FOOD SCIENCE AND TECHNIQUES

Reports of the Scientific Committee for Food

(Thirty-fifth series)

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food science and techniques

Reports of the Scientific Committee for Food

(Thirty-fifth series)

Opinions of the Scientific Committee for Food on:

Propylene glycol

Alternatively refined carrageenan produced from *Eucheuma cottonii* and *Eucheuma spinosum*

p-Hydroxybenzoic acid alkyl esters and their sodium salts

Specifications for food additives

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Invertase derived from *Saccharomyces cerevisiae*

Aflatoxins, Ochratoxin A and Patulin

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OPIN ONNIO PROPYLENE GLYCOL

EXPRESSED ON 9 DECEMBER 1993

1. Terms of reference

To re-evaluate the safety in use of propylene glycol (PG) in the light of the presently available toxicity data and indications of changing patterns of use.

2. Background

PG is currently listed in Community legislation relating to food only as a permitted diluent and carrier solvent for antioxidants. A proposal for a directive on food additives other than colours and sweeteners presently before the Council of Ministers would restrict the use of PG as a diluent and carrier to colours, emulsifiers, antioxidants and enzymes.

In its 11th Series of Reports on Solvents (EUR 7421, 1981) the Scientific Committee for Food expressed the following opinion in relation to PG:

> "There are sufficient data available from oral toxicity studies, including long-term studies in rats and dogs, to establish an ADI of 0-25 mg/kg b.w. (JECFA, 1973). The Committee agrees with the ADI established by JECFA and considers the use of this substance acceptable as solvent for food. Because of the information submitted on the extensive use of this substance in food technology the Committee recommended that the intake from all sources should be reviewed in relation to the established ADI".

At its 75th meeting, in October 1990, the Committee evaluated propylene glycol alginate. It allocated an ADI "not specified" for alginic acid and its sodium, potassium and calcium salts but maintained the ADI of 25 mg/kg b.w., expressed as PG, for the ester. The Committee also expressed the wish to re-evaluate the ADI for PG at a future stage and reiterated the request that the intake from all sources of this solvent should be reviewed in relation to the ADI.

Preliminary results of surveys undertaken in Denmark indicate a large use of PG in some food commodities and to an extent not fully explainable by its use as a carrier solvent.

In addition, some recent information has become available on the toxicity of PG in cats and reviews of previous results suggest a need for revision of the present ADI. In relation to pet food, PG is presently allowed in dog food up to a maximum of 53 g/kg and for cat food up to a maximum of 75 g/kg complete feeding stuff. A review of the toxicity data relating to cats has prompted the Scientific Committee for Animal Nutrition to give the following provisional advice:

> "addition of 1,2-propanediol to feedingstuffs for cats to be reduced to 40 g/kg complete feeding stuff. Adequate dose-response relationships for various strains of cats and an NEL for the observed reduced life span of erythrocytes is to be provided. In the light of this information a review will be undertaken."

3. Discussion

PG is rapidly absorbed from the gastro-intestinal tract of mammals, quickly distributes in the whole body water, and is partially rapidly excreted and partially metabolised to lactic acid, pyruvic acid and carbon dioxide, thus essentially contributing well-known intermediates of mammalian carbohydrate metabolism. The reported pharmacological or biochemical reactions only occur with high parenteral doses and are of little relevance to the safety assessment of PG when ingested through food.

PG has a very low acute oral toxicity in laboratory animals and only very high oral doses have produced central nervous system depression and minimal renal and liver changes. For these reasons there has been a wide use of PG as a solvent carrier in pharmaceutical preparations and those intended for topical application. No systemic injuries to humans have been reported following dermal applications, the main effects being irritation of the skin in some people possibly due to dehydration effects. There is no evidence for PG being a primary sensitizer in man.

Short-term studies in rats, rabbits and dogs showed no adverse effects at levels approximating 10% in the diet. Long-term feeding studies in rats, with 5% in the diet being the highest level tested, showed no adverse effects. In a 2-year feeding study in dogs the no-adverse-effect level was 2 g/kg b.w. Reproductive effects were noted in rats only at dietary doses which caused maternal toxicity. No teratogenic effects were observed in mice, rats, rabbits and hamsters. No convincing evidence for a genotoxic potential has been demonstrated in several *in vitro* and *in vivo* assays for different mutagenicity end-points but one *in vivo* assay at germ cell level was suggestive of a possible potential to induce chromosomal aberrations in spermatocytes.

Cats appear to be uniquely sensitive to haematological effects of ingested PG, responding with a highly significant increase of Heinz bodies in circulating erythrocytes at PG concentrations found in commercially available cat food. The half-life of circulating erythrocytes was also reduced. Due to the failure of the feline spleen to cull Heinz body-containing erythrocytes these remain in the circulating blood with little haemolysis occurring. The latter is easily compensated by increased reticulocytosis. These adverse effects are considered minimal in healthy cats, but may cause anaemia under severe endogenous or exogenous oxidant stress or with concomitant inflammation or other processes depressing erythropoiesis. Dogs show similar haematological effects but at much higher doses. No haematological signs have been reported in humans receiving oral or intra-venous medication containing PG as vehicle.

The present ADI of 25 mg/kg b.w. is based on the no-adverse effect level in the long-term rat studies in which, however, the maximum tolerated dose has not been reached. A safety factor of 100 was used to establish this ADI on the basis of the metabolism of PG, its total toxicity profile, and the large human experience with oral and parenteral pharmaceutical preparations containing PG as a vehicle.

4. Conclusion

There are no new toxicological studies which would persuade the Committee to increase the present ADI. Moreover, the haematological findings in cats, and to a lesser degree in dogs, constitute additional arguments for maintaining this view.

The uncertainty with regard to potential mutagenic effects at the germ cell level, the fact that most studies at the chromosomal level used limited protocols, that there is no *in vitro* assay for gene mutation in cultured mammalian cells as well as the absence of a carcinogenicity study in a second species led the Committee to change the established full ADI into a temporary ADI of 25 mg/kg b.w.

To clarify the existing doubts, the Committee recommended that the results of an *in vitro* mouse lymphoma cell assay, which is known to be sensitive both to gene mutations and chromosomal effects, be provided. Alternatively, the results of *in vitro* chromosomal aberration and gene mutation assays in cultured mammalian cells, preferably human peripheral lymphocytes, carried out using the most recent recommended international protocols, would be acceptable.

The Committee re-iterates its wish that the intake of PG from all sources in the Community be reviewed in order to enable maximum limits to be set for its uses in food technology should it become apparent that the intake is exceeding the ADI on a regular basis.

OPINION ON ALTERNATIVELY REFINED CARRAGEENAN PRODUCED FROM EUCHEUMA COTTONII AND EUCHEUMA SPINOSUM

EXPRESSED ON 25 FEBRUARY 1994

1. Terms of reference

To advise on the safety in use of alternatively refined carrageenan produced from *Eucheuma cottonii* and *Eucheuma spinosum*.

2. Background

In October 1991 the Commission received an application from the Seaweed Industry Association and the Government of the Philippines (Seaweed Industry Association of the Philippines, 1991) to assess the specification and safety-to-health of carrageenan manufactured by an alternative process (alternatively refined carrageenan-ARC) to that used for the production of conventionally refined carrageenan (CRC).

ARC is claimed to be prepared only from *Eucheuma cottonii* and *Eucheuma spinosum*, the commonest sources of commercial carrageenan. The process of manufacture consists essentially of the treatment of washed seaweed with strong alkali to coagulate carrageenan inside the plant cells without extracting it. After some clean-up steps the coagulated carrageenan together with cellulosic debris from the cell wall and other cell constituents is converted into a powdery end product.

In contrast, the CRC is extracted from the seaweed cells with mild alkali. The extracted carrageenan is then cleaned up and precipitated with alcohol, the precipitated material being eventually converted into a powdery end product consisting essentially of carrageenan and mineral salt. It therefore does not contain any cell wall debris and needs a separate purity specification to distinguish it from ARC.

The table below compares the major parameters of the specifications for purity of CRC and ARC.

Parameter	CRC	ARC
Carrageenan polysaccharides	74%	73.3%
Total ash (dry basis)	36.3%	20.9%
Acid insoluble matter (a.i.m.) less than	0.1%	11.2%
Crude fibre	0.2%	6.5%
Viscosity (filtered)	12 cps	105 cps
Viscosity (as is)	15 cps	215 cps
Heavy metals less than	1.4 mg/kg	3.9 mg/kg

The method of production of ARC is sufficiently different from that used for the production of CRC, so that a clear distinction can be made in the specifications for purity of the two products. The main polysaccharide is kappa-carrageenan in both cases, there being little difference in the respective concentrations. The presence of 10-12% acid insoluble matter (a.i.m.) in ARC constitutes the major difference from CRC. Carrageenan has been a permitted food additive in the EU, appearing in the Directive 74/329/EEC.

Because of the incompleteness of the data in the original submission of ARC and the unavoidable delay in obtaining the additional information required by the Committee to complete the assessment of the safety of ARC, some considerable time has elapsed before a final evaluation could be made.

The recent additional data now provided relate to the chemical nature of the a.i.m., the molecular weight distribution of the carrageenan in ARC and the microbiological status of the ARC. The results of a 90-day feeding study in rats, of several *in vitro* and *in vivo* genotoxicity tests, and of cytotoxicity tests using bone marrow mononuclear cell and hepatocyte cultures have also been supplied.

3. Evaluation

The toxicology of carrageenan (CRC) has been evaluated previously by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), last in 1984, and is summarized in the relevant JECFA monograph (IPCS 1984). The SCF reviewed carrageenan in 1978 (Scientific Committee for Food, 1978) and at that time agreed with the then existing ADI for man of 0-75 mg/kg bw previously established by JECFA. The Committee re-evaluated carrageenan (CRC) in 1992 when it confirmed the ADI of 0-75 mg/kg bw (Scientific Committee for Food, in press).

The analytical data on ARC show that the molecular weight distribution of the kappa-carrageenan component was similar for ARC and CRC and that the low molecular weight fractions are also similar. The microbiological data for ARC show the absence of pathogenic organisms in the large number of samples examined. X-ray diffraction powder analysis suggests that the a.i.m. is probably similar to amorphous cellulose. This is apparently confirmed by infrared absorbance measurements on kappa-carrageenan films from ARC. These measurements also detected the presence of calcium carbonate as a component of a.i.m. No bands characteristic of a long-chain carboxylic acid, originally suspected to be present, could be found in films of washed and unwashed ARC from 2 sources nor in a film of a commercial CRC.

The subchronic rat study on ARC shows no obvious adverse effects, thus excluding the presence of toxic compounds. The NOEL in this study is 5% ARC in the diet, the highest level tested. The *in vitro* genotoxicity tests using bacterial test systems, including an additional Salmonella reverse mutation test, are negative, thereby excluding the presence of genotoxic contaminants. The highest level of a.i.m. in the samples examined is 112 µg/plate. The two available *in vivo* genotoxicity tests confirm the absence of any genotoxic or clastogenic activity. The doses of ARC examined are estimated to have been equivalent to an exposure of approximately 8 mg a.i.m. per animal. The interpretation of the results of the submitted cytotoxicity tests remain unclear.

4. Conclusion

The details supplied on the specification, the chemical analysis and the microbiological status of ARC, the identification of the nature of the a.i.m. as amorphous cellulose and the absence of other toxic contaminants enable the Committee to consider ARC to be an acceptable carrageenan preparation. The absence of toxic effects in the 90-day study and the evidence for the absence of genotoxicity additionally support this opinion of the Committee and its conclusion that further toxicological testing is not needed.

Although JECFA eventually established an ADI "not specified" for CRC, the Committee wishes to maintain the original group ADI for all carrageenans (CRC and ARC) of 0-75 mg/kg bw for man because of some remaining uncertainty over the general immunoreactive potential of the various carrageenans now in use as food additives.

The Committee also concluded that separated specifications of purity should be developed for ARC and CRC, each specification being formulated to reflect the respective method of production. It also noted information indicating that traces of ethylene oxide had been found in batches of ARC circulating in commerce in North America and Europe. It affirmed that the treatment with ethylene oxide of ARC intended for food use is not acceptable. The Committee advised the Commission that, should it not prove to be the case that the existing law prohibits the use of ethylene oxide on carrageenan products, the specifications for purity should be formulated in such a way as to preclude this use whilst at the same time requiring the carrageenan product to meet adequate microbiological criteria.

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OPINION ON *P*-HYDROXYBENZOIC ACID ALKYL ESTERS AND THEIR SODIUM SALTS

EXPRESSED ON 25 FEBRUARY 1994

1. Terms of reference

To advise on the safety in use of *p*-hydroxybenzoic acid alkyl esters and their sodium salts as food additives.

2. Introduction

p-Hydroxybenzoic acid alkyl esters and their sodium salts (parabens, PBs) have been extensively used as preservatives in food over many years. In 1974 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated several parabens and established an ADI of 0-10 mg/kg bw, as the sum of ethyl, methyl and propyl *p*-hydroxybenzoic acid and their sodium salts. JECFA was unable to establish an ADI for the butyl ester of *p*-hydroxybenzoic acid. The Scientific Committee for Food (SCF) has not previously established an ADI for any of the parabens. However, the SCF did consider one of the parabens, sodium methyl *p*-hydroxybenzoate, in 1975 and confirmed its agreement with the JECFA evaluation. Accordingly, sodium methyl *p*-hydroxybenzoate was added to the EC list of permitted food preservatives which already included methyl *p*-hydroxybenzoic acid and ethyl *p*-hydroxybenzoic acid and its sodium salt.

3. Summary of metabolism and toxicity data

Many of the pharmacokinetic observations and toxicological studies on the parabens were carried out some years ago and would not fulfil present day criteria for conduct of studies. However, considering the parabens as whole, there is a considerable range of studies available and the Committee regards most of them as helpful for safety evaluation purposes.

Absorption, metabolism and excretion has been studied in rats, rabbits, dogs and humans. The methyl, ethyl and propyl esters of *p*-hydroxybenzoic acid (Me-PB, Et-PB and Pr-PB) are well absorbed and the ester linkage is readily hydrolysed, as indicated by high plasma levels and early urinary excretion of free *p*-hydroxybenzoic acid, *p*-hydroxyhippuric acid and other metabolites such as ester glucuronides and ether sulphates. Urinary excretion of unchanged esters of *p*-hydroxybenzoic acid is very low, usually less than 1% of the administered dose. Limited *in vitro* data on the butyl ester (Bu-PB) suggest it may follow a different metabolic pathway. Studies with prolonged dosing in dogs show no evidence of accumulation of either parent compounds or metabolites in the tissues.

Acute toxicity is only seen at high doses. All the parabens produce similar symptoms with rapid onset of ataxia, paralysis and central nervous system depression, resembling anaesthesia, suggesting their toxicity is related mainly to the free acid. With non-fatal doses recovery is prompt.

Subchronic toxicity studies on Me-PB, Et-PB and Bu-PB and chronic toxicity studies on Me-PB, Et-PB and Pr-PB have been conducted in rats. The no-effect level for all four parabens was 2% in the diet, equivalent to 0.9-1.2 g/kg bw/day. Effects occurring at a much higher dietary inclusion level of 8% were decreased weight gain (Me-PB and Pr-PB) accompanied by depression and death (Et-PB and Bu-PB). Doses intermediate to 2% and 8% were not tested. Me-PB and Pr-PB have also been tested at 500 and 1000 mg/kg bw/day given for approximately one year in the dog with a no-effect level of 1000 mg/kg bw/day for both esters. Bu-PB has been tested in the mouse at levels up to 10% in the diet for 6 weeks. The no-effect level in the mouse was 0.6% (equivalent to around 0.9 g/kg bw/day).

Several *in vitro* mutagenicity studies covering both point mutations and chromosome aberrations, and an *in vivo* host mediated assay and dominant lethal assay provided no evidence of genotoxicity of Me-PB. Pr-PB and Bu-PB were not mutagenic *in vitro*. No mutagenicity data are available for Et-PB.

The only long-term study specifically designed to address carcinogenicity was conducted on Bu-PB in mice, given up to 0.6% in the diet for two years. It reported no significant difference in tumour rates between treated and control animals but was inadequate for assessment due to early deaths in treated and control groups and relatively high incidence of some tumours in the control group.

Reproduction and teratogenicity studies in the rat using Et-PB at levels up to 10% in the diet found no adverse effects on reproductive performance but the findings with respect to fetal anomalies were equivocal, the reported anomalies showing no clear dose-response relationship. There are no other reproduction studies available for the parabens.

A number of special studies on cell proliferation in the forestomach and glandular stomach of rats have been carried out using finely ground powdered parabens, fed for 9 days at up to 4% in the diet. Me-PB was without activity, Et-PB showed minimal activity, whilst Pr-PB and Bu-PB induced cell proliferation in the pre-fundic region of the forestomach. The potency depended on the alkyl chain length; 4% Pr-PB and Bu-PB had activities equivalent to 0.5% and 2% dietary BHA respectively.

4. Conclusions and recommendations

The data available give adequate reassurance that use of the methyl, ethyl and propyl esters of *p*-hydroxybenzoic acid and their sodium salts as food preservatives is temporarily acceptable. However, the toxicological information available shows some inadequacies and uncertainties and further studies along the following lines are needed:

- Since cell proliferation effects in the forestomach similar to those produced by BHA have been observed when certain alkyl esters of *p*-hydroxybenzoic acid were given in the diet in the form of a ground powder, a cell proliferation study in the rat on the propyl ester of *p*-hydroxybenzoic acid given as a solution should be carried out.

- In view of the equivocal findings in the existing oral teratogenicity study, a new oral teratogenicity study in the rat using either free *p*-hydroxybenzoic acid or its methyl, ethyl or propyl ester.

An overall no-effect level of 1000 mg/kg bw can be taken from the toxicity studies. The Committee considers that a 100-fold safety factor is appropriate, giving a temporary ADI of 0-10 mg/kg bw, as the sum of methyl, ethyl and propyl *p*-hydroxybenzoic acid and their sodium salts. The Committee was not required to establish an ADI for the butyl ester of *p*-hydroxybenzoic acid since it is not used as a food additive. The t-ADI will be reviewed in 3 years time in the light of any new toxicological studies, along the lines suggested above, together with information on consumer intakes of parabens, which we understand will then be available.

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OPINION ON SPECIFICATIONS FOR FOOD ADDITIVES

EXPRESSED ON 25 FEBRUARY 1994

1. Introduction

The Commission has responsibility for drawing up and agreeing with Member States specifications for additives which are permitted for use in the EU. The Commission has asked the Scientific Committee for Food (SCF) if there are any comments it wishes to make about the setting of specifications in general, or on the draft specifications recently prepared for colours and sweeteners. The SCF offers the following general advice on toxicological aspects of specifications and a few comments on the draft specifications for colours and sweeteners but the Committee has not examined each individual draft specification in detail. Additional expertise to that of the SCF is required for the detailed examination of individual specifications. Thus the absence of comments on particular substances should not be taken as endorsement of the draft specifications by the Committee and the SCF urges the Commission to seek such advice from appropriate specialists in the Member States. Should any new questions arise from such consultations which have health implications, then the Committee would be happy to advise the Commission further.

2. Value of specifications

The SCF has stressed the importance of specifications in safety evaluation in an earlier report[1]. We reiterate the views expressed then, which still hold good today, that the material subject to toxicological testing should correspond to the food additive to be used in practice by the food industry. To achieve this, draft specifications are needed at an early stage. Tests carried out on samples for which there are inadequate specifications may later be found to be valueless and tests carried out unidentified material are of no value. Specifications are therefore an essential prerequisite for a sound evaluation of the safety in use of any additive; they ensure that the batches of material used in the toxicity and other safety tests on a particular additive are similar in composition and, whilst they may vary to a small extent within acceptable limits, they do not differ in any way that is biologically significant from the product which is eventually marketed. Any differences in the proportion of the major component which performs the technological function in the food or in the nature or amounts of any impurities present in the final product may alter the outcome of safety tests. For Committees such as the SCF which evaluate the safety aspects of new and existing additives, draft specifications should be available at the time of the evaluation. Draft specifications may also need to be amended in the light of the results of toxicity tests.

Similar views have been expressed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) some years ago in its Eighth and Tenth Reports[2,3] and elaborated more recently in "Principles for the Safety Assessment of Food Additives and Contaminants in Food". JECFA has emphasised the need to establish internationally agreed specifications for the identity and purity of food additives. The SCF endorses this view and urges the Commission, in drawing up EU specifications, to base them where possible on existing Member State or FAO/WHO agreed Codex specifications, provided these are considered satisfactory by current day standards.

As JECFA noted in its early reports[2,3], agreed specifications are an essential aid in consumer protection, ensuring that any additives used are of known composition and purity. They assist in the regulatory control and identification of additives and ensure that what is marketed does not differ significantly from the grade or quality of the substances that has been evaluated for its safety in use. They are of value to industry in ensuring that products of an agreed standard are traded between chemical manufacturers of the additives and those utilising them in processed foods.

3. Information which should be included in specifications

Specifications should include information to enable the additive to be properly identified and, where appropriate, should include identification tests. They should include both general purity criteria and specific purity criteria decided on a case-by-case basis. In case-by-case considerations any opinions already expressed by the SCF or JECFA concerning particular toxicological problems with known impurities should be taken into account by limiting the level of the impurities to that which is considered safe.

As mentioned in the Tenth Report of the SCF[1], consideration of a substance's chemical structure and the route by which it has been synthesised will enable a search to be made for specific potential impurities whose presence might otherwise have escaped detection. It is important to scrutinise carefully not only the source of the raw materials but also the method(s) of production for persisting intermediates or impurities. Consideration should also be given to degradation products which may arise during formulation or storage.

For some additives, methods of production may vary or new methods of production may be proposed for additives already on the market. For example, new methods involving biotechnology are being used now or will be increasingly in the future. This is particularly true, for example, for enzyme preparations, including preparations derived from genetically modified organisms. In cases where chemical or biotechnological methods of production for a particular additive vary, it may be necessary to evaluate separately products made by different production processes. It may also be necessary in some instances to have separate specifications for such products even though the major component performing the technological function is the same.

4. Food additives derived from natural sources

Additives derived from natural sources may contain only relatively small proportions of the active principles. In the SCF safety evaluations of natural source additives, for which there is little specific toxicity information, the Committee has stressed that they should be derived from edible parts of plants normally used for food, extracted by physical processes, and that their use as additives should not lead to intakes which are significantly increased compared with intakes from natural food sources generally. These principles need to be taken into account when drawing up specifications. Additives from natural but non-food sources, or from food sources used in parts of the world other than Europe, need to be considered on a case by case basis, both from the point of view of their toxicity and the specification. For food additives derived from natural sources, there may be a need to include limits for microbial contaminants and inherent natural toxicants.

5. Heavy metals

Limits for heavy metals are useful in ensuring that avoidable sources of toxic metals, especially arsenic, lead, mercury and cadmium, do not contribute unnecessarily to intakes of metals from the diet. We wish to see such individual limits continue to be included in specifications where there is a possibility of heavy metal contamination. Existing specifications for many additives include limits for the content of arsenic and lead and, less commonly, cadmium and mercury. These limits may vary depending on the quantities of particular additives which need to be added to foods to achieve their technological function. For example, the limits may need to be lower for additives used in larger quantities in individual foods (e.g. bulk sweeteners, chewing gum bases, thickeners, stabilisers and emulsifiers), than for additives present in small amounts. The Committee recommends that limits for individual metals should be revised, if necessary, using the principle that the higher the maximum permitted level of addition of the additive to food, the lower the heavy metal limit needs to be. However, we do not consider it necessary to strive to reduce limits to the lowest achievable limit of detection since the high dilution of additives in food is such that the overall contribution of additives to metal intakes is generally negligible compared with other sources.

Many existing specifications also include an overall limit for total heavy metals, expressed as lead. It has been brought to the Committee's attention that there may be some confusion about what the term "total heavy metals" includes and that the "catch-all" test currently recommended in JECFA specifications[5] (all metals giving a colour with hydrogen sulphide) is widely regarded as unsatisfactory and generally is no longer used. In discussing this problem, the Commission may wish to note that the Committee considers that since heavy metals are not known to be additive or synergistic in their actions, there is no need on toxicological grounds for an overall limit on heavy metals provided there is control of the individual toxic heavy metals, as discussed above. Thus, the currently recommended method, which expresses total heavy metals as lead, has neither an analytical not a health basis.

Further specific comments about heavy metals in colours and sweeteners will be found below in the relevant paragraphs on the draft specifications for these categories of additives.

6. Solvent residues

SCF opinions on a number of individual solvents are available and these should be borne in mind when setting purity criteria for individual and overall solvent residues in additive preparations, but taking account of the likely dilution of any solvent residues when the additive is incorporated into foods.

Comments on draft specifications for colours

1. General

We note that some of the specifications deviate from **Codex** ones. Such changes may be appropriate but we recommend that the draft EU specifications be compared to the existing Codex ones to ensure that any differences are necessary and that the changes are logical and consistent.

A number of **unsulphonated primary aromatic amines** are known to be carcinogenic in animals and a few are proven human carcinogens. Limiting the amount of unsulphonated primary aromatic amines in any azo colour to not more than 0.01% (calculated as aniline) is recommended. Aromatic amines sulphonated on all aromatic or conjugated ring structures are considered of low risk and do not normally need to be controlled by limits. Sulphonated aromatic amines other than the principal coloured components should be limited to not more than 0.5% of the total.

Limits for **heavy metals** in colours (and perhaps other categories of additive) need some rationalisation. For example, not all colours are likely to be contaminated with mercury yet all have limits for mercury. Conversely, none of the draft specifications for colours have any limits for cadmium. Sources and methods of production need to be examined for each individual colour to see whether limits for mercury and cadmium are needed. A re-examination of the mercury limits for Caramel is particularly needed; whilst the limits for arsenic and lead in Caramel are lower than for other colours, the limit for mercury in Caramel is twice that for other colours, yet this colour is often added to food in much higher quantities than are other colours (see also general comments relevant to quantities used under "Heavy metals" above).

2. Colours derived from natural sources

We note that, in a number of cases, the draft specifications for natural source colours do not make it clear which food sources may be used or include sources which are not normally consumed by man. These should be reviewed in the light of the general comments made under "Food additives derived from natural sources" above.

3. Ammonia caramel

The context of **2-acetyl-4-tetrahydroxybutylimidazole (THI)** in Ammonia caramel should not exceed 10 mg/kg (SCF opinion, in press).

4. Vegetable carbon

Polycylic aromatic hydrocarbons should not be detectable in Vegetable carbon using an agreed sensitive method.

Comments on draft specifications for sweeteners

1. General

We note that some of the specifications deviate from **Codex** ones. Such changes may be appropriate but we recommend that the draft EU specifications be compared to the existing Codex ones to ensure that any differences are necessary and that the changes are logical and consistent.

Limits for **heavy metals** in sweeteners need some rationalisation. None of the draft specifications for sweeteners have limits for mercury or cadmium. Some do not have limits for lead (Cyclamic acid and Saccharin and its salts), whilst limits for lead in others vary between 1, 3 and 10 mg/kg. Depending on sources and methods of production specific limits may not be necessary in some cases, but this should be checked. Similarly, there may be acceptable reasons for varying lead limits (e.g. related to the maximum amount of the additive permitted in foods) but this too should be checked and a consistent limit applied wherever possible.

2. Aspartame

The SCF has commented on the breakdown product of aspartame, diketopiperazine (5 benzyl-3, 6-dioxo-2-piperazineacetic acid), and set an ADI of 0 - 7.5 mg/kg bw[6]. The content of diketopiperazine in the product aspartame as supplied should not exceed 1.5%.

3. Cyclamic acid and its salts

Whilst the majority of the toxic substance cyclohexylamine, derived from cyclamic acid, is generated from metabolic conversion *in vivo* after ingestion, there is a need to limit the content of the cyclohexylamine in cyclamic acid and its sodium and calcium salts as manufactured to not more than 25 mg/kg, and to limit the content of dicyclohexylamine to not more than 2 mg/kg. This should ensure that the exposure to cyclohexylamine from that ingested and that which may be formed *in vivo* is within the temporary ADI of 0 - 11 mg/kg bw, expressed as cyclamic acid, which is based on the toxicity of cyclohexylamine[6, 7].

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OPINION ON SORBIC ACID AND ITS CALCIUM AND POTASSIUM SALTS

EXPRESSED ON 25 FEBRUARY 1994

1. Terms of reference

To advise on the safety in use of sorbic acid and its salts as preservatives for foodstuffs.

2. Background

These compounds have not been previously evaluated by the Scientific Committee for Food although they are already included in the Directive 64/54/EEC. Sorbic acid and its calcium, potassium and sodium salts have been evaluated, however, by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at several meetings since 1961 and sodium sorbate was specifically reevaluated in 1985. Most of the toxicological data, on which the present evaluation is based, have been previously summarized in a monograph published by WHO in 1974. Additional data were culled from a literature search carried out by the National Food Agency of Denmark. These data included new investigations on biochemical aspects, a long-term chronic toxicity/carcinogenicity study on sorbic acid in rats, 2-year studies in mice and rats with potassium sorbate, teratogenicity studies in mice and rats with potassium sorbate, a large number of *in vitro* and *in vivo* genotoxicity studies with sorbic acid, potassium sorbate and sodium sorbate, special studies on the reactions of sorbates with nitrite, and six further studies in humans with particular emphasis on the allergenicity of sorbates.

3. Evaluation

Sorbic acid is readily metabolised like other short-chain fatty acids. Both man and the rat appear to utilise identical metabolic mechanisms for the oxidation of sorbates. Under normal conditions there is almost complete oxidation of sorbic acid to carbon dioxide and water.

Long-term carcinogenicity studies with sorbic acid up to 10% in the animal feed have been conducted in mice and rats without showing any carcinogenic effects. The rather high and widely spaced selected dosages used in the newer studies only permit the establishment of 1,5% in the rat and 1% in the mouse as approximate NOELs, while the earlier studies with lower and more closely spaced dosages showed the more accurately determined NOEL for potassium sorbate and sorbic acid to be 5% in both species. In the newer mouse study a statistically significant increase in liver weight was found in females at all dose levels tested but this was not accompanied by histopathological changes and therefore not considered to be treatment-related.

The only study reporting a carcinogenic affect on the liver of mice used a diet containing 15% sorbic acid. Only a summary abstract of this study is available for evaluation and it does not give enough details to interpret the reported findings of the study. The number of animals per group was too small by modern standards, only one dose level appears to have been tested and no information is given on the historical incidence of liver tumours in the mouse strain used. In the light of the results of all the other long-term studies it is reasonable to set aside these results.

Sorbic acid and potassium sorbate complying with the appropriate specifications do not cause tumours when administered orally or subcutaneously. Long-term studies with sorbic acid containing also parasorbic acid showed no evidence of any carcinogenic potential when administered orally to mice and rats.

Experimental studies to elucidate the probable mechanism by which sorbic acid may be involved in the induction of hepatomas, which had been reported in studies on mice, have indicated that high doses (15%) of sorbic acid in the diet may reduce the levels of lipid peroxides and glutathione in the liver and induce hepatic peroxisomal enzyme activities. However, final explanations cannot yet be based on these studies.

Sorbic acid has been tested for genotoxic activity *in vitro* and *in vivo* in various test systems. Most of the *in vitro* results have been negative. Some *in vivo* tests have yielded positive results, but it should be noted that in these experiments the control values were unusually low.

Sorbic acid has caused hypersensitivity reactions, particularly contact urticaria, in certain population subgroups.

No toxicological studies have been carried out with calcium sorbate.

Long-term carcinogenicity studies with potassium sorbate have been conducted in mice and rats with doses up to 5% in the diet. However only summary interim reports are available for scrutiny. Potassium sorbate inclusion in the diet up to the dose level of 5% caused no carcinogenic effects in rats. The final data of the mouse study have not been supplied.

Potassium sorbate has been tested for genotoxic activity *in vitro* and *in vivo* in various test systems. The *in vitro* results have been almost exclusively negative, and all the *in vivo* tests gave negative results. No teratogenic effects were noted after dosing mice with up to 460 mg/kg b.w. and rats with up to 340 mg/kg b.w.

Potassium sorbate has caused hypersensitivity reactions, particularly contact urticaria, in certain population subgroups.

Sodium sorbate has been tested for genotoxic activity in various *in vitro* and *in vivo* systems, both as freshly prepared and as stored solutions. These data indicate, that sodium sorbate is genotoxic in some *in vitro* tests and, after storage, in some *in vivo* tests, although the potency appears to be weak. The mechanism of the genotoxicity of sodium sorbate is unclear but is likely to be related to breakdown products formed in stored aqueous solutions. This instability does not occur with potassium sorbate or calcium sorbate solutions, which are the only salts with established technological use. These findings with sodium sorbate can therefore be set aside in the overall safety assessment of the other sorbates and sorbic acid.

In 1985 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) stated, that the use of the sodium salt instead of the calcium and potassium salts did not introduce any new toxicological problems, and extended the group ADI for sorbic acid and its salts to include also the sodium salt. However, the ADI of sorbic acid and its salts already covered the sodium salt according to the 1974 JECFA report. It is therefore not completely clear why sodium sorbate was on the agenda of the 1985 JECFA meeting as no additional toxicological report or specification was prepared.

The use of sodium sorbate appears to be very limited, and for that reason it is not included in the present proposal for a Community positive list of food additives nor in the title of this report. It is also reported not to be used any longer in Japan and it is proposed to remove it from the list of GRAS substances in the USA because of lack of use data.

Overall, the lack of evidence of carcinogenicity, demonstrated in several adequate studies in 2 species, allows the setting aside of the occasional positive result in the genotoxicity studies on sorbates except in the case of sodium sorbate. The latter has not been tested for carcinogenicity in any laboratory animal species.

The safety in use of the combination of sorbates and nitrites has been questioned. The results of studies on the formation of potentially mutagenic or DNA-damaging reaction products, when sorbic acid or potassium sorbate are present together with nitrite, are to some extent conflicting and not convincing. In some of the studies with positive results, even low concentrations of nitrite alone have given rise to positive results. Experimental data have shown that under normal conditions of use no hazard to human health arises.

4. Conclusion

In 1973 JECFA established an acceptable daily intake (ADI) for man of 0-25 mg/kg b.w. as the sum of sorbic acid and its calcium, potassium and sodium salt expressed as sorbic acid. This estimate was based on the then available data, particularly the NOEL of 2500 mg/kg b.w./day in the long-term study in rats, and using a safety factor of 100. The more recent long-term study in rats, using however only the two dose levels 750 mg/kg b.w. and 5000 mg/kg b.w., showed that at 5000 mg/kg b.w./day changes in the relative weights of some organs occurred. In this study the dose level of 750 mg/kg b.w. would thus be the apparent NOEL.

In the more recent long-term study in mice changes in organ weights were noted at the two highest dose levels of 7000 and 14000 mg/kg b.w. but none at the dose level of 1400 mg/kg b.w. The apparent NOEL in this study was therefore 1400 mg/kg b.w.

Neither of these studies unfortunately included the intermediate dose level of 2500 mg/kg b.w. Taking into account the dose levels used in the studies evaluated by JECFA and those used in the more recent studies, there is no reason to alter the conclusion of the 1974 JECFA assessment, that 2500 mg/kg b.w./day is the best approximation to the NOEL for both mice and rats.

Level causing no toxicological effect:	Rat: 50000 ppm (5%) in the diet, equivalent to 2500 mg/kg b.w./day
Estimate of acceptable daily intake for man of sorbic acid and its calcium and potassium salts:	0-25 (*) mg/kg b.w.

(*) group ADI expressed as sorbic acid

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OPINION ON SULPHUR DIOXIDE AND OTHER SULPHITING AGENTS USED AS FOOD PRESERVATIVES

EXPRESSED ON 25 FEBRUARY 1994

1. Background

Sulphur dioxide (E220) and various salts of sulphur (IV) oxoanions have been widely used as food preservatives for many years. These compounds (listed below) are commonly referred to as the "sulphiting agents".

Sulphur dioxide	E220
Sodium sulphite	E221
Sodium sulphite	E222
Sodium metabisulphite	E223
Potassium metabisulphite	E224
Calcium sulphite	E226
Calcium bisulphite	E227

The SCF reviewed the toxicology of these compounds in 1981[1]. The Committee then noted that there was a large endogenous metabolic turnover of sulphite (ca 20-40 fold the estimated dietary intake) and considered that it was not necessary to set an ADI at that point in time. The SCF concluded that for the great majority of people no hazard to health would arise from the use of sulphiting agents to preserve food. Since that time, there have been a number of publications which have reported bronchoconstriction and bronchospasm in asthmatics challenged with acidified drinks containing sulphite[2]. It is therefore timely to further review these chemicals.

2. Animal toxicology

The Committee noted that there are extensive toxicological data available in animals which indicate that sulphiting agents have little or no systemic toxicity[3]. These results are not surprising in view of the rapid metabolism to sulphate and elimination of these compounds in laboratory animals. Sulphiting agents are not carcinogenic, mutagenic or reproductive toxicants in animals. Furthermore, no evidence of any systemic effects attributable to sulphites has been reported in short term studies in rats with an induced deficiency in sulphite metabolism[4].

The only treatment-related effects in animals attributable to sulphiting agents are localised changes in the stomach, of dose-related severity, which have been noted in several species including, in early studies, dogs, rabbits, rats, mice, guinea pigs and cattle[7, 8]. It is possible that in the early studies some of these gastric changes and related toxic effects may have been attributable to thiamine deficiency since sulphites destroy thiamine in the diet. However, similar though less marked changes have also been observed in more recent studies in pigs and rats in which thiamine deficiency was prevented by addition of a thiamine supplement to the diet[5-10]. They included inflammatory changes and hyperplasia in the stomach. At very high doses, decreases in growth rates and food consumption were also seen in pigs, probably due to unpalatability of the diet, and anaemia secondary to severe haemorrhage from stomach erosions was seen in rats.

An important consideration regarding the early tissue changes seen in the stomach in animals at lower doses is whether they are caused by repeated direct exposure of the stomach to sulphites, or whether they may be a specific but secondary change in the stomach which perhaps occurs after systemic absorption of sulphites. The gastric effects have been studied in some detail in rats and pigs and there is some evidence of a slight difference in the type of response seen in the rat and the pig[5-10]. In the rat, hyperkeratosis, acanthosis ulceration and intraepithelial microabscesses were seen in the forestomach. In the glandular stomach, inflammatory changes, necrotic cells and haemorrhagic erosions were observed, together with an unusual hyperplasia of the fundic glands in a small number of animals. The hyperplasia was limited to the chief (pepsin-secreting) cells at the base of the glands, an effect which is not seen with other known gastric irritants in rodents. In the pig, hyperkeratosis and hyperplasia of the epithelium were observed in the pars oesophagea and hyperplasia of the mucosal glands and surface epithelium were observed in the cardiac and pyloric regions of the stomach. Thus there are qualitative differences in the types of hyperplasia seen in rats and pigs exposed to sulphiting agents.

The No-Observed-Effect-Level (NOEL) for gastric irritation in long-term feeding studies in both rats and pigs was 70 mg/kg bw/day (expressed as sulphur dioxide equivalents)[5, 6]. After high dose treatments the effects on gastric pathology were reversible[6]. Lesions were no more numerous or pronounced in long-term than in short-term experiments, there was no evidence of stomach tumours in long-term studies on rodents or pigs, and some of the regressive changes (erosions and necrosis) seen in short-term experiments were not observed in long-term studies[5-10]. No evidence of any systemic effects was seen in either the rat or pig at doses of approximately 8 times the NOEL, the highest doses tested.

3. Human toxicology

Gastric reactions are also known to occur in man; with very high doses of sulphites, abdominal pains and vomiting have been observed in human volunteers[8, 11]. The possible gastric effects of lower doses of sulphite have not been studied.

Occasional severe asthmatic reactions, including deaths, have been recorded following the use of sulphiting agents in proprietary salad fresheners on vegetables[2, 13, 14]. Salad fresheners were popular in the USA for some time, but have not to our knowledge been used in Europe. There is one published case report of an asthmatic reaction following consumption of dried apricots[2]. Challenge studies showed that this individual reacted to sulphur dioxide vapour released from the apricots, which suggests that the release of sulphur dioxide vapour is an important step in the process leading to an asthmatic reaction following ingestion of foodstuffs preserved with sulphiting agents.

Dose-related respiratory hyper-reactivity has also been documented in a small number of individuals following consumption of potassium bisulphite-treated red wine and wine which contained smaller amounts of sulphites formed naturally as products of fermentation[12]. Although no confirmed case reports are available, the possibility cannot be discounted of asthmatic reactions to other alcoholic beverages, such as cider and beer, and to non-alcoholic beverages such as fruit juices, all of which can contain sulphiting agents.

The aetiology of the asthmatic reaction induced by sulphites present in foodstuffs has not been established, but a number of clinical studies in patients have provided evidence that this response may be mediated through stimulation of an oro-bronchial reflex by sulphur dioxide vapour released from foods, rather than by an immunological mechanism[15]. However, the occurrence of rare, immunologically-mediated anaphylactic reactions to ingested sulphites has also been reported[3].

Much of the sulphite in foods is bound in stable or unstable combined forms[2]. Further information on the relative proportion of free sulphur dioxide in foods and beverages at the point of consumption or released from combined forms, and which might potentially form a vapour following consumption would be valuable in assessing the relative risks associated with individual foodstuffs.

4. Discussion

The Committee reiterates its view that the use of sulphite preservatives in food products poses no health hazards to the great majority of people. It is possible that the gastric effects observed in animals could also occur in man if there were exposure to high levels of sulphite via the diet, but it should be noted that no such gastric reactions in response to levels currently found in food have been reported.

The gastric effects observed in rats, pigs and other species were similar but not identical. The reasons for these small species differences are unclear and may in part be due to differences in their anatomy. The majority of gastric changes could be described as irritant effects. However, the hyperplasia of the chief cells seen in the rat is not consistent with a simple irritant effect and it has been suggested that this might be a primary response to sulphites, rather than a reaction to injury, particularly as hyperplasia of mucous gland cells, which is common in the recovery of gastric irritancy lesions, was not seen in sulphite treated rats [9]. Such lesions could be caused by direct contact of sulphites with the chief cells or, possibly but less likely, caused by an indirect mechanism, perhaps by impairment of an entero-hormone feedback system[9]. The toxicological significance of these chief cell changes is unknown. Despite the observed histopathological differences, there is no difference between rats and pigs in the threshold dose level for gastric changes.

Whilst a number of the gastric changes described above might well be due solely to local direct contact with sulphite, it is not possible to rule out an indirect mechanism for some of the effects. This indicates that an Acceptable Daily Intake (ADI) should be set based on the no-effect level of 70 mg sulphur dioxide/kg bw/day in rats and pigs. In view of the unknown mechanism for the effect on the chief cells, and the observation of treatment-related occult blood in the faeces of rats at high doses and sporadically at lower doses (including at the no-effect level for stomach effects), there is no reason to deviate from the usual safety factor of 100, giving an ADI of 0 - 0.7 mg sulphur dioxide/kg bw. This should ensure that gastric reactions will not occur in man.

However, the Committee is concerned that occasional, severe, asthmatic reactions can occur, even at comparatively low levels of exposure. A numerical ADI would not prevent the occurrence of sulphite-induced asthma; ingestion of any food containing sulphites may be sufficient to trigger a reaction in those who are susceptible. The Committee considers that it is appropriate to view this reaction as a food intolerance reaction. It is not possible to estimate the number of individuals in the European Community that might be susceptible to asthmatic reactions from dietary sulphites with any degree of accuracy, as data on the prevalence of asthma in the Community as whole are not available.

The estimates of the proportions of asthmatics that may be sensitive to sulphites reported in the literature (1-4% of all asthmatics and 5-10% of steroid dependent asthmatics) are probably overestimates, since these figures were based on small studies using people referred from allergy clinics who had severe asthma and thus were not a random cross-section of the asthmatic or normal population[2,3].

Nevertheless, the Committee is concerned that there might be in excess of several thousands of individuals living in the European Community who are potentially at risk of experiencing asthmatic reactions to dietary sulphites. While the actual risk of a severe reaction may be very low, in view of the seriousness of the effects reported in some asthmatics and the likelihood that several thousands may be at risk of lesser reactions, the Committee considers that the uses of sulphites in food should not be greatly extended, even though the ADI might not be exceeded with further added uses. The Commission is urged to limit the use of sulphites where possible to those foods in which its technological actions are essential, so that the number of sulphite-containing foods asthmatics are likely to encounter does not further increase.

An important safeguard for those who know they are sensitive to sulphites is the facility to consult the labels on foods and beverages. The Committee considers that EC labelling regulations should ensure that the presence of added sulphite in foods and non-alcoholic beverages is always indicated in the list of ingredients. We do not consider that any additional warning about the presence of sulphites is necessary. However, the Committee is concerned that no such labelling is required for alcoholic beverages and recommends that the presence of added sulphite should be declared on labels of alcoholic beverages. In addition, action should be taken to disseminate information about possible reactions to sulphites in foods and beverages to the medical profession and those suffering from asthma.

5. Recommendations

The following recommendations were agreed:

i) An ADI of 0-0.7 mg sulphur dioxide/kg bw is appropriate.

ii) Whilst sulphites do not pose a health hazard to the great majority of people, they may pose a serious hazard to some people suffering from asthma. The use of sulphites should therefore be limited where possible to those foods where there is a sound technological justification for their inclusion, in order that the number of sulphite-containing foods asthmatics are likely to encounter does not increase.

iii) Sensitive individuals should be able to identify the presence of added sulphites in foods and non-alcoholic beverages from labelling of ingredients and, if sulphites have been added to foods or beverages, they should be listed in the ingredients label irrespective of the final amount present. However, alcoholic beverages are currently exempt from such labelling. Since some wines are known to contain added sulphites or sulphites carried over from their use as production/processing aids, the Committee recommends that labelling be extended to include the declaration of the presence of sulphites in alcoholic beverages.

iv) The attention of the medical profession should be brought to the possibility of respiratory hyper-reactivity in a small proportion of mainly steroid dependent asthmatics following exposure to sulphur dioxide and other sulphiting agents. Sensitive individuals should be made aware of the possibility of asthmatic reactions to sulphites in foods and beverages.

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OPINION ON BENZOIC ACID AND ITS SALTS

EXPRESSED ON 25 FEBRUARY 1994

1. Terms of reference

To advise on the safety in use of benzoic acid and its salts as food additives.

2. Introduction

Benzoic acid and its salts have been extensively used as preservatives in food over many years. The Scientific Committee for Food has not previously evaluated or established an ADI for benzoic acid and its salts. Benzoic acid and its salts have been previously evaluated in 1974 by the Joint FAO/WHO Expert Committee on Food Additives, which established an ADI of 0-5 mg/kg bw, as a sum of benzoic acid and its salts (expressed as benzoic acid).

Benzoic acid occurs naturally in plants, especially fruits and berries, but intakes from natural sources are low in comparison with potential intakes from food additive uses. The Committee therefore considered it important to evaluate carefully the toxicological information on benzoic acid and its salts in relation to their food additive uses.

3. Summary of metabolism and toxicity data

Many of the pharmacokinetic observations and toxicological studies on benzoic acid and its salts were carried out some years ago and would not fulfil present day criteria. However, there is reasonable consistency in the repeat-dose toxicity data and the Committee regards most of the studies as helpful for safety evaluation purposes. The more recent studies on benzoate-induced depletion of glycine and of glycine metabolism in humans and animals may also explain some of the adverse effects observed in earlier studies.

Benzoate is a normal product of intermediary metabolism of phenylalanine and tyrosine and this results in human urinary excretion of a few tens of milligrams of benzoate/kg bw/day. Benzoate administered orally to man is rapidly absorbed and excreted in the urine within 14 hours. The main metabolite is its glycine conjugate, hippuric acid, with the glucuronyl conjugate and free benzoic acid as minor pathways of excretion. The rate limiting step in excretion of hippuric acid is the availability of glycine and this accounts for the glycine depletion which can occur when high doses of benzoate are administered. For example, in man the bolus dose of sodium benzoate causing 80% saturation of the maximal rate of hippuric acid secretion was found to be 28 mg/kg bw (expressed as benzoic acid).

In rats, a single intraperitoneal injection of sodium benzoate, at doses equivalent to 305, 610 or 1220 mg benzoic acid/kg bw caused depletion of plasma glycine levels to 50%, 47% and 34% respectively of control values. Early sub-chronic studies showed adverse effects on body weight and survival when high doses of 1-5% sodium benzoate in the diet were given. Addition of glycine to the diet reduced the severity of the body weight loss induced by benzoate administration.

An early long-term study in which 1.5% benzoic acid was given in the diet to rats for 18 months revealed no adverse effects other than a reduction in food intake and body weight, but was limited in scope and did not include histopathological observations. A later life-span carcinogenicity study in mice given sodium benzoate in the drinking water at a single dose equivalent to 3.4 g benzoic acid/kg bw/day showed an equivocal increase in mammary tumours. A study in rats given 1% or 2% sodium benzoate in the diet for 18-24 months, a dose equivalent to 425 or 840 mg benzoic acid/kg bw/day, was negative. However, there is some doubt as to whether either of these carcinogenicity studies adequately addressed histopathological changes other than neoplastic ones.

Benzoic acid was negative in gene mutation tests in bacteria (*Salmonella typhimurium*) and in yeast (*Saccharomyces cerevisiae*), with and without metabolic activation. However it was positive in tests for chromosomal aberrations in cultured rat cells *in vitro* and in a recombination (REC) assay. It also caused cytological effects in *Vicia faba* root mitotic cells including inhibition of DNA synthesis, induction of anaphase bridges and subsequent micronuclei.

A single teratogenicity study, reported in abstract only, in which sodium benzoate was given intraperitoneally up to 1000 mg/kg bw/day on selected days of pregnancy was said to have produced adverse effects at the top dose, but was inadequate for evaluation and the route of administration was inappropriate for assessment of the effects of dietary benzoate. An early multigeneration study in which rats were given the equivalent of 250 or 500 mg benzoic acid/kg bw/day over 4 generations was reported to be without effects on growth, fertility, lactation or survival.

In humans, acute toxicity symptoms from high doses are gastro-intestinal irritation, central nervous system effects and convulsions. These effects are rapidly reversible and attributable to disturbance of acid-base balance. In very early studies bolus doses of 25 mg/kg bw/day for 20 days caused irritation, discomfort and malaise, whilst doses up to 14 mg/kg bw/day for 88-92 days were said to be without visible effect and doses of 4.3-5.7 mg/kg bw/day for 62 days had no effect on haematology, urine composition or nitrogen balance. Glycine deficiency, as detected by measurement of urinary 5-oxo-proline, accompanied by nausea, bloating and epigastric discomfort, has been observed in humans within 2-3 hours of administration of sodium benzoate at doses ranging from 21-135 mg/kg bw (expressed as benzoic acid).

4. Conclusions and recommendations

The data available give adequate reassurance that the use of benzoic acid and its salts as food preservatives is temporarily acceptable. However, the role of glycine in the rate limiting step for hippuric acid formation from benzoic acid suggests that there may be a narrow margin between the metabolic demand for glycine and the rate at which glycine is formed or made available in the body. Glycine is not generally regarded as an essential amino acid but it has been suggested that in rapidly growing organisms glycine may be a conditionally essential amino acid and that this fine balance might be disturbed by benzoic acid. An adequate teratogenicity study using a dietary route of administration is therefore desirable.

The observations of clastogenic activity of benzoic acid *in vitro* indicate that it should be tested for clastogenic activity *in vivo* in peripheral lymphocytes or bone marrow in animals and that blood or bone marrow levels respectively of benzoic acid should be measured in such a study.

An overall no-effect level of 500 mg/kg bw can be taken from the long-term and multigeneration studies. The Committee considers that a 100-fold safety factor is appropriate, giving a temporary ADI of 0-5 mg/kg bw, as the sum of benzoic acid and its salts, expressed as benzoic acid. This t-ADI is below doses causing symptomatic effects in humans. Intolerance to benzoic acid in patients with asthma has been recorded but such observations are not relevant to the setting of an ADI. We wish to review the situation in 3 years time in the light of any new toxicological studies, along the lines suggested above, together with information on consumer intakes of benzoic acid and its salts, which we understand will then be available.

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OPINION ON HEXANE USED AS AN EXTRACTION SOLVENT

EXPRESSED ON 17 JUNE 1994

1. Background

In its second report on extraction solvents (Scientific Committee for Food, 1992) the Scientific Committee for Food had been presented with the results of a 90-day toxicity study on technical hexane (light petroleum) with a content of 58% n-hexane (TNO, 1989). In its report the Committee was unable to establish whether the no observed effect level (NOEL) of 40 mg/kg bw claimed by the study authors was truly a NOEL or a minimal effect level and therefore only gave the substance a temporary acceptance. At the same time the Committee recommended that information be sought on actual residues occurring in food to allow an evaluation as to whether the maximum residue limits in Community legislation remained appropriate and also that the limits should in future be specified in terms of n-hexane (the most toxic of the isomers).

2. Discussion

The Committee has now had the opportunity to evaluate the original slides from the 90-day toxicity study together with an additional analysis by the study authors (TNO, 1992) and is now satisfied that 40 mg/kg bw is a true NOEL.

The extraction solvents directive (EEC, 1988) currently provides for the use of technical hexane in the production or fractionation of fats and oils and the production of cocoa butter with a maximum residue limit of 5 mg/kg, in the preparation of protein products and defatted flours with a maximum residue limit of 10 mg/kg in foods containing them, in the preparation of defatted cereal germs with a maximum residue limit of 5 mg/kg, and in the manufacture of defatted soya products with a maximum residue limit of 30 mg/kg in the soya product as sold to the consumer. The Committee has been informed that for fats and oils, residues of less than 1 mg/kg can now be achieved. With respect to the remaining categories of foodstuffs, industry has asked that the existing legal limits be maintained but has provided only imprecise information concerning actual residues.

The latest 90-day study was performed with a technical hexane with an n-hexane content of 58% and had a NOEL of 40 mg/kg bw. If it is assumed that the effects seen were due only to n-hexane (which has been shown to be the most toxic of the isomers) this would lead to a calculated NOEL for the n-isomer of 23 mg/kg bw. On the assumption that the maximum content of hexane in any item of food to which consumers would be exposed is 30 mg/kg and that such foods might be consumed at a rate of 200 g per day (an extreme figure for total daily protein intake), the intake of hexane would equate to 0.1 mg/kg bw/day for a 60 kg person. Even if all of this hexane consisted of the n-isomer, a safety margin of around 200 would still exist between the level of exposure and the NOEL.

3. Conclusion

Given that a clear NOEL has now been established and that the present maximum levels as expressed in the extraction solvents directive give an adequate margin between the potential exposure and the NOEL, the Committee considers the continued use of hexane as an extraction solvent acceptable and sees no need from a toxicological point of view to change the limits in the directive.

The Committee notes that for fats and oils a lower residue limit of 1 mg/kg is now achievable and welcomes this development as a contribution to good manufacturing practice. In view of the imprecision of the residue data in relation to the other categories of foodstuffs for which hexane is permitted as an extraction solvent **the Committee recommends that confirmation be sought that in no instance will residues exceed 30 mg/kg in products sold to the consumer**.

With a confirmed NOEL and in view of the fact that the toxicity study from which it was derived was carried out on technical hexane, the Committee no longer sees a need to express the maximum limits as n-hexane as recommended in its second report on solvents.

The Committee wishes to re-iterate its statement from its first (Scientific Committee for Food, 1981) and second reports on solvents that specifications are required to limit the presence of unsaturated aliphatic hydrocarbons and polycyclic aromatic hydrocarbons.

The Committee also wishes to re-iterate its statement from its first report that as ethylmethylketone significantly increases the potential for n-hexane to induce neurotic effects and thereby to reduce the otherwise adequate safety margin, these substances should not be used together.

References

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OPINION ON LINDANE IN BABY FOODS

EXPRESSED ON 23 SEPTEMBER 1994

1. Terms of reference

The Committee is asked to advise whether there is any special need on health grounds for maximum limits for pesticide residues in relation to foods prepared for infants and young children.

2. Background

Limits on residues of pesticides are established at Community level in relation to certain primary agricultural products. There are no Community rules setting limits on pesticide residues in processed foods. Member States are therefore free to apply national rules, provided they are consistent with Member States' obligations under the Treaty of Rome with respect to intra-Community trade.

With respect to foods specially prepared for infants and young children ("baby foods"), some Member States adopt a general, across-the-board policy of requiring such foods to be free from pesticide residues. In such cases, the Member States have set limits in their legislation which reflect the limits of analytical detection; - i.e. although their legislation provides for certain, very low residues, the limits equate to, and are intended to represent, a zero tolerance. Other Member States have adopted a policy which provides that, unless other more specific limits apply, pesticide residues may be present in baby foods at levels determined by proportional carry over from the pesticides legally present in the primary, unprocessed ingredients.

The question arises, in relation to lindane and to pesticides in general, whether maximum residue limits established for foods in general, which take into account the ADI's and are implemented through a consideration of carry over, are acceptable and sufficient for foods prepared specially for infants and young children; whether specific limits should be set for pesticide residues in baby foods; or whether the concept of the ADI is not applicable to infants and young children and cannot therefore be used to establish any safe or acceptable level for pesticides in this category of foods.

3. Conclusions

The Committee has previously only considered those pesticides which also can be used as food additives. Thus lindane has never been evaluated by SCF.

The Committee noted, however, that lindane was evaluated on several occasions by JMPR, most recently in 1989, when an ADI of 0.008 mg/kg bw was allocated. The substance was evaluated on the basis of a wide range of toxicity tests including reproduction studies and studies where very young animals were exposed.

With a residue level of 0.04 mg/kg in baby food for example a child of 10 kg, would have to consume 2 kg of that food per day, an amount which is physiologically impossible, to reach a dose equalling the ADI. The Committee, therefore, has no reason to believe that a content of 0.04 mg lindane/kg baby food would cause reason for concern.

The Committee is presently reviewing the scientific basis for establishing ADI's in general and their applicability to infants and young children. The Committee intends to issue a report on this matter.

OPINION ON CROSS-LINKED SODIUM CARBOXYMETHYL CELLULOSE

(MODIFIED CELLULOSE GUM)

EXPRESSED ON 23 SEPTEMBER 1994

1. Terms of reference

The Committee was asked to consider the safety-in-use of the modified cellulose gum, cross-linked sodium carboxymethylcellulose (also known as Croscarmellose) as a disintegrant in sweetener tablets.

2. Discussion

The substance requested is an internally cross-linked form of the currently permitted food additive, sodium carboxymethylcellulose (E466). In considering the submission, the Committee took into account not only the toxicological data available on the substance itself but also that available on the "parent" gum, sodium carboxymethylcellulose. The parent substance, sodium carboxymethylcellulose is of low toxicity and has a long history of safe use. It is only poorly absorbed in man and laboratory animals and shows little degradation in the gastrointestinal tract. When sodium carboxymethylcellulose is modified to form Croscarmellose, the cross-linking makes it less soluble in water and in simulated gastric and intestinal environments than the parent substance. It is even less likely to be absorbed and degraded than the parent substance.

The available toxicity data on Croscarmellose itself include acute oral toxicity studies in rats and mice, a 13-week oral feeding study in rats, and a gene mutation study in bacteria. None of these indicated any significant toxic effects. The only effects observed (reduced body weight gain, reduced efficiency of food utilisation and intermittent incidence of soft, moist faeces) were caracteristic for rodents fed high amounts of non-nutritious, non-absorbed, high molecular weight materials. Similar effects were seen in animals given the parent substance, sodium carboxymethylcellulose, at similar doses.

It can be estimated that consumption of Croscarmellose would be no more than around 1 mg/kg bw/day for a 60 kg person using 12 sweetener tablets per day, each tablet weighing 90 mg and containing 6% Croscarmellose. It can be further estimated from the solubility data for Croscarmellose in simulated gastrointestinal fluids that a maximum of 0.05 mg/kg bw/day would be available for absorption.

The Committee was also provided with a specification and details of the method of production of Croscarmellose, including information on the identity of the reagents used for production and their residues in the finished product. The Committee was satisfied that these gave no cause for concern.

3. Conclusion

The extent of the toxicological data available is insufficient to establish an ADI but in the light of the limited intake that would result, the Committee agreed that sodium carboxymethylcellulose, cross-linked, is acceptable for use as a disintegrant for sweetener tablets.

References

Modified cellulose gum, a disintegrant for use in sweetener tablets. Submitted by FMC Corporation NV, Belgium, May 1991.

Supplementary information on modified cellulose gum (sodium carboxymethylcellulose, cross-linked). Submitted by FMC Corporation NV, Belgium, August 1992.

Further supplementary information on modified cellulose gum (sodium carboxymethylcellulose, cross linked). Submitted by FMC Corporation NV, Belgium, January 1994.

OPINION ON INVERTASE DERIVED FROM SACCHAROMYCES CEREVISIAE

EXPRESSED ON 23 SEPTEMBER 1994

1. Terms of reference

The Committee was asked to review two dossiers on invertase. The dossiers were presented as required in the SCF "Guidelines for the presentation of data on food enzymes" (27th Report Series) and contained information on the enzyme, the source material, manufacturing process and purification methods. The enzyme is prepared by good manufacturing practice and is being tested for content of contaminants.

2. Conclusion

The enzyme preparations concerned are derived from *Saccharomyces cerevisiae*. On the grounds that the source organism has a long history of safe food use and, by virtue of this fact is considered to be acceptable by the Committee in its Guidelines, the Committee agreed that invertase preparations derived from *Saccharomyces cerevisiae* are acceptable for food use. Members stressed that while this acceptance applies to all invertase preparations from *Saccharomyces cerevisiae*, it is subject to the commercial product being in compliance with the general requirements and specifications set out in the Committee's Guidelines (27th Series of Reports, pages 18-19) and the fact that the source organism has not been subjected to any recombinant genetic engineering (i.e. is not one in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination).

References

Reports of the Scientific Committee for Food (Twenty-seventh series), EUR 14181, Office for Official Publications of the European Communities, Luxembourg, 1992.

OPINION ON AFLATOXINS, OCHRATOXIN A AND PATULIN

EXPRESSED ON 23 SEPTEMBER 1994

1. Terms of reference

The Committee is asked to make a preliminary evaluation of risk to public health resulting from dietary exposure to the following contaminants:

- Aflatoxins B_1, B2, G_1 and G_2
- Ochratoxin A
- Patulin

2. Background

In the light of moves in some Member States to introduce national limits for certain mycotoxins in food, the Committee was asked to carryout an urgent evaluation of the status of the recommendations of international bodies concerning the toxicology of these substances. In particular, the Committee was requested to advise the Commission on the possibility of using for Community purposes, possibly on a provisional basis, the tolerable intakes or other recommendations established by JECFA or other international organisations.

The urgency of the question did not permit the Committee to estimate dietary exposure to these mycotoxins which will be addressed at a later stage.

During the course of the evaluation, the Committee also reviewed recommendations of international organisations in relation to aflatoxin M_1. The conclusions for this substance are included under the section dealing with aflatoxins for the sake of completeness.

3. Conclusions

Aflatoxins B_1, B_2, G_1 and G_2

Aflatoxins are produced by three *Aspergillus* species, i.e. *A. flavus*, *A. parasiticus* and the rare species *A. nomius*. It is generally considered that *A. flavus* produces aflatoxins B_1 and B2, whereas *A. parasiticus* produces aflatoxins B_1, B2, G_1 and G_2.

The aflatoxin producing *Aspergillus* species, and consequently dietary aflatoxins contamination, are ubiquitous in areas of the world with hot, humid climates. Since countries in colder climatic areas import food from areas where aflatoxin levels are high, however, aflatoxins are of world-wide concern. Aflatoxins are frequent contaminants of corn, peanuts, dried figs, brazil nuts and other agricultural products from subtropic and tropic areas. There are many surveys on the occurrence of aflatoxins in foods and feeds.

Aflatoxin B_1 is the most frequent type present in contaminated samples (60-80% of the total aflatoxin content) and aflatoxins B_2, G_1 and G_2 are generally not reported in the absence of aflatoxin B_1. Aflatoxins B_2 and G_2 are typically present in much lower quantities. Depending on the method of analysis, the detection limit is 0.01 - 10 µg/kg. The limits of detection of routine methods used internationally are in the range of 5 - 10 µg/kg.

Intake of a large quantity (milligrams) of aflatoxins has a number of toxic effects (primarily an acute toxic effect in the liver) and intake of large or smaller quantities has a carcinogenic effect on the liver.

Very extensive assessments of the toxic effects of these substances have been carried out among others by the World Health Organisation (WHO) (1987), Danish report (1989), Kuiper and Goodman (1991), International Agency for Research on Cancer (IARC) (1993).

The potential carcinogenicity of aflatoxins has been examined in a large number of population studies, both cohort and correlation studies. Most of them were carried out in Africa and Asia, where substantial quantities of aflatoxins occur in basic foodstuffs. It can be concluded that there is good accordance between the results of practically all the existing population studies, even though these have been carried out in population groups where other known risk factors for liver cancer such as hepatitis B-virus and alcohol, vary considerably. IARC concluded in 1993 that there is sufficient evidence in humans for the carcinogenicity of naturally occurring mixtures of aflatoxins, and for the carcinogenicity of aflatoxin B_1. The overall evaluation of IARC was: "Naturally occurring aflatoxins are carcinogenic to humans (Group 1)".

Aflatoxins are extremely potent carcinogens in animal experiments and they are potent in all animal species investigated, i.e. mice, rats, hamsters, fish, duck, tree shrews and monkeys, and in several organs, the liver being the primary target.

A linear dose-response relationship has been demonstrated for aflatoxin B_1 in at least two animal species down to doses of less than 0.1 µg/kg b.w./day. Although aflatoxin G_1 has been tested less extensively, it appeared to be toxicologically similar to aflatoxin B_1. It is a slightly less potent liver carcinogen, with a comparable carcinogenic potency to aflatoxin B_1, i.e. within a factor of 10.

Much less data are available describing the toxic/carcinogenic potential of aflatoxin B_2 and aflatoxin G_2.

IARC concluded in 1993 that there is sufficient evidence in experimental animals for the carcinogenicity of naturally occurring mixtures of aflatoxins and aflatoxin B_1 and G_1; in experimental animals there is limited evidence for carcinogenicity of aflatoxin B_2 and inadequate evidence for the carcinogenicity of aflatoxin G_2.

Aflatoxins, especially B_1, have been tested extensiveley for genotoxicity. Aflatoxin B_1 is consistently found to be genotoxic, producing adducts in humans and animals *in vivo* and chromosomal anomalies in rodents and, in a single study, in rhesus monkeys *in vivo*. It induces DNA damage, gene mutation, chromosomal anomalies and cell transformation in mammalian cells *in vitro*, in insects, lower eukaryotes and bacteria. Fewer studies have been performed, in descending order, with aflatoxins B_2, G_1 and G_2 but these showed a comparable genotoxic profile.

Aflatoxins are genotoxic carcinogens. For this type of carcinogen, it is generally felt that there is no threshold dose below which no tumour formation would occur. In other words, only a zero level of exposure will result in no risk.

Several mathematical and biological models have been used by different organisations to approximate the risk of tumour formation at low levels of aflatoxin exposure, based on human and animal data primarily for aflatoxin B_1. It should be noted that the decision as to which risk level is judged to be acceptable or tolerable is socio-political and goes beyond scientific assessment.

Similarity in the toxicological profile of aflatoxins B_1, B_2, G_1 and G_2, despite a restricted data base for the latter three and especially aflatoxins B_2 and G_2, justifies a risk assessment for all these aflatoxins as a group, based on the data of aflatoxin B_1. This group approach is moreover supported by the fact that aflatoxins G_1, B_2, and G_2 are generally not detected in the absence of aflatoxin B_1 and, if present, occur at lower quantities in the food.

In summary, based on the vast amount of data and recent evaluations on aflatoxins, the Committee agreed that there was no need for it to do further work in the area of toxicological assessment. It agreed with the recent evaluations of IARC (1993) with respect to the carcinogenicity and genotoxicity of the aflatoxins. From the many reports on risk assessment, it can be concluded that even very low levels of exposure to aflatoxins, i.e. 1 ng/kg b.w./day or less still contribute to the risk of liver cancer.

Aflatoxin M_1

Aflatoxin M_1 is a metabolic hydroxylation product of aflatoxin B_1. It can occur in the absence of the other aflatoxins. Human exposure occurs primarily via milk and milk products from animals that have consumed contaminated feed; it has also been found in human milk samples.

Aflatoxin M_1 produced DNA damage in rodent cells *in vitro* and gene mutation in bacteria. With respect to the carcinogenicity of aflatoxin M_1, IARC (1993) concluded that there is inadequate evidence in humans, but sufficient evidence in experimental animals (liver tumours). The overall evaluation of IARC was: "Aflatoxin M_1 is possibly carcinogenic to humans (Group 2B)".

The Committee concluded that there is sufficient evidence that aflatoxin M_1 is a genotoxic carcinogen; its carcinogenic potency is estimated to be approximately 10 times lower than aflatoxin B_1.

Patulin

Patulin is a mycotoxin produced by fungi belonging to several genera, including *Penicillium*, *Aspergillus* and *Byssochlamys* species. Although patulin can occur in many mouldy fruits, grains and other foods, the major sources of patulin contamination are apples and apple products.

Patulin is characterised by its strong affinity for sulphhydryl groups. Patulin adducts formed with, for example, cysteine in the diet are less toxic than the unmodified compound in acute toxicity studies, teratogenicity and mutagenicity studies. The affinity of patulin for sulphhydryl groups explains its inhibitory activity on many enzymes.

As patulin adducts are formed in the diet and the major exposure of humans to patulin is via fruit drinks such as apple juice and apple cider, the most relevant studies to consider for toxicological evaluation are those in which patulin was administered in solution by gavage or dissolved in drinking water.

In acute and short-term studies, patulin caused gastrointestinal hyperaemia, distension, haemorrhage and ulceration. In long-term studies (at lower dose levels) these effects were not observed. Short-term *in vitro* studies revealed that patulin is not mutagenic, but that it has clastogenic activity in some test systems. No clear teratogenic effects were published. In a combined reproduction/long-term/carcinogenicity studies in rats and in long-term studies in mice and rats no carcinogenic properties were established (JECFA, 1988). Based on these combined reproduction/long-term/carcinogenicity studies, JECFA allocated a Provisional Tolerable Weekly Intake (PTWI) of 7 µg/kg b.w. (JECFA, 1988). The JECFA review did not include the results of a subchronic study in rats with patulin administered through drinking water in which the most sensitive effect was impairment of the kidney function (decreased creatinine clearance) and hyperaemia in the duodenum. The no observed adverse effect level in this study was 0.8 mg/kg b.w. In the same study, a different response was found in conventional and in specific pathogen free laboratory animals based on the antibiotic effect of patulin in gut micro flora, leading to mortality at a much lower dose level in conventional animals (Speijers et al. 1988).

IARC (1986) concluded that no evaluation could be made of the carcinogenicity of patulin to humans and that there is inadequate evidence in experimental animals. The overall evaluation of IARC (1987) was: "Patulin is not classifiable as to its carcinogenicity to humans (Group 3)".

The Committee agrees for the time being with the JECFA and IARC conclusions. It proposes to reconsider its opinion in the light of new information.

Ochratoxin A

Ochratoxin A is a mycotoxin produced by several fungi (*Penicillium* and *Aspergillus* species), and occurs naturally in a variety of plant products such as cereals, cereal products, coffee beans, beans and pulses all over the world. It occurs also by transfer from feed in animal products especially in organ meat (kidney, liver, blood) and it is even detected in human blood.

Ochratoxin A causes a number of toxic effects in laboratory animals, primarily of a teratogenic, immunological, nephrotoxic and carcinogenic (mainly urinary tract tumours) nature. The most sensitive and notable effects are the nephrotoxicity and the kidney tumours (Dirheimer and Creppy, 1991). According to IARC (1993), there is sufficient evidence in animals for carcinogenicity of ochratoxin A and inadequate evidence in humans for carcinogenicity. The overall evaluation of IARC was: "Ochratoxin A is possibly carcinogenic to humans (Group 2B)". Ochratoxin A does not induce mutations in *in vitro* systems, but it induces DNA-damage in rodent cells *in vitro* and in rodents *in vivo*. A rat hepatocyte culture medium mediated mutagenic response was demonstrated in *S. typhimurium*:. Ochratoxin A also formed DNA-adducts in mouse kidney and to a lesser extent in liver and spleen (IARC 1993).

Ochratoxin A has also been associated with the nephropathy in humans from the Balkan area (Balkan Endemic Nephropathy - BEN), and with the occurrence of kidney tumours (Ceovic et al. 1992). JECFA (1991) concluded that the epidemiological data on BEN and kidney tumours were not conclusive with respect to the role of ochratoxin A since other factors might also be involved. Ochratoxin A was evaluated by Kuiper-Goodman (1989), by JECFA (1991), and by the Nordic Working Group on Food Toxicology and Risk Evaluation (1991).

JECFA (1991) has based a provisional tolerable weekly intake value on the lowest observed effect level (0.008 mg ochratoxin A/kg b.w.) for kidney damage in pigs (the most sensitive species) and a 500-fold margin of safety. The PTWI was allocated at 112 ng/kg b.w./week (16 ng/kg b.w./day). The Kuiper-Goodman review and the report of the Nordic Group regard the carcinogenic property as the most important. The Nordic Working Group has estimated an acceptable safe level for ochratoxin A at 5 ng/kg b.w./day based on a lifetime risk level of $1:10^6$. Kuiper-Goodman estimated a safe level for ochratoxin A at 0.2 ng/kg b.w./day at the same risk level.

The Committee agrees that ochratoxin A is a potent nephrotoxic agent, a carcinogen and that it has genotoxic properties. The genotoxic effect may also be explained by an indirect mechanism involving impaired protein synthesis.

The Committee concluded that although the risk assessments are on different toxicological end points there was a broad agreement between the calculated values (16, 5 and 0.2 ng/kg b.w./day) and provisionally supports the conclusion that an acceptable safe level of daily exposure would fall in the range of a few ng/kg b.w./day.

The Committee proposes to reconsider its opinion in the light of new information.

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The members are independent persons, highly qualified in the fields associated with medicine, nutrition, toxicology, biology, chemistry, or other similar disciplines.

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