rats and mice, the kidney was shown to be the target organ for toxicity. In a 4-week study in rats treated by gavage at 30 mg/kg bw per day, 3-chloro-1,2-propanediol increased the relative kidney weights. In a 13-week study in rats given an oral dose of 9 mg/kg bw per day a similar effect was seen.

In the pivotal long-term study in Fischer 344 rats, the absolute weight of the kidney was reported to be significantly increased by administration of 3-chloro-1,2-propanediol in drinking-water at all doses. Also at all doses tested, the incidence of tubule hyperplasia in the kidneys of animals of each sex was higher than in controls. Although the incidence did not reach statistical significance at the lowest dose tested (1.1 mg/kg bw per day), the Committee concluded that it represented part of a compound-related, dose-response relationship. Overt nephrotoxicity was seen at higher doses (5.2 and 28 mg/kg bw per day).

The results of most assays for mutagenicity in bacteria in vitro were reported to be positive, although negative results were obtained in the presence of an exogenous metabolic activation system from mammalian tissue. The results of assays in mammalian cells in vitro were also reported to be generally positive. It should be noted, however, that the concentrations used in all these assays were very high (0.1-9

mg/ml), raising serious questions about their relevance. The weight of the evidence indicates that 3-chloro-1,2-propanediol is not genotoxic in vitro at concentrations that do not cause toxicity. The results of assays conducted in vivo, including a test for micronucleus formation in mouse bone marrow and an assay for unscheduled DNA synthesis in rats, were negative. The Committee concluded that 3-chloro-1,2-propanediol was not genotoxic in vivo.

Altogether four long-term studies of toxicity and carcinogenicity were available; three (two with mice and one with rats) did not meet modern standards of quality. Nevertheless, none of the three studies indicated carcinogenic activity. In the fourth study, conducted in Fischer 344 rats, 3-chloro-1,2-propanediol was associated with increased incidences of benign tumours in some organs. These tumours occurred only at doses greater than those causing renal tubule hyperplasia, which was selected as the most sensitive end-point.

Occurrence

3-Chloro-1,2-propanediol has been detected at concentrations in excess of 1 mg/kg in only two food ingredients: acid-hydrolysed vegetable protein and soya sauce. In both ingredients, a range of concentrations has been reported, from below the limit of quantification (0.01 mg/kg with a method that has been validated in a range of foods and food ingredients) up to 100 mg/kg in some samples of acid-hydrolysed vegetable protein and more than 300 mg/kg in some samples of soya sauce.

Formation of 3-chloro-1,2-propanediol in acid-hydrolysed vegetable protein has been found to be related to production processes, and the concentration can be reduced markedly with suitable modifications. The source of 3-chloro-1,2-propanediol in soya sauce is being investigated; by analogy with hydrolysed vegetable protein, however, it may arise during acid hydrolysis in the manufacture of some products. Traditionally fermented soya sauces would not be contaminated with 3-chloro-1,2-propanediol.

3-Chloro-1,2-propanediol has also been quantified at low concentrations in a range of other foods and food ingredients, notably a number of cereal products that have been subjected to high temperatures, e.g., roasting or toasting. The concentrations are generally less than 0.1 mg/kg. Slightly higher concentrations (up to 0.5 mg/kg) have been found in food ingredients such as malt extracts, but the resulting concentrations in finished foods are below 0.01 mg/kg.

Estimates of dietary intake

Information on the concentrations of 3-chloro-1,2-propanediol in food, food ingredients, and protein hydrolysates was submitted by the United Kingdom, the USA, and the International Hydrolyzed Protein Council. The USA supplied a national estimate of the intake of 3-chloro-1,2-propanediol. Information on the consumption of soya sauce in Australia, Japan and the USA was also received.

At any level of intake that might reasonably be expected, 3-chloro-1,2-propanediol would not be expected to have acute effects. This analysis therefore addresses only long-term intake of 3-chloro-1,2-propanediol from its presence in foods.

The data submitted by the United Kingdom showed that 3-chloro-1,2-propanediol is found in some savoury foods, about 30% of samples containing concentrations above the limit of detection of 0.01 mg/kg. The mean residual concentration in these savoury foods was 0.012 mg/kg.

In a survey of 90 samples of commercially obtained soya sauces, 50 samples contained less than 1 mg/kg; the average concentration in the 90 samples was 18 mg/kg. The results of this survey were taken as representative of all soya sauces for the purpose of the intake assessment. Intake of 3-chloro-1,2-propanediol would be dominated by consumption of soya sauces contaminated with the compound.

When estimating the intake of 3-chloro-1,2-propanediol from food other than soya sauce, it was assumed that about one-eighth of the diet, 180 g (on the basis of 1500 g/day of solid food), consists of savoury foods that might contain 3-chloro-1,2-propanediol and that the mean residual concentration of

the compound in those foods is 0.012 mg/kg. On this basis, the intake of 3-chloro-1,2-propanediol from foods other than soya sauces is approximately 2 µg/person per day.

The mean and 90th percentile consumption of soya sauce that was used in the USA intake assessment were 8 and 16 g/person per day, respectively (consumers only), and the resulting estimate of intake of 3-chloro-1,2-propanediol was 140 µg/person per day for mean consumption and 290 µg/person per day for consumption at the 90th percentile. The mean consumption of soya sauce in Australia (consumers only) was approximately 11 g/person per day, and for consumers at the 95th percentile it was approximately 35 g/person per day, resulting in intake of 3-chloro-1,2-propanediol of 200 µg/person per day for mean consumption of soya sauce and 630 µg/person per day at the 90th percentile of consumption. Per-capita consumption of soya sauce in Japan (approximating a consumers-only consumption) was approximately 30 g/person per day, resulting in intake in Japan of 3-chloro-1,2-propanediol of approximately 540 µg/person per day for mean consumption of soya sauce. Intake at the 95th percentile in Japan would be 1100 µg/person per day by assuming consumption of soya sauce that is twice the mean.

Evaluation

The Committee chose tubule hyperplasia in the kidney as the most sensitive end-point for deriving a tolerable intake. This effect was seen in the long-term study of toxicity and carcinogenicity in rats in a dose-related manner, although the effect did not reach statistical significance at the lowest dose. The Committee concluded that the lowest-observed-effect level (LOEL) was 1.1 mg/kg bw per day and that this was close to a NOEL.

The Committee established a provisional maximum tolerable daily intake (PMTDI) of 2 µg/kg bw for 3-chloro-1,2-propanediol based on the LOEL of 1.1 mg/kg bw per day and a safety factor of 500, which included a factor of 5 for extrapolation from a LOEL to a NOEL. This factor was considered to be adequate to allow for the absence of a clear NOEL and to account for the effects on male fertility and for inadequacies in the studies of reproductive toxicity. Data available to the Committee indicated that the estimated mean intake of 3-chloro-1,2-propanediol by consumers of soya sauce would be at or above this PMTDI.

Impact of regulatory limits

As 3-chloro-1,2-propanediol is found infrequently in foods, a regulatory limit would be unlikely to have much effect on the overall intake of non-consumers of soya sauces. However, because the distribution of residual 3-chloro-1,2-propanediol in soya sauce is highly skewed and because it is likely that brand loyalty could result in regular consumption of highly contaminated brands of soya sauce, a regulatory limit on the concentration of 3-chloro-1,2-propanediol in soya sauce could markedly reduce the intake by soya sauce consumers.

2. 1,3-Dichloro-2-propanol

Since the time of the evaluation of the chloropropanols at the forty-first meeting, new data have become available, and the Codex Committee on Food Additives and Contaminants asked the Expert Committee to re-evaluate 1,3-dichloro-2-propanol.

Absorption, distribution, metabolism, and excretion

Approximately 5% of an oral dose of 1,3-dichloro-2-propanol was excreted in the urine of rats as ß-chlorolactate. About 1% of the dose was excreted as 2-propanol-1,3-dimercapturic acid. In another experiment, the urine of rats contained the parent compound (2.4% of the dose), 3-chloro-1,2-propanediol (0.35% of the dose), and 1,2-propanediol (0.43% of the dose). Epoxy-chloropropane (epichlorohydrin) was postulated to be an intermediate, which may either undergo conjugation with glutathione to form mercapturic acid or be hydrolysed to 3-chloro-1,2-propanediol. The latter undergoes oxidation to ß-chlorolactate, which is further oxidized to oxalic acid.

Toxicological studies

The oral LD_{50} of 1,3-dichloro-2-propanol in rats is 120-140 mg/kg bw. In several short-term rat studies, 1,3-dichloro-2-propanol at doses of 10 mg/kg bw per day and higher caused significant hepatic toxicity. This was associated with oxidative metabolism, which yielded intermediates that reacted with and depleted glutathione.

In a 13-week study in rats, overt hepatotoxicity, including increased liver weights, histological changes, and/or increased activity of serum alanine and aspartate transaminases, was seen after oral administration of 1,3-dichloro-2-propanol at 10 mg/kg bw per day and above. These doses also caused histopathological changes in the kidney, increased kidney weights, and alterations in urinary parameters. The NOEL was 1 mg/kg bw per day.

1,3-Dichloro-2-propanol has been reported to be hepatotoxic in humans exposed occupationally.

1,3-Dichloro-2-propanol was clearly mutagenic and genotoxic in various bacterial and mammalian test systems in vitro. The only available study in vivo showed no effect in a wing spot test in *Drosophila melanogaster*.

The results of the one long-term study of toxicity and carcinogenicity in rats confirmed the hepatotoxicity and the nephrotoxicity seen in the 13-week study. Furthermore, it demonstrated a clear carcinogenic effect of 1,3-dichloro-2-propanol at the highest dose tested, 19 mg/kg bw per day. The tumors (adenomas and carcinomas) occurred in liver, kidney, the oral epithelium and tongue, and the thyroid gland. No increase in tumour incidence was seen at the lowest dose tested, 2.1 mg/kg bw per day. Treatment-related non-neoplastic lesions of the liver were observed, sinusoidal peliosis being found in all treated groups.

Occurrence

Information on the concentrations of 1,3-dichloro-2-propanol in soya sauce was submitted by the USA. Additional information was derived from a published report on the concomitant occurrence of 3-chloro-1,2-propanediol and 1,3-dichloro-2-propanol in soya sauces. This information showed that 1,3-dichloro-2-propanol may be present in samples of hydrolysed vegetable protein and soya sauce that contain 3-chloro-1,2-propanediol at concentrations greater than 1 mg/kg. In those products in which 1,3-dichloro-2-propanol was quantifiable, the ratio of concentrations of 3-chloro-1,2-propanediol to 1,3-dichloro-2-propanol was at least 20.

Estimates of dietary intake

A report from the USA was used by the Committee to estimate the intake of 1,3-dichloro-2-propanol due to its presence in soya sauces. Information about the consumption of soya sauce was received from Australia, Japan, and the USA.

At any level of intake that might reasonably be expected, 1,3-dichloro-2-propanol would not be expected to have acute effects. This analysis therefore addresses only long-term intake of the compound from its presence in foods.

The intake of 1,3-dichloro-2-propanol from food other than soya sauce can be estimated roughly from data on residual concentrations of 3-chloro-1,2-propanediol in savory foods and the upper-bound 20:1 ratio of 3-chloro-1,2-propanediol:1,3-dichloro-2-propanol. If it is assumed that about one-eighth of the diet, 180 g (on the basis of 1500 g/day of solid food), consists of savory foods that might contain 1,3-dichloro-2-propanol and that the mean residual concentration of the compound in those foods is 0.0006 mg/kg, the background intake is approximately 0.1 µg/person per day.

The upper-bound 20:1 ratio of 3-chloro-1,2-propanediol concentration to that of 1,3-dichloro-2-propanol was used by the Committee to estimate the intake of 1,3-dichloro-2-propanol from consumption of soya sauce. The average concentration of 3-chloro-1,2-propanediol in a survey of 90 commercially

obtained soya sauce samples was 18 mg/kg; the residual concentration of 1,3-dichloro-2-propanol was therefore assumed to be 0.9 mg/kg.

The mean and 90th percentile consumption of soya sauce in the USA (consumers-only) is 8 and 16 g/person per day, respectively. The resulting estimate of the intake of 1,3-dichloro-2-propanol would be 7 µg/person per day at the mean level of consumption and 14 µg/person per day at the 90th percentile of consumption. The mean and 95th percentile consumption of soya sauces in Australia is approximately 11 and 35 g/person per day, respectively, resulting in estimates of intake of 10 and 30 µg/person per day for consumers at the mean and 90th percentiles, respectively. Per-capita intake of soya sauce in Japan (approximating a consumers-only intake) is 30 g/person per day, resulting in an estimate of intake for 1,3-dichloro-2-propanol of 27 µg/person per day. An upper percentile intake of 55 µg/person per day was estimated by assuming a consumption of soya sauce of two times the mean.

Evaluation

Although only a few studies of kinetics, metabolism, short- and long-term toxicity, and reproductive toxicity were available for evaluation, they clearly indicated that 1,3-dichloro-2-propanol was genotoxic in vitro, was hepatotoxic, and induced a variety of tumours in various organs in rats. The Committee concluded that the estimation of a tolerable intake was inappropriate because of the nature of the toxicity based on the following considerations:

- The results of the long-term toxicity/carcinogenicity study showed significant increases in the incidences of both benign and malignant neoplasms in at least three independent tissues.
- It has been shown unequivocally that this contaminant can interact with chromosomes and/or DNA; however, the tests were confined to bacterial and mammalian test systems in vitro, and there were no data on intact mammalian organisms or humans.

The Committee noted that the dose that caused tumours in rats (19 mg/kg bw per day) was about 20 000 times the highest estimated intake of 1,3-dichloro-2-propanol by consumers of soya sauce (1 µg/kg bw per day).

The available evidence suggests that 1,3-dichloro-2-propanol is associated with high concentrations of 3-chloro-1,2-propandiol in food. Regulatory control of the latter would therefore obviate the need for specific controls on 1,3-dichloro-2-propanol.

3. Polychlorinated dibenzodioxins, polychlorinated dibenzofurans, and coplanar polychlorinated biphenyls

Introduction

Polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are by-products of combustion and of various industrial processes, and they are widely present in the environment. Polychlorinated biphenyls (PCBs) were manufactured in the past for a variety of industrial uses, notably as electrical insulators or dielectric fluids and specialized hydraulic fluids. Most countries banned manufacture and use of PCBs in the 1970s; however, past improper handling of PCBs constitutes a continuing source of PCBs in the environment, and disposal of equipment now in use poses some risk of further contamination.

Neither PCDDs nor PCDFs have been evaluated previously by the Committee. PCBs were evaluated at the thirty-fifth meeting, when a provisional tolerable weekly intake (PTWI) could not be established because of the limitations of the available data and the ill-defined nature of the materials that were used in feeding studies.

PCDDs, PCDFs, and coplanar PCBs were evaluated at the present meeting on the basis of a request by the Codex Committee on Food Additives and Contaminants to evaluate the risks associated with their presence in food.

The Committee evaluated the PCDDs, PCDFs, and coplanar PCBs for which toxic equivalency factors (TEFs) for mammals have been derived by WHO. Table 1 summarizes the compounds that were considered and their assigned TEF values. The TEF approach relates the toxicity of all chemicals in the series to 2,3,7,8-TCDD, one of the most potent of the chemicals on which most toxicological and epidemiological information was available. Use of the TEF concept rests on the assumption that PCDDs, PCDFs, and coplanar PCBs have a common mechanism of action, which involves binding to the aryl hydrocarbon (Ah) receptor, an intracellular receptor protein. This binding is considered to be the necessary first, but not sufficient, step in expressing the toxicity of these compounds. Many uncertainties exist in use of the TEF approach for human risk assessment, but pragmatically it is the most feasible approach that is available.

Table 1. Compounds considered and their assigned TEFs

Compound	WHO TEF value	Compound	WHO TEF value
Dibenzodioxins		*"Non-ortho" PCBs*	
2,3,7,8-TCDD	1	3,3'4,4'-TCB (PCB #77)	0.0001
1,2,3,7,8-PeCDD	1	3,4,4'5-TCB (#81)	0.0001
1,2,3,4,7,8-HxCDD	0.1	3,3,'4,4,'5-PeCB (#126)	0.1
1,2,3,6,7,8-HxCDD	0.1	3,3',4,4'5,5'-HxCB (#169)	0.01
1,2,3,6,7,9-HxCDD	0.1		
1,2,3,4,6,7,8-HpCDD	0.01	*"Mono-ortho" PCBs*	
OCDD	0.0001	2,3,3',4,4'-PeCB (#105)	0.0001
Dibenzofurans		2,3,4,4',5-PeCB (#114)	0.0005
2,3,7,8-TCDF	0.1	2,3',4,4',5-PeCB (#118)	0.0001
1,2,3,7,8-PeCDF	0.05	2,3',4,4',5'-PeCB (#123)	0.0001
2,3,4,7,8-PeCDF	0.5	2,3,3',4,4',5-HxCB (#156)	0.0005
1,2,3,4,7,8-HxCDF	0.1	2,3,3',4,4',5'-HxCB (#157)	0.0005
1,2,3,6,7,8-HxCDF	0.1	2,3',4,4',5,5'-HxCB (#167)	0.00001
1,2,3,7,8,9-HxCDF	0.1	2,3,3',4,4',5,5'-HbCB (#189)	0.00001
2,3,4,6,7,8-HxCDF	0.1		
1,2,3,4,6,7,8-HpCDF	0.01		
1,2,3,4,7,8,9-HpCDF	0.01		
OCDF	0.0001		

A WHO consultation held in 1998 established a tolerable daily intake (TDI) of 1-4 pg/kg bw, which was applied to the toxic equivalents (TEQs) of PCDDs, PCDFs, and coplanar PCBs. The TDI was based on a number of studies of developmental toxicity, in which pregnant rats were given 2,3,7,8-TCDD by gavage, and immunological toxicity. The present Committee used this assessment as the starting point for its evaluation, taking into account newer studies that provided information on:

- toxicokinetics in a comparison of the fetal transfer of TCDD after bolus and repeated dosing;
- two new studies of developmental toxicity; and
- new information on the study in rhesus monkeys that placed its results in question.

Toxicokinetics

Coplanar compounds in dietary fat pass easily from the gastrointestinal tract into the blood. Indeed, experiments in humans and animals show 50-90% absorption of orally administered 2,3,7,8-TCDD. This figure is comparable with the near-complete absorption of PCDDs, PCDFs, and PCBs by nursing infants from their mothers' milk.

After absorption from the gastrointestinal tract, 2,3,7,8-TCDD enters the lymph in the form of chylomicrons and is then cleared from the blood within 1 h. Cleared 2,3,7,8-TCDD appears mainly (74-81% of an administered dose) in the liver and adipose tissue. After clearance of chylomicrons, coplanar

compounds remain mainly in serum lipoproteins (very low density, low density, and high density) and some are bound to serum proteins.

The Committee used the results of a study in which the radiolabel was measured in the tissues of pregnant Long-Evans rats one day after administration of 50, 200, 800, or 1000 ng/kg bw [^{3}H]2,3,7,8-TCDD by gavage on day 15 of gestation. The average maternal body burdens (with the percentage of the dose) were 31 (60%), 97 (48%), 520 (65%), and 580 (59%) ng/kg bw, respectively. On the basis of this study, the Committee used a value of 60% for the amount of 2,3,7,8-TCDD retained in pregnant rats one day after administration of a single dose by gavage on day 15 of gestation.

The distribution of PCDDs and PCDFs between the serum and organs is governed by lipid partitioning and protein binding. The concentrations of PCDDs and PCDFs in blood and adipose tissue correlate well. TCDD is distributed between plasma or blood and adipose tissue by lipid partitioning, whereas the distribution of HxCDD/HxCDF and OCDD/OCDF are governed by both lipid partitioning and plasma protein binding.

In the liver, protein binding plays an important role in the uptake of coplanar compounds from the blood, even for lower chlorinated congeners. When rodents are exposed to increasing doses of 2,3,7,8-TCDD, it is preferentially sequestered in the liver, so that the concentration in the liver exceeds that in adipose tissue by many times. The biochemistry of this phenomenon is as follows: After entering liver cells, 2,3,7,8-TCDD either dissolves in the lipid fraction or binds to the Ah receptor or cytochrome P450 (CYP) proteins, probably microsomal P4501A2. As the amount of CYP1A and 1B proteins in cells is regulated by formation of the TCDD-Ah receptor complex, exposure to increasing amounts of TCDD triggers a cascade of events involving increased TCDD entering the cell, increased formation of the TCDD-Ah receptor complex, increased formation of CYP1A and 1B mRNA and protein (enzyme induction), and accumulation of TCDD by increased binding to the induced CYPs. Similar sequestration has been observed with higher chlorinated PCDDs and PCDFs and with coplanar PCBs.

The hepatic sequestration of coplanar compounds markedly affects the distribution of these compounds in the body. For example, whereas the liver contributes 10% and the adipose tissue 60% to the body burden of TCDD in uninduced mice containing only constitutive concentrations of hepatic CYP, these fractions may increase to 67% in liver and decrease to 23% in fully induced mice containing both constitutive and induced hepatic CYP protein concentrations. Similar results were found in rats, clearly indicating the non-linear character of the kinetics of TCDD at concentrations that induce hepatic CYP proteins.

As in rodents, preferential sequestration of PCDDs and PCDFs in the liver rather than in adipose tissue has been observed in humans exposed to background concentrations of these compounds. This sequestration is probably due to binding to constitutive CYP proteins for, although Ah receptor-dependent CYP induction has been observed in human liver cells in vitro after exposure to TCDD (induction at 1 pmol/L; EC_{50} ~ 100 pmol/L), it occurred at concentrations that were several orders of magnitude higher than those observed in human blood.

Metabolism and excretion

In experimental animals, PCDDs and PCDFs are excreted almost exclusively in the bile, excretion in the urine being a minor route. Whereas the parent compound is found primarily in the organs of rodents, only metabolites of PCDD and PCDF occur in bile, indicating hepatic metabolism, including hydroxylation and conjugation, of these compounds. Similar reactions have been found in vitro in incubated recombinant human liver enzyme (metabolism of 2,3,7,8-TCDF by CYP1A1). Faecal excretion of unmetabolized PCDDs and PCDFs is also an important route of elimination in humans.

In rodents, the terminal half-time of 2,3,7,8-TCDD ranges from 8-24 days in mice to 16-28 days in rats. Humans eliminate PCDDs and PCDFs more slowly, the estimated mean half-time of TCDD ranging from 5.5 to 11 years. The half-lives of other PCDD congeners and of PCDFs and coplanar PCBs

vary widely. The TEFs (Table 1) take into account, to some extent, the differences in half-time between different congeners.

Relationship between human intake and doses used in animal studies

The biochemical and toxicological effects of PCDDs, PCDFs, and coplanar PCBs are directly related to tissue concentrations and not to the daily dose. The most appropriate dose measure would therefore be the concentration at the target tissue; however, this is seldom known. The body burden, which is strongly correlated with tissue and serum concentrations, integrates the differences in half-lives between species. Thus, rodents require appreciably higher daily doses (100-200-fold) to reach a body burden at steady state equivalent to that recorded in humans exposed to background concentrations. Toxicokinetically, estimates of body burden are therefore more appropriate measures of dose for interspecies comparisons than daily dose.

The long half-lives of PCDDs, PCDFs, and coplanar PCBs has several implications for the period of intake of relevance to the assessment. First, the TEQs in the body (or the internal TEQs to which a target organ is exposed) will rise over time as more of the compounds are ingested. Second, after cessation of exposure, the body's stored TEQs (and the exposure of internal organs) will decline slowly, only half of the accumulated TEQs disappearing over about 7 years, resulting in a pseudo steady state only after decades. Third, because of this long-term storage in the body and the consequent daily exposure to the body's stored TEQs, a person's ingestion on a particular day will have a small or even negligible effect on the overall body burden. For example, food contamination that leads to an intake 100 times the amount in a typical meal – an event not expected to occur – would result in less than a 3% increase in the body burden of an adult eating that meal. The rest of the person’s body burden would be made up of the PCDDs, PCDFs, and coplanar PCBs consumed in the many thousands of past meals over the previous decade or more.

Therefore, the Committee concluded that the appropriate averaging period for evaluating intake of these compounds is one month or more.

In order to transform an animal body burden into an equivalent human monthly intake (EHMI) that on a long-term basis would lead to a similar body burden (at steady state), simple, classical toxicokinetic calculations can be used. The elimination of PCDDs at low doses was considered to follow first-order kinetics and to be independent of the body burden or dose. Equation 1 describes the relationship between the total steady-state body burden and intake assumed by the Committee.

Equation 1
Body burden at steady state (ng/kg bw) = f * intake (ng/kg bw per day) * half-time in days/ln(2)

where f is the fraction of dose absorbed (assumed to be 50% for absorption from food for humans) and the estimated half-time of 2,3,7,8-TCDD is 2774 days (7.6 years). For compounds that follow first-order kinetics, four to five half-lives will be required to approach steady state. For TCDD, this would be equivalent to more than 30 years.

This model is based on the assumption that PCDDs are distributed in only one compartment (the whole body). Although most of the body burden of PCDD is distributed in the lipid stores, at higher doses the liver also sequesters these compounds to some extent in both humans and animals. Predictions of body burden that are based on lipid concentrations after intake of high concentrations may therefore underestimate the total body burden (and the intake leading to that body burden) because of hepatic sequestration. Use of physiologically based pharmacokinetic models may be more appropriate under these circumstances. For the low concentrations to which the general human population is exposed and for the low doses used in the relevant pivotal toxicological studies, the Committee considered use of a less complicated, classical pharmacokinetic model appropriate for transformation of body burdens into estimated human daily intakes.

Exposure of the fetus in developmental toxicity studies

The time of dosing in several of the studies considered by the Committee, day 15 of gestation, marks the onset of the sensitive phase of sexual differentiation in rats and represents a critical time of fetal exposure. The determinant of the reproductive effects is the fetal concentration on day 15-16 of gestation. As placental transfer is mediated *via* the blood, the extent of fetal exposure is determined by the serum concentration, which may differ with a bolus dose (as in these studies) and with repeated doses providing the same total intake. As the serum concentration of 2,3,7,8-TCDD after a bolus dose rises before redistribution to the tissue compartments, the serum concentration is likely to be higher than after long-term intake of a lower concentration.

The difference in the fetal body burden after a single bolus dose and after repeated administration of a low dose resulting in a similar maternal body burden was addressed in a study in which radiolabel was measured in both maternal and fetal tissues of Long Evans dams at day 16 of gestation (Hurst et al, 2000a,b). The rats were dosed by gavage with [^{3}H]2,3,7,8-TCDD at 1, 10, or 30 ng/kg bw per day in corn oil, 5 days per week, for 13 weeks. They were then mated, and dosing was continued daily throughout gestation. The regimen produced a steady-state concentration of 2,3,7,8-TCDD in the dams. The average maternal and fetal body burdens at day 16 of gestation after this treatment and after a single administration of 2,3,7,8-TCDD by gavage on day 15 of gestation are shown in Table 2.

Table 2. Average maternal and fetal body burdens after a single dose and after administration of repeated doses of 2,3,7,8-TCDD to pregnant rats

Single dose on day 15 of gestation			Administration of repeated doses		
Single dose (ng/kg bw)	Body burden measured at day 16 of gestation		Adjusted daily dose (ng/kg bw per day) [a]	Body burden measured at day 16 of gestation	
	Maternal (ng/kg bw)	Fetal (ng/kg bw)		Maternal (ng/kg bw)	Fetal (ng/kg bw)
50	30	5.3	0.71	20	1.4
200	97	13	7.1	120	7.5
800	520	39	21	300	15
1000	590	56			

From Hurst *et al*., 2000a,b

[a]Adjusted for continuous administration from 5 to 7 days per week

As expected, a single dose on day 15 of gestation by gavage resulted in considerably higher fetal concentrations on day 16 than short-term administration of low daily doses leading to maternal steady-state body burdens of similar magnitude.

Using the data in Table 2, the Committee conducted least-squares linear fits of dose versus maternal and fetal body burdens. Since radiolabelled 2,3,7,8-TCDD was used in both studies, a zero intercept was assumed for the fitted line. None of these fits showed what appeared to be any significant deviation from linearity. These data indicate that the ratio of fetal to maternal body burden would be 1.7 times higher from a bolus dose than from repeated dosing that providing the same total dose. Kinetic data indicate that a linear dose relationship would be expected at the dose ranges used in these studies. The fetal versus maternal body burdens in both data sets could also be fit to power equations, which provided a better fit of the data in the lower dose range of the single-dose experiments. The factor used to convert maternal body burden following acute dosing into a corresponding steady-state body burden using the power equations was 2.6.

Toxicological and epidemiological studies

Acute toxicity

In experimental animals, the acute toxicity of TCDD and related PCDDs and PCDFs substituted in at least the 2, 3, 7, and 8 positions varies widely between and among species. For example, the oral LD_{50} in

guinea-pigs was 0.6 µg/kg bw, while that in hamsters was greater than 5000 µg/kg bw. Explanations for this variation include Ah receptor functionality (size, transformation, and PCDD response element binding), toxicokinetics (metabolic capacity and tissue distribution), and body fat content. While data on acute toxicity were available for various commercial PCB mixtures (LD_{50} values usually greater than 100 mg/kg bw), the data on the individual coplanar PCB congeners in mammals were limited. Ah-responsive rodent species tend to have lower LD_{50} values.

One of the commoner symptoms associated with PCDD-induced acute lethality is a generalized delayed wasting syndrome characterized by inhibition of gluconeogenesis, reduced feed intake, and loss of body weight. Although some species differences exist, other toxic effects observed after acute exposure to PCDDs include haemorrhages in a number of organs, thymic atrophy, reduced bone-marrow cellularity, and loss of body fat and lean muscle mass.

Developmental toxicity

A number of biochemical changes, such as enzyme induction, altered expression of growth factors and enhanced oxidative stress, have been noted in experimental animals with 2,3,7,8-TCDD body burdens within a lower range of 3-10 ng/kg bw. The Committee considered these biochemical effects to be early markers of exposure to PCDDs, PCDFs, and coplanar PCBs or events induced by these compounds in animals and in humans that may or may not result in adverse effects at higher body burdens.

The Committee reviewed the relevant studies included in the 1998 WHO evaluation published in *Food Additives and Contaminants*, 2000 (Gehrs et al., 1997; Gehrs & Smailowicz, 1999; Gray et al., 1997a,b; Mably et al., 1992a,b,c; Rier et al., 1993;) and identified two additional recent studies (Faqi et al., 1998; Ohsako et al., 2001). The Committee noted that the most sensitive adverse effects reported were on development in the male offspring of rats and immunological deficits after prenatal exposure to 2,3,7,8-TCDD (see Table 3).

Table 3. Summaries of the studies presenting the lowest NOELs and LOELs for the most sensitive adverse effects of 2,3,7,8-TCDD on developmental end-points in experimental animals.[a]

Study/ Rat strain	**End-point**	**Dosing regimen**	**NOEL body burden (ng/kg bw)**	**LOEL body burden (ng/kg bw)**
Ohsako et al. (2001) Holtzman	Ventral prostate weight; decreased anogenital distance in male offspring	Single oral gavage bolus gestation day 15	13	51
Faqi et al. (1998) Wistar	Decreased sperm production and altered sexual behavior in male offspring	Loading dose/maintenance dose by subcutaneous injections		25
Gray et al. (1997) Long Evans	Accelerated eye opening and decreased sperm count in offspring	Single oral gavage bolus gestation day 15		28
Mably et al. (1992c) Holtzman	Decreased sperm count in offspring	Single oral gavage bolus gestation day 15		28
Gehrs et al (1997); Gehrs and Smailowicz (1998) F344	Immune suppression in offspring	Single oral gavage bolus gestation day 14		50

[a] Body burdens estimated using a linear fit to the data in Table 2.

The 1998 WHO consultation identified a study that found endometriosis after long-term administration of TCDD to rhesus monkeys. The Committee stressed that the reported findings in this study

should be interpreted with caution, as the daily intake was not adequately reported. In addition, analyses conducted 13 years after termination of exposure identified increased concentrations of coplanar PCBs in the blood of the monkeys with endometriosis, possibly due to an unknown source of PCB. The Committee also noted that some of the pivotal studies in rats (Table 3) would result in similar or lower equivalent EHMIs than that obtained from the LOEL for endometriosis in monkeys.

In a recent study (Ohsako et al., 2001), pregnant Holtzman rats were given a single oral dose of 2,3,7,8-TCDD at 0-800 ng/kg bw on day 15 of gestation, and the male offspring were examined on days 49 and 120 after birth. No changes were seen in testicular or epididymal weight nor in daily sperm production or sperm reserve at any dose. However, the weight of the urogenital complex, including the ventral prostate, was significantly reduced at doses of 200 and 800 ng/kg bw in rats killed on day 120. Moreover, the anogenital distance of male rats receiving doses of 50 ng/kg bw or above and killed at this time was significantly decreased. The Committee noted that administration of 2,3,7,8-TCDD at any dose resulted in a dose-dependent increase in 5a-reductase type 2 mRNA and a decrease in androgen receptor mRNA in the ventral prostate of rats killed at day 49 but not in those killed at day 120, with no adverse sequelae at the lowest dose of 12.5 ng/kg bw. On the basis of 60% absorption and an assumption of a linear relationship estimated for the data in Table 2, the equivalent maternal body burden after multiple doses at this NOEL would be 13 ng/kg bw. Using the power model to fit the data in Table 2, the body burden LOAEL was estimated to be 19 ng/kg bw. The LOEL of 50 ng/kg bw per day corresponds to an equivalent body burden of 51 ng/kg bw using the linear and 76 ng/kg bw using the power model.

The lowest LOAEL reported for the reproductive system of the male offspring used Wistar rats (Faqi et al 1998). In this study, the dams were treated subcutaneously prior to mating and throughout mating, pregnancy and lactation. They received an initial loading dose of 25, 60, or 300 ng ^{14}C-2,3,7,8-TCDD/kg bw 2 weeks prior to mating, followed by weekly maintenance doses of 5, 12, or 60 ng 2,3,7,8-TCDD/kg bw. The size of the maintenance doses was based on a reported elimination half-time of 3 weeks for adult rats. Effects on male reproduction were studied on postnatal days 70 and 170. The number of sperm per cauda epididymis was reduced in all treated groups at puberty and at adulthood. Daily sperm production was permanently decreased, as was the sperm transit rate in the male offspring that were administered 2,3,7,8-TCDD, thus increasing the time required by the sperm to pass through the cauda epididymis. Moreover, the male offspring of the treated groups showed an increased number of abnormal sperm when investigated at adulthood. Mounting and intromission latencies were significantly increased in the low- and high-dose groups, but not in the mid-dose group. The Committee noted the lack of a clear dose-response relationship for most of these effects in the treated groups. In the high-dose group, the concentration of serum testosterone was decreased at adulthood and permanent changes in the testicular tubuli included pyknotic nuclei and the occurrence of cell debris in the lumen. The fertility of the male offspring was not affected in any of the treated groups.

To compute the long-term dose required to produce the fetal concentration in the dose group given the initial loading dose of 25 ng/kg bw, it should be noted that this dosing pattern would have been reduced to 20 ng/kg bw prior to the maintenance dose of 5 ng/kg bw given on day 14. Based on the linear fit to the data in Table 2, the fetal body burden resulting from the maternal body burden of 20 ng/kg bw would be 1.04 ng /kg bw. The maintenance dose of 5 ng/kg bw administered on gestation day 14 would produce an additional contribution to the fetal body burden of 0.27 ng/kg bw resulting in a total fetal body burden of 1.31 ng/kg bw. Based on a linear fit to the data in Table 2, a maternal body burden of 25 ng 2,3,7,8-TCDD/kg bw at steady state would be needed to produce this fetal body burden.

The studies described in Table 3 provide evidence of adverse effects on the reproductive system in the male offspring of pregnant rats administered 2,3,7,8-TCDD. The studies demonstrate reduction in daily sperm production, cauda epididymal sperm number and epididymis weight as well as accelerated eye opening, reduction in anogenital distance and feminised sexual behaviour in the male offspring associated with maternal steady-state body burdens in the range of 25 ng 2,3,7,8-TCDD/kg bw and above. Reductions in the weights of testes and the size of sex-accessory glands, such as the ventral prostate in the male offspring, and development of external malformations of genitalia in female offspring as well as reduced male and/or female fertility require higher maternal body burdens. The Committee noted that the

most sensitive end-points identified differed between studies. This might reflect strain differences in sensitivity and/or even minor differences in the experimental conditions, e.g. the diet. The Committee also noted that in one study a single maternal gavage dose of 12.5 ng 2,3,7,8-TCDD/kg bw produced a decrease in the androgen receptor mRNA level in the ventral prostate at puberty on post-natal day 49, indicative of reduced androgenic responsiveness. However, at this dose level none of the above-mentioned adverse effects were seen in the male offspring. This dose corresponds to an estimated maternal steady-state body burden of approximately 19 ng 2,3,7,8-TCDD/kg bw (Table 3). As with enzyme induction, altered expression of growth factors and enhanced oxidative stress, the Committee considered this effect to be an early marker of exposure to 2,3,7,8-TCDD or an event induced in animals that may or may not result in adverse effects at higher body burdens.

Genotoxicity

Several short–term assays for genotoxicity with 2,3,7,8-TCDD covering various end-points were primarily negative. Furthermore, TCDD does not bind covalently to DNA from the liver of mice. The Committee concluded that TCDD is not an initatiator of carcinogenesis.

Carcinogenicity studies in animals

2,3,7,8-TCDD and other PCDDs induced tumours at multiple sites in studies with multiple animal species in both sexes. In a series of in vivo and in vitro assays TCDD displayed the capacity to promote growth of transformed cells (e.g. rat tracheal epithelium cells treated with *N*-methyl-*N*'-nitro-*N*-nitrosoguianidine), consistent with observations of cancer promotion in whole animals. In a long-term study of carcinogenicity of TCDD in rats, the LOEL for hepatic adenomas in females was 10 ng/kg bw per day. The NOEL was 1 ng/kg bw per day. Several studies have shown that 2,3,7,8-TCDD promotes tumours in experimental animals, in particular liver tumours. Several other PCDDs, PCDFs, and non-ortho- and mono-ortho-PCBs also promoted liver tumours. In a long-term study in rats in which the incidence of liver tumours was increased, the LOEL (10 ng/kg bw per day) corresponded to a steady-state body burden of 290 ng/kg bw. In order for humans to attain a similar steady-state body burden, they would have to have a daily intake of 150 pg/kg bw (Equation 1).

Non-cancer effects in humans

In two episodes of food poisoning in Japan and Taiwan in which infants were exposed *in utero* to heat-degraded PCBs a variety of adverse physical developmental abnormalities were observed, such as decreased penis length and alterations of spermatozoa; neurodevelopmental abnormalities were also seen. The affected children in Taiwan were born to mothers with estimated TEQ body burdens of 2-3 µg/kg bw.

For cohorts of infants in Germany, the Netherlands, and the USA, effects of exposures that could be considered environmental or background were evaluated at the time the studies were conducted; for example, the mean concentration of TEQs in human milk was 60 pg/g of lipid (range 25–155) in a study in Rotterdam and Groningen. Low birth weight and detriments in neurological development were observed in several of these studies, and alterations in thyroid hormones, lymphocyte subpopulations, infections, and respiratory symptoms were observed in specific studies. The observed neurodevelopmental deficits were subtle, were within the normal range, and their potential consequences for future intellectual function are unknown. The associations observed were considered to be due to prenatal (*in utero*) exposure rather than to postnatal intake (human milk). In one study of breast-fed and bottle-fed infants, the intake of PCDDs and PCBs was inversely related to performance in neurobehavioural tests; breast-fed infants performed better in neurobehavioural tests than bottle-fed infants. The studies of low exposure primarily addressed PCBs, while fewer data were available on the effects of PCDDs and PCDFs.

In adults, most of the non-cancer effects observed after exposure to PCDDs, PCDFs, and coplanar PCBs, such as chloracne, appeared only at doses several orders of magnitude greater than those generally due to background contamination of foods. In Seveso, more female children than expected were born to fathers with serum TCDD concentrations > 80 pg/g of lipid (16-20 ng/kg bw) at the time of conception.

Carcinogenicity in humans

A working group convened by the International Agency for Research on Cancer (IARC) classified 2,3,7,8-TCDD as a human carcinogen (Group 1) on the basis of limited evidence in humans and sufficient evidence in experimental animals as well as on mechanistic considerations. The other PCDDs and PCDFs were considered not to be classifiable as to their carcinogenicity to humans (Group 3).

The most informative studies for an evaluation of the carcinogenicity of 2,3,7,8-TCDD are four cohort studies of herbicide producers (two in Germany and one each in the Netherlands and the USA) and one cohort study of residents of a contaminated area in Seveso, Italy. A multi-country cohort study from IARC included three of these four cohorts and other industrial cohorts, many of which had not been reported in separate publications, as well as some professional herbicide applicators.

In most of the epidemiological studies considered, exposure had been primarily to TCDD, with some exposure to mixtures of other PCDDs, as contaminants of phenoxy herbicides and chlorophenols. The studies involved persons with the highest recorded exposure to 2,3,7,8-TCDD, with estimated geometric mean blood lipid concentrations after the last exposure ranging from 1100 to 2300 pg/g of lipid in the industrial cohorts and lower average concentrations among persons exposed in Seveso.

Low excess risks on the order of 40% were found for all neoplasms combined in all the studies of industrial cohorts in which the exposure assessment was adequate. Risks for cancers at specific sites were increased in some of the studies, but the results are not consistent between studies and no single cancer site seemed to predominate. Tests for trends to increasing excess risks for all neoplasms combined with increasing intensity of exposure were statistically significant. Increased risks for all neoplasms with time since first exposure were observed in those studies in which latency was evaluated. The follow-up of the Seveso cohort has been shorter than for the industrial cohorts; however, the rate of death from all cancers has not differed significantly from that expected in the general population. Excess risks were seen for cancers at some specific sites in the most heavily contaminated zones, but the numbers of cases are small.

In these well-conducted cohort studies, therefore, increasing intensity of exposure could be ascertained with precision because of the long biological half-time of TCDD in human tissues, and the relative risks increased significantly with increasing exposure. Although the excess cancer risk at the highest exposure was statistically significant, these results must be evaluated with caution, as the overall risks are not high and the strongest evidence is for industrial populations with two to three orders of magnitude greater exposure than the general population who also had heavy exposure to other chemicals; furthermore, lifestyle factors such as smoking were not evaluated. There are few precedents of carcinogens that cause an increase the risk of cancer for all tumours combined, without an excess risk for any tumour predominating.

The calculation of a “benchmark dose” was explored (e.g., the ED_{01} (effective dose), the dose estimated to result in a 1% increase in cancer mortality), on the basis of a meta-analysis of data on three industrial cohorts with well-documented exposure, for comparison with non-cancer effects. A statistically significant linear trend in risk with exposure was observed, which persisted even after exclusion of groups with the highest exposure. Within the range of reasonable assumptions, the ED_{01} ranged quite widely and strongly depended on the assumptions made. Furthermore, a number of uncertainties exist that would influence the predicted ED_{01}, including the exposure of the occupational cohorts and, to a lesser extent, potential confounding effects of factors not considered in the studies.

Sampling and analytical methods

No specific guidelines have been drawn up for sampling foods to be analysed for their PCDD, PCDF, and coplanar PCB content. The basic guidelines for sampling of organic contaminants or pesticides should therefore be used. The objective is to obtain a representative, homogeneous laboratory sample without introducing secondary contamination. Although PCDDs, PCDFs, and coplanar PCBs are chemically stable, the storage and transport of samples should ensure that they do not deteriorate. PCDDs, PCDFs, and coplanar PCBs are usually found as complex mixtures of varying composition in different matrices.

Their identification and quantification requires a highly sophisticated analysis, because the toxic congeners as presented in Table 1 must be separated from the more prevalent and less toxic congeners. Usually, PCDDs, PCDFs, and coplanar PCBs are determined by capillary gas chromatography with mass spectrometry (GC/MS).

No official method exists for the determination of these compounds in food. Reliable results can be obtained in the absence of official methods if the method used has been shown to fit the purpose and to fulfil analytical quality criteria developed in other fields of residue analyses. The methods used to determine PCDDs and PCDFs in food must be capable of providing sufficient information to calculate results as TEQs at 0.1-1 pg/g of fat in milk, meat, and eggs, around 10 pg/g of fat in fish or up to 100 pg/g of fat or more in cases of higher contamination, and 0.1-0.5 pg/g of dry matter for TEQs in food of vegetable origin. The patterns of congeners can vary between regions and foods.

Particularly when the method used is of insufficient sensitivity, the concentrations of PCDDs, PCDFs, and coplanar PCBs in many foods may be near or below the limit of quantification. The method used to derive the concentrations of undetected congeners (the imputation method) can therefore have a variable effect on the summary TEQ value for a food sample. The most commonly used imputation methods calculate the contribution of each non-detected congener to the TEQ either as zero (“lower bound concentrations”), as the limit of detection/limit of quantification ("upper bound concentrations") or as half the limit of detection/determination. For methods with insufficient sensitivity, the factor for differences between lower- and upper-bound concentrations can be in the range of 10 to 100, in extreme cases even higher. If sensitivity is appropriate, there are negligible differences between lower- and upper-bound concentrations in the relevant ranges. Therefore, low estimates of PCDDs, PCDFs, and coplanar PCBs may represent truly low concentrations in the sample or be the result of use of zero as the factor for undetected congeners in a food sample. Conversely, high estimates may be the result of a real contamination or of application of the upper-bound concept with insufficient sensitivity.

Application of upper-bound concentrations leads to an overestimate of intake and application of lower-bound concentrations to an underestimate of intake. Therefore, the Committee recommended that laboratories report their results as lower-bound, upper-bound, and half-detection limits, in addition to values for individual congeners. In that way, all the necessary information is available for interpreting the results for specific requirements. At a minimum, it should be clear which concept was used. Experts who are summarizing results based on TEQs should consider the way in which the TEQs were calculated and indicate this in their reports.

For reliable analysis of food samples with normal background contamination, gas chromatography/high-resolution mass spectrometry (GC/HRMS) has been validated in collaborative studies and has been shown to provide the required sensitivity and specificity. Bioanalytical assays have been developed for rapid screening in sediments, soil, fly ash, and various foods, but only the chemical-activated luciferase gene expression (CALUX) assay has been used for food, and first steps of validation have been undertaken. While GC/MS is the most powerful method for identification and quantification of congeners and recognition of congener-specific patterns, it does not provide in a matrix a direct measure of the total toxicity of all congeners that act through the Ah-receptor pathway. The CALUX assay provides an indication of the TEQs present in a certain matrix, including interactive (synergistic or antagonistic) effects; however, it cannot provide information on the congener pattern.

The Committee recognized that the available analytical data for PCDDs, PCDFs, and coplanar PCBs are hampered by the lack of generally accepted criteria for intra-laboratory validation and validation procedures that would permit comparison of results from different laboratories. A mutual acceptance of analytical methods would be facilitated by collaborative studies and proficiency testing programs on an international level. For reliable analysis in the range of normal background contamination, laboratories must use sufficiently sensitive methods for control. General statistical parameters that have been established in other fields of residue analysis could provide orientation. The requirements for acceptable analytical methods clearly need to be harmonized so that data are comparable and may be used for risk management purposes.

Levels and patterns of contamination of food commodities

Data were submitted by Belgium, Canada, Japan, New Zealand, Poland, and the USA and by the European Commission in a report based on data for Belgium, Denmark, Finland, France, Germany, Italy, the Netherlands, Norway, Sweden, and the United Kingdom. In all countries in which a substantial number of samples had been analysed, the concentrations of PCDDs, PCDFs, and coplanar PCBs in food decreased until the late 1990s, but this decrease had slowed or was even partly reversed recently in some food categories in several countries owing to contamination of animal feed. In addition, at the end of the 1990s, the measures taken to reduce contamination at source initiated at the beginning of the decade had a weaker effect than they had earlier. For the present assessment of intake at the international level, only data collected after 1995 were considered.

As the Committee did not have access to the original analytical results, the concentrations used in the assessment were expressed as the sum of congeners. Consequently it was not possible to identify whether the results were obtained by the lower- or upper-bound approach (see previous section).

Insufficient individual data were available from most countries for construction of a full distribution curve of concentrations, and most were submitted in an aggregated format. As recommended by a FAO/WHO workshop on assessing exposure to contaminants, aggregated data were weighted as a function of the number of initial samples and then used to obtain a weighted mean concentration of PCDD/PCDFs and PCBs in 6 major food groups – meat and meat products, eggs, fish and fish products, milk and milk products, vegetables and vegetable products, and fats and oils. National data were therefore aggregated by region or country (Western Europe, North America, New Zealand, Japan), which are summarized in Table 4. Insufficient data were available for the rest of the world to permit a realistic estimate of distribution of contaminants. The Committee recognized that significant differences occur within the food categories in Table 4, and that the data used in this analysis may not reflect the true mean for a food category. For example, mean PCDD, PCDF, and coplanar PCB levels as well as consumption rates vary considerably across fish species, and it was not possible to determine if the mean represents the fish species most commonly consumed. However the data received were not sufficient to allow a more detailed analysis to adequately account for this variation.

In a second step, a log-normal distribution of contaminants in foods was assumed, and a model of distribution was constructed from the weighted mean and a geometric standard deviation of 3 derived from concentrations in six broad food groups. Based on these derived distributions, the percentiles were determined and the derived median values (50^{th} percentile) are presented in Table 4.

Food consumption and dietary intake assessment

Because of the long half-lives of PCDDs, PCDFs, and coplanar PCBs, their health hazard can be estimated only after consideration of intake over a period of months. Short-term variations in PCDD, PCDF, and coplanar PCB concentrations in foods have much less effect on overall intake than might be the case for other food contaminants.

The distribution of long-term mean intake in various populations was calculated using the following procedure:

(1) The distributions of concentrations were constructed for various regions and food groups from the available data. The distributions were assumed to be log-normal.

(2) Data on food consumption from the GEMS/Foods regional diets and national surveys were used to estimate mean consumption for six major food groups for each different diet. A log normal distribution was constructed from these data with a geometric standard deviation of 1.3 extrapolated from the Dutch food consumption survey to account for inter-individual variation in consumption. The average contribution of the six basic food groups to the total food consumption were also derived for each diet.

(3) The dietary intake of a particular population was assessed by combining the concentration and food consumption distributions for that population in a Monte Carlo approach. In each Monte Carlo trial, dietary intake was estimated by multiplying random values for food consumption and concentrations in various food groups. The concentrations were weighted according to the fraction that the food group contributed to total food consumption. The collected intake estimates thus formed a distribution of long-term mean dietary intake for each population studied. The distributions are characterized by the median and the 90th percentile intake. The calculations were performed for the sums of the TEQs of PCDDs/PCDFs and of coplanar PCBs separately, because the data on occurrence were obtained independently.

Table 4. Weighted mean and derived median of concentrations of PCDDs, PCDFs, and coplanar PCBs in six food groups expressed as TEQs (pg/g whole food)

Region or country	Food category	PCDD/PCDFs		coplanar PCBs	
		Weighted mean	Derived median	Weighted mean	Derived median
Western Europe	Dairy	0.07	0.04	0.08	0.07
	Eggs	0.16	0.15	0.07	0.06
	Fish	0.47	0.31	2.55	0.90
	Meat	0.08	0.06	0.41	0.08
	Vegetable products	0.04	0.03	0.04	0.00
Japan	Dairy	0.06	0.04	0.04	0.02
	Eggs	0.07	0.03	0.06	0.04
	Fish	0.37	0.11	0.69	0.19
	Meat	0.09	0.01	0.04	0.009
	Vegetable products	0.003	0.002	0.02	0.003
North America	Dairy	0.10	0.07	0.02[a]	0.01[a]
	Eggs	0.17	0.14	0.04[a]	0.02[a]
	Fish	0.56	0.28	0.13[a]	0.08[a]
	Meat	0.13	0.10	0.14[a]	0.05[a]
New Zealand	Dairy	0.02	0.02	0.01	0.008
	Fish	0.06	0.05	0.09	0.07
	Meat	0.01	0.01	0.02	0.01
	Vegetable products	0.008	0.008	-	-
All	Fats and oils	0.21	0.10	0.07[a]	0.02[a]

[a] PCB data frequently did not include mono-ortho PCBs

The simulated intakes of PCDD/PCDFs and coplanar PCBs based on the GEMS/Food diets are presented in Table 5. However, in general the estimated intakes in Table 5 overestimate the real intake levels, because the concentration data partly consist of surveillance data (surveillance data are not randomly sampled), and GEMS/Food diets are based on food supply (apparent consumption) data which are known to overestimate food consumption by at least 15%.

More reliable estimates of intake (Table 6) were obtained by using national food consumption data rather than food supply (apparent consumption) data from the GEMS/Foods regional diets. The simulated intakes presented in Table 6 are not strictly national estimates and are somewhat higher than the national estimates submitted by European Union Member States.

The calculated contributions of various food categories to the intake of PCDDs, PCDFs, and coplanar PCBs showed that the largest fraction (> 70%) is from food of animal origin in both the GEMS/Foods regional and national diets.

Table 5 Median and 90th percentile of estimated long term intakes of TEQs (pg/kg bw per month, assuming 60 kg bw) based on the GEMS/Foods regional diets

Source of concentration data[a]	Source of food consumption data	PCDD/PCDFs		Coplanar PCBs	
		Median	90th percentile	Median	90th percentile
Western Europe	European	54	130	57	150
North America	European	68	160	14	35
New Zealand	European	18	36	10	22
Japan	Far Eastern	7	15	7	19

[a]for North America the concentration data of vegetables from western Europe were used; for New Zealand the concentration data of eggs from Japan were used.

Table 6. Median and 90th percentile of estimated long term intakes of TEQs (pg/kg bw per month, assuming 60 kg bw) based on national food consumption data

Source of concentration data[a]	Source of food consumption data	PCDD/PCDFs		coplanar PCBs	
		Median	90th percentile	Median	90th percentile
North America	USA	42	100	9	25
Western Europe	Netherlands	33	81	30	82
Western Europe	France	40	94	47	130
Western Europe	United Kingdom	39	91	41	110

[a]for North America the concentration data of vegetables from western Europe were used.

Information was lacking on both the quality and geographic representativeness for some regions. More data are required on the occurrence of coplanar compounds in food products, particularly from geographic regions other than Europe for more representative intake estimates for all regions.

Breast-fed infants have higher intakes of these compounds on a body-weight basis, although for a small portion of their life-spans. Breast milk has beneficial effects, despite the contaminants present. WHO has therefore repeatedly evaluated the health significance of contamination of breast milk with coplanar compounds. WHO recommends and supports breast feeding but has concluded that continued and enhanced efforts should be directed towards identifying and controlling environmental sources of these substances.

Evaluation

In view of the long half-times of PCDDs, PCDFs, and coplanar PCBs, the Committee concluded that it would not be appropriate to establish an acute reference dose for these compounds.

The Committee concluded that a tolerable intake could be established for 2,3,7,8-TCDD on the basis of the assumption that there is a threshold for all effects, including cancer. Carcinogenicity due to 2,3,7,8-TCDD was not linked to mutagenicity or DNA binding, and it occurred at higher body burdens in animals than other toxic effects. The Committee concluded that the establishment of a tolerable intake based on non-cancer effects would also address any carcinogenic risk.

The studies listed in Table 3 were those considered by the Committee in choosing the lowest LOELs and NOELs for assessment of tolerable intake. The lowest LOEL was provided by the study of Faqi et al. (1998) and a NOEL was provided by the study of Ohsako et al. (2001). With the toxicokinetic conversions described in Table 7, these two studies indicate maternal body-burden LOELs and NOELs for effects on male rat offspring of 25 ng/kg bw and 13 ng/kg bw, respectively. The conversion is shown in full in Table 7.

Background body burdens in laboratory animals

In the studies used to estimate body burden on the basis of the distribution of TCDD after multiple dosing, radiolabelled material was used. Therefore, the known background concentrations of TCDD and other PCDDs and PCDFs in the tissues of laboratory rodents resulting from traces of these compounds in rat feed were ignored. The Committee identified two studies that could be used to predict body burdens of rats resulting from the presence of coplanar compounds in laboratory feed. These studies were mutually consistent and predicted that 'unexposed' laboratory rats had TEQ body burdens of 3-12 ng/kg bw, depending on age. Thus, the maternal body burdens of TCDD based on studies with radiolabelled material should be adjusted upward by a minimum of 3 ng/kg bw to account for the background of unlabelled PCDDs and PCDFs. This may still tend to underestimate the maternal TEQ body burden, since 3 ng/kg bw was the minimum in the two studies, and in one of the studies coplanar PCB compounds were not included.

Addition of 3 ng/kg bw to the body burdens calculated using the linear model for the data in Table 2 results in estimated total TEQ body burdens of 16 ng/kg bw for the NOEL of Ohsako et al. (2001) and 28 ng/kg bw for the LOEL identified by Faqi et al. (1998). These body burdens correspond to equivalent human monthly intakes (EHMI) of 240 and 420 pg/kg bw, respectively. Using the power model for the data in Table 2 the EHMIs were 330 pg/kg and 630 pg/kg, respectively.

Identification of safety factors

Safety factors typically considered in establishing acceptable levels of intake on the basis of results of animal studies usually include 1) a factor to convert a LOEL to a NOEL (if needed), 2) a factor to extrapolate from animals to humans, 3) and factors to account for inter-individual variations in susceptibility. Factors of 10 have been used traditionally for interspecies extrapolation and human variability and a factor of 3 to 10 for extrapolating from a LOEL to a NOEL.

A NOEL was identified for effects in the offspring of male rats; thus, no factor for conversion from NOEL to LOEL was needed for the EHMI derived from the Ohsako et al. 2001 study.

As concluded by the 1998 WHO consultation, use of body burdens to scale doses from animal studies to equivalent human levels removes the need for safety factors for toxicokinetic differences between animals and humans.

To account for inter-individual differences in toxicokinetics among humans, a safety factor should be applied. The Committee noted that limited data were available on the toxicokinetics of 2,3,7,8-TCDD in humans, and considered that the default factor of 3.2 was appropriate.

The Committee observed that humans may be less sensitive than rats to some effects, but the conclusion is less certain for others, and it cannot be excluded that the most sensitive humans might be as sensitive to the adverse effects of 2,3,7,8-TCDD as rats were in the pivotal studies. Therefore, the Committee concluded that no safety factor in either direction needs to be applied for differences in toxicodynamics among humans.

Use of a LOEL instead of a NOEL indicates the need for an additional safety factor. As the LOEL reported by Faqi et al. (1998) for the sensitive end-point was considered to be close to a NOEL and represented marginal effects, the Committee applied a factor of 3 to account for use of a LOEL instead of a NOEL. This leads to an overall safety factor of 9.6 (3 x 3.2).

The Committee concluded that a total safety factor of 3.2 should be applied to the EHMI associated with the NOEL identified by Ohsako et al. (2001) and a total safety factor of 9.6 should be applied to the EHMI associated with the LOEL identified by Faqi et al. (1998).

Tolerable intake

As stated previously in the discussion of toxicokinetics, the long half-times of PCDDs, PCDFs, and coplanar PCBs result in each daily ingestion having a small or even negligible effect on overall intake. Only after consideration of the total or average intake of PCDDs, PCDFs, and coplanar PCBs over months can their long- or short-term risk to health be assessed. The tolerable intake should therefore be assessed over 1 month or longer. To encourage this view, the Committee decided to express the tolerable intake as a monthly value in the form of a *provisional tolerable monthly intake*[2] (PTMI).

As shown in Table 7, use of the linear model to extrapolate the maternal body burden at the NOEL in the study of Ohsako et al. (2001) with a single dose to that expected at multiple doses shows that the EHMI expected to produce a body burden that is below that which had effects in animals is 237 pg/kg bw. The PTMI derived by application of the safety factor of 3.2 to this EHMI is 74 pg/kg bw.

Similarly, as presented in Table 7, the PTMI derived by application of the safety factor of 9.6 to the EHMI derived from the study by Faqi et al. (1998) is 44 pg/kg bw.

As also shown in Table 7, use of the power model to extrapolate the maternal body burden with single doses to multiple doses would result in PTMIs of 103 pg/kg bw for the NOEL of Ohsako et al. (2001) and 66 pg/kg bw for the LOEL of Faqi et al. (1998).

The range of PTMIs derived from the two studies, with either the linear or the power model to extrapolate the maternal body burden with single to multiple doses, is 40 to 100 pg/kg bw per month. The Committee chose the midpoint of this range, 70 pg/kg bw per month, for the PTMI. Furthermore, on the basis of the 1998 WHO consultation the Committee concluded that this tolerable intake should be applied to intake of PCDDs, PCDFs, and coplanar compounds expressed as TEFs.

Table 7. Summary of four calculations of PTMI

	Linear model		Power model	
	Ohsako	Faqi	Ohsako	Faqi
Administered dose (ng/kg bw)	12.5[a]		12.5[a]	
Maternal body burden (ng/kg bw)	7.6	25	7.6	25
Equivalent Maternal BB with long-term dosing (ng/kg bw)	13[c]	25[c]	19[d]	39[d]
Body burden from feed (ng/kg bw)	3	3	3	3
Total body burden (ng/kg bw)	16	28	22	42
EHMI (pg/kg bw/month)	237	423	330	630
Safety factor	3.2	9.6	3.2	9.6
PTMI (pg/kg bw/month)	74	44	103	66

[a]Bolus dose (NOEL).
[b]Target maternal body burden from repeated dosing (LOEL).
[c]Assumes a linear relationship between fetal and maternal body burden (based on data in Table 2).
[d]Assumes a non-linear relationship between fetal and maternal body burden (based on data in Table 2).
[e] Assumes, for humans, 7.6 year half-time and 50% uptake from food (Equation 1).

Comparison of PTMI with estimated intake from food

In the GEMS/Food regional diets, the range of estimated intake of TEQs for PCDDs and PCDFs is 7-68 pg/kg bw per month at the median and 15-160 pg/kg bw per month at the 90th percentile of mean lifetime exposure, and those for coplanar PCBs were 7-57 pg/kg bw per month at the median and 19-150 pg/kg bw per month at the 90th percentile of consumption. The intakes estimated from national food consumption data were lower: 33-42 pg/kg bw per month at the median and 81-100 pg/kg bw per month at the 90th percentile for PCDDs and PCDFs, and 9-47 pg/kg bw per month at the median and 25-130 pg/kg bw per

[2] By analogy with the PTWI, the end-point used for safety evaluations by JECFA for food contaminants with cumulative properties. Its value represents permissible human monthly exposure to those contaminants unavoidably associated with otherwise wholesome and nutritious foods.

month at the 90th percentile for coplanar PCBs. Estimates could not be made for the sum of PCDDs, PCDFs, and PCBs, because data on concentrations were submitted separately by countries.

The median and 90th percentile of the derived distribution of intakes were considered to describe long-term intake. A Monte Carlo calculation was used to predict these intakes for coplanar compounds on the basis of two sets of distribution curves generated from information on mean concentrations in six major food groups and corresponding data on mean food consumption from several sources, by applying geometric standard deviations of 3 and 1.3 to the respective means. The geometric standard deviation for the food consumption curves accounted for long-term consumption patterns. As the mean intakes of the whole population tend not to change with the duration of a survey, use of mean consumer intakes to generate the curves for major food groups, rather than individual commodities, approximates the mean intakes of the whole population, as nearly all respondents were consumers.

Uncertainties

Several sources of uncertainty were identified in the data used to assess intake, which suggest that they are likely to be overestimates at both the median and the 90th percentile level of consumption. Despite the uncertainties, the results suggest that a considerable fraction of the population will have long-term mean intake above the PTMI.

Furthermore, despite the large amount of information on toxicity, substantial uncertainties remain which should be considered in applying the risk assessment and interpreting the estimates of intake of PCDDs, PCDFs, and coplanar PCBs. The Committee used the overall data to identify a level of intake of coplanar compounds in food that represents no appreciable risk to humans. The safety assessment includes adjustment for a number of uncertainties, including estimates of TEF values within orders of magnitude to relate the potency of 28 relatively poorly studied compounds to that of one well-studied compound, 2,3,7,8-TCDD. Moreover, the proportion of 2,3,7,8-TCDD in relation to the other 28 compounds varies, typically constituting a small percentage of the total TEQ exposure in foods.

The PTMI is not a limit of toxicity and does not represent a boundary between safe intake and intake associated with a significant increase in body burden or risk. Long-term intakes slightly above the PTMI would not necessarily result in adverse health effects but would erode the safety factor built into the calculations of the PTMI. It is not possible given our current knowledge to define the magnitude and duration of excess intake that would be associated with adverse health effects.

Effect of maximum limits on intake, risk, and food availability

The concentrations of PCDDs, PCDFs, and coplanar PCBs vary within foods. In establishing regulatory limits for them, the possible undesired consequences of their enforcement should be taken into account, for example reductions in the food supply. The Committee explored the theoretical effect of various maximum regulatory limits on compliance and on long-term average reduction of intake require. On the basis of this analysis the Committee concluded that to achieve, for example, a 20% reduction in food-based intake of coplanar compounds one would need to decrease intake of a wide range of foods by a similar percentage. This relationship exists because these contaminants are present at relatively high levels across major food types. Furthermore, in view of the half-times of these compounds in humans, setting regulatory limits on the basis of the PTMI would have no discernible effect on body burdens for several years.

In contrast, long-term reductions could be gained by identifying and eliminating pathways from the environment to food supplies. The Committee was informed that in several countries studies of environmental levels over time suggest that measures taken to control emissions to the environment generally have had a substantial impact on both the amounts of PCDDs and PCDFs present in the environment and the body burdens of the general public.

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