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REPORTS OF THE SCIENTIFIC COMMITTEE FOR FOOD

(forty-first series)

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OPINIONS OF THE SCIENTIFIC COMMITTEE FOR FOOD ON:

Opinion on colours in foods for special medical purposes for young children

Opinion on maximum limits for vitamins and minerals in processed cereal-based foods and baby foods

Opinion on the potential for adverse health effects from the consumption of genetically modified maize (zea mays l)

Opinion on β-cyclodextrin manufactured by the action of the enzyme cycloglycosyltransferase obtained from *bacillus circulans* on partially hydrolysed starch

Opinion on foods for special medical purposes (FSMPS)

Opinion on the safety in use of konjac gum as a food additive

Opinion on the safety in use of konjac glucomannan as a food additive

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OPINION ON COLOURS IN FOODS FOR SPECIAL MEDICAL PURPOSES FOR YOUNG CHILDREN
(expressed on 13 December 1996)

Terms of reference

To advise on the acceptability of the use of Carotenes (E 160a), Beetroot red (E162), Chlorophylls (E140) and Anthocyanins (E163) in foods for special medical purposes (FSMP) for young children aged 1-3 years.

Background

According to Council Directive 91/321/EC, "infants" means those below 12 months of age and "young children" those between 12 and 36 months of age (1). The use of additives in FSMP for infants and young children is controlled under Part 4 of Annex VI of the European Parliament and Council Directive 95/2/EC on food additives other than colours and sweeteners (2). This restricts the additives permitted in FSMP to those listed in Parts 1-3 of Annex VI to this same Directive which cover additives permitted in infant formulae, follow-on formulae and weaning foods for children in good health. None of these permitted additives are colours. Annex II of European Parliament and Council Directive 94/36/EC on colours for use in foodstuffs (3) lists foods for infants and young children including those not in good health as one of the categories of foodstuffs which may not contain added colours. The four colours requested are however all permitted at quantum satis levels in foodstuffs generally, other than those foodstuffs listed in the colours Directive as having restrictions.

General considerations

In considering the request to use four colours in FSMP for young children, the Committee was mindful of the principle set out in its earlier reports that it is prudent to keep the number of additives used in foods for infants and young children to the minimum necessary (4, 5). The Committee continues to endorse this principle, but has also acknowledged earlier that nutritional adequacy requires that food be palatable to children (4). In this context, the Committee considered that the use of colours in FSMP for young children could represent a special case.

The Committee noted that for some young children FSMP will form the major source of nutrients and that it is important to ensure that there is good compliance with these strictly controlled diets. The roles which added colours and flavours may play in the acceptability and

palatability of FSMP, which would otherwise generally be rather bland, is difficult for the Committee to judge.

The Committee was presented with limited evidence from acceptability trials (6). Thirty-three children (age not stated) suffering from a variety of conditions and receiving different FSMP in UK hospitals were given first uncoloured, unflavoured products for a short period. Compliance ranged from 0-11%. They were then given equivalent coloured, flavoured products and compliance improved to 79-100%. Two other studies in France and the UK have investigated long-term compliance with coloured, flavoured products in children aged from 2 years upwards who had been showing poor compliance with diets containing uncoloured, unflavoured products. In both studies product acceptability and long-term compliance improved. The Committee notes these findings and the fact that there is laboratory evidence that colour plays an important role in determining the perception of flavour and the acceptability of foods in general (6, 9). However, no acceptability trials on FSMP have been presented which examine separately the roles of added colour and of added flavour. The Committee is further unable to judge whether colours are required to ensure good compliance from as young as 12 months of age.

FSMP are intended for use for a very heterogeneous group of medical conditions and it should be noted that the views on safety which the Committee offers in this opinion can only be based on general considerations rather than considerations of the precise nature of the various illnesses and conditions for which they are intended for use.

Relevant existing Scientific Committee for Food (SCF) opinions

These colours have all been considered by the SCF in 1975 (7) and deemed either acceptable without establishing an Acceptable Daily Intake (ADI) or, in the case of beta-carotene, an ADI has been established. The SCF views from 1975 may be summarised as follows:-

Carotenes	E160a(i)	mixed carotenes from natural food sources: acceptable for food use generally
	E160a(ii)	synthetic beta-carotene: ADI of 0-5 mg/kg bodyweight
Beetroot red	E162	acceptable for food use generally
Chlorophylls	E140	chlorophylls and chlorophyllins obtained from natural sources: acceptable for food use generally

Anthocyanins	E163	anthocyanins prepared by physical processes from natural foods: acceptable for food use generally

Intake considerations

The maximum levels of use requested for these colours in FSMP in dilute products as consumed are as follows:-

Carotenes	E160a	30 mg/L
Beetroot red	E162	20 mg/L
Chlorophylls	E140	20 mg/L
Anthocyanins	E163	20 mg/L

Utilising the maximum levels of use requested and the recommended energy intake and fluid requirements for 1-3 year-olds, the maximum intake of each colour would be about 3 mg/kg bodyweight/day for Carotenes, and about 2 mg/kg bodyweight/day for each of Beetroot red, Chlorophylls and Anthocyanins. These estimates may be compared with estimates of intakes of these colours from all sources by normal children aged 1.5 - 4.5 years of age, derived from the British National Diet and Nutrition Survey, in which the 97.5th percentile intakes were estimated as 0.9. 1.3, 3.2 and 3.7 mg/kg bodyweight/day for Carotenes, Beetroot red, Chlorophylls and Anthocyanins respectively (8). Thus the estimated maximum intakes of colours for children consuming FSMP would not be significantly greater than the estimates for normal children for Carotenes and Beetroot red and lesser for Chlorophylls and Anthocyanins. The Committee notes that typical levels of use of colour in FSMP is around 10 mg/L rather than the maximum levels requested.

Toxicological considerations

Given these four colours have all been deemed acceptable for food use generally, the additional relevant safety consideration for the use of colours in FSMP is whether there is any likelihood that young children with particular medical conditions might react to these colours differently from normal children and adults. With the exception of children suffering from allergy to cow's milk or other whole proteins, who may be at higher risk of intolerance to other dietary constituents, there is no evidence to suggest that children requiring FSMP would

be more likely to experience adverse effects from these colours. The Committee also notes that these colours have been found to exhibit no or very low toxicity in studies incorporating high doses into the feed of rats, mice and dogs.

Beta-carotene

The applicants in different parts of the submissions (6, 9) have requested use of Carotenes and Beta-carotene. Beta-carotene has been administered in doses up to 1000 mg/kg bodyweight/day in sub-chronic, long-term, multigeneration and teratogenicity studies with minimal or no effects. It is negative in in vitro and in vivo mutagenicity studies (10, 11). Earlier literature on Beta-carotene suggested that is poorly absorbed unless given in oil (10). Some is stored in the liver and epithelial tissue. Some is converted to vitamin A and stored in the liver as vitamin A. Absorption of Beta-carotene has been quoted as lower in babies (2.6%) than in adults (11%) and conversion of Beta-carotene to vitamin A is said to be reduced in gastrointestinal, liver and kidney disease (12). However, there may be considerable individual variation in the absorption of Beta-carotene and in the extent of its transformation to vitamin A (13, 14). These and other aspects of synthetic Beta-carotene are being considered separately by the Committee. The Committee therefore considers that Mixed carotenes derived from natural foods normally consumed are acceptable for use in FSMP, but that until the separate review of Beta-carotene is completed, the Committee is unable to give a view on the use of synthetic Beta-carotene in FSMP.

Beetroot red

The major colouring principle of Beetroot red, betanine, has been reviewed by the SCF and by the FAO/WHO Joint Expert Committee on Food Additives (JECFA) (7, 15). It is poorly absorbed in the rat and mostly metabolised in the gastro-intestinal tract. Betanine was not carcinogenic when given to rats at 50-78 mg/kg bw/day in the drinking water or via subcutaneous injection. Neither did four different preparations of beet pigment initiate or promote hepato-carcinogenesis when given with N-nitrosodiethylamine. Beet red was not mutagenic in in vitro tests. JECFA considered an important safety consideration was to control the nitrate component of beet red via its specification because there was a need to limit nitrate in foods produced for infants and young children (16). The Committee notes that such a limitation would be necessary whether or not infants were in good health. The EU specification (Commission Directive 95/36/EC) limits nitrate to not more than 2g nitrate anion/g of red colour (17).

Chlorophyll

Chlorophylls are obtained by solvent extraction of grass or lucerne. The colour requested (E 140) refers to chlorophylls and chlorophyllins obtained from natural sources. In 1975, the SCF noted that no biological data were available for natural chlorophylls and did not establish an ADI but agreed that their use in food generally was acceptable (7). The Committee notes that in 1975 only chlorophylls obtained by physical processes from natural food sources normally consumed were discussed, whereas under the current EU Directive on colours, (3) chlorophylls from non-human food sources (e.g. grass) are allowed.

Anthocyanins

Anthocyanins represent a large group of 200 or more water-soluble plant pigments which occur naturally in a wide range of fruits and vegetables. They normally exist as glycosides, chemically combined to a sugar moiety (glucose, rhamnose, galactose, xylose and arabinose). In 1975, the SCF noted that no biological data were available and did not establish an ADI, but anthocyanins prepared by physical processes from natural foods were regarded as acceptable for use in food without further investigations (7).

Anthocyanins appear to be poorly absorbed from the gastro-intestinal tract in rats, dogs, rabbits and man, and do not appear to be metabolised. The coloured parent compounds are not found in human urine as measured by the comparatively insensitive test of colouration. In rats, some anthocyanins may be degraded by bacteria in the gut and their breakdown products (mostly unidentified) absorbed to a limited extent (15).

No effects have been observed in subchronic studies in rats, guinea pigs and dogs, nor in a multigeneration reproduction study in rats in which anthocyanin or grape-skin extracts were given up to high doses in the diet (15). Anthocyanin extracts were not teratogenic in mice, rats or rabbits and were not mutagenic in in vitro tests (15).

Two of the most common anthocyanins, pelargonidin and delphinidin have been shown to inhibit aldoreductase in the lens of rats. Other anthocyanins extracted from grapes were shown to increase the activity of of alpha-glucan phosphorylase and glutamic acid decarboxylase, but inhibit glycerol dehydrogenase, malate dehydrogenase and hexokinase. The Committee notes that it is perhaps possible that these interactions with enzymes could have an effect in certain inborn errors of carbohydrate metabolism.[15]

Conclusions

Where toxicological data exist, it can be seen that the intrinsic toxicity of the four colours requested is extremely low. It has been estimated that extreme intakes of these four colours in FSMP by young children with medical conditions would be comparable to or less than those of normal children who are high consumers of foods containing these colours naturally.
Considering the question of whether any of these colours may pose special problems if present in FSMP because of the nature of the medical conditions of the children consuming them, there does not appear to be any obvious cause for concern from the information available. Although the biological information is limited, it is not anticipated that conditions such as inborn errors of metabolism, intestinal, liver or kidney disease, or general feeding difficulties, would predispose to problems with the use of any of these four colours. Only in the case of allergy might there be a predisposition to intolerance to colours (18). The Committee therefore considers that use of colours may be inadvisable in products for children with cows' milk or other protein allergies.

It is noted that there has already been considerable experience of the use of FSMP to which these colours have been added in young children whilst under close medical supervision and observation. FSMP can form the major part of their diet and no untoward effects have been attributed to the presence of colours in FSMP to date.

The Committee concludes that mixed carotenes, beetroot red, chlorophylls and anthocyanins are acceptable from the safety point of view for use in FSMP for young children from 12 months of age upwards, up to the maximum levels requested in the diluted product as consumed (mixed carotenes 30 mg/L, chlorophyll 20 mg/L, beetroot red 20 mg/L and anthocyanins 20 mg/L).

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OPINION ON MAXIMUM LIMITS FOR VITAMINS AND MINERALS IN PROCESSED CEREAL-BASED FOODS AND BABY FOODS

(expressed on 13 December 1996)

Mandate

To advise on the maximum levels, where necessary, for vitamins, minerals, trace elements and other nutritional substances added to processed cereal-based foods and baby foods.

Regulatory situation

1. In conformity with the provisions of article 5 of Commission Directive 96/5/EC of 16 February 1996 on processed cereal-based foods and baby foods, only the vitamins, minerals, trace elements, amino acids and other nitrogen containing compounts listed in Annex IV of the directive may be used in the manufacturing of these products [1].

2. The only specifications with respect to these substances to be found in Annex I (cereal based foods) and Annex II (baby foods) of the directive concern sodium, calcium, thiamin, vitamin C, vitamin A and vitamin D. Minimum values are stipulated for calcium in products mentioned in articles 1(2)(a)(ii) (cereals which are to be reconstituted with water) and 1(2)(a)(iv) (milk biscuits), for thiamin in processed cereal-based products, for vitamin C in fruit juices, nectars or vegetable juices, and for vitamin A in vegetable juices. Maximum limits are set for sodium in cereal-based foods and baby foods. Minimum and maximum limits, identical to those for follow-on formulae (Directive 91/321/EEC), are provided for vitamins A and D in cereal-based foods (the addition of vitamin D in baby foods is not permitted). Further, it is stressed that the addition of amino acids is only allowed to improve the nutritional value of the protein mixtures and only in quantities necessary for that purpose (point 2.4 of annex I and point 1.5 of annex II).

General principles

3. Maximal values, when needed, should be fixed on the basis of two sets of considerations : 1) toxicological reasons when a demonstrated threshold of toxicity has been defined ; 2) and nutritional balance taking into account the paucity of knowledge about the long-term effects of chronic exposure to supra-physiogical intakes in infants and young children. Thus, an upper limit may be fixed for all added vitamins and minerals, even for the apparently non-toxic ones, or those whose toxicity appears to be extremely low. Indeed, it seems an equitable principle that if a specific nutrient is added

to a food, rather than being naturally present in the food, then a maximum should be set [2].

4. Data in food composition tables normally reflects the average statistical values rather than the true content. Baby foods are very often a combination of different types of food (for example, beef, carrots and potatoes). This variability leads to the large range of variations within the nutrient content. The usual calculation of the nutrient content, based on recipes, only provides rough information. Processing (e.g. heat treatment), other factors like pH, presence or absence of reactive elements (such as iron or copper), and storage conditions are further contributory factors. Moreover, the amount added should garantie that the declared amount of the nutrient, mainly vitamins, is still present on expiry of the minimum shelf life.

5. In assessing the maximum value, we have to consider that it is very improbable that the intake of baby foods by infants over the age of 4 months and young children (1-3 years) would exceed 6 jars of 200 g per day (i.e. 850 kcal/d based on an average energy content of about 70 kcal/100 g) [3].

Water-soluble vitamins

6. When the amount of a water-soluble vitamin consumed is greater than the required by the body, the excess is excreted. This explains why their toxicity generally is low and that informations available in adults suggest that the ratio between PRI and toxic levels is about 100 (Table I). However, no long-term exposure data are available for small babies, and it has been suggested that a reasonable cautious upper limit for infants should be set not higher than 5 times the present US RDAs [4].

7. Possible exceptions are thiamin, niacin and vitamin B_6 for which it has bee suggested that maximum levels in baby foods ready for consumption should not exceed 4 times, 2 times and 5 times respectively the EC Population Reference Intake (PRI) for infants per 100 g. Based on a consumption of 6 jars of 200 g per day containing such levels the daily intake of these three vitamins would not exceed one tenth of doses, as extrapolated from the adult to 4 month infants, suspected to have toxic effects if ingested over a long period [3].

8. Vitamin C could also be a matter of concern if added in very large quantities in baby foods. There also it has been suggested that vitamin C levels should not exceed twice

the PRI per 100 g of solid foods and 4 times for fruit/vegetable juices, since the total amount of juice consumed is likely to be less than the amount of solids [3].

9. The toxicity risk of adding water soluble vitamins to baby foods and processed cereal-based foods for infants and young children is negligible. However setting maximum limits is prudent for nutritional considerations and we recommend the nutrient density of the water-soluble vitamins in baby's foods, expressed for 100 kcal of foods as ready for use, does not exceed about 50 % of the EC PRI for young children, i.e. approximately one half of the references values for nutrition labelling (annex V) of the directive 96/5/EC. The same reasoning should apply to biotin and pantothenic acid although no PRI has been established for them ; the upper limit should be inspired in these cases by the safe and adequate daily intakes for 1-3 year-old children suggested by the Food and Nutrition Board [5], i.e. 20 µg and 3 mg, respectively (Table II).

10. An exception should however be made for thiamin in cereals, for vitamin C in products enriched with iron, and for vitamin C in fruit juices, nectars and vegetable juices. Article 6.1 of annex I of directive 96/5/EC stipulates that the amount of thiamin shall not be less than 100 µg per 100 kcal for processed cereal-based foods ; it seems therefore reasonable in this case that the maximum limit does not exceed 5 times the minimum, i.e. 0.5 mg/100 kcal. As for vitamin C, article 5 of annex II of the above mentioned directive provides that in fruit juices and nectars and vegetable juices the content should not be less than 25 mg per 100 g or per 100 kcal, that is 100 % of the PRI for this age group ; given the relatively low consumption of these products by infants and young children a maximum value of 125 mg per 100 kcal could be recommended. We have also to take into consideration the fact that iron absorption is improved by ascorbic acid; it is therefore necessary to set the maximum vitamin C level in such a way that optimum iron absorption is possible, which justify a maximum of 25 mg/100 kcal in products fortified with iron. The same vitamin C maximum of 25 mg/100 kcal also could be allowed for fruit-based dishes (Table II).

Fat-soluble vitamins

11. Maximum levels of vitamins A and D (180 µg RE and 3 µg cholecalciferol, respectively, per 100 kcal) that may be added to processed cereal-based foods which have to be reconstituded with milk of other appropriate nutritious liquids are already covered in the 96/5/EC directive. These limits are also applicable if vitamins A and D are added to other processed cereal-based foods. Vitamins A and D may not be added to other baby foods, the only exception being vegetable juices, in which the final

content of vitamin A in the product should be not less than 100 µg RE per 100 kcal [1].

12. Regular daily intakes of retinol should not exceed 900 µg/day in 6-12 months old infants and 1800 µg/day in children aged 1-3 years [6]. Vitamin A naturally present in vegetable juices is as carotenoids. An appropriate maximum for the amount of retinol added to vegetable juices, as suggested by the UK IDFA, whould be 90 µg/100 ml as consumed (one tenth of the maximum retinol intake per 100 ml). Infants with very high intakes of such juices, and consuming some retinol from other sources, should therefore be unlikely to exceed a total retinol of 900 µg/day [3]. The Committee agrees with this approach but suggests that the upper limit for vitamin A in vegetable juices should be set at the same level as for infant formulae and follow-on formulae, i.e. 180 µg RE per 100 kcal.

13. Vitamin E *per os* is practically not toxic. Few adverse effects were reported and none consistently. Most adults appear to tolerate doses of 200 to 600 mg/day, without evidence of toxicity. Some adults complain of gastrointestinal disturbances (diarrhoea and cramps) and interference with anticoagulation therapy has been reported [7,8]. Nevertheless, vitamine E supplementation is not generaly encouraged [5] and it has been recommended to limit the amount of vitamin E added to infant foods [9]. It is therefore more safe to set a maximum value for vitamin E in processed cereal-based foods and other baby foods. Under the circumstances the Committee recommends that the vitamin E content does not exceed half the NAS/NRC RDA, i.e. 3 mg a-TE per 100 kcal of the product ready to eat (Table II).

14. The situation is less clear with respect to vitamin K. "Recommended intakes" are set at 5 µg/day for the first 6 months, at 10 µg/day for the following 6 months and at 15 µg/day between 1 and 3 years [5]. A minimum of 4 µg/100 kcal has been suggested for infant formulae , adopted by the Codex Alimentarius [CODEX STAN 72-1981] and specified in directive 91/321/EEC on infant formulae and follow-on formulae. On the other hand, the COMA and the SCF considered that they had not enough data to make a recommendation but that an intake of 1 µg/kg body weight per day appears adequate and would be provided by a normal mixed diet [6]. Since no adverse sign has ever been reported from large oral doses of vitamin K, the Committee sees no reason to make a recommandation.

Minerals and trace elements

15. Calcium excess arising from dietary intakes is rare because of the effectiveness of the homeostatic mechanisms. In healthy adults intakes of 2.5 g are well tolerated but above this, as may occur after ingestion of supplements of calcium (and sometimes of vitamins) or of antacids there is a risk of renal stones, hypercalcaemia and impaired renal function. An upper limit of intake 2.5 g/d has been therefore advised, i.e. approximately 3-4 times the PRI for adult men [6]. Minimum values for calcium content of complete cereals to be reconstituted with water and milk biscuits have been set to 80 mg and 50 mg/100 kcal, respectively, in the above mentioned Directive. Even if there is little evidence of toxic effects of high calcium intakes in infants and young children, the Committee recommend that the calcium content of cereals which are to be reconstituted with water and milk biscuits does not exceed twice the minimum values required by the Directive, i.e. 160 and 100 mg/100 kcal, respectively. On the other hand, when calcium is added to other processed cereal-based products and to baby foods, which are not usually an important source of calcium for infants and young children, the Committee recommends that, if calcium is added, the total calcium content does not exceed one fifth the PRI for 1-3 year- old children i.e. 80 mg/100 kcal of the product as sold.

16. The very effective regulation of iron absorption prevents overload of the tissues by iron from a normal diet, except in individuals genetically susceptible (e.g. idiopathic haemochromatosis) [6]. The acute toxic dose in infants and young children has been estimated to approximately 20 mg/kg body weight and the lethal dose to about 200-300 mg ferrous iron/kg body weight. This gives an acute toxic dose of about 10-20 times the PRI for 1-3 year-old children (4 mg/d, assuming a bioavailability of iron from all the diet of 15 %). Nevertheless infants and young children normally receive already iron-fortified foods (e.g. infant and follow-on formulae). It is therefore appropriate to set a maximum level for the iron content of weaning foods [3]. Taking into account the fact that the absorbability of iron in weaning foods is probably lower than 15 %, possibly down to 10 % or even less if ferric salts are used, an upper limit of 75 % the PRI, i.e. 3 mg/100 kcal of the ready to eat product is advised.

17. Magnesium is ubiquitous in the diet ; both plants and meats are good dietary sources. Additionally the improved efficiency, on restricted intakes, of both intestinal absorption and renal conservation of magnesium has made it difficult for the SCF to propose any PRI with confidence. An acceptable range of intakes of 150-500 mg/d was therefore proposed. The difficulties in proposing a reliable PRI for adults are more pronounced in children for whom data are even more scarce. However, the quasi-PRI for the 1-3

year-old children, calculated on the basis of body weight, has been estimated by the Committee to 85 mg/d [6], a value which does not really differ from the NAS/NRC RDA for the same age group, i.e. 80 mg/d [5]. Since there is no evidence that large dietary intakes are harmful to humans with normal renal function, except for the induction of intestinal secretion and diarrhoea with amounts exceeding 10 times the acceptable range of intakes for adults, an upper limit of 50 % the present NAS/NRC RDA of 1-3 year-old-children, i.e. 40 mg/100 kcal, could be set [3].

18. A similar opinion can be adopted for manganese since manganese is particularly abundant in vegetable-based foods. If a manganese salt is added, however, in harmony with our general approach, it would be safe to set as upper limit 50 % of the NAS/NRC "estimated safe and adequate daily dietary intake" of 1-3 year-old-children, i.e. 0.6 mg/100 kcal.

19. A PRI of about 3 g/d has been proposed for potassium by the SCF and PRIs for ages up to 17 were estimated factorially (800 mg/d for 1-3 year-old children). In adults intakes of 17.5 g/d may induce symptomatic hyperkalaemia in some otherwise normal individuals. However, intakes above 6 g/d could be dangerous for individuals with undetected renal dysfunction. Since there is no apparent benefit of exceeding such intakes, this has bee proposed as an upper safe level of intake [6]. Since the safe level of intake in adults is only twice the PRI, the Committee recommends to set an upper limit for potassium when it is added to weaning foods. This upper limit, if potassium is added, could be set to one fifth the PRI of 1-3 year-old children, i.e. 160 mg/100 kcal of the product as sold.

20. High intakes of copper are toxic. The PRI for adults has been set by the Committee to 1.1 mg/d and for safety reasons an upper lmit of 10 mg/d has been proposed. The PRI for children has been calculated factorially, the value for 1-3 year-old children being set to 0.4 mg/d [6]. Daily intakes of copper for infants are 0.3-0.6 mg/d ; intakes for most infants should therefore be satisfactory, and fortification of weaning foods with copper is not only unnecessary, but is also inadvisable [3]. For all these reasons, the Committee recommends to limit strictly the addition of copper to weaning foods and to set an upper limit which not exceed one tenth the PRI of 1-3 year-old children, i.e. 40 µg/100 kcal of the ready to eat product.

21. Gross acute zinc toxicity has been described following the use of water which has been stored in galvanised containers. Prolonged intakes of 75-300 mg/d in adults have been associated with impaired copper utilisation, producing features such as microcytic anaemia and neutropenia, but even short term intakes of about 50 mg zinc daily interfere with the metabolism of both iron and copper. The PRI for zinc has been set to

9.5 mg/d but, to avoid interactions with iron and copper, the Committee has considered that it would be unwise to exceed a daily zinc intake of 30 mg in adults, i.e. three times the PRI. The PRI for 1-3 year-old children calculated factorially is 4 mg/d, a value possibly over generous [6]. In the absence of data on toxicity in infants, if the data for adults are extrapolated to infants and young children, then it would be appropriate to set a maximum for the zinc content of savoury weaning foods[1] to 50 % the PRI, i.e. 2 mg/100 kcal of the ready to eat product.

22. High iodine intakes cause toxic nodular goitre and hyperthyroidism. Such toxicity is rare in normal populations or individuals with an intake less than 5 mg/d but those with pre-existent iodine deficiency may be susceptible to developing toxic goitre at intakes below this. The SCF has set the PRI for iodine to 130 µg/d and intakes of 1-2 mg/d, i.e. 10 times the PRI, appear to be safe in adults. PRI for 1-3 year-old children, calculated from adult values on the basis of energy requirements, is 70 µg/d. The maximum limit for iodine in weaning foods should therefore be set to one half the PRI, i.e. 35 µg/100 kcal of ready to use products (Table III).

Other compounds

23. Carnitine, choline and inositol are listed in Annex IV, 3 of the Directive 96/5/EC. Indeed it has been suggested that newborn infants, especially the low-birth-weight infants, may have insufficient biosynthetic capacity to produce their full requirement. There is however no indication of any need for these compounds to be supplied in the diet of healthy individuals above the age of six months [6]) and no RDA can be established [5]. On the other side, there is no evidence of toxicity and no untoward effects after supplementation have been reported so far in humans. It seems therefore unnecessary to set an upper limit for these three compounds.

Vitamins and minerals in foods for infants and young children

24. According to the informations provided by the Association of the Food Industries for Particular Nutritional Uses of the European Union (IDACE), the practices of the industry regarding cereal-based foods and baby foods in the European Union are summarised below (IDACE, 95/647, 18 September 1995).

[1] According to the UK IFDA, addition of zinc to dessert/non savoury baby foods is inappropriate [3].

25. In the various Member States, the manufacturers tend to add a full range of vitamins and minerals to milk-based baby cereals. The rationale for these fortifications is that milk-based baby cereals are frequently used as early weaning foods when the diet first becomes diversified. Manufacturers try to provide a certain amount of vitamins and minerals to give similar levels of nutrients to those found in a milk-only diet (breast milk or infant formula).

26. Simple cereals, rusks and biscuits, and/or baby pasta are often, but not always fortified. In most European countries simple cereals are reconstituted with milk and are fortified with water-soluble vitamins (sometimes also with the fat-soluble vitamins A, D and E) and iron. Calcium is not often added. In Germany iodine is added due to paediatric recommendations. In Italy, on the contrary, simple cereals are generally reconstituted with a nutritious liquid, like a pure meat baby-food and water, to prepare a savoury dish. They are supplemented with calcium, but iron is not usually added. Baby pastas are practically only sold on the Italian market ; they have the same nutritional properties as simple cereals, but a different physical structure ; the fortification is similar to that of simple cereals and generally includes vitamins of the B group, calcium and iron. Rusks and biscuits can be compared with simple cereals (they are generally consumed as an accompaniment to a milk-based meal) and are usually fortified with vitamins of the B group and sometimes calcium and iron.

27. Savoury meals/dishes are the most important category of baby foods because they are the basis of the baby's diet. Generally they are not supplemented with vitamins and minerals, although a few are fortified with iron. Fruit-based products (fruit-only dishes, fruit juices and nectars) are all supplemented with vitamin C. Vegetable dishes sometimes contain high amounts of carotene from vegetable sources, and are supplemented in few cases with vitamin C, calcium and iron.

28. Vitamins and minerals are supplemented both in order to restore the natural content lost in the manufacturing process and to guarantee a minimum vitamin content at the end of the products life. A survey of compositional data, provided by the manufacturers (March 1996), is reassuring in indicating that, with few exceptions, the vitamin and mineral contents of products as consumed do not exceed the proposed maximum limits.

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Table I. Relative toxicity of water-soluble vitamins in adult [from 6].

	RDA[1]	PRI [2]	Toxicity
Thiamin (mg)	1.5	1.1	No evidence of toxicity of thiamin taken by mouth, at intakes up to 500 mg/day (for 1 month)
Riboflavin (mg)	1.7	1.6	No evidence of any toxicity of B_2 taken by mouth
Niacin (mg NE)	19	18	Nicotinic acid, but not nicotinamide, at doses of 500 mg/day, may result in liver damage
Vit B_6 (mg)	2	1.5	Intakes of more than 50 mg/day must be regarded as potentially harmful
Folate (µg)	200	200	Up to 5 mg per day would seem to be well tolerated
Vit B_{12} (µg)	2	1.4	A daily intake greater than 200 µg should be discouraged
Vit C (mg)	60	45	Up to 10 g/day seem not to be unsafe for healthy individuals

[1] for males 25 - 50 yrs

[2] for males 18+ yrs

Table II. Maximum limits for vitamins, if added, in cereal-based foods and baby foods for infants and young children.

	Labelling reference value [1]	Maximum per 100 kcal
Vitamin A (µg RE)	400	180 [4]
Vitamin E (mg a-TE)	6 [2]	3
Vitamin K (µg)	15 [2]	NS[5]
Vitamin C (mg)	25	12.5 [6] (125)[7]
Thiamin (mg)	0.5	0.25(0.5) [8]
Riboflavin (mg)	0.8	0.4
Niacin (mg NE)	9	4.5
Vitamin B_6 (mg)	0.7	0.35
Folate (µg)	100	50
Vitamin B_{12} (µg)	0.7	0.35
Pantothenic acid (mg)	3 [3]	1.5
Biotin (µg)	20 [3]	10

[1] Annex V of the directive 96/5/EC

[2] Recommended Dietary Allowances [5]

[3] Safe and adequate daily intake [5]

[4] Maximum value for vegetable juices

[5] NS : not specified

[6] Except for fruit-based dishes and for products fortified with iron for which the maximum should be 25 mg/100 kcal

[7] Maximum value for fruit juices, nectars and vegetable juices, in parentheses

[8] Maximum value for cereal-based foods, in parentheses

Table III. Maximum limits for minerals and trace elements, if added, in cereals-based foods and baby foods for infants and young children.

	Labelling reference value [1]	Maximum per 100 kcal
Potassium (mg)	800 [2]	160
Calcium (mg)	400	80(160/100) [3]
Magnesium (mg)	85 [2]	40
Iron (mg)	4	3
Zinc (mg)	4	2
Copper (µg)	400	40
Iodine (µg)	70	35
Manganese (mg)	NS [4]	0.6

[1] Annex V of the directive 96/5/EC

[2] PRI for 1-3 year-old children [6]

[3] Maximum values for products mentioned in article 1(2)(a)(i) and (iv), respectively, in parentheses

[4] NS : non specified

OPINION ON THE POTENTIAL FOR ADVERSE HEALTH EFFECTS FROM THE CONSUMPTION OF GENETICALLY MODIFIED MAIZE (ZEA MAYS L)

(Opinion expressed on 13 December 1996)

Terms of Reference

To consider whether there is reason to believe that the genetic modification of the maize lines of Zea mays L. will have adverse effects on the health of human consumers of the maize. The Committee is asked to give particular attention to the concerns raised by certain Member States with respect to any potential toxic or allergenic effects associated with the introduced genes and any potential adverse effects from the non-expressed β-lactamase gene.

Background

The Commission has submitted a proposal for a Council Decision concerning the placing on the market of genetically modified maize (Zea mays L.) with the combined modification for insecticidal properties conferred by the Bt-endotoxin gene and increased tolerance to the herbicide glufosinate ammonium pursuant to Council Directive 90/220/EEC.

Member States have expressed a variety of concerns which have led the Commission to request the opinions of the Scientific Committee for Food, the Scientific Committee on Animal Nutrition (SCAN) and the Scientific Committee on Pesticides to examine the dossier as concerns safety matters within their remits.

Evaluation

This evaluation addresses transgenic maize CG-00526-176. The submission included the administrative data necessary for its unique identification and for record keeping purposes.

1. Characterisation of the inserted genes and their expression

On the basis of the information provided, the inserted genes (*CRY1A(b)*, *bar* and *bla*) and their expression are characterised as follows:

- two plasmids have been inserted into the same locus in two to five gene copies.Their presence has been demonstrated by southern blotting.

- the product of the inserted genes, the CRYIA(b) protein (Bt-delta-endotoxin) from the two genes is expressed in the leaves and in pollen respectively but its concentration is below 5 ppb. However the toxin is apparently expressed in the kernel since in a bioassay study with the European corn borer, insecticidal activity was observed in fresh, but not in dried or re-hydrated kernels. Phosphinothricin acetyl transferase (PAT), responsible for the increased tolerance to the herbicide glufosinate ammonium, was not detectable in kernels, but traces were found in the plant. On the basis of current knowledge the prokaryotic *bla* gene construct (β-lactam antibiotic resistance) would not be expected to be expressed in the maize plant.

2. Toxicological assessment

2.1 Products of the *cryIA(b)* gene encoding Bt-delta-endotoxin:

Both the truncated maize *cryIA(b)* gene and the native *cryIA(b)* gene produce protoxins which undergo proteolytic cleavage in the mid-gut of insects resulting in the same active toxin. The native CRYIA(b) protein and the corresponding protein from transgenic maize, have similar target range effects thereby demonstrating the likelihood of similar biological properties for the two proteins.

The native CRYIA(b) protein (65% purity) has been tested for acute toxicity in mice and no mortality has been reported at a dose of 5 g per kg body weight. Furthermore, reports in the literature[1] of a 28 day study with mice on CRYIA(b) protein, did not reveal any mammalian toxicity at 1.5 g per kg body weight, the only dose level tested. Moreover, it was demonstrated that CRYIA(b) was rapidly degraded *in vitro* in simulated gastric fluid containing pepsin at pH 1-1.2. Since the CRYIA(b) product level in kernels is below 5 ppb, dietary exposure to CRYIA(b) from maize kernels is expected to be very low.

2.2 Products of the *bar* gene encoding phosphinothricin acetyl transferase (PAT)

The enzyme phosphinothricin acetyl transferase (PAT) is not likely to present safety problems. The quantitative level of PAT in kernels is very low. Its enzymatic function is specific to a substrate which is not naturally present in humans, namely phosphinothricin, and furthermore, it is degraded and inactivated in simulated gastric fluid containing pepsin at pH 1-1.2. It is therefore unlikely to retain any enzymatic activity *in vivo*. Furthermore, no sequence homology between the PAT protein and known toxins has been found. The native PAT protein (51% purity) has been tested for acute toxicity in mice and no toxicity has been reported at a dose of 5 g per kg body weight.

[1] Noteborn HUB, Kuiper HA 1995: Safety evaluation of transgenic tomatoes expressing *Bt* endotoxin. In: Application of the principles of substantial equivalence to the safety evaluation of foods or food components from plants derived by modern biotechnology. Report of a WHO workshop, Copenhagen, World Health Organisation, Food Safety Unit

3. Nutritional assessment

The newly expressed proteins have no nutritional significance and the composition of the transgenic maize is within the known biological variation of the composition of the host plant.

It is concluded that transgenic maize (event 176) is substantially equivalent to the corresponding non-transgenic maize from a nutritional point of view.

4. Allergenicity

The Committee expressed an opinion covering general aspects of food intolerance including allergenicity at its 98th Meeting (September 1995).

The amino acid sequences of the proteins CRY1A(b) and PAT do not show any homology with proteins of known allergenic potential. Moreover, the new gene products appear to be readily degraded by simulated gastric fluid *in vitro*. Comparison of the protein profiles of the transgenic maize and the native maize by SDS gel electrophoresis and iso-electric focusing give no indication that the maize protein have been changed. There are no indications that the prolamine proteins have been altered which is of relevance for patients with coeliac disease, but this possibility cannot be excluded. It is therefore concluded that it is unlikely that the genetic modification changes the potential for allergenicity in the kernel of the transgenic maize. This does not exclude the possibility that there will be individuals allergic to this variant of maize, just as there are individuals who are allergic to traditionally produced variants of maize.

5. Horizontal gene transfer

Studies of the transfer of intact genes from plant materials to micro-organisms have demonstrated an extremely low likelihood of transfer, suggesting that the probability of this event occurring in practice is very small. There is no evidence that genes from plants have ever been transferred under natural conditions to bacteria. In addition, the degradation of DNA occurring during processing of maize and its intestinal passage reduces this possibility even further. Bacteria with natural ampicillin resistance exist in the environment as well as in human intestines. Nevertheless, in the view of the SCF, the acquisition of additional resistance from this transgenic maize by intestinal bacteria needed special attention. The Commission convened an expert consultation on the subject, where SCF and SCAN together posed a number of questions to the specialised experts. From this consultation it is confirmed that the degeneration of DNA through processing of maize and its products and the enzymatic

decomposition of DNA in the gastrointestinal tract of man and animal makes the residual amount of intact DNA which could contain a gene very small. Furthermore, the probability for transfer of plant DNA by transformation to bacteria is small,–as is the chance for the transformed DNA to become functional in the bacteria. Even if this unlikely sequence of events, each of which has a very low probability, were to take place, it would have no detectable additional effect as the *bla* gene is already widely spread in nature including human and animal gastrointestinal tracts. Should transformed bacteria harbouring the high copy plasmid pUC18 of the transgenic maize arise, they would not have a competitive advantage and therefore would not lead to their spread and interference with therapy by beta-lactam antibiotics.

6. Assessment of secondary changes

In addition to the products of the inserted genes, a number of comparisons between the transgenic maize plant and the equivalent non-transgenic plant have been performed. A series of morphological parameters were examined as well as yield. DIMBOA (2,4- dihydroxy-7-methoxy-1,4-benzoxazin-3-one), one of the natural defence compounds of the maize plant towards, for example, the European corn borer was also examined. No significant differences in morphology, yield or DIMBOA content were observed. Differences between the concentrations of other components of toxicological or nutritional relevance in the genetically modified plant and the parent plant were statistically significant in some instances but, even so, the measured levels were still within the published reference biological variation for maize. Animal feeding studies with the genetically modified maize supported its substantial equivalence.

Conclusions

On the basis of the information provided the Committee draws the following conclusions:

- The transgenic maize is, except for the inserted traits, substantially equivalent to maize presently on the market.
- Animal feeding studies with the genetically modified maize support its substantial equivalence to the parent plant.
- No nutritional concerns are associated with the use of this transgenic maize.
- It is unlikely that the genetic changes introduce any new potential for allergenicity.

- No human toxicological concerns arise regarding the inserted traits based upon the toxicological and degradation data considered.
- The possibility that the product would add significantly to the already widespread occurrence of ampicillin resistant bacteria in animals and man is remote.

The latter conclusion was based on the balance of evidence available at this time to the Committee, which derived from theoretical considerations and laboratory studies. A stepwise assessment regarding the gene construct itself, its distribution and persistence in maize and its products, the possibility of its transfer from maize to gram negative bacteria, and the possibility that it would function in such bacteria led to the conclusion that the risk of bacterial transformation is extremely low.

The Committee was conscious of the general question of the use of genes coding for antibiotic resistance in marker gene constructs in the development of novel foods and proposes to scrutinise the future needs and application of marker genes.

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- SCF opinions on the assessment of novel foods III. Recommendations concerning the scientific aspects of the preparation of the initial assessment reports on applications for placing on the market of novel foods and novel food ingredients [CS/NF/GUID/4 Rev.2]

The scientific basis for the classification of the PUC-plasmids according to Directive 90/219/EEC. [CS/NF/MAIZE/18]

OPINION ON ß-CYCLODEXTRIN MANUFACTURED BY THE ACTION OF THE ENZYME CYCLOGLYCOSYLTRANSFERASE OBTAINED FROM *BACILLUS CIRCULANS* ON PARTIALLY HYDROLYSED STARCH
(expressed on 13 December 1996)

Terms of Reference

To evaluate the safety-in-use of ß-cyclodextrin (BCD) as a carrier and stabiliser, manufactured by the action of the enzyme cycloglycosyltransferase (CGTase) obtained from Bacillus circulans on partially hydrolysed starch.

Background

BCD is a food additive with potential application as a carrier and stabiliser of food flavours, food colours and some vitamins. It is a cyclic heptamer composed of seven glucose units joined "head-to-tail" by α-1,4 links. It is manufactured by the action of the enzyme CGTase on partially hydrolysed starch. JECFA has also evaluated BCD manufactured by the action of CGTase, obtained from Bacillus macerans, Bacillus circulans or related strains of Bacillus, on hydrolysed starch syrups, in 1993 and 1995 and has allocated an ADI of 0-5 mg/kg bw (1,2).

Evaluation

The manufacturing method described in the petition (3) uses a crude CGTase preparation, which is produced by heat treatment of the non-pathogenic microorganism Bacillus circulans. The information supplied on the process controls in the manufacturing process for the enzyme preparation, on the batch to batch consistency of the preparation and on its toxicity was limited (3-6). However, since the final BCD product is processed to a high level of purity before use and has been subject to extensive toxicological testing, the Committee considered that the data provided on the enzyme preparation were sufficient. However, the Committee considered that the source of the enzyme used to manufacture BCD should be cited in the specification for BCD.

The metabolism data submitted on BCD are limited, but indicate that, in both animals and humans, at low dietary concentrations BCD is probably not absorbed in the stomach or small intestine, but is degraded in the colon by gut microflora and possibly endogenous enzymic activity (7-14). There is evidence from a toxicity study in dogs (15-16) that, at a high dietary

concentration of 5% BCD, up to 6% of administered BCD is absorbed and excreted unchanged in the urine in this species.

The toxicity studies supplied on BCD manufactured as described in the application comprise a number of in vitro and in vivo mutagenicity studies (17-20), a 13-week study in the rat (21-22), one-year studies in the rat and dog (15-16, 23), a teratology study in the rat (24), a multigeneration study in the rat (25-26) and chronic toxicity/carcinogenicity studies in the rat and mouse (27-28). All studies were conducted and reported to an acceptable standard.

The results of these studies indicate that BCD has little systemic activity in laboratory animals. In the one-year study in the dog, in which BCD was administered at dietary concentrations of 0, 0.62, 1.25 and 5.0%, the authors identified a No Observed Adverse Effect Level (NOAEL) of 5% BCD in the diet, on the assumption that certain urinary effects reported at this level were not indicative of systemic toxicity in the absence of adverse histopathological findings. The Committee considered that, since the kidney is known to be the target organ for parenterally administered BCD (1) and there is evidence that BCD is absorbed at high levels of administration, 5% BCD is a minimal effect level and the true NOAEL in dogs is 1.25% in the diet, equivalent to an intake of 466 mg/kg bw/day.

In a teratology study, BCD was given by gavage at doses of 0, 1.25, 2.5 and 5.0 g/kg bw/day and no adverse effects were observed. In a multigeneration study, BCD was given at dietary doses of 0, 1.25, 2.5 and 5.0%. The results indicated that 1.25% in the diet, equivalent to around 550-3000 mg/kg bw/day over the different phases of the study, is a NOAEL for reproductive effects in the rat. Minor effects reported at higher doses are considered to be secondary to mild depression of food consumption and body weight gain in the dams. In view of the low absorption of BCD, the Committee considered that a teratology study in a second species was not required.

BCD was negative in in vitro tests for point mutations in bacterial and mammalian cells, in an in vitro test for chromosome aberrations in human lymphocytes, and in an in vivo micronucleus test. In the chronic toxicity/carcinogenicity study in the rat, BCD was given in the diet to provide nominal doses of 0, 25, 75, 225 and 675 mg/kg bw/day. In the chronic toxicity/carcinogenicity study in the mouse, BCD was given in the diet to provide nominal doses of 0, 75, 225 and 675 mg/kg bw/day. In the mouse BCD caused occasional, non-neoplastic lesions in the lower gastrointestinal tract which were considered by the authors to be the cause of death in a few mice and to be related to administration of high doses of a unabsorbed material. The Committee considered these lesions to be unusual but of no significance for humans. The Committee noted that both in the mouse and the rat carcinogenicity studies, a few tumour types were of slightly higher incidence in BCD-treated groups than in concurrent controls and/or historical controls. However, in the absence of any

clear dose-related effects, these were judged to be of no toxicological significance. The Committee concluded that BCD showed no carcinogenic potential.

Conclusion

The Committee considered that an ADI of 0-5 mg/kg bw can be set for β-cyclodextrin, derived from a NOAEL of 466 mg/kg bw/day in the one-year dog study and a safety factor of 100. This evaluation and ADI applies only to β-cyclodextrin manufactured using a CGTase preparation from Bacillus circulans.

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OPINION ON FOODS FOR SPECIAL MEDICAL PURPOSES (FSMPS)
(expressed on 13 December 1996)

Mandate

To advise on the nature, the essential compositional requirements, where necessary, and any specific requirements concerning the labelling and appropriate use of FSMPs.

Documents consulted

1. In drafting its opinion the Committee considered extensively the relevant Codex Standard (CODEX STAN 180-1991 on Labelling of and Claims for Foods for Special Medical Purposes). It considered also information on the nature and use of such products in different Community Member States provided by the Association of Dietetic Food Industries of the EU (IDACE).

Background

2. FSMPs are foods for particular nutritional uses (dietetic foods) covered by the framework directive 89/398/EEC. This latter defines dietetic foods in general and lays down labelling requirements for them. It also foresees the adoption of a specific directive for FSMPs in which the necessary specific provisions shall be laid down. The opinion of the Committee has been requested in order to help the Commission to develop such a directive.

3. At the international level FSMPs are described in the Codex Alimentarius Standard as a category of foods for special dietary uses which are specially processed or formulated and presented for the dietary management of patients and may be used only under medical supervision. They are intended for the exclusive or partial feeding of patients with limited or impaired capacity to take, digest, absorb or metabolize ordinary foodstuffs or certain nutrients contained therein, or who have other special medically-determined nutrient requirements, whose dietary management cannot be achieved only by modification of the normal diet, by other foods for special dietary uses, or by a combination of the two. In addition the Codex Standard lays down labelling provisions specific to these products. The Codex Standard does not lay down compositional criteria for these products.

4. The above description appears to the Committee a reasonable one. However it is obvious that FSMPs comprise a vast range of products intended for a variety of conditions difficult or almost impossible to categorise accordingly. A broad distinction can however be made between: (i) products which can be qualified as *nutritionally complete*, which are formulated and presented as able to cover the entire spectrum of nutritional requirements, if necessary during prolonged periods, whether they constitute the only source of food for patients, are designed to cover only part of daily requirements or to supplement a diet which is inadequate and (ii) products which aim to cover the special requirements of various categories of patients, such as those which compensate for an enzyme deficiency (e.g. phenylketonuria, maple syrup urine disease), or which supplement a nutritional deficiency or enable the patient to adapt to a particular situation (such as renal insufficiency) and which are, in essence, *nutritionally incomplete.*

5. Given the nature and the diversity of the products in question the Committee can understand why the Codex Alimentarius Standard does not include provisions for the composition of FSMPs. Firstly it would be very difficult to establish such criteria for such diverse products. Second it would be even more difficult to keep such criteria under review and up to date. However it may be very useful within the European union and for the purpose of protecting public health and for avoiding barriers to trade to refer to what may reasonably be considered as normal requirements for a number of nutrients, a concept which can then be used, where applicable, to determine essential compositional requirements for some of these products, taking into account also their distinction into nutritionally complete and incomplete products.

Classification of foods for special medical purposes

6. The Committee fully endorses the labelling provisions laid down in the Codex Standard, namely that the labelling mentions unambiguously whether the product is supposed or not to be the sole source of energy and nutrients for the patients for whom it has been conceived, and that there is a prominent warning to consumers if the product is likely to be hazardous for individuals for whom it is not designed, as would be the case of dietetic products with a reduced phenylalanine content intended for patients suffering from phenylketonuria, but which would provoke serious metabolic disorders if they were to be consumed by infants, children or adults in good health.

7. The Committee also fully subscribes to the idea that these products are to be used under medical supervision and that such a warning should appear on the label.

However it fears that such mandatory notice might not fully protect consumers from misuse of some FSMPs. The Committee suggests therefore that the availability or sale of products likely to be hazardous for persons for whom such products are not intended should be restricted to health care services or pharmacies.

8. The existence or not of undesirable effects and, whether or not the food is able to cover on its own the energy and nutrient requirements of the patients for whom it has been designed, should be the criteria which should serve as a basis for classifying products and for deciding on the warnings which should be included on their labels. Consequently, the Committee recommends distinguishing between:

(i) *nutritionally complete foods* which, if they are consumed orally or administered enterally in appropriate quantities cover by themselves the normal nutritional requirements of the people of the age group for which the product has been formulated; these products may be the only source of food for such people, may substitute for a part of their diet or be used as supplements; and

(ii) *nutritionally incomplete foods*, whose essential features of composition diverge to a greater or lesser extent from the limits listed in Annex I, whether they are proposed as the only source of food for the patients for whom they have been formulated, as a partial substitute for their diet or as a supplement to their diet; mixtures of amino acids, whether equilibrated or not, modules which, according to various combinations (proteins, lipids, carbohydrates, minerals and vitamins) make it possible to meet the special nutritional requirements of patients and supplements of various kinds aimed at the dietetic treatment of an illness or a specific disorder all come under this category.

Essential composition criteria of nutritionally complete foods for special medical purposes

9. It would be completely unrealistic to want to define the essential composition criteria of foods designed for special medical purposes to treat specific diseases or disorders or formulated to respond to particular situations. Any attempt at harmonization in this area would meet with insurmountable technical obstacles. Such a step would also constitute a major stumbling block to innovation. The Committee proposes therefore abandoning this idea, including for foodstuffs intended for premature and/or low-birthweigth infants, which are excluded from the scope of Directive 91/321/EEC.

10. There would nevertheless be every advantage in defining the composition criteria for the products under category (i), designed to cover alone or in association with others the normal nutritional requirements of a defined age group. The adoption by the Member States of individual regulations would in effect constitute an obstacle to the free circulation of such products, whereas nothing would justify such differences of interpretation concerning the mineral, vitamin or trace element content of such products since they are, by definition, *nutritionally complete.* An analysis of the state of this market reveals that such obstacles do exist nowadays and that, as a result, it is necessary to harmonize the rules.

11. The nutritional requirements of children over ten years of age, of adolescents and adults do not differ in any appreciable way. Therefore, in order to be sure that the needs of almost the entire (97.5%) population will be covered, the Committee has based its recommendations for these age groups on the PRIs (Population Reference Intakes) of adult males (>18 years), as defined in the report by the Committee on Nutrient and Energy Intakes for the European Community (SCF, 31st series). These PRIs, divided by 20 to arrive at 100 kcal, assuming 2000 kcal as the average energy intakes of these age groups, constitute the minimum values for each vitamin, mineral and trace element. In the case of nutrients for which PRIs have not yet been proposed, (i.e. vitamins D, E and K, biotin, pantothenic acid, sodium, chloride, magnesium, manganese, chromium, molybdenum and fluoride), the Committee recommends using as a basis, when they exist, the recommended dietary allowances (NAS/NRC RDAs) for men over 18 years of age; the US recommended dietary allowances set for vitamins D, E and K were therefore used in this report as reference values. In other cases, the Committee suggests using as limits the "acceptable range of intakes" proposed in the above mentioned SCF report, or in their absence the "acceptable range of intakes" proposed in the above mentioned SCF report, or in their absence the "estimated safe and adequate daily dietary intakes" (ESADDI) suggested by the Food and Nutrition Board. The "acceptable range of intakes" for potassium has also been retained in this report, despite the existence of a PRI, both for reasons of coherence with respect to the other electrolytes (sodium and chloride) and for the upper safe level of intake for potassium (5.9 g/d) recommended by the SCF not to exceed twice the PRI (3.1 g/d) (Annex 1).

12. The Committee also felt that it would be wiser to fix upper limits, too. Indeed, prolonged excessive intakes could have undesirable consequences. What is more, if there were no upper limits, it would not be possible to draw a demarcation line between nutritionally complete foods (category i) and those aimed at covering special requirements (category ii). The general rule suggested by the Committee would be to fix this upper limit to about 3 times (300%) the PRI or, in its absence,

the RDA, where such values have been defined by the SCF or the NAS/NRC committee. The Committee appreciates that this approach is in part arbitrary but it is felt that this provides a prudent limit above which there would be no nutritional benefits and which also minimises the risk of toxicity. An exception should however be made for Vitamin C due to discrepancies between the PRI for adults (45 mg/d) and the minimum value set for infants in the directive 91/321/EEC (8 mg/100 kcal) (see below para 13); in that case the upper limit should be fixed to 5 times the PRI. In the case of nutrients for which only "acceptable range of intakes" or "safe and adequate intakes" have been proposed, the upper and lower values should be retained as such. However for fluoride, whose addition to these products should not be compulsory, only an upper limit should be retained, it being the upper limit of the NAS/NRC "safe and adequate intake", divided by 20, given the choice to express the limits per 100 kcal. Finally where other nutrients not included in Annex I were to be included in these products, especially substances considered "conditionally essential" (e.g., choline, taurine, carnitine, inositol, nucléotides, etc), their levels should be in line with those to be found in the usual average diet (CX/NFSDU 96/11).

Nutritionally complete foods for infants and children up to age 10 years

13. Complete formula foods for infants (0-12 months) should comply with the provisions of the directive 91/321/EEC as modified by directive 96/4/EC. In the absence of minimum values for selenium specified in the above directives the selenium PRI for infants of 6-12 months could serve as basis for their establishment. The "safe and adequate intakes" for infants of 0-6 months of age defined by the NAS/NRC could serve as the basis for defining the limits for manganese, chromium and molybdenum for which the Committee had considered that requirements cannot be established reliably. Finally, maximum levels should be established for all nutrients, including fluoride, where such maximum levels are not stipulated in directive 91/321/EEC. This is the case for all water-soluble vitamins, vitamin E, vitamin K and iodine. Principles which are analogous to those defined in § 12 could be used for this age category, i.e. 3 times the minimum value for the group B vitamins, vitamin C and vitamin E. The maximum values for vitamins D and K could also be fixed at 3 times the minimum values given the needs of young infants for these vitamins and the fact that the maximum value for vitamin A defined in directive 91/321/EEC is 180 μg RE, that is 3 times the minimum values of 60 μg RE (Annex II).

14. The needs of young children and children aged from 1 to 10 years, expressed per 100 kcal, do not differ substantially from those of children above 10 years of age,

adolescents and adults. Complete formula foods for young children and school-children (up to age 10 years) should therefore contain per 100 kcal the same amounts of nutrients as those for adults, except for the following deviations:

Vitamin D: 1 - 3 μg/100 kcal

Calcium: 50 - 100 mg/100 kcal.

Similar proposals are currently under discussion in the Codex Committee on Nutrition and Foods for Special Dietary Uses (CX/NFSDU 96/11).

Disease-specific diets

15. Complete special formula foods often differ in only one or a few points - to a greater or lesser extent - from the composition of a complete formula food, in order to respond to disease-specific requirements. In these cases, deviations from the established minimum and maximum levels are admissible if specific dietary requirements call for adjustments to be made. As a consequence, these deviations should be explicitly stated and the underlying reasons given (such as no added vitamin A and lower phosphate and potassium levels for patients with renal insufficiency). However, any nutrients contained in these complete special diets that are not subject to disease-specific adjustments shall be covered by the same recommendations as those applying to complete formula foods (CX/NFSDU 96/11).

16. In accordance with the Committee's conclusions on the lists of nutrients and additives likely to be used in formulating products for special medical purposes, the Committee is of the opinion that the future legislation on this subject should provide the possibility for manufacturers to use, where necessary, nutrients or additives which do not feature on their respective positive lists, provided that they can scientifically justify the reasons for so doing.

Annex I: Suggested limit values per 100 kcal for vitamins, minerals and trace elements in nutritionally complete foods intended for use by children over 10 years of age, adolescents and adults.

	PRI/RDA[1]	"Acceptable range"[2]	Limits/100 kcal
Vitamins:			
Vitamin A (μg RE)	700		35 - 100
Vitamin D (μg)	10*		0.5 - 1.5
Vitamin E (mg α-TE)	10*		0.5 - 1.5
Vitamin K (μg)	70*		3.5 - 10
Vitamin C (mg)	45		2.25 - 12
Thiamin (mg)	1.1		0.06 - 0.18
Riboflavin (mg)	1.6		0.08 - 0.24
Niacin (mg NE)	18		0.9 - 2.7
Vitamin B_6 (mg)	1.5		0.08 - 0.24
Folic acid (μg)	200		10 - 30
Vitamin B_{12} (μg)	1.4		0.07 - 0.21
Pantothenic acid (mg)		3 - 12	0.15 - 0.6
Biotin (μg)		15 - 100	0.75 - 5
Minerals:			
Sodium (mg)		575 - 3500	30 - 175
Chloride (mg)		575 - 3500	30 - 175
Potassium (mg)		1600 - 5900	80 - 150
Calcium (mg)	700		35 - 100
Phosphorus (mg)	550		30 - 80
Magnesium (mg)		150 - 500	7.5 - 2.5
Trace Elements:			
Iron (mg)	9		0.5 - 1.5
Zinc (mg)	9.5		0.5 - 1.5
Copper (mg)	1.1		0.06 - 0.18
Iodine (μg)	130		6.5 - 20
Selenium (μg)	55		2.5 - 7.5
Manganese (mg)		1 - 10	0.05 - 0.5
Chromium (μg)		50 - 200*	2.5 - 10
Molybdene (μg)		75-250*	3.5 - 12
Fluoride (mg)		1.5 - 4.0*	<0.2

1 Population Reference Intake (PRI) or Recommended Daily Allowances (RDA)*, if no PRI has been proposed by the SCF (31st series).

2 "Acceptable range of intakes", according to the SCF report (31st series) or "estimated safe and adequate daily dietary intakes "according to the 1989 NAS/NRC report* (RDA,1989) when no "acceptable range" has been proposed by the SCF.

Annex II: Suggested limit for vitamins, mineral and trace elements in nutritionally complete foods values per 100 kcal for infants (0-12 months).

Vitamins:

Vitamin A (μg RE)	60 - 180
Vitamine D (μg)	1 - 3
Vitamine E (mg α-TE)	0.5 - 1.5
Vitamin K (μg)	4 - 12
Vitamin C (mg)	8 - 25
Thiamin (mg)	0.04 - 0.12
Riboflavin (mg)	0.06 - 0.18
Niacin (mg NE)	0.8 - 2.4
Vitamin B_6 (mg)	0.035 - 0.1
Folic acid (μg)	4 - 12
Vitamin B_{12} (μg)	0.1 - 0.3
Pantothenic acid (mg)	0.3 - 0.9
Biotin (μg)	1.5 - 4.5

Minerals:

Sodium (mg)	20 - 60
Chloride (mg)	50 - 125
Potassium (mg)	60 - 145
Calcium (mg)	50 - 125 [1]
Phosphorus (mg)	25 - 90
Magnesium (mg)	5 - 15

Trace elements:

Iron (mg)	0.5 - 1.5
Zinc (mg)	0.5 - 1.5
Copper (μg)	20 - 80
Iodine (μg)	5 - 15
Selenium (μg)	1 - 3
Manganese (mg)	0.05 - 0.1
Chromium (μg)	2 - 8
Molybdene (μg)	3 - 6
Fluoride (mg)	< 0.2

[1] The calcium/phosphorus ratio shall not be less than 1.2 nor greater than 2.0

OPINION ON THE SAFETY IN USE OF KONJAC GUM AS A FOOD ADDITIVE (expressed on 13 December 1996)

Terms of reference

The Committee was asked to consider the safety-in-use of Konjac gum as food additive.

Background

Konjac gum is a water-soluble hydrocolloid obtained from the root of the perennial plant Amorphophallus konjac which is cultivated in Asian countries. It has been used for centuries as a traditional foodstuff in Far East countries, for example in Japan and China, in the production of gels and noodles. According to the petitioner, the term Konjac gum is used for a refined product and corresponds to the material evaluated by JECFA under the name Konjac flour (1).

The table below shows the major parameters of the specifications of Konjac gum:

Water-soluble fibres	not less than 75 %
Moisture	not more than 12 %
Starch	not more than 3 %
Protein	not more than 3 %
Ether-soluble material	not more than 0.1 %
Ash	not more than 5.0 %
Arsenic	not more than 3 ppm
Lead	not more than 2 ppm
Total heavy metals	not more than 10 ppm
Viscosity (1% solution)	at least 3000 cps
Salmonella	negative
Escherichia coli	not more than 3 mpn/g

The main component is the water-soluble high-molecular-weight polysaccharide gluco-mannan which consists of D-mannose and D-glucose units at a molar ratio of 1.6:1.0, connected by ß(1-4)-glycosidic bonds. Shorter side chains are attached through ß(1-3)-glycosidic bonds, and acetyl groups occur at random at a ratio of about 1 group per 9 to 19 sugar units. The molecular weight of glucomannan ranges from 200.000 to 2.000.000 dalton. Minor components are insoluble fibres, starch and proteins, which have not been further specified (2, 3).

Konjac gum has been proposed for use as gelling agent, thickener, emulsifier and stabilizer in foodstuffs, for example pasta, baked goods, sausages, salad dressings, ice creams, des-serts,

jams, mayonnaise, soups and beverages. It is intended to be used at levels ranging between 0.02 and 1.0 % (2). The anticipated maximum intake from food additive uses was estimated to be about 3 g/person/day. When consumed in traditional food in Japan and China, intakes may be up to 4 g/person/day (1).

Toxicological evaluation

The available toxicity data on Konjac gum/flour (specifications not given in most cases) include data from an acute oral toxicity study in rats, a study on skin sensitization in guinea pigs (Buehler test), sub-acute and sub-chronic feeding studies in rats addressing specific questions e.g. effects on intestine, colonic microflora and protein absorption, an 18-month study with rats performed to examine the effect on cell-ageing, and an embryotoxicity study with domestic cats (2, 4-8). None of these studies indicated any relevant toxic effects. The observed reduction in body weight, and caecum/colon enlargement are typical of the administration of high doses of non-digestible, non-absorbed, high-molecular-weight materials, e.g. cellulose, pectin or guar gum. The studies, however, can not be regarded as adequate in terms of modern standards. They do not allow to derive a no-effect level.
Genotoxicity tests with Konjac gum/flour (Ames test, mouse lymphoma assay, mouse bone-marrow micronucleus test) were negative (2).

According to published data, studies in humans with Konjac flour did not reveal any toxic effects. Only diarrhoea, flatulence and slight abdominal pain have been reported at high doses. Limited human studies suggest that these effects on the gastrointestinal tract occur with single doses in excess of about 5 g/person/day (9). In addition, a reduction of the absorption of fat-soluble vitamins E and A has been reported. The absorption of water-soluble vitamins B_{12} and B_1, respectively, was not or only slightly influenced (10, 11). There was no influence on mineral absorption. Some findings suggested a positive influence on human health, for example reduction of the gastrointestinal transit time, body weight and blood lipid levels, effects which are also observed after the ingestion of other types of dietary fibres.
The main component glucomannan seems to be non-digestible by human intestinal enzymes, but is susceptible to digestion by enzymes of the colonic microflora. It has not been clearly demonstrated, however, to what extent the material is decomposed in the intestine.
Seven cases of oesophageal obstruction caused by the swelling of tablets containing non-hydrated glucomannan after ingestion were reported from Australia where the substance has been marketed as a dietary aid. However, no cases of oesophageal obstruction caused by hydrated Konjac materials have become known (12).

Conclusion

The toxicological data on Konjac gum are insufficient to establish an ADI. Adequate sub-chronic and long-term feeding studies with this material are lacking and a no-observed-effect level can not be derived. In addition, it has not been clarified to what extent the main component glucomannan is digested in the human intestine.

On the other hand, the existing data (including genotoxicity studies) as well as human experience do not give reason for concern. Konjac materials have a long history as tradi-tional food in Far East countries. Apart from diarrhoea, abdominal pain and an effect on vitamin absorption after ingestion of high doses, no adverse effects of oral ingestion have been reported in humans.

The Committee considers that the use of Konjac gum as an additive at the intended levels up to 1% in food is acceptable provided that the total intake from all sources did not exceed 3 g/d. This upper limit should be taken into account when setting the conditions of use.

The Committee notes that directive 95/2/EC includes a footnote in relation to similar products which points out that these substances should not be used to produce dehydrated foodstuffs intended to rehydrate on ingestion (13). The Committee considers that a similar remark would be applicable to Konjac gum.

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OPINION ON THE SAFETY IN USE OF KONJAC GLUCOMANNAN AS A FOOD ADDITIVE
(expressed on 13 December 1996)

Terms of reference

The Committee was asked to consider the safety-in-use of Konjac glucomannan as food additive.

Background

Konjac glucomannan is a water-soluble hydrocolloid obtained from Konjac flour by washing with water-containing ethanol. Konjac flour, the unpurified raw product isolated from the root of the perennial plant Amorphophallus konjac, has been used for centuries as a traditional foodstuff in Far East countries, for example in Japan and China, in the production of gels and noodles.

The table below shows the major parameters of the specifications of Konjac glucomannan:

Total dietary fibre	not less than 95 % (on a dry weight basis)
Loss on drying	not more than 8 %
Ash	not more than 2 %
Protein	not more than 1.5 %
Starch	not detected (detection limit ?)
Ether-soluble material	not more than 0.5 %
Arsenic	not detected (detection limit 0.2 ppm)
Heavy metals	not detected (detection limit 1 ppm)
Sulfite	not more than 4 ppm
Chloride	not more than 0.02 %
50 % Alcohol-soluble material	not more than 2 %
Viscosity (1 % solution)	not less than 60 000 cps
Salmonella	negative
Escherichia coli	negative

The main component glucomannan is a water-soluble high-molecular-weight polysaccharide consisting of D-mannose and D-glucose at a molar ratio of 1.6:1.0, connected by ß(1-4)-glycosidic bonds with a branch at about each 50th or 60th unit. About each 20th sugar residue is acetylated. The molecular weight ranges from about 500.000 to 2.000.000 dalton. Minor components are insoluble fibres, proteins and lipids, which are not further specified (1, 2).

Konjac glucomannan has been proposed for use in foodstuffs, for example as thickener, emulsifier, stabilizer and gelling agent in baked goods, fish and meat products, pasta, jam and soups.

It is intended to be used at levels ranging between 0.01 and 2 % depending on the food-stuff, and a daily intake of approximately 4g is considered realistic by the producer (1). That is the same intake reported for Konjac flour which can be reached by traditional use (3).

Toxicological evaluation

The available toxicity data on Konjac glucomannan include data from acute oral toxicity studies in mice and rats, a study on skin sensitization (guinea pig maximization test), a sub-acute feeding study (28 days) in rats and sub-chronic feeding studies (90 days) in beagle dogs and rats, the latter combined with a study on reproduction toxicity (1, 4-6). The 90-day studies did not reveal relevant toxic effects. Reduction in food consumption and body weight and caecum/colon enlargement are commonly observed in feeding studies with non-digestible dietary fibres and were found as well as some changes in hematological and clinical chemical parameters only at high doses. The no-observed-effect level (NOEL) was 2.5 % Konjac glucomannan in the diet, corresponding to 1.25 g/kg body weight/day.

Genotoxicity tests in bacteria (Ames test and gene mutation assay with E.coli) were negative (7).

According to published data on Konjac flour, studies in humans did not reveal any toxic effects. Only diarrhoea, flatulence and slight abdominal pain have been reported at high doses. Limited human studies suggest that these effects on the gastrointestinal tract occur with single doses in excess of about 5 g/person/day (8). In addition, a reduction of the absorption of fat-soluble vitamins E and A has been reported. The absorption of water-soluble vitamins B_{12} and B_1, respectively, was not or only slightly influenced (9, 10). There was no influence on mineral absorption. Some findings suggested a positive influence on human health, for example reduction of the gastrointestinal transit time, body weight and blood lipid levels, effects which are also observed after the ingestion of other types of dietary fibres.

The main component, glucomannan, seems to be non-digestible by human intestinal enzymes, but is susceptible to digestion by enzymes of the colonic microflora. It has not been clearly demonstrated, however, to what degree the material is decomposed in the intestine.

Seven cases of oesophageal obstruction caused by the swelling of tablets containing non-hydrated glucomannan after ingestion were reported from Australia where the substance has been marketed as a dietary aid. However, no cases of oesophageal obstruction caused by hydrated Konjac materials have become known (11).

Conclusion

Konjac glucomannan was tested adequately in 90-day feeding studies with rats and beagle dogs. These studies did not reveal any relevant toxic effects and a no-observed-effect level of 2.5 % glucomannan in the diet can be derived, corresponding to 1.25 g/kg body weight/day. However, a long-term toxicity/carcinogenicity study is lacking and only gene mutation tests in bacteria were performed with a negative result. In addition, it has not been clarified to what extent the glucomannan is digested in the human intestine. Therefore an ADI can not be established.

On the other hand, the existing experimental data as well as human experience do not give reason for concern. Konjac glucomannan is consumed as a component of Konjac flour in Far East countries where the Konjac materials have a long history as traditional food. Apart from diarrhoea, abdominal pain and an influence on vitamin absorption after ingestion of high doses of Konjac materials, no adverse effects have become known from studies in humans.

Therefore, the Committee considers that the use of Konjac glucomannan as an additive up to 1 % in food is acceptable provided that the total intake from all sources did not exceed 3 g/d. This upper limit should be taken into account when setting the conditions of use.

The Committee notes that directive 95/2/EC includes a footnote in relation to similar products which points out that these substances should not be used to produce dehydrated foodstuffs intended to rehydrate on ingestion (12). The Committee considers that a similar remark would be applicable to Konjac glucomannan.

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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.

Reponsibility for the Secretariat of the Scientific Committee for Food was transferred form Directorate-General III "Industry" to Directorate General XXIV "Consumer Policy and Consumer Health Protection" with effect from 1st April 1997.

The present report deals with:

* Opinion on colours in foods for special medical purposes for young children
* Opinion on maximum limits for vitamins and minerals in processed cereal-based foods and baby foods
* Opinion on the potential for adverse health effects from the consumption of genetically modified maize (zea mays l)
* Opinion on β-cyclodextrin manufactured by the action of the enzyme cycloglycosyltransferase obtained from *bacillus circulans* on partially hydrolysed starch
* Opinion on foods for special medical purposes (FSMPS)
* Opinion on the safety in use of konjac gum as a food additive
* Opinion on the safety in use of konjac glucomannan as a food additive