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food - science and techniques

Reports of the Scientific Committee for Food

(Fourteenth series)

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REPORT OF THE SCIENTIFIC COMMITTEE FOR FOOD
ON DI-2-ETHYLHEXYLPHTHALATE AND DI-2-ETHYLHEXYLADIPATE

(opinion expressed 1 October 1982)

TERMS OF REFERENCE

To evaluate the hazards to human health arising from the migration into food of di-2-ethylhexylphthalate (DEHP) and di-2-ethylhexyladipate (DEHA) present in certain plastic materials and articles intended to come into contact with foodstuffs.

DISCUSSION

These substances were evaluated by the Committee (6th Report, 1978) as part of the review of the positive list of substances to be authorised in the manufacture of regenerated cellulose films intended to come into contact with foodstuffs. Since then the Committee has been provided with a considerable amount of documentation on recent studies on these compounds, including the entire proceedings of the United States' National Toxicology Program (NTP) Conference on Phthalates held in June 1981[1], the NCI/NTP study on DEHP[2] and on DEHA[3].

CARCINOGENESIS

a) Di-2-ethylhexylphthalate (DEHP)

A recent study[2] using two dose levels of 6,000 ppm and 12,000 ppm in the diet of rats and 3,000 ppm and 6,000 ppm in the diet of mice, has produced tumour induction under the conditions of these experiments not observed in previous studies[4,5] considered inadequate by present day standards. These effects were an increase in hepatic adenomas and/or hepatic carcinomas in both sexes of both species. No increase was noted in other types of tumours.

In an attempt to elucidate the mechanism underlying the hepato-carcinogenic activity of DEHP, 2 experiments were performed in rats[8] and one in marmosets[9] to determine the time and dose relationship of intracellular alterations. At all dose levels (50, 200 and 1000 mg/kg b.w./day for up to 28 days and 2000 mg/kg/day for 14 days), dose-related changes were observed in rats. By electron microscopy from the third day, these changes affected the smooth and rough endoplasmic reticulum and the peroxisomes. These morphological changes were mirrored by corresponding changes in enzyme activities. Marmosets receiving 2000 mg/kg b.w./day for 14 days showed slight increase in peroxisomes only. Therefore an epigenetic mechanism might be considered a possible explanation of the tumour induction observed in the carcinogenicity studies in rats. The marmoset appears to be a less sensitive species. Covalent binding to DNA was not observed in the rat.

b) Di-2-ethylhexyladipate (DEHA)

A recent study[3] using 12,000 and 25,000 ppm in the diet of rats and mice over a period of 103 weeks has shown some evidence of tumour induction in mice only. Compound administration was not associated with tumour formation in the rat.

In the male mouse a significant increase in the incidence of hepatocellular adenoma was observed only at the highest dose level.

In the female mouse there was an increased incidence of hepatocecullar-carcinoma at both dose levels.

MUTAGENESIS

a) DEHP

A dominant lethal test in the mouse (i.p.) was positive for DEHP[6], but not dose-dependent. In vitro tests with DEHP for bacterial reversion[10,11] gave conflicting results depending on the methodology employed. In vitro tests[12,13,14,15] on various cultured cell systems showed no chromosomal aberrations. In view of these conflicting results it is not possible to express a definite opinion on the mutagenic potential of DEHP.

b) DEHA

A dominant lethal test in the mouse (i.p.) showed that DEHA[7] produced dose-dependent mutagenic effects. No other mutagenicity tests have been reported.

TERATOGENESIS

a) DEHP

DEHP induces embryotoxic and teratogenic effects in the mouse when given in doses at 180 mg/kg b.w./day or more in the diet throughout pregnancy. 50 mg/kg b.w./day, appeared to be the no-effect level for embryo-toxicity and teratogenicity.

Two studies in rats[16,17] using oral and i.p. administration showed increased resorptions but no malformations.

Monoethylhexylphthalate (the principal metabolite of DEHP), administered intravenously to rabbits at doses up to 11.3 mg/kg/day for 13 consecutive days, produced no toxic effects in the embryo and foetus[1]. An oral study in rats showed some early foetotoxicity[11] but no teratogenicity.

These results suggest that DEHP may be teratogenic in the mouse only and at oral doses of 180 mg/kg b.w./day or more[18].

b) DEHA

DEHA produced teratogenic and embryotoxic effects in one study when administered intraperitoneally at doses above 1 ml/kg b.w.

CONCLUSIONS AND RECOMMENDATIONS

a) DEHP

DEHP has produced benign and malignant tumours of the liver in the mouse and the rat at the high doses used in the NCI/NTP studies.

Although the in vitro mutagenicity tests gave conflicting results, the dominant lethal study (i.p.) indicates a possible mutagenic effect which was, however, not dose-dependent. High oral doses in mice produced embryotoxic and teratogenic effects.

b) DEHA

The evidence regarding carcinogenicity is inconclusive because only in the mouse was there evidence of liver tumour induction. The only available mutagenicity test indicated mutagenic potential. Teratogenic and embryotoxic effects were only seen at high dose levels.

In view of these new findings including the studies on mechanism and covalent binding for both DEHP and DEHA, the Committee excluded DEHP from the previously established TDI for phthalates and excluded DEHA from the ADI previously established for three adipates. It recommended that the exposure levels for man from all sources, including migration from food packaging, be reduced as much as possible. The Committee also recommended that further mutagenicity tests be carried out to elucidate the genotoxic potential, if any, of these two substances and to receive the results of ongoing studies.

c) Other phthalates and adipates

Like other groups of chemicals having similar technological applications the biological properties of adipates and of phthalates may vary considerably both within each chemical group and between the groups, although certain similarities also exist.

This is also in this case reflected in their individual toxicological profile of effect, and their corresponding potencies. The effects of DEHP and DEHA do not allow for extrapolation of the same toxicity profile to other phthalates or adipates. Each substance has to be assessed on its own merits based upon the individual characteristics determined by the toxicity studies.

REFERENCES

1. National Toxicology Program Conference on Phthalates, Washington, June 10, 1981.

2. Report on the carcinogenesis of di-2-ethylhexylphthalate, NIP, 80-37, HHS Publication (NIH) 81-1773.

3. Report on carcinogenesis bioassay of di-2-ethylhexyladipate, NIP, Technical report series No. 212.

4. Carpenter et al., 1953. Chronic oral toxicity of di-2-ethylhexylphthalate for rats, guinea pigs and dogs. AMA Arch. Ind. Hyg. Occup. Med., 8, 219-226.

5. Harris et al., 1956. Chronic oral toxicity of di-2-ethylhexylphthalate in rats and dogs. AMA Arch. Ind. Health 13, 259-264.

6. Singh, A.R., et al., 1974. Mutagenic and antifertility sensitivities of mice to DEHP and dimethoxyethylphthalate (DMEP). Toxicol. Appl. Pharmacol. 29, 35-46.

7. Singh, A.R. et al., 1975. Dominant lethal mutations and antifertility effects of DEHP and DEHA in male mice. Toxicol. Appl. Pharmacol. 32, 566-576.

8. Bridges, J.W. et al 1982. Study 5/81/TX submitted by CEFIC.

9. Nixon, J. and Jackson, S.J. 1982. Report No.CTL/P/690 submitted by CEFIC.

10. Simmon et al., 1977. Mutagenic activity of chemicals identified in drinking water. Dev. toxicol. Environ. Sci. 2, 249.

11. Ruddick, J.A. et al., 1981. An assessment of the teratogenicity in the rat and mutagenicity in Salmonella of mono-2-ethylhexylphthalate. Bull. Environ. Contam. Toxicol. 27, 181-186.

12. Abe, S. and Sasaki, M. 1977. Chromosome aberrations and sister chromatid exchanges in Chinese hamster cells exposed to various chemicals. J. Natl. Cancer Inst., 58, 1635-1641.

13. Ishidate, M. and Odashima, S., 1977. Chromosome tests with 137 compounds on Chinese hamster cells in vitro in a screening for chemical carcinogens. Mutation Res., 48, 337-354.

14. Turner, J.H. et al., 1974. An evaluation of the effects of the Diethylhexylphthalate (DEHP) on mitotically capable cells in blood packs. Transfusion, 14, 560-566.

15. Stenchever, M.A. et al., (1976) Effects of bis(2-ethylhexyl)phthalate on chromosomes of human leukocytes and human fetal lung cells. J. Pharmac. Sci., 65, 1648-1651.

16. Singh, A.R., Lawrence, W.H. and Autian, J. (1972) Teratogenicity of Phthalate esters in rats. J. Pharmac. Sci., 61, 51-55.

17. Nikonorow, M. et atl, (1973). Effect of orally administered plasticisers and polyvinylchloride stabilisers in the rat. Toxicol. appl. Pharmacol., 26, 253-259.

18. Singh, A.R., Lawrence, W.H. and Autian, J. (1973). Embryonicfetal toxicity and teratogenic effects of adipic acid esters in rats. J. Pharmac. Sci. 62, 1596-1600.

REPORT OF THE SCIENTIFIC COMMITTEE FOR FOOD ON CERTAIN ANABOLIC AGENTS USED IN ANIMAL PRODUCTION

(Opinion expressed 3/4 February 1983)

TERMS OF REFERENCE

To give an opinion on whether the use for fattening purposes in animals of the following substances: oestradiol-17β , testosterone, progesterone, trenbolone and zeranol present any harmful effect to health.

BACKGROUND

On the 31 July 1981 the Council adopted Directive 81/602/EEC[1] concerning the prohibition of certain substances having a hormonal action and of any substance having a thyreostatic action.

By Article 5 of that Directive the Council decided to take a decision as soon as possible on the administering to farm animals of oestradiol-17β , testosterone, progesterone, trenbolone and zeranol, for fattening purposes. It also noted the Commission's intention to consult the competent Scientific Committees on the matter.

The Commission asked the Scientific Veterinary Committee, the Scientific Committee for Animal Nutrition and the Scientific Committee for Food the same question, recognizing that the three committees would lay emphasis in their separate reports on the aspects of the question which fell within their terms of reference.

The three Committees established a joint working group composed of members of the committees with special knowledge of the subject.

A number of eminent specialists in the field joined the working group which met several times under the auspices of the Commission Services as a "scientific working group on anabolic agents in animal production".

The Scientific Committee for Food, together with the Scientific Committee for Animal Nutrition and Scientific Veterinary Committee, was requested to consider the report of the Scientific Working Group and to comment on its conclusions and recommendations during the Committee's own review.

The Committee has been informed that the Commission intends to make public the report of the Scientific Working Group. The Committee also notes that the Scientific Veterinary Committee and the Scientific Committee for Animal Nutrition have both already made recommendations to the Commission on the proper use of these anabolic agents.

CURRENT REVIEW

The Committee is impressed by the thoroughness with which the report of the joint working group has been prepared. There appears no reason in the present report to rehearse all the reasoning included therein, particularly in view of its imminent publication.

The conclusions of the Scientific Working Group were as follows:-

1. The Scientific Working Group is of the opinion that the use of oestradiol-17β , testosterone and progesterone, and those derivatives which readily yield the parent compound on hydrolysis after absorption from the site of application, would not present any harmful effects to the health of the consumer when used under the appropriate conditions as growth promoters in farm animals.

[1] OJ No L222 of 7.8.1981, p. 32.

2. Evaluation of the data on "trenbolone" and "zeranol" revealed that some data on the hormonal no-effect level and the toxicology of these compounds and their metabolites are still missing.

3. The Scientific Working Group considers it necessary that additional information be provided before a final conclusion can be given on trenbolone and zeranol.

4. Proper programmes to control and monitor the use of anabolic agents are essential.

5. It is necessary to continue scientific investigations on the relevance of the present use of the "no-hormone effect" level when related to the harmful effects of anabolic agents.

CONCLUSIONS AND RECOMMENDATIONS

In general, the Scientific Committee for Food agrees with the conclusions and recommendations of the Scientific Working Group. It also wishes to put forward the following comments for consideration by the Commission:-

i) In relation to Recommendation 2 it would be of interest to generate data on the threshold values for the toxic and hormonal activities of trenbolone and zeranol.

ii) The Committee endorses the Recommendation 4 on the importance of implementing programmes for control and monitoring of anabolic agents. It emphasizes that proposals should be made by the Commission for implementation. In connection with this aim it would be necessary to encourage the development and standardisation of appropriate analytical methods.

iii) The Committee recognises that the possible misuse of naturally occurring hormones or their derivatives, which easily yield the original hormone, may be difficult to detect analytically. Information is therefore needed on the levels of naturally occurring hormones likely to engender a health hazard. It is an important public health consideration to prevent the illegal use of banned products and the misuse of authorised products by efficient monitoring and proper controls.

SUMMARY OF CONCLUSIONS AND RECOMMENDATIONS

1. The Committee agrees, in general, with the conclusions and recommendations of the Scientific Working Group.

2. The Committee offers the following comments for further activity:

   a) the determination of the thresholds for toxic and hormonal actions of trenbolone and zeranol,

   b) the importance of implementing programmes for control and monitoring, including development of analytical methods,

   c) the prevention of misuse of these substances and their derivatives,

   d) information on the levels of natural hormones likely to endanger health.

FIRST REPORT OF THE SCIENTIFIC COMMITTEE FOR FOOD ON THE ESSENTIAL REQUIREMENTS OF INFANT FORMULAE AND FOLLOW-UP MILKS BASED ON COWS' MILK PROTEINS

(opinion expressed on 27 April 1983)

TERMS OF REFERENCE

To advise on the essential requirements of products intended for the feeding of infants and young children.

INTRODUCTION

1. Scope

This opinion relates only to milk preparations intended for infants and young children, when manufactured from cows' milk proteins to the exclusion of all other sources of protein.

The Committee has decided that, in order to fulfil its mandate, it would be preferable to approach the subject in stages and that processed cereal products and diversified children's foods should be the subject of subsequent opinions.

The Committee was fully aware that there exist on the Community market products manufactured using protein sources other than cows' milk, but in view of the time at its disposal it decided to postpone specific recommendations for such products to a later date.

Lastly, for the same reasons, products intended for low birth-weight infants and for infants and young children suffering from nutritional or metabolic disorders are also excluded from the scope of this opinion.

2. Definitions and conventional values

The following definitions have been adopted in line with the Codex Alimentarius[1]:

"Infants" : children aged less than 12 months;
"Young children" : children aged between 1 and 3 years.

The following conversion factors were adopted for the purpose of calculating the amount of available energy from the main nutrients, in accordance with the report of the U.K. Committee on Medical Aspects of Food Policy (COMA) (1980)[2]:

protein (Total N x 6.38)	17 kJ/g	4 kcal/g*
carbohydrates (expressed as monosaccharides or lactose monohydrate)	16 kJ/g	3.75 kcal/g
lipids	37 kJ/g	9 kcal/g

3. Documents consulted

In drafting this opinion the Committee drew heavily on several very detailed reports on the feeding of infants and young children published in recent years. These include in particular three reports by COMA:

Present-day practice in infant feeding (1974);
The composition of mature human milk (1977);
Artificial feeds for the young infant (1980);

and the Guidelines adopted by the Committee on Nutrition of the European Society for Paediatric Gastroenterology and Nutrition (ESPGAN)

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* 1 kilocalorie equals 4.184 kilojoules; the two sets of values are given in round numbers for the sake of simplicity.

I. Recommendations for the composition of an adapted formula (1977);
II. Recommendations for the composition of follow-up formula and Beikost (1981);
III. Recommendations for infant feeding (1982).

The Committee also had the benefit of the standards laid down by the Codex Alimentarius and the work of a variety of national committees. The Committee has not reproduced all the arguments for the conclusions set out in these documents and in some cases has found it necessary to refine the recommendations of these committees to accord more closely with current opinion in the Community.

4. Bacteriological standards and contaminants

The Committee was not asked to include in its present review the questions raised by the presence of contaminants in infant foods (e.g. antibiotics, compounds with hormonal activity, pesticides, mycotoxins and pharmacologically active substances).

Furthermore, the Committee was not asked to advise on the question of microbiological standards.

The Committee suggests that at a later date its advice on such matters should be requested.

GENERAL CONSIDERATIONS

5. Provided it is available in sufficient quantities, human milk is best able to satisfy the needs of virtually all healthy infants during the first 4-6 months of post-natal life as its composition is adapted to he immaturity of the neonate and its limited tolerance with respect to certain nutrients during its first few months of life. However when mothers do not breast-feed, or only do so partially, there is a legitimate market for infant formulae.

6. In several EEC Member States a distinction has been made between products intended for the first few months of life and those to be included in a diversified diet. For this reason the Commission asked the Committee to include both groups of products in its review. The Committee recognises that this field is changing constantly in the light of new scientific knowledge. The Committee appreciates the need for making recommendations for specific purposes in this sensitive area and stresses the importance of keeping the subject continuously under review and of being sufficiently adaptable in its recommendations to allow for future developments.

7. A number of physiological processes involved in the digestion, absorption, hepatic functions and renal clearance are not fully-developed at birth. The food of neonates and young infants should therefore not only supply them with all the materials and energy needed for growth and for the development of various tissues, particularly of the brain, but also be capable of being metabolized and anything given in excess to be eliminated. A reasonable safety margin should also be selected to allow for a possible further reduction in their tolerance in the event of illness and possible errors made by parents in the preparation (reconstitution) of bottled feeds[3, 4, 5].

8. After the age of about 4 months, the mechanisms that ensure homeostasis are sufficiently developed to cope with much wider variations in the concentration of nutrients[6].

9. Generally speaking, non-milk foods, i.e. solid foods such as cereals, vegetables, fruit, meat, fish, eggs, etc., and fruit juices should in principle not be introduced into the infant diet before the age of three months and not later than the age of six months.

10. As soon as the diet is sufficiently varied and the risks of metabolic disorders have been minimized, little or no benefit derives from the prolongued use of milk preparations of the kind which, in the absence of breast-feeding, are necessary during the first 4-6 months of life[6, 7].

11. In view of the foregoing, recommendations which included a range of preparations intended for infants from birth to the age of one year (e.g. by the Codex Alimentarius[1] and the American Academy of Pediatrics (AAP)[8] do not appear to be entirely satisfactory.

In the case of preparations intended for young infants ("starting formulae" in the terminology of ESPGAN[3]), the permitted limits for a number of nutrients, in particular proteins (4 g/100 kcal in the Codex standards and 4.5 g/100 kcal in the AAP guidelines) greatly exceed those recommended by ESPGAN (2.8 g/100 kcal)[3] and COMA (3 g/100 kcal)[2]. Furthermore, with the exception of sodium, potassium and chloride - the limits for which are again higher on the whole than in the European recommendations[2,3] and in certain national regulations[9] - no maximum limits are laid down either by Codex Alimentarius or by the AAP for a number of mineral salts, in particular phosphorus[1,8]. The risks of tetany, acidosis and disturbances in the fluid balance, which can appear in young infants as a results of an excessive intake of proteins and mineral salts with a consequential excessive renal solute load[3,7], could therefore probably not be discounted in some cases.

12. It appears, on the other hand, that there are too many requirements in the Codex Alimentarius and the AAP guidelines regarding the vitamins and trace elements in milk-based preparations intended for older children as part of a diversified diet. Cows' milk is a good source of zinc, iodine and manganese[10], and non-milk foods supply sufficient copper and water-soluble vitamins[6] for it not to be essential to add any to such preparations, but iron requirements, which reach their highest point between the ages of six months and one year, are not in some cases fully satisfied by a varied diet that is not enriched with iron[6].

13. The Committee therefore considered that a distinction should be drawn between:

- products intended where necessary to replace human milk and to meet by themselves the normal nutritional needs of infants during the first 4-6 months of life (i.e. "infant formulae");

- products intended to constitute the basic milk element in the progressively diversified diet of infants aged over 4 months (i.e. "follow-up milks").

14. Nevertheless, the Committee agrees with the Codes Alimentarius[1] and the AAP recommendations[8] that "infant formulae"* can satisfactorily be used for feeding infants aged over 4-6 months provided they are suitably enriched with iron (see § 39). On the other hand, it stresses, in line with the ESPGAN recommendations[6], that "follow-up milks" should not be given to infants of less than four months[11]. It therefore recommends that a statement, clearly differentiating the two types of product, should appear on the label:

- for "infant formulae", the words "suitable for infants from birth to one year of age" or any other equivalent statement unless the product is not enriched in iron in which case the time period should be from birth to six months;

- for "follow-up milks", the words "suitable for infants over the age of four months and young children" or any other equivalent wording accompanied by a statement to the effect that the product should form part of a diversified diet.

15. In view of the foregoing, in particular the fact that either of the two products can be given without specific reservations to infants between the age of 4 months and one year provided the iron requirement is fulfilled and the diet is sufficiently varied, the Committee took the view that there was no need to draw a clear distinction between the two types as regards protein content, as had been proposed by the ESPGAN Committee[6].

16. Lastly, the Committee's attention was drawn to the fact that infant formulae described as "adapted", "humanized", "maternalized"or the like, which meet special compositional critieria, currently exist on the market in several Member States. It considered that such distinctions were not justified, since all infant formulae have to be specifically adapted to the needs of infants and the special conditions of their metabolism. It therefore concluded that the concept of "extra quality", which seems to imply that one type of preparation is better than another, should be abolished. The abovementioned terms, which are in effect liable to lead consumers to believe that such preparations are equivalent to human milk, would thus in effect be banned, in accordance with the Recommendation of the 34th World Health Assembly and the International Code of Marketing of breast-milk substitutes[12].

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* Although the term "infant formulae" is widely adopted internationally, in Ireland and the United Kingdom, these products are often referred to as "baby milks".

The Committee nevertheless accepted the idea that, in order to better inform the purchaser, certain approved claims could be allowed to appear next to the name of the product, where the latter's composition satisfied specific requirements. The Committee has approved a list of specific compositional criteria for infant formulae warranting a corresponding claim.

ESSENTIAL COMPOSITION

17. In line with all the committees whose reports were consulted [1-8], the Committee took the view that the average composition of human milk should be used as a reference for determining the formulae of breast-milk substitutes. The Committeee allowed for the fact that the composition of human milk is not constant, but varies not only according to the stage of lactation and the nutritional condition of the mother, but also at different times in the day and especially during feeding.[4]. On the other hand, no data were available to the Committee that would justify any great departure from this average composition, which, except for special cases (calcium, phosphorus, iron and vitamin D) was therefore adopted as a minimum standard for infant formulae.

18. As a general rule maximum values were chosen to be 2 to 3 times the minimum values. Some variation with respect to the average energy density of human milk (65 to 70 kcal/100 ml) was accepted in order to make allowance for the variability of the published figures[2], a larger variability being generally speaking accepted in the case of follow-up milks than for infant formulae. On the whole, the same principles were followed for infant formulae both by the Codex Alimentarius[1] and ESPGAN[3], so that the values adopted are very similar, if not identical, for the majority of nutrients.

19. As far as proteins are concerned, the Committee took the view that there was no need to depart from previous recommendations concerning minimum concentrations in infant formulae. It was, however, well aware of the fact that the figure of 1.8 g/100 kcal (1.2 g/100 ml) corresponds to the total nitrogen concentration of the milk x 6.38*, while the actual protein content of human milk, when calculated by multiplying by 6.38 the total α-amino N (i.e. the sum of the free amino acids and of those derived from the hydrolysis of the proteins), amounts to only approximately 1 - 1.1 g/100 ml, i.e. 1.5 g/100 kcal[2]. Non-protein nitrogen (mainly in the form of urea, creatinine, uric acid, amino acids and nucleotides) accounts for more than 20% of total nitrogen in human milk, whereas for cows' milk is not more than 7-10% although their concentrations are very similar (400-500 mg N/l) in both milks[2-4]. There is however no doubt, that a proportion of the non-protein N in human milk is used for the maintenance and growth of infants, so that this fraction should be taken into consideration when calculating nitrogen requirements at that age.

20. In line with the report of the Joint FAO/WHO ad hoc Expert Committee on energy and protein requirements (1973)[13] and the recommendations of COMA and ESPGAN[2,3], the Committee consider that the only reference protein acceptable for infants less than six months old was the protein of human milk thus rejecting for infant formulae any reference to casein[1,8] or the FAO protein[13]. The Committee emphasises that, irrespective of the protein content of these preparations, they still had to supply the infant with at least as much of each essential and semi-essential amino acid as is provided by an equivalent amount (in energy terms) of human milk. It also wished to ensure adequate protein quality in all cases by specifying that the chemical index of the protein should always be not less than 80% of that of the reference protein. In order to enable enforcement of these requirements, which do allow for a possible deterioration in protein quality through processing (e.g. inadequate heat treatment) or during storage, the Committee particularly specified in the Annexes not only the percentage composition of this reference protein, but also the minimum quantities of sulphur amino acids and of each other essential amino aicd (expressed per 100 kJ and 100 kcal) that these preparations must contain**. These values were taken from the FAO data on the amino-acid content of foods (1970)[14], which differ very little from the results of the most recent UK studies[4].

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* The protein content of a food is usually obtained by multiplying its total nitrogen content by a given factor, which varies according to the amino-acid composition of the protein. In the case of milk and milk products, this factor is 6.38 [13,14].

** The minimum values for the amino-acid content of human milk adopted in the Annex are those laid down by the FAO (expressed in mg/100 ml) multiplied by a coefficient of 0.36 or 1.5 according to whether they are expressed per 100kJ or per 100 kcal respectively. This coefficient is obtained by dividing the real protein content (α-amino N x 6.38) by the energy density[4].

21. The Committee finally took into account previously mentioned considerations on the risk of an excessive intake of proteins during the first few weeks of life, particularly the possible consequences of even a temporary increase in the blood levels of urea, ammonia and certain amino acids on brain development[5]. It therefore stipulated that the maximum protein content in this type of preparation should not exceed twice the real protein content of human milk (3 g/100 kcal)[2], a concentration that would not contribute excessively to the renal solute load[1]. These proposals, although departing appreciably from those of the Codex Alimentarius[1] and the AAP[8], are in practice very close to those of ESPGAN[3] and COMA[2]. Both bodies stipulate that a concentration of 1.8 g/100 kcal is only acceptable if the casein/whey protein ratio is adjusted so that the proteins have a nutritional value equivalent to that of human milk (ratio less than 1:1). They also advised that this concentration should be at least 2.25 g/100 kcal[2] where the casein/whey protein ratio has not been changed from that of cows' milk (82:18)[2] or when the chemical index of the proteins does not exceed 80% of that of human milk[3].

22. Because difficulties related to the immaturity of the renal function and the metabolic interconversion system no longer arise after the age of four months the Committee believed that the minimum value for protein could be raised and that casein can be chosen as reference protein without any risk. The Committee adopted the figure of 2.25 g/100 kcal as the minimum value for follow-up milks and a figure of twice that value (i.e. 4.5 g/100kcal) as the maximum concentration, this latter in accordance with the recommendations of the AAP[8], on the grounds that the protein content of cows' milk (5.5 g/100 kcal), which had been suggested for follow-up milks by ESPGAN[6], could be excessive before the age of one year.

23. The Committee sees no reason for the minimum and maximum values for the fat content of infant formulae and follow-up milks to differ as between one type of preparation and another and proposes a range of 3.3 to 6.5 g/100 kcal. With few small exceptions* these values are accepted by other committees[1-3,6,8,11].

24. The Committee also agreed with COMA and ESPGAN[2,3], that the absorption of fats depended on the nature of the fatty acids and on their position in the glycerol molecule, rather than on whether they were of animal or vegetable origin. Nevertheless it considered that, specifications relating to the origin and quality of the fats and oils used to make up infant formulae and follow-up milks were desirable. The Committee was unable to put forward any irrefutable argument in support of the assertion that milk fats, whether on their own or in mixtures, were in some way superior to other fats.

25. The Committee recommended to ban cottonseed oil, which contains cyclopentenic fatty acids that have the property of reducing the permeability of membranes, and sesame seed oil, whose unsaponifiable fraction contains a goitrogenic factor. An alternative could be to ban oils containing cyclopentenic acids or goitrogenic factors; this would mean that if cottonseed oil were used, it would have to satisfy appropriate quality criteria. The Committee believes that the former approach is more appropriate. It has been also established that a high intake of trans isomers of fatty acids could lead to excessive blood cholesterol levels[15], was liable to inhibit Δ 6-desaturase and to reduce the production of arachidonic acid and prostaglandins, especially if the supply of essential fatty acids was insufficient[16]. An atherogenic role for trans fatty acids is also suspected, but this has not yet been confirmed[15]. The 8% limit proposed by the Committee takes into consideration both the amounts of trans isomers normally present in human milk (2 - 6% of total fatty acids) and cows' milk (1 - 9%) and also the fact that it has been recommended that their concentration should not exceed 5% of the fats in a diversified diet[15,16].

26. The irrefutable hypercholesterolemic effect of saturated fatty acids - in particular myristic acid - in man together with observations in animals using lauric acid and myristic acid[3] also prompted the Committee to recommend imposing a limit not only on coconut oil, but also on all "lauric" oils, and oils that are particularly rich in C14 fatty acids[17].

    Nevertheless the Committee considered it to be a more realistic proposal that the content of each of these two fatty acids should not exceed 15% of the total fatty acids in the mixtures of oils and fats used in manufacture.

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*The Codex Alimentarius has not laid down a maximum value for the fats and essential fatty acids content of infant formulae.

27. The Committee agreed that linoleic acid (18:2, n-6) should account for at least 2.7% of the energy content of infant formulae (i.e. 300 mg/100 kcal), in accordance with the Codex Alimentarius standards[1] and the AAP recommendations[8]. It was, however, aware of the fact that a deficiency of essential fatty acids had been described only in infants receiving extremely low-fat diets by either enteral or parenteral administration, and also that many studies had failed to reveal signs of an essential fatty acid deficiency during the first few months of life when linoleic acid accounted for more than 1% (100 mg/kg) of the energy supply[2, 3]. These facts justified this value being adopted as a minimum by COMA[2]. The Committee felt however that it could not overlook the fact that, in most regions of the world and in the Community in particular, the linoleic acid content of human milk ranges between 7% and 12% of the total fatty acids content (3 - 6% of the energy content)[2, 3], which corresponds to the range suggested by the ESPGAN Committee for adapted formulae[3].

28. It is now clearly established that linoleic acid, together with its trans isomers (see paragraph 25), can interfere with the formation of very long-chain polyunsaturated fatty acids[2], the production of prostaglandings[18] and certain immunological reactions[19]. Although the Committee was fully aware that no undesriable clinical effect had been reported in infants fed during the first few months with preparations composed exclusively of vegetable fats (corn oil) extremely rich in linoleic acid, it endorsed the findings of COMA[2], that it was more prudent for linoleic acid not to account for more than 20% of the total fatty acids in infant formulae, i.e. a maximum of 1 200 mg/100 kcal.

29. On the other hand in view of the intakes of essential fatty acids derived from non-milk foods, in particular cereals[6], the Committee did not think it vital to specify minimum and maximum contents for follow-up milks. In its opinion, there was no justification at that age for these products not to be manufactured exclusively from milk fats. Nevertheless, it considered the imposition of the lower limit adopted for infant formulae (300 mg/100 kcal) unobjectionable once vegetable fats were in the formula. This attitude, which agrees with that of ESPGAN[6], is embodied in the draft (step 3) Codex standard on follow-up milks[11].

30. No requirement has been laid down for α-linolenic aicd (18:3, n. 3). The Committee did not feel in a position to do so, although recent studies have clearly suggested that α-linolenic acid is indeed essential for man at about 0.5% of total energy supply[20] and it is established that excessive intakes of one of these polyunsaturated fatty acids can interfere with the metabolism and use of the other family[20].

31. The limits within which the content of carbohydrate can vary in infant formulae and follow-up milks derive more or less directly from those adopted for fats and proteins (see sections 19, 21, 22 and 23). The Committee agrees with COMA that the carbohydrate content of the two types of product should neither fall below that of cows' milk (i.e. 4.8 g/100 ml (or 7 g/100 kcal) expressed as monosaccharides) nor exceed twice that value (i.e. a range of 7-14 g/100 kcal)[2]; this is a little less restrictive than the ESPGAN recommendations (8-12 g/100 kcal)[3, 6]. Neither the Codex Alimentarius[1] nor the AAP[8] have laid down any requirements for carbohydrates.

32. The Committee was aware of the fact tha lactose accounts for approximately 40% of the total energy content of human milk and approximately 26% of that of cows' milk[2], and that ideally it should constitute most if not all of the sugars that go to make up infant formulae and follow-up mils[3, 6]. Nevertheless it recognised that there is a limit to the amount of lactose that can be absorbed by the young infant[2] and that preparations with a lactose content barely exceeding 2 g/100 kcal exist on the Community market. It also took into account the increased frequency of lactose intolerance among certain ethnic groups[21], but that lactase activity continues in such subjects as a rule until the age of 18 months to 3 years[22]. It concluded that the lactose content of infant formulae should not fall below half their minimum carbohydrate content (i.e. 3.5 g/100 kcal), it being understood that foods intended for infants and young children suffering from nutritional metabolic disorders are excluded from the scope fo this opinion*. Given the possible decline of lactase activity after 12-18 months, the Committee decided it could accept a minimum concentration of 1.8 g/100 kcal in follow-up milks.

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*This viewpoint was not accepted by one of the experts assisting the Committee who felt that the lower limit laid down for infant formulae was too high and wished the figure of 1.8 g/100 kcal also to be adopted for this type of product.

33. The Committee agreed with the ESPGAN recommendations that the addition of sucrose to infant formulae and follow-up milks should be discouraged while malto-dextrins (and possibly maltose) should preferably be used[3,6]. The Committee also considered that even though the use of fructose in follow-up milks might be acceptable, it was preferable not to incorporate fructose into infant formulae, because no results of any relevant clinical tests were available. It also noted that in the concentrations used and under the usual temperature conditions (i.e. 37°C), the sweetening effect of fructose was no greater than that of sucrose[23]; furthermore, as a result of its slower absorption in the free form due to the loss of the kinetic advantage conferred by the hydrolysis of sucrose, fructose gave rise to virtually no insulin secretion[24]. The fact that fructose promotes the formation of dental plaque to a lesser extent than sucrose was also underlined[25].

34. Blood glucose and insulin levels rise much less after the ingestion of starch than of malto-dextrins and sucrose and, although the activity of pancreatic α-amylase is virtually zero at birth, several studies have demonstrated that neonates and young infants are perfectly capable of digesting a certain amount of starch[26,27]. For these reasons the Committee could accept the addition of starch without limits to follow-up milks, and for infant formulae up to a concentration of 2 g/100 ml of the mixture ready for use but not exceeding 30% of the total carbohydrate content. Experience has moreover amply demonstrated, that infants can from the first few weeks onwards absorb greater amounts of starch without any apparent disorder. On the other hand the use of other thickening agents has not been recommended for infant formulae[3]. The addition of cereals that could contain gluten was rejected in the case of both infant formulae and follow-up milks.

35. Breast-fed infants receive an adequate intake of sodium, potassium and chloride, and the concentrations of these elements in infant formulae should be not lower than those present in mature human milk[1,2,3,8] (see paragraph 17). The maximum limits recommended by the several committees concerned show some variation. However, all the calculations are based on the accepted maximum protein content. The Committee has followed the same principle for potassium and chloride (see Annex 1), but considered that for sodium there was not sufficient justification for departing from the internationally accepted figure of 2.6 mEq (60 mg)/100 kcal. Although intakes of sodium close to the minima were propably preferable, the Committee considered that the sodium/potassium and (sodium + potassium)/ chloride ratios should be as similar as possible to those of human milk[8], but that such restrictions were undoubtedly too stringent to be included in standards.

36. The same approach was adopted in the case of calcium, phosphorus, magnesium and a number of trace elements (iodine, copper, zinc), for which minimum requirements were laid down. The values adopted in the various reports are, moreover, either identical[1,8] or only slightly different[2,3]. The Committee considered such values to be on the whole perfectly acceptable for calcium, phosphorus, the calcium/phosphorus ratio and iodine; for the sake of uniformity, it therefore endorsed the Codex requirements[1] for these substances. For magnesium and zinc it adopted a slightly lower value in order to take into account the results of the most recent studies[7], and a much lower figure for copper (20 instead of 60 μg/100 kcal), in view of the much lower concentrations often found in human milk and cows' milks[2] and the technological problems (oxidation of the fatty acids) that can arise as a result of an excessive concentration of this element in the formulae[3]. At all events, with the exception of specific disorders and observations made in cases of malnutrition, the Committee noted that clinical indications of copper or zinc deficiency have rarely been reported in infants fed with products manufactured from cows' milk which has not been enriched with copper or zinc.

37. The quantity of manganese in human milk is very low (in some cases undetectable[4]). In addition the concentrations of this element in cows' milk are between four and five times greater than in human milk. Under these circumstances and although it was put forward that manganese could be partly eliminated during the demineralization process[2] the Committee considered that no requirement was necessary for manganese in milk-based infant formulae. However they would not object to its addition should circumstances dictate. The Committee noted that manganese may be present in the water used for making up the feed[28].

38. For the reasons explained above, however, the Committee preferred to specify a maximum limit for phosphorus (and therefore indirectly for calcium and magnesium) and copper in infant formulae.

39. The Committee took the view that enrichment of infant formulae with iron should be allowed but that this should not be compulsory. Acceptable limits for enriched infant formulae at

that age could in its opinion range between 0.5 and 1.5 mg/100 kcal.

40. For follow-up milks the desirable range for iron is between 1 and 2 mg/100 kcal. The Committee subscribed to the idea that milk probably constituted the best vehicle for iron in a diversified diet and that on the basis of a consumption of approximately half a litre a day, concentrations exceeding 1 mg/100 kcal were necessary in order to satisfy requirements[6].

41. As regards the other requirements for follow-up milks, the Committee was content with endorsing the Codex standard for zinc (0.5 mg/100 kcal)[1], which it felt to be more appropriate to a food the protein content of which was less limited. The Committee also stated that in general the concentration of mineral elements (sodium, potassium,chloride, calcium,phosphorus, magnesium and copper) should be approximately equivalent to that normally present in cows' milk, reduced where applicable in the same proportion as the protein concentration. In order to facilitate the application of this principle, the Committee nevertheless listed in an annex, for guidance only, the figures corresponding to the most recent data[29], expressed per 100 g of solids-non-fat* and per gram of protein.

42. Lastly, it considered that, in view of the vitamins supplied by milk itself and by non-milk foods, the vitamin requirements for follow-up milks could be the same as those for vitamins A, D, E and C proposed in this document for infant formulae. With the exception of vitamin A, for which less stringent limits were preferred, these requirements - in line with all those adopted for infant formulae - conform with the Codex standard. In the case of fat-soluble vitamins and niacin, they are expressed in a slightly different but more appropriate form that takes account of changes in concepts and terminology[30, 31]. The most up-to-date analytical methods (mass spectrometry and HPLC) have demonstrated that the phylloquinone contents of human milk and cows' milk are nearly ten times lower than the concentrations hitherto reported. Therefore even if the composition of infant formulae included only vegetable fats, it would still have a higher vitamin K_1 concentration than human milk[32]. However, the Committee would have no objection to the optional addition of vitamin K_1 to infant formulae. Once the neonatal period has elapsed, the intestinal flora contributes as a rule sufficiently to the synthesis of this vitamin for requirements to be satisfied. Therefore the addition of vitamin K_1 to follow-up milks should not be considered.

    The Committee did not propose specifications for choline which is available from both exogenous and endogenous sources and cannot be considered as a vitamin for man.

    However, neonates utilize a great deal of choline in membrane synthesis chiefly into lecithin, and several biochemical pathways, in which choline is a precursor, are crucial for them. Therefore the possibility cannot be excluded that feeding formulae with choline content much lower than human milk may have long term consequences. Supplementation should be allowed but is not compulsory (see Annex).

43. The Committee examined the list of proposed additives and essential nutrients from the point of view of their safety and their nutritional necessity. The Committee endorses the principle that technological additives should not be used in food for infants and young children, but nutritional adequacy requires that the food be palatable to the child. Although the amount of additives in these foods should be limited as far as possible, the Committee recognises that technological additives may contribute to the total nutrient content and acknowledges that manufacturers do require a certain amount of choice to meet both objectives. For this reason the list of additives is somewhat longer than at first sight might seem compatible with the principles of non-use of additives in food for this age group.

    Furthermore the normal principles apply and no additive should be authorized in infant formulae and follow-up milks unless a suitable specification is available.

44. Once the physiological role and essential character of substances which are normally present in human milk have been established provision should be made to include these substances on a positive list as rapidly as possible.

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*The solids-non-fat residue is calculated by subtracting the weight of water and fat content from the total weight of the milk; it is approximately 9 g/100 ml in the case of whole milk[2].

CONCLUSIONS

1. The Committee considers that a distinction should be drawn between infant formulae, intended to cover on their own the nutritional requirements of infants in good health during the first 4-6 months after birth but which could be used up to one year of age if enriched in iron and follow-up milks intended to form the basic milk element in a diversified diet of infants older than 4 months and of young children.

   Appropriate statements arising from these principles should appear on the labels of these two types of products.

2. For infant formulae and follow-up milks minimal compositional requirements, which take into account not only the essential nutrients but also the quality of the raw materials and the technological processes applied, should be fixed and a certain number of maximum limits also specified.

3. The concept of "extra" quality should be abolished for infant formulae and the terms "maternalized", "humanized", "adapted" or other similar terms should not be used in trade descriptions.

   To provide better information for the purchaser, only claims corresponding to well defined compositional criteria and appearing on a positive list should be authorized by competent authorities.

4. The Committee's proposals on the composition of infant formulae and follow-up milks mentioned above are set out in Annexes 1 and 2 of this docuemnt, accompanied by explanatory notes. The compositional criteria which could be the basis of specific claims are defined in Annex 3. The list of vitamins, mineral substances, amino acids and acceptable nitrogen compounds is specified in Annex 4, the list of technological additives is specified in Annex 5. The minimum level of available amino acids for infant formulae and the composition of the reference proteins adopted are given in Annexes 6 and 7 and in Annex 8 the level of mineral elements of cows' milk is given as an indication for follow-up milks.

5. Substances normally present in human milk may be included in infant formulae if their essential character has been established.

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REFERENCES

1. Codex Alimentarius Commission. Recommended international standards relating to foods intended for infants and young children. Joint FAO/WHO programme on food standards; CAC/RS 72/74. Rome, FAO and WHO, 1976.

2. Report of the Working Party on the Composition of Foods for Infants and Young Children, Committee on Medical Aspects of Food Policy. Artificial feeds for the young infnat. Report on Health and Social Subjects, No. 18. London : HMSO, 1980.

3. ESPGAN Commmittee on Nutrition. Guidelines on infant nutrition. I. Recommendations for the composition of an adapted formula. Acta Paediatr Scand 1977 ; suppl. 262.

4. Report of a Working Party of the Committee on Medical Aspects of Food Policy. The composition of mature human milk. Report on Health and Socail Subjects, No. 12. London : HMSO, 1977.

5. ESPGAN Committee on Nutrition. Guidelines on infant nutrition. III. Recommendations for infant feeding. Acta Paediatr Scand 1982 : suppl. 302.

6. ESPGAN Committee on Nutrition. Guidelines on infant nutrition. II. Recommendations for the composition of follow-up formula and Beikost. Acta Paediatr Scand 1981 : suppl. 287.

7. Report of a Working Party of the Panel on Child Nutrition, Committee on Medical Aspects of Food Policy. Present-day practice in infant feeding. 3rd. ed. Report on Health and Social Subjects, No. 9. London : HMSO, 1975.

8. American Academy of Pediatrics - Committee on Nutrition. Commentary on breast-feeding and infant formulas including proposed standards for formulas. Pediatrics 1976 ; 57 : 278-85.

9. Arrêté du 1er juillet 1976 relatif aux aliments diététiques et de régime de l'enfance. Journal officiel de la République française 1976 ; 108 (n°215) : 5519-24.

10. LÖNNERDAL B, KEEN CL, HURLEY LS. Iron, copper, zinc, and manganese in milk. Ann Rev Nutr 1981 ; 1 : 149-74.

11. Codex Alimentarius Commission. Proposals for the standardization of follow-up or supplementary milks. Document CX/FSDU 82/10 (July 1982).

12. WHO/UNICEF. International code of marketing of breast-milk substitutes. Geneva : WHO, 1981.

13. Joint FAO/WHO ad hoc Expert Committee. Energy and protein requirements. FAO Technical Report Series, N° 52. Rome : FAO, 1973.

14. FAO, Food Policy and Food Science Service, Nutrition Division. Amino-acid content of foods and biological data on proteins. Rome : FAO Nutritional Studies N°24, 1970.

15. HECKERS H. MELCHER FW. Trans-isomeric acids present in West German margarines, shortenings, frying and cooking fats. Am J. Clin Nutr 1978; 31 : 1041-9.

16. KINSELLA JE, BRUCKNER G, MAI J, SHIMP J. Metabolism of trans fatty acids with emphasis on the effects of trans, trans-octadecadienoate on lipid composition, essential fatty acid, and prostaglanding : an overview. Am J Clin Nutr 1981; 34 : 2307-18.

17. BRIGNOLI CA, KINSELLA JE, WEIHRAUCH JL. Comprehensive evaluation of fatty acids in foods. V. Unhydrogenated fats and oils. J Am Diet Assoc 1976 ; 67 : 224-9.

18. FRIEDMAN Z, FROLICH JC. Essential fatty aicds and the major urinary metabolites of the E prostaglandins in thriving neonates and in infants receiving parenteral fat emulsions. Pediatr Res. 1979 ; 13 : 932-6.

19. BEISEL WR. Single nutrients and immunity. Am J. Clin Nutr 1982 ; 35 : 417-68.

20. HOLMAN RT, JOHNSON SB, HATCH TF. A case of human linolenic acid deficiency involving neurological abnormalities. Am J Clin Nutr 1982 ; 35 : 617-23.

21. SIMOONS FJ. The geographic hypothesis and lactose malabsorption. A weighing of the evidence. Am J. Dig Dis 1978 ; 23 : 963-80.

22. KRETCHMER N. Malabsorption of lactose : biochemistry and genetics of intestinal lactase. Mod Probl Paediatr 1975 ; 15 : 177-85.

23. HYVÖNEN L, KURKALA R., KOIVISTOINEN P., MERIMAA P.. Effects of temperature and concentration on the relative sweetness of fructose, glucose and xylitol. Lebensmittelwiss Technol 1977 ; 10 : 316-20.

24. CRAPO PA, KOLTERMAN OG, OLEFSKY JM. Effects of oral fructose in normal, diabetic and impaired glucose tolerance subjects. Diabetes Care 1980 ; 3 : 578-81.

25. SCHEININ A,MÄKINEN KK, eds. Turku sugar studies 1-21. Acta Odontol Scand 1975; 70 : 1-348.

26. DE VISIA B, CICCIMARRA F, DE CICCO N, AURICCHIO S. Digestibility of starches in infants and children. J Pediatr 1975 ; 86 : 50-5.

27. SENTERRE J. Net absorption of starch in low birth weight infants. Acta Paediatr Scand 1980 ; 69 : 653-7.

28. ERICSSON Y, RIBELIUS U. Wide variabtions of fluoride supply to infants and their effect. Caries Res 1971 ; 5 : 78-88.

29. Paul AA, SOUTHGATE DAT. McCance and Widdowson's - The composition of foods. 4th ed. Amsterdam ; Elsevier/North-Holland Biomedical Press, 1978.

30. BIERI JG, McKENNA MC. Expressing dietary values for fat-soluble vitamins : changes in concepts and terminology. Am J Clin Nutr 1981 ; 34 : 289-95.

31. HORWITT MK, HARPER AE, HENDERSON LM. Niacin-tryptophan relationships for evaluating niacin equivalents. Am J Clin Nutr 1981 ; 34 : 423-7.

32. HAROON Y, SHEARER MJ, RAHIM S, GUNN WG, McENERY G, BARKHAN P. The content of phylloquinone (Vitamin K_1) in human milk, cows' milk and infant formula foods determined by high-performance liquid chromatography. J Nutr 1982 ; 112 : 1105-17.

ANNEX 1

INFANT FORMULAE - ESSENTIAL COMPOSITION OF THE PRODUCT READY FOR USE

WHEN RECONSTITUTED AS INSTRUCTED BY THE MANUFACTURERS

1. ENERGY

60-75 kcal (250-315 kJ)/100 ml.

2. PROTEINS

- When the protein in a formula consists of unmodified cows' milk protein, the protein content[1] must not be less than 0.56 g/100 kJ (2.25 g/100 kcal) nor more than 0.7 g/100 kJ (3 g/100 kcal). The chemical index of the proteins must be equal to at least 80% of that of human milk[2, 3] allowing methionine and cystine to be added together.

- When the protein in a formula consists of cows' milk protein which has been modified (e.g. alteration of the casein/whey protein ratio) then the limits must not be less than 0.45 g/100 kJ (1.8 g/kcal) nor more than 0.7 g/100 kJ (3 g/100 kcal). With an equal energy value, the formula must provide an available quantity of each essential and semi essential amino acid at least equal to that contained in human milk [4].

- The addition of amino acids is permitted solely for the purpose of improving the nutritional value of the proteins, and then only in the proportion necessary.

3. LIPIDS AND LINOLEATE

- The lipid content must not be less than 0.8 g/100 kJ (3.3 g/100 kcal) nor more than 1.5 g/100 kJ (6.5 g/100 kcal).

- The use of cotton oil, sesame oil and fats containing more than 8% trans isomers of fatty acids is prohibited.

- The percentage of lauric acid must not exceed 15% of the total fat content.

- The percentage of myristic acid must not exceed 15% of the total fat content.

- The content of linoleic acid (in the form of glycerides = linoleates) must not be less than 70 mg/100 kJ (300 mf/100 kcal) nor more than 285 mg/100 kJ (1200 mg/100 kcal).

4. CARBOHYDRATES

- The carbonhydrate content must not be less than 1.7 g/100 kJ (7 g/100 kcal) nor more than 3.4 g/100 kJ (14 g/100 kcal).

- The lacose content must not be less than 0.85 g/100 kJ (3.5 g/100 kcal).

- The addition of malto-dextrins, of maltose and of sucrose is permitted; the sucrose content must not exceed 20% of the total carbohydrate content.

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1 Protein content = Nitrogen content X 6.38

2 The chemical index is the lowest of the ratios between each individual essential amino acid of the test protein and the corresponding amino acid of the reference protein.

3 See Annex 7

4 See Annex 6

- Starch may be added so long as it does not exceed a maximum of

  a) 2 g/100 ml of the reconstituted product, and

  b) 30% of the total carbohydrate content.

  No native or chemically modified starches but only precooked or gelatinized starches should be added. They should be naturally free of gluten.

- No other carbohydrates and no other thickening agents should be added.

5. MINERAL SALTS

	per 100 kJ[1]		per 100 kcal	
	Minimum	Maximum	Minimum	Maximum
Sodium (mEq)	0.25	0.6	1	2.6
Potassium (mEq)	0.4	0.9	1.6	3.8
Chloride (mEq)	0.35	0.8	1.4	3.5
Calcium (mg)	12	-	50	-
Phosphorus (mg)	6	22	25	90
Calcium/Phosphorus ratio	1.2	2.0	1.2	2.0
Magnesium (mg)	1.2	3.6	5	15
Iron (mg)[2]	0.12	0.36	0.5	1.5
Zinc (mg)	0.07	-	0.3	-
Copper (µg)	4.8	19	20	80
Iodine (µg)	1.2	-	5	-

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[1] 1 kJ = 0.239 kcal

[2] Limits applicable to formulae with added iron.

6. VITAMINS

	per 100 kJ		per 100 kcal[1]	
	Minimum	Maximum	Minimum	Maximum
Vitamin A (µg RE)[2]	14	43	60	180
Vitamin D (µg)[3]	0.25	0.5	1	2
Thiamine (µg)	10	-	40	-
Riboflavin (µg)	14	-	60	-
Nicotinamide (µg-NE)[4]	60	-	250	-
Pantothenic acid (µg)	70	-	300	-
Vitamin B_6 (µg)	9	-	35	-
Biotin (µg)	0.4	-	1.5	-
Folic acid (µg)	1	-	4	-
Vitamin B_{12} (µg)	0.025	-	0.1	-
Vitamin C (mg)	1.9	-	8	-
Vitamin E (mgα-TE)[5]	0.5/g of poly unsaturated fatty acids expressed as linoleic acid but in no case less than 0.1 mg/100 available kJ	-	0.5/g of poly unsaturated fatty acids expressed as linoleic acid but in no case less than 0.5 mg/100 available kcal	-

[1] 1kJ = 0.239 kcal

[2] RE = all trans retinol equivalent

[3] In the form of cholecalciferol, of which 10 µg = 400 i.u. of vitamin D

[4] NE = Niacin equivalent = mg nicotinic acid + mg tryptophan/60

[5] α-TE = d-α-tocopherol equivalent

ANNEX 2

FOLLOW- UP MILKS
ESSENTIAL COMPOSITION OF THE PRODUCT READY FOR USE
WHEN RECONSTITUTED AS INSTRUCTED BY THE MANUFACTURERS

1. ENERGY

60-80 kcal (250-335 kJ)/100 ml

2. PROTEINS

- The protein content[1] must not be less than 0.5 g/100 kJ (2.25 g/100 kcal) nor more than 1 g/100 kJ (4.5 g/100 kcal).
- The chemical index must at least be equal to that of casein[2, 3].
- The addition of amino acids is permitted solely for the purpose of improving the nutritional value of the proteins, and then only in the proportions necessary for that purpose.

3. LIPIDS AND LINOLEATE

- The lipid content must not be less than 0.8 g/100 kJ (3.3 g/100 kcal) nor more than 1.5 g/100 kJ (6.5 g/100 kcal).
- The use of cotton oil, sesame oil and fats containing more than 8% trans isomers of fatty acids is prohibited.
- The percentage of lauric aicd must not exceed 15% of the total fat content.
- The percentage of myristic acid must not exceed 15% of the total fat content.
- The content of linoleic acid (in the form of glycerides = linoleates) must not be less than 70 mg/100 kJ (300 mg/100 kcal) where vegetable oils are part of the composition.

4. CARBOHYDRATES

- The carbohydrate content must not be less than 1.7 g/100 kJ (7 g/100 kcal) nor more than 3.4 g/100 kJ (14 g/100 kcal).
- The lactose content must not be less than 0.45 g/100 kJ (1.8 g/100 kcal).
- The sucrose and/or fructose and/or honey content must not exceed 20% of the total carbohydrate content.
- The addition of products containing gluten is prohibited.

5. MINERAL SALTS

- The iron content must not be less than 0.25 mg/100 kJ (1 mg/100 kcal) nor more than 0.5 mg/100 kJ (2 mg/100 kcal).

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[1] Protein content = Nitrogen content x 6.38

[2] The chemical index is the lowest of the ratios between each individual essential amino acid of the test protein and the corresponding amino acid of the reference protein

[3] See Annex 7

- The zinc content must not be less than 0.12 mg/100 kJ (0.5 mg/100 kcal).

- The content of other mineral salts must be in the same range as that normally found in cows' milk, where appropriate reduced by the same proportions as the protein concentration[1].

- The calcium/phosphorus ratio must not exceed 2:1.

6. VITAMINS

	per 100 kJ[2]		per 100 kcal	
	Minimum	Maximum	Minimum	Maximum
Vitamins A (µg RE)[3]	14	43	60	180
Vitamin D (µg)[4]	0.25	0.5	1	2
Vitamin C (mg)	1.9	-	8	-
Vitamin E (mg α-TE)[5]	0.5/g of poly unsaturated fatty acids expressed as linoleic acid but in no case less than 0.1 mg/100 available kJ	-	0.5/g of poly unsaturated fatty acids expressed as linoleic acid but in no case less than 0.5 mg/100 available kcal.	-

[1] See Annex 8

[2] 1 kJ = 0.239 kcal

[3] RE = all trans retinol equivalent

[4] In the form of cholecalciferol, of which 10 µg = 400 i.u. of vitamin D

[5] TE = d-α-tocopherol equivalent

ANNEX 3

SPECIFIC COMPOSITIONAL CRITERIA FOR INFANT FORMULAE,

WARRANTING A CORRESPONDING CLAIM

- PROTEINS

  Content less than 0.6 g/100 kJ (2.5 g/100 kcal).

  Whey protein/casein ratio not less than 1.0.

- SODIUM

  Content less than 0.4 mEq/100 kJ (1.7 mEq/100 kcal).

- SUCROSE

  Absence

- LACTOSE

  Only carbohydrate used

- IRON

  If added

DIETETIC ADDITIVES

1. VITAMINS

Vitamin	Vitamin formulation
Vitamin A	Vitamin A acetate Vitamin A palmitate Beta-carotene (Provitamin A)
Vitamin D	Vitamin D_2 (Ergocalciferol) Vitamin D_3 (Cholecalciferol) Vitamin D_3 Cholesterol
Vitamin B_1	Thiamine hydrochloride Thiamine mononitrate
Vitamin B_2	Riboflavin Riboflavin 5'-phosphate sodium
Nicotinamide	Niacinamide Nicotinic acid (niacin)
Vitamin B_6	Pyridoxine hydrochloride Pyridoxal-5'-phosphate
Folic acid	Folic acid
Pantothenic acid	D-calcium pantothenate D-sodium pantothenate D-panthenol
Vitamin B_{12}	Cyanocobalamin Hydroxocobalamin
Vitamin H	d-Biotin
Vitamin C	l-ascorbic acid Sodium-l-ascorbate Calcium-l-ascorbate l-ascorbyl-6-palmitate Potassium ascorbate
Vitamin E	d-alpha-tocopherol dl-alpha-tocopherol d-alpha-tocopherol acetate dl-alpha-tocopherol acetate
Vitamin K	Vitamin k_1

Special vitamin formulations

For reasons of stability and handling, some vitamins have to be converted into suitable preparations, for example stabilised oily solutions, gelatine-coated products and fat-coated preparations. For this purpose, edible substances and additives recommended in the present document may be used. In addition, the following substances are permitted:

Gelatine; gum arabic (acacia gum); silicon dioxide (as an anti-caking agent): maximum level 10 g/kg in the vitamin preparation.

2. MINERAL SUBSTANCES

Mineral substances	Permitted salts
Calcium (Ca)	Calcium carbonate Calcium chloride Calcium citrate Calcium gluconate Calcium glycerophosphate Calcium lactate Calcium phosphate, monobasic Calcium phosphate, dibasic Calcium phosphate, tribasic Calcium hydroxide
Phosphorus (P)	Calcium phosphate, monobasic Calcium phosphate, dibasic Calcium phosphate, tribasic Magnesium phosphate, dibasic Magnesium phosphate, tribasic Potassium phosphate, monobasic Potassium phosphate, dibasic Sodium phosphate, dibasic
Magnesium (Mg)	Magnesium carbonate Magnesium chloride Magnesium oxide Magnesium phosphate, dibasic Magnesium phosphate, tribasic Magnesium sulphate Magnesium gluconate
Iron (Fe)	Ferrous citrate Ferrous gluconate Ferrous lactate Ferrous sulphate Ferric ammonium citrate
Copper (Cu)	Cupric citrate Cupric gluconate Cupric sulphate Copper Lysine Complex Copper carbonate
Iodine (I)	Potassium iodide Sodium iodide Potassium iodate
Zinc (Zn)	Zinc acetate Zinc chloride Zinc lactate Zinc sulphate Zinc citrate
Manganese (Mn)	Manganese carbonate Manganese chloride Manganese citrate Manganese sulphate Manganese gluconate
Sodium (Na)	Sodium bicarbonate Sodium chloride Sodium citrate Sodium gluconate Sodium carbonate Sodium lactate Sodium phosphate, monobasic

(continued)	Sodium phosphate, dibasic Sodium phosphate, tribasic Sodium hydroxide
Potassium (K)	Potassium bicarbonate Potassium carbonate Potassium chloride Potassium citrate Potassium gluconate Potassium lactate Potassium phosphate, monobasic Potassium phosphate, dibasic Potassium phosphate, tribasic Potassium hydroxide

3. AMINO ACIDS AND OTHER NITROGEN COMPOUNDS

L arginine and its hydrochloride
L cystine and its hydrochloride
L histidine and its hydrochloride
L isoleucine
L leucine
L lysine
L and DL methionine
L phenylalanine
L threonine
L tryptophan
L tyrosine
L valine

taurine

4. CHOLINE (choline chloride)

ANNEX 5

TECHNOLOGICAL ADDITIVES

	Maximum level in 100 ml of the product ready for use when reconstituted as instructed by the manufacturer
Thickening agents	
E 407 Carrageenan	0.03 g in follow-up milks
E 410 Gum carob	0.1 g in follow-up milks
E 412 Gum guar	0.1 g in follow-up milks
Emulsifiers	
E 322 Lecithin	0.5 g in infant formulae and follow-up milks
E 471 Mono- and di-glycerides	0.4 g in infant formulae and follow-up milks
Antioxidants	
E 300 L-Ascorbic acid	1 mg in infant formulae and follow-up milks
E 301 Sodium-L-ascorbate	" " " " " " "
E 302 Calcium-L-ascorbate	" " " " " " "
E 304 Ascorbyl palmitate	" " " " " " "
E 306 Tocopherol-rich extracts of natural origin	" " " " " " "
E 307 Alpha-tocopherol	" " " " " " "
E 308 Gamma-tocopherol	" " " " " " "
E 309 Delta-tocopherol	" " " " " " "
Acidified milks	

For the manufacture of acidified milks, the addition of citric acid, l (+) lactic acid or the culture method, producing lactic acid in situ, can be used.

For the purpose of this report, the contents of essential and semi-essential amino acids of human milk, expressed in mg per 100 kJ and 100 kcal, are the following:

	per 100 kJ[1]	per 100 kcal
Arginine	16	69
Cystine	6	24
Histidine	11	45
Isoleucine	17	72
Leucine	37	156
Lysine	29	122
Methionine	7	29
Phenylalanine	15	62
Threonine	19	80
Tryptophan	7	30
Tyrosine	14	59
Valine	19	80

[1] 1 kJ = 0.239 kcal

ANNEX 7

AMINO ACID COMPOSITION OF

CASEIN AND HUMAN MILK PROTEIN (g/100 g OF PROTEIN)

	Casein [1]	Human milk [1]
Arginine	3.7	3.8
Cystine	0.3	1.3
Histidine	2.9	2.5
Isoleucine	5.4	4.0
Leucine	9.5	8.5
Lysine	8.1	6.7
Menthionine	2.8	1.6
Phenylalanine	5.2	3.4
Threonine	4.7	4.4
Tryptophan	1.6	1.7
Tyrosine	5.8	3.2
Valine	6.7	4.5

[1] Amino acid content of foods and biological data on protein. FAO Nutritional Studies, n° 24, Rome 1970, items 375 and 383.

As a reference, the contents of mineral elements in cow's milk expressed per 100 g of solids-non-fat and per g of proteins are the following:

	per 100 g SNF [1]	per g of proteins
Sodium (mEq)	24	0.65
Potassium (mEq)	43	1.1
Chloride (mEq)	30	0.8
Calcium (mg)	1350	35
Phopshorus (mg)	1070	28
Magnesium (mg)	135	3.5
Copper (µg)	225	6
Iodine	NS [2]	NS

[1] SNF: "solids-not fats"

[2] NS: Non specified, varies widely according to season and stock farming conditions.

REPORT OF THE SCIENTIFIC COMMITTEE FOR FOOD

ON BUTYLATED HYDROXYANISOLE

(Opinion expressed 29 April 1983)

TERMS OF REFERENCE

To assess the results of recent studies on the safety of Butylated hydroxyanisole (BHA) and to advise on requirements for further action within the European Economic Community.

BACKGROUND

Following a Japanese study which has recently been published (1) purporting to show that BHA was carcinogenic to rats and the consequential decision by the Japanese authorities to effectively ban the use of BHA in food, the Commission asked the Scientific Committee for Food to evaluate the data on the safety of BHA. As national authorities had also been involved in a similar exercise, it was decided that it would be desirable for members of the Scientific Committee for Food's working group on BHA and EEC Government experts to discuss the interpretation of these results in the knowledge of already evaluated data with a view to a coordinated EEC decision on their relevance to the acceptability of the additive as an antioxidant in food. The deliberations of the working party took place in Heidelberg, 11 October 1982. The report of the working party is annexed.

PRESENT REVIEW

The Committee has considered the report of the Heidelberg working party and the data presented at that meeting, and has already endorsed the conclusions drawn by the experts participating in that discussion. Since then, other research has been undertaken on the basis of the Heidelberg report and suggestions emanating from similar working groups in other parts of the world. Results are available in respect of some of these experiments and these have enabled the Committee to specify priority areas of research which should be undertaken rapidly so that any doubts on the immediate effects on health by the use of BHA in food can be dissipated. These priorities for research do not negate the necessity for the results of the other research mentioned in the Heidelberg report.

The original Japanese data suggested that the amount of BHA fed was reduced by about 50% during processing, which suggested the possibility that degradation products may have been involved in the effect on the forestomach. Recent studies carried out in the UK (unpublished data submitted by the UK Ministry of Agriculture, Fisheries and Food using isotopic (^{14}C-BHA) and analytical techniques),on diets of the same formulation and processed in the same way have shown different results, as follows:

(a) the amounts of BHA added to the diet before pelleting are not significantly reduced by the pelleting process. The explanation for the Japanese finding that the levels of BHA in the pelleted rat diets as fed were reduced from 2.0 to 1.07% and 0.5 to 0.24%, lies in the poor extraction of BHA from the diet into hexane (49.7%), compared with the results obtained using 5% water in ethanol (87.0% extraction);

(b) within a limit of 0.1% of added BHA no transformation products were detectable; and

(c) the pasteurisation and pelleting processes (using a UK diet of the same formulation as that used in Prof. Ito's study) resulted in a reduction in vitamin A level from that added initially (20,000 i.u./kg) to 9,000 and finally 1,700 i.u./kg. (For comparison, the level found in a sample of the diet used in Professor Ito's laboratory was between 8 and 9,000 i.u./kg).

Therefore it was concluded that:

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(1) Carcinogenicity of Butylated Hydroxyanisole in F344 Rats
Nobuyuki Ito et al JNCI, Vol. 70, No. 2 February 1983.

1. the amount of BHA in the pelleted rat diets was substantially the same as the 2% and 1% initially added,

2. the effects on the rat forestomach must be attributed to BHA and not to any transformation product(s).

The Committee was also informed about a number of toxicological studies:

(i) Short-term studies carried out by the Health Protection Branch of Health and Welfare Canada, in which BHA, in powdered diets at levels from 2% to 0%, was fed to rats for 9 days, have demonstrated:

a) at 1% and 2% BHA, irritative changes in the forestomach, visible to the naked eye,

b) at 0.5% and higher levels of BHA, proliferative changes in the squamous epithelium of the forestomach, on histopathological examination,

c) DNA synthesis as measured by thymidine uptake, was increased in the forestomach epithelium at 0.5% BHA and above.

The changes were confined to the squamous epithelium of the forestomach proximate to its junction with the glandular stomach and were dose-related, with a no-effect level of 0.25% BHA.

(ii) Studies with BHA carried out jointly by the Bundesgesundheitsamt (Berlin) and the Rijksinstituut voor de Volksgezondheid (Bilthoven) have shown similar effects in the rat. TBHQ appears to have less effect.

(iii) Professor Ito has also reported that hamsters fed 2% BHA in a pelleted and 1% in a powdered diet had all developed papilloma of the forestomach - when examined after 16 weeks. A similar concurrent study in F344 rats produced no papilloma in rats fed 1% BHA, but papilloma in 8/10 rats in the 2% group. A further study is under way in F344 rats, using doses of from 0.125 to 2% BHA in powdered diets.

(iv) Studies are under way at the BGA (Berlin) on the mechanism of action of BHA and other antioxidants with respect to their effect on oxygen metabolism and free radical formation and on the distribution and excretion of orally administered ^{14}C-BHA in the rat.

None of this more recent information invalidates the need for further research indicated in the working party's initial report.

It is likely that work currently in progress in Japan, EEC Member States and Canada will provide data on the threshold level for induction of hyperplasia in the rat forestomach by BHA, the reversibility of this process and the potential of other phenolic antioxidants to produce similar effects.

As there is evidence that the effects on the forestomach are not species specific, but it is not yet known whether BHA can produce similar proliferative changes in species without a forestomach, efforts should now be directed primarily towards establishing:

1) whether or not BHA can produce epithelial hyperplasia in the oesophagus and /or glandular stomach of species of animal without a forestomach - such as the dog, pig and monkey, and

2) the mechanisms underlying the biological action of BHA on the forestomach epithelium.

CONCLUSIONS

1. The Committee endorses the conclusions of the EEC working party on BHA of 11 October 1982.

2. Information should be supplied without delay on whether BHA can produce epithelial hyperplasia in the oesophagus and/or glandular stomach of species of animal without a forestomach.

3. The situation in respect of the use of BHA as a food additive should be kept continuously under review.

REPORT OF AN EEC WORKING PARTY ON BUTYLATED HYDROXYANISOLE (BHA)

Heidelberg, 11 October 1982

TERMS OF REFERENCE

To assess the results of recent studies on the safety of BHA at national and Community level and to advise on requirements for action in the European Economic Community (EEC).

BACKGROUND

Following a recent Japanese study purporting to show that BHA was carcinogenic to rats and the consequential decision by the Japanese authorities to effectively ban the use of BHA in food, the Commission of the European Communities (Commission) asked the EEC Scientific Committee for Food to evaluate the data on the safety of BHA. National authorities have also been involved in a similar exercise. It was therefore decided that it would be desirable for members of the Scientific Committee for Food's working group on BHA and EEC Government experts to discuss the interpretations of these results in the knowledge of already evaluated data with a view to a coordinated EEC decision on their relevance to the acceptability of the additive as an antioxidant in food. The deliberations of the working party were facilitated by the presence of the author of the study, Professor Nobyuki ITO, Nagoya City University Medical School, Japan in Heidelbergand his participation in the meeting.

PRESENTATION OF RESULTS BY PROFESSOR ITO

Professor Ito reviewed the study, which had been commissioned by the Japanese government, and which indicates that the addition of BHA to the diet of Fischer F 344 rats caused hyperplasia, papillomas and squamous call carcinomas of the forestomach of the rats. Thirty four percent of males and twenty nine percent of females fed for 112 weeks on a diet containing 2 % BHA developed carcinomas of the forestomach ; no cancers were observed in the forestomach of rats receiving 0.5 % BHA in the diet, nor in the controls. Papillomas were seen in 2 % of males and females fed 0.5 %. BHA in the diet and 100 % and 96 % respectively in animals fed 2.0 % BHA in the diet. Hyperplasia of the forestomach epthelium was seen in both sexes at both dose levels, the incidence being clearly dose related. Neither papillomas nor hyperplasia were found in the control animals. The incidence of tumours in other organs was not increased significantly by BHA and was comparable to that observed in the controls. Bile duct proliferation in treated animals was reduced in a dose-related manner compared to control animals.

In a 12 week range finding study carried out prior to the carcinogenicity study the levels of BHA added to the diet were 4 %, 2 %, 1 %, 0.5 % and 0.25 %. On the basis of the observed reduction in body weight, the test level of 2 % BHA in the diet was chosen as the maximum tolerated dose for the long term study. Hyperplasia of the forestomach was noted at the higher dose levels. (Information on the no-effect-level for this finding was not made available to the working party).

Subsequent to the carcinogenicity study investigations were undertaken by Prof. Ito and coworkers to determine a possible promoting action of BHA. In a model experiment extending over 36 weeks the bladder carcinogen BBN* was administered for 4 weeks in the drinking water to rats at 0.01 % or 0.05 %. From the 4th to the 36th weeks BHA (2 %), BHT (1 %) and L-ascorbic acid (5 % or 1 %) were given in the diet. Both BHA and BHT enhanced hyperplasia of bladder epithelium following 0.01 % BBN and enhanced bladder papilloma and cancer incidence following 0.05 BBN. Five percent ascorbate enhanced hyperplasia following 0.01 % BBN but enhanced papilloma formation after 0.05 % BBN. One percent ascorbate had no promoting effect irrespective of the concentration of BBN. From these results the authors concluded that BHA, BHT and L-ascorbate show promoting activity under these experimental conditions.

In another experiment using the induction of gamma-glutamyl transferase foci by 200 mg/kg DEN** in partially hepatectomised rats it was found that 2 % BHA, 1 % BHT and 1 % ethoxyquin

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* butylbutanolnitrosamine

** diethylnitrosamine

inhibited the formation of foci in the liver, but 5 % ascorbate or 0.5 % disulfuram had no effect. In conjunction with all these results further promotion and chronic feeding studies are being conducted.

DISCUSSION

The working party noted that the BHA content of the tested material was 98.8 % and that a typical analysis of a similar diet to that used by Professor Ito showed no detectable levels of contaminants likely to be present in such laboratory diets. The BHA tested was produced by butylation of the starting phenolic materials, followed by methylation. However, in Europe BHA is frequently produced by a process involving initial methylation followed by butylation. The working party could not exclude that the nature and amount of impurities might differ in the two processes.

Professor Ito stated that the treatment of BHA by wet pelleting and heat drying was not intended to mimic the conditions in food processing but was adopted in the present study merely for practical convenience in the preparation of the rats diet.

A pelleted diet was also used in a similar long term feeding study on mice on BHA and in long term feeding studies with BHT and other compounds. In this procedure the test substance is added to the basal powdered diet which after pelleting is dried at 90-100°C for 2-2.5 hours. Analysis of test diets on five occasions showed that actual levels of BHA in the animal diet were 0.24 % and 1.07 % and not the 0.5 % and 2 %, respectively, initially expected. It has been claimed that the main product was a dimer of BHA and that "no unusual products" were formed although no direct evidence of this was available. The working party noted an experiment on BHA in pellets in which the BHA had been added at definite concentrations. The authors claim that the observed loss of about half the BHA originally present was probably caused by sublimation during the manufacturing process. There was no evidence available on the identity of the degradation products of BHA or reaction products with components of the rat diet. Additionally the working party was not prepared to make any assumptions in respect of the identity of reaction and degradation products of BHA in the human diet.

With respect to the toxicological findings the working party noted that a similar Japanese 112 week feeding study in rats using BHT had produced no adverse findings. The results with BHA were therefore somewhat unexpected particularly as another recent Japanese feeding study in mice was negative although the same pelleted diet was used. An unpublished Japanese study carried out in rats of the Wistar strain, using dose levels of 0.1 % and 0.5 % BHA in the pelleted diet produced only papillomas of the forestomach but no increase in general tumour incidence. However, the number of animals per group was too small for reliable interpretation. The working party also recalled that as recently as 1980 JECFA had reviewed the then existing data on BHA including a number of long term studies in various species which had not revealed any carcinogenic activity although the experimental protocols were not fully adequate by modern standards (JECFA 1980).

Members of the working party with appropriate expertise were not convinced that the forestomach of the rat served as a reservoir for food. Therefore the suggestion that irritation caused by prolonged contact with the lining of the epithelium might be an important factor in tumour production at this site was not so easily sustained and requires further elucidation. The forestomach of the rat has no counterpart in the human stomach and is structurally comparable to the human oesophagus and therefore the kinetics and mechanisms of action may differ in rat and human situations. Mutagenic studies have not revealed any obvious genotoxic potential for BHA but this too requires confirmation.

There is a need to judge the acceptability of the alternative currently permitted antioxidants in comparison with BHA, bearing in mind the fact that the technological information provided indicates that BHA, BHT and the gallates (e.g. propyl gallate) are frequently used in combination to take into account their synergistic action on each other. BHA is considered by the food industry to be indispensible at the moment as no investigations have yet been completed on how antioxidant mixtures could be reformulated to give a similar technological effect. The predominant utilisation of antioxidants in foodstuffs is in oils and fats as commodities in their own right or in fat containing foods. Such fats and oils may be rendered unpalatable by the production of flavoured or odoriferous substances (i.e. rancidity). The nutritional value of foodstuffs may be decreased by the diminution of the content of lipid soluble vitamins A and E. Indeed foodstuffs may be rendered injurious to health by the

production, by oxidation, of toxic substances. Oxidative attacks on lipids proceed through the mediation of free radicals which are eliminated by antioxidants.

In trying to assess likely intake of BHA from foodstuffs the working party was faced with the problem that actual permissibility of BHA varied significantly from Member State to Member State and from product to product. However, except for specialised food ingredients (e.g. essential oils, vitamin preparations, etc) levels range normally from 100:200 mg/kg (e.g. in oils for retail sale) to 400 mg/kg (e.g. in oils for manufacturing purposes). Assuming a daily intake of about 100 g fat containing the maximum permissible amounts of BHA, it may be calculated that the dose used in the Japanese rat experiment is some 500-1000 fold the maximum likely human intake.

Information was reported to the working party on the extensive coordination of activities in progress in the USA, Canada, Japan and the UK on a full consideration and comparitive evaluation of the chemistry, toxicology, metabolism and pathology of BHA, BHT, TBHQ, propyl gallate, ascorbate, and tocopherols. The working party was also informed of ongoing studies in the Netherlands and the UK and elsewhere on various elements of the questions that had been raised. To avoid duplication of effort, coordination of these activities is essential. The goods offices of the Commission could be used to ensure that the relevant information is made available to those parties active in the field.

CONCLUSIONS

1. Although the results of the recent Japanese study in rats give cause for concern, in the context of all available data on BHA no short term risk to health is evident by the continued use of BHA until the results of the proposed further studies become available. These studies should be completed in every case within 3 years but should be evaluated as they become available.

2. Further information is required before firm conclusions can be drawn on the chemical nature of possible degradation and reaction products of commercial BHA, in the laboratory animal diet and in human food, and their biological significance.

3. The following studies should be completed within 3 years :

   a) reversibility of the early hyperplastic forestomach lesions ;

   b) further mutagenicity studies on heated and non heated BHA to evaluate possible genotoxicity ;

   c) determination of a clear no-effect level for the induction of forestomach hyperplasia ;

   d) investigation of the kinetics and mechanism of action of BHA and its decomposition and reaction products and the significance of the forestomach of the rat as a target organ relative to carcinogenisis in man.

4. A safety evaluation of BHA, compared with other permitted antioxidants should be undertaken.

5. To avoid duplication of studies and to avoid unnecessary effort and expenditure within the EEC Member States or between EEC and other countries coordination is essential.

ACKNOWLEDGEMENTS

The Commission of the European Communities and the members of the working party are grateful for the assistance given by Professor N. Ito, Nagoya City university Medical School, Japan and to Professor D. Schmähl and the staff of the Institute of Toxicology and Chemotherapy, Heidelberg for providing the facilities to enable the meeting to be a success.

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REPORT OF THE SCIENTIFIC COMMITTEE FOR FOOD ON CAFFEINE

(Opinion expressed 7 July 1983)

TERMS OF REFERENCE

To evaluate the possible harm to health from the consumption of caffeine from food and beverages.

BACKGROUND

During the latter part of 1978 a number of reports were published concerning the safety of caffeine. These reports raised doubts as to the safety of drinks containing caffeine as a naturally occurring constituent (e.g. coffee), and were a source of concern to the EEC Commission, particularly since caffeine-containing drinks could be consumed by children.

Of particular historical relevance was the Report published in 1978 by FASEB (Federation of American Societies for Experimental Biology) under an FDA contract. That report concluded on the basis of an evaluation of then available data, that uncertainties existed regarding the use of caffeine in cola beverages. The report stated that although no evidence existed for a hazard to the public when used at current levels and practices in beverages, additional studies should be conducted. Information was provided showing that the current levels of consumption of cola-type beverages led to an intake of caffeine approximating that known to induce certain pharmacological effects, e.g. central nervous stimulation. Concern was also expressed about the potential teratogenic, behavioural and carcinogenic effects of caffeine. The Food and Drug Administration (FDA) of the USA proposed in 1980 as a result of the FASEB report that a number of additional problems required further study, in particular the comparative metabolism and pharmacokinetics in experimental animals and man, the potential behavioural effects in children and the potential reproductive effects.

Recently published studies by the FDA on the effects and pharmacokinetics of caffeine, given by gavage to rats, and on its teratological effects in rats have also given grounds for some concern at least in the USA. There are also recent epidemiological reports claiming an association between coffee consumption and cancer of various organs in man. For these reasons the Commission considered it advisable to request the opinion of this Committee on these issues.

DISCUSSION

Introduction:

Caffeine has been consumed widely as a constituent of food for many years. It occurs naturally in coffee beans, tea leaves, Kola nuts and cocoa beans and is therefore consumed in coffee, tea and chocolate, unless specific steps have been taken to remove it (e.g. decaffeination). It is also an added ingredient *per se* in a variety of foods, e.g. baked goods, ice creams, soft candy, and predominantly in beverages, principally of the cola-type, ostensibly as a flavouring despite its well known stimulating properties of the central nervous system.

Chemically, caffeine (1,3,7-trimethylxanthine) is a stable, unionised alkaloid and one of several related methylxanthines, principally theobromine (3,7-dimethylxanthine) and theophylline (1,3-dimethylxanthine).

Caffeine is a pharmacologically active substance that has been used as a drug because of its stimulating action on the central nervous system, the heart, and the kidneys. It maintains wakefulness, increases alertness, motor activity and cardiac output, stimulates diuresis and depresses appetite and fatigue.

Summary of relevant toxicology:

Absorption, distribution in the body, and excretion of caffeine itself is similar in all animals studied, although metabolism differs between species. Absorption is rapid by any

route of administration, however, the presence of fibre in the diet appears to increase faecal excretion and to lower the toxicity of caffeine, making caffeine availability diet dependent.

Caffeine is widely distributed in all tissues in proportion to their water content and in total body water. It traverses virtually all biological membranes and does not accumulate. It crosses the blood/brain, mammary and the placental barrier.

Late pregnancy increases the half-life up to 3 times the normal value though with large individual variation, thus leading to increased plasma concentrations for any given intake. The relationship of this effect to hormonal status is unclear. These pharmacokinetic data suggested to the author of the study that excessive intake of caffeine by women in the later trimesters of pregnancy should be avoided.

Premature and new born full-time infants eliminate caffeine more slowly than adults. Children up to 10 years eliminate caffeine rapidly (half-life 2 hours) while adolescents and adults are slower (half-life 3 hours for smokers, 5-6 hours for non-smokers) and thus develop higher plasma levels for the same dose. Renal excretion is inefficient because almost all unmetabolized caffeine is reabsorbed in the renal tubules. It is cleared from the body principally through biotransformation. The rate of metabolism is similar for most species but route and pattern of metabolites are species specific. Metabolism occurs primarily in the liver. Some metabolites are common to most laboratory species and man, but human metabolism occurs primarily through demethylation to various xanthines, oxidation to uric acid derivatives and hydration to uracils. The rat produces similar metabolites to man but with quantitative differences and also metabolites not found in man. The main primary metabolite in man is paraxanthine through 3-demethylation, while the rat and mouse produce theobromine and theophylline, the main metabolite of the dog being theophylline.

The most relevant data for human metabolism derive from experiments with caffeine radiolabelled with ^{14}C at the 1-methyl and 2-C position. The half-life of caffeine itself in the plasma and saliva is approximately 3 hours, while that of caffeine plus metabolites is over 8 hours in plasma and about 7 hours in saliva. The salivary concentration is between 65-85% of the plasma concentration, the concentration in human milk also parallels the plasma concentration.

Human metabolism of radiolabelled caffeine results in some 86% of the radioactivity appearing in the urine, 2 - 5% in the faeces and 13% in the exhailed CO_2. The fraction of CO_2 due to oxidation of the 1-methyl group represents only 1% of the absorbed caffeine. About 11% of absorbed caffeine enters the 1-C pool.

Immediately after administration caffeine appears in the plasma. The major plasma and salivary metabolites are paraxanthine (1,7-dimethylxanthine) (72%), theobromine (3,7-dimethylxanthine) (20%) and theophylline (2,3-dimethylxanthine) (8%).

The major urinary metabolites are 1-methylxanthine (18%), 1-methyluric acid (15%) 5-acetylamino-6-formylamino-3-methyluracil (15%), 7-methylxanthine (10%), paraxanthine (6%), 1,7-dimethyluracil (5%), 3-methylxanthine (4%) an unidentified polar compound (3%), unchanged caffeine (1%), 6-amino-5-/N-formylmethylamino/-1,3-dimethyluracil (1-2%) and other identified compounds in quantities below 1% or as traces.

The major faecal metabolites are 1-methyluric acid (38%), 1,7-dimethyluric acid (44%), 1,3-dimethyluric acid (14%), 1,3,7-trimethyluric acid (6%) and unchanged caffeine (2%).

Man metabolises paraxanthine to 1-methylxanthine and 5-acetyl-6-formylamino-3-methyluracil. The extent of acetylation varies from 7-35% and appears to be genetically controlled. Smoking doubles the clearance of caffeine but age has no effect once adulthood is reached.

A recent review of reproduction and teratology studies on caffeine in various laboratory animal species and strains, using different methods of dosing, indicated that caffeine may have foetotoxic and teratogenic potential at dose levels which are toxic to the mother. However, most of these studies had serious deficiencies but they demonstrated, that at high levels of exposure, well above human intakes, caffeine can cause birth defects in animals. The studies were inadequate to determine a no-effect level. Two further studies in rats were initiated in 1979 by the FDA. The study using gavage has been completed and suggests a no-effect level of 40 mg/kg b.w., at higher doses there were skeletal abnormalities considered related to caffeine administration. The preliminary results of the study using exposure to caffeine in the drinking water suggest that caffeine may not cause birth defects although more incomplete ossification than in

controls was still noted at 75 mg/kg b.w.. In other studies doses of the order of 80 mg/kg caused slight delays in mineralization of sternebrae without accompanying effects on plasma calcium levels, alkaline phosphatase or corticosteroid levels. However, caffeine does produce a dose-related decrease in foetal weight if administered as one dose rather than distributed over several administrations. Because of the difficulties in extrapolating the results of the teratological studies to man, due to the differences in the metabolism of caffeine in the rat and in man, it is not possible to determine with confidence the potential teratogenic risk for man. The available human epidemiological studies on congenital malformations, infant mortality, and other suggestive parameters do not determine conclusively any relationship between caffeine consumption and teratological effects. The data raise, however, questions needing resolution.

The available human and animal data on the effect of caffeine on the reproductive function are variable. In most animal studies effects were only seen at exposure levels much above human experience. The human studies are equally inconclusive. There is no clear evidence to conclude that caffeine affects human reproductive function adversely.

The effects of caffeine on the central nervous system are complex. Caffeine stimulates the CNS enhancing some activities and depressing others. Doses ranging from fractions of 1 mg to 10 mg exert a stimulatory response of the order of 10-15%. Caffeine will counteract fatigue but does not improve intellectual or learning performance. Caffeine has been shown to enhance dopamine metabolism in some specific brain areas with corresponding changes in paradoxical sleep pattern and haloperidol catalepsy without affecting motor activity.

Caffeine also stimulates the pituitary secretion of β-endorphins in rats. This effect is blocked by pretreatment with the opiate antagonist naloxone. Elevation of β-endorphin secretion occurs at blood levels of 15-18 μg/ml comparable to those achieved by humans ingesting 250 mg or more of caffeine. Whether this effect also occurs in man is not known. However, caffeine, in doses of 10 mg/kg enhanced withdrawal symptoms and signs to morphine in non-addict volunteers, if given before i.v. naloxone to precipitate opiate withdrawal effects. Caffeine therefore appears to enhance morphine dependence in man.

The mechanisms underlying the behavioural effects of caffeine and other methylxanthines have been investigated by the use of radiolabelled material including analogues. It probably acts by blocking adenosine receptor sites since micro-molar quantities are effective for blocking action and behavioural effects, while millimolar quantities are needed for phosphodiesterase inhibition and benzodiazepine receptor blockage, neither of which correlates consistently with behavioural stimulation. Blockage of adenosine receptors may be reflected in the action of caffeine on the cardiovascular system. Clinical studies have shown mild but unsustained rises in the blood pressure, paralleled by rises in plasma levels and urinary excretion levels of catechol amines. Tolerance develops to these effects and they show large individual variations. They are therefore likely to be no greater than similar changes induced by postural variation and need plasma levels of 1-2 mg/L. Curiously little diuretic effects have been reported in animal studies using comparatively high doses.

The available animal studies do not point to any clear permanent and adverse neurobehavioural effects in rodents up to dose levels causing severe toxicity. The effects in adult man, due to normal or overdose, are known but transient. However, practically no information exists on the effects of chronic exposure to caffeine in soft drinks on the behaviour of children. However, the administration of theophylline to asthmatic children has shown that at therapeutic doses neurological effects can be observed.

The earlier data in experimental animals on the potential carcinogenicity of caffeine have been clarified by the results of two recently completed lifespan studies in rats. No effect on tumour incidence in terms of enhancement, promotion or initiation could be detected. There is therefore no toxicological basis for concluding that caffeine poses any risk of cancer in man. This is supported by the absence of any direct alkylating potential of caffeine, by the failure to bind covalently to DNA or other cellular macromolecules, and by the absence of any biological activity as modifier of known carcinogens.

Caffeine in high concentrations has been shown to be mutagenic in some bacteria, yeasts, plants and in cultured mammalian cells and increases the chromosomal and cytotoxic effects of other mutagenic agents. It is clastogenic for chromosomes. This activity is due to its inhibitory effect on post-replication repair mechanisms by decreasing the size of replicons and inhibiting the elongation of nascent DNA. Mutagenicity for Drosophila is contradictory. In *in vivo* systems caffeine does not appear to be mutagenic.

Human epidemiological studies have been directed mainly towards associations between coffee drinking and cancer of various organs, but have also explored the suspected teratogenicity of caffeine and its effect on calcium metabolism. Correlations have been perceived between coffee consumption and bladder cancer, cancer of the pancreas and ovarian cancer. Most of these studies have been criticised because of inadequate control of confounding factors, inappropriate selection of controls, or small numbers surveyed. However, two recent prospective studies in Norway on about 16 000 persons do not support any association between coffee consumption and cancer of the pancreas. The available studies therefore do not permit a firm conclusion that there is a causal association between coffee consumption and cancer at various sites in man. Nevertheless, the reports indicate that some of these areas deserve further clarification by adequate investigations.

Two studies on pregnancy outcome and coffee consumption do not support any association between coffee drinking, teratogenic effects in the neonate, or other adverse reproductive effects. However, a recent nutritional study with coffee and caffeine disclosed a negative calcium balance in post-menopausal women contributing to osteoporosis.

Human exposure

When caffeine forms part of a therapeutic preparation, it is usually taken in doses of 100 to 200 mg, if used for maintaining wakefulness. For diuretic or appetite-controlling purposes single doses vary from 30-200 mg. Caffeine consumption from cola-type beverages is estimated at 0.18 to 0.31 mg/kg/day for the average U.S. consumer but could rise to 0.66 - 1.1 mg/kg/day for heavy consumers. For an average EEC consumer, the intake from similar beverages ranges from 0.013 mg/kg/day to 0.48 mg/kg/day. Caffeine exposure from all sources, including coffee, tea, chocolate, etc. for a consumer in the EEC ranges from 2.4 mg/kg/day to 4.5 mg/kg/day and averages 3.5 mg/kg/day. Pregnant women are estimated to have exposures of 2.9 mg/kg/day on average in the USA and 2.0 mg/kg/day on average in Europe. Plasma concentrations (µg/ml) in man following oral administration are approximately 1.4 x the dose in mg/kg b.w..

Strong coffee contains about 200 mg caffeine/cup, filtered brewed coffee about 85-150 mg/cup and instant coffee about 85 mg/cup. A cup of tea contains 50 mg caffeine and a 12 oz. cola can about 45 mg caffeine.

CONCLUSIONS

Caffeine has been shown to be a substance possessing certain pharmacological and biological activities demonstrated in animal models and in man. Its metabolic fate in man has been clarified but the exact mechanisms for its CNS-stimulating activity, its action on neurotransmitter and opiate receptors, and behavioural effects in experimental animals and man have not yet been completely elucidated. Caffeine in comparatively high doses shows some weak teratogenic effects in experimental animals and mutagenic effects in *in vitro* system involving chromosomal events.

Nevertheless, considering the total available evidence, there appears to be no reason for concern over carcinogenic, teratogenic, or mutagenic effects of caffeine in man at normal levels of intake. The available human epidemiological evidence for a causal association between coffee consumption and human cancer is conflicting and further research would be desirable. Recent human epidemiological studies have shown no evidence for any association between coffeeconsumption and congenital defects.

SELECTED REFERENCES

General

Department of Health and Human Services, USA (1980) Federal Register, 45, (205) 69835-69837

Heim, F. and Ammon, H.P.T. (1968) Coffein und andere Methylxanthine, F.K. Schattauer, Stuttgart.

Eichler, O. (1976) Kaffee und Coffein, Springer-Verlag, Berlin.

Turnbull, A.J. (1981) Coffee, BNF Nutrition Bulletin, 6 (3), 153-165.

Goodman, L.S. and Gilman, A. (1975) The Pharmacological Basis of Therapeutics, chapter 19, 354-366, Macmillan Publishing Co., Inc. New York.

Third International Caffeine Workshop (1981) Nutr. Reviews, 39 (4) 183-191.

Graham, D.M. (1978) Nutr. Reviews, 36(4), 97-102.

Czok, G. and Lang, K. (1972) Physiologische, Pharmakologische und klinische Wirkungen des Kaffees, Dr. D. Steinkopff Verlag.

Lachance, M.P. (1982) The Pharmacology and Toxicology of Caffeine (1982) J. Food Safety, 4 (2), 71-112.

Metabolism, Pharmacokinetics

Burg, A.W. (1975) Drug Metabolism Reviews 4 (2), 199-288.

Axelrod, J. and Reichenthal, J. (1953) J. Pharmacol exptl. Therap., 107, 519-523.

Rothweiler, H. Knütti, R. and Schlatter, Ch. (1981) Mitt. Gebiete Lebensm. Hyg. 72, 88-98.

Manion, C.V., Whitsett, T.L. and Christensen, H.D. (1981) Fed. Proceed. (Part I) 40, (3), 2352.

Weiss, B. and Hait, W.N. (1977) Ann. Rev. Pharmacol, Toxicol. 17, 441-77.

Newton, R., Broughton, L.J., Lind, M.J., Morrison, P.J., Rogers, H.J. and Bradbrook, I.D. (1981) Europ. J. Clin. Pharmacol. 21, 45-52.

Rafter, J.J. and Nilsson, I. (1981) Xenobiotica, 11 (11) 771-778.

Callahan, M.M., Robertson, R.S., Arnaud, M.J., Branfman, A.R., McComish, M.F. and Yesair, D.W. (1982) Drug Metabolism and Disposition, 10 (4) 417.

Knütti, R. Rothweiler, H. and Schlatter, Ch (1981) Eur. J. Clin. Pharmacol., 21, 121-126.

Brazier, J.L. and Ribon, B. (1982) Med. et Nutr., 18(5), 295-301.

Heaney, R.P. and Recker, R.R. (1982) J. Lab. Clin. Med., 90 (1), 46-55.

Grant, D.M., Tang, B.K. and Kalow, W. (1983) Clin. Pharm. Therap., 33 , 355-359.

Neurobehavioural Effects

Czok, G. (1980) Z. Ernährungswiss., 19, 290-295.

Anon (1981) Science, 211, 27/3/1981.

Arnold, M.A., Carr, D.B., Togasaki, D.M., Pian, M.C. and Martin, J.B. (1982) Life Sciences, 31, 1017-1024.

Reproduction, Teratology

Thayer, P.S. and Kensler, C.J. (1973) Toxicol. Appl. Pharmacol., 25, 169-179.

Vogel, F., Krüger, J., Kurth, M. and Schroeder, T.M. (1966) Humangenetik, 2, 119-132.

Linn, S., Schoenbaum, S.C. Monson, R.R., Rosner, B., Stubblefield, P.G. and Ryan, K.J. (1982) New Engld. J. Med. 306 (3), 141-144.

Mau, G. (1977) Gynäkologe, 10, 45-48

Mau, G. and Netter, P. (1974) Geburtsh. u. Frauenheilk., 34, 1018-1022

Elmayar, M.M.A., McElhatton, P.R. and Sullivan, F.M. (1982), Toxicology, 23, 57.

Leviton, A. (1982) Report on Harvard Medical School study on caffeine consumption - adverse effects on pregnancy and a similar study at Boston University. 4th Int. Caff. Workshop, Athens, Oct. 17-21, 1982.

Chronic Toxicity, Carcinogenicity

Johansson, S. (1981) Acta path. microbiol. Scan. Sect. A. 89, 185-191.

Heyden, S. (1972) Z. für Ernährungsw., Supplement 14, 11-18.

Stocks, P. (1970) Brit. J. Cancer, 24, 215-224.

Trichopoulos, D., Papapostolou, M. and Polychronopoulou, A. (1981) Brit. J. Cancer, 28, 691-693.

Morrison, A.S., Büring, J.E., Verboek, W.G., Aoki, K., Leck, I., Ohno, Y. and Obata, K. (1982) JNCI, 68, 91-94.

MacMahon, B., Yen, S., Trichopoulos, D., Warren, K. and Nardi, G. (1981) New Engld. J. Med., 304, 630-633.

Pegel, K.H. (1981) Chemical and Engineering News, 20th July 1981, 4.

Takayama, S. and Kuwabara, N. (1982) Gann. 73, 365-371.

Mohr, U., Althoff, J. and Morgareidge, K. (1982) Unpublished report presented at 4th International Caffeine Workshop.

Mutagenicity

Tinson, J. (1977) Mutation Res. 47, 1-52.

Thayer, P.S. and Kensler, C.J. (1973) Tox. Appl. Pharmacol. 25, 157-163.

Kihlman, B.A. (1974) Mutation Res., 26, 53-71.

Ostertag, W. (1966) Mutation Res., 3, 249-267.

Weinstein, D. Mauer, I. and Solomon, H.M. (1972) Mutation Res., 16, 391-399.

Kuhlmann, W., Fromme, H.G., Heege, E.M. and Ostertag, W. (1968) Cancer Res., 28, 2375-2389.

Ostertag, W., Duisberg, E. and Stürmann, M. (1965) Mutation Res., 2, 293-296.

Aeschbacher, H.U., Chappuis, C. and Würzner, H.P. (1980) Fd. Cosmet Toxicol, 18, 605-613.

REPORT OF THE SCIENTIFIC COMMITTEE FOR FOOD ON COLOURING MATTERS AUTHORIZED FOR USE IN FOODSTUFFS INTENDED FOR HUMAN CONSUMPTION

(Opinion expressed 7 July 1983)

TERMS OF REFERENCE

To review the safety in use of certain colouring matters, particularly those previously classed by the Committee as temporarily acceptable.

BACKGROUND

1. In its first report on colouring matters, issued in June 1975[1] the Committee reviewed the safety in use of all colouring matters proposed for inclusion in a revised Community list of substances and materials to be authorized in foodstuffs intended for human consumption.

   At that time a number of colouring matters were found to be unacceptable and have since ceased to be used in the Community. (Subsequently, for one of these, Allura Red AC, data have been provided which have made it possible to reevaluate its status.)

   For other colouring matters, the Committee requested additional toxicological data within specified periods. These data have been reviewed as they became available[2,3]. The Commission has now been advised that all outstanding studies have been made available and has asked the Committee to complete its review on these substances.

2. In 1975 many of the colouring matters were considered acceptable from the point of view of safety. The Committee has now been informed about a number of studies on some of these substances and therefore it was thought appropriate to include these new data in a reevaluation of the substances. (This applies particularly to substances referred to in paragraph 7.)

3. In addition to the substances submitted for review in 1975 the Committee in September 1977 also advised on a second submission[2]. At which time it was agreed that riboflavin-5'-phosphate was acceptable for use in food. A number of other colouring matters were considered to be not toxicologically acceptable.

4. Finally, the Committee has advised in October 1981 on the question of the sensitivity of individuals to food components and food additives[4].

CURRENT REVIEW

5. The present report is intended to provide a comprehensive summary of colouring matters evaluated since 1975 and to complete in detail the revisions referred to above, necessary for an up-to-date assessment of all colouring matters used in foodstuffs in the Community.

6. The Committee has already established an acceptable daily intake (ADI) for a number of colouring matters in its 1975 or 1979 reports, and has not reevaluated the data on which these assessments were made, nor has any new data become available which would require reevaluation of these colouring matters.

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[1] Reports of the Scientific Committee for Food 1st Series 1975.

[2] Reports of the Scientific Committee for Food 4th Series 1977.

[3] Reports of the Scientific Committee for Food 8th Series 1979.

[4] Reports of the Scientific Committee for Food 12th Series EUR 7823 1982.

These are as follows:

Beta-apo-8'-carotenal Beta-apo-8'-carotenoic acid, ethyl ester Beta carotene	(1975) ADI 0-5 mg/kg b.w. as sum of the three carotenoids
Canthaxanthin	(1975) ADI 0-25 mg/kg b.w.
Chlorophyllin copper complex Chlorophyll copper complex (sodium or potassium)	(1975) ADI 0-15 mg/kg b.w. as sum of both complexes
Iron oxides and hydroxides	(1975) ADI no upper limit specified
Red 2G	(1975 and 1977) (see Red 10B) ADI 0-0.1 mg/kg b.w. (not to be used under conditions in which significant hydrolysis to Red 10B occurs)
Annatto extract	(1979) ADI 0-2.5 mg/kg b.w. of annatto extract

7. The following colouring matters were accepted in 1975 and 1977 without a formal ADI:-

Anthocyanins	(1975)
Beet Red	(1975)
Chlorophyll	(1975)
Curcumin	(1975)
Lycopene	(1975)
Mixed alpha, beta, gamma carotenes	(1975)
Xanthophylls	(1975)
Riboflavin	(1975 and 1977)
Riboflavin-5'-phosphate	(1977)
Titanium dioxide	(1975 and 1977)
Vegetable carbon	(1975 and 1977)

The Committee sees no reason to change these evaluations now, but draws attention to the fact that the original acceptance in 1975 was limited to situations under which the use of colouring matters extracted from foods would not be expected to result in ingestion differing substantially from the amounts likely to be ingested from the normal consumption of the foods in which they occur. It was also concluded in 1975 that colouring matters derived from natural sources which are not natural foods or their synthetic equivalents, or colouring matters found in foods, prepared synthetically, require suitable testing.

The Committee continues to hold the same opinion. It is also aware that there have been significant technological advances in the isolation, preparation and extraction of colouring matters from natural sources and recommends that a review should be undertaken to assess the impact of these changes on the evaluation of these materials and to improve the specifications of purity.

In addition, aluminium, calcium carbonate, gold and silver were in 1975 considered acceptable only for the external colouring and decorating of food and the Committee sees no reason to modify its view. Other uses of calcium carbonate are under review.

8. In the context of the acceptability of colouring matters, as indeed for other food additives, the Committee reiterates the advice given to the Commission in 1978[5] that where the Committee finds an additive acceptable, even though an ADI has not been allocated, this is because the Committee considers that its use does not raise any health problem.

[5] Reports of the Scientific Committee for Food 5th Series 1978.

9. During the present review the Committee has examined data not only on colouring matters on which it was earlier unable to give a complete answer, but also on certain other colouring matters for which new data had become available.

10. Thus, on the basis of new evidence the Committee has re-evaluated the following colouring matters for which an ADI was established previously:

Brilliant Blue FCF, Erythrosine, Indigotine, Sunset Yellow FCF and Tartrazine.

11. The group of colouring matters falling under the heading "caramels" has been the subject of a continuing review that has enveloped even those caramels considered acceptable by the Committee in 1975 and 1979.

12. Of the colouring matters submitted for evaluation by the Committee in its first review of 1975 and subsequently, for which the Committee was unable to provide a definitive opinion, the EEC Industry Colours Steering group has provided data on the following:

Amaranth

Brilliant Blue FCF (see also above)

Brown HT

Carmoisine

Green S

Patent Blue V

Ponceau 4R

Quinoline Yellow

and, as mentioned in paragraph 1, Allura Red AC.

Data have also been provided on Brown FK, cochineal/carminic acid and lithol rubine BK.

13. For ease of reference, Annex 1 to this report lists colouring matters which have been submitted to the Committee since it started evaluating food colouring matters in 1975 for which the data are insufficient to assess the safety of the substance, or for which the data show cause for concern. Annex 2 summarizes the decisions taken by the Committee on individual colouring matters during the present review. Annex 3 summarizes the data on which the assessments on individual colouring matters were made by the Committee.

14. All substances reevaluated by the Committee met adequate purity specifications provided in the submission. Colouring matters which do not comply with these specifications must be evaluated separately and this may require further toxicological testing if the differences in specification are significant.

15. The Committee has accepted that sodium, potassium, calcium salts of colouring matters are to be considered as equivalent, in general, for the purposes of the evaluations in this report.

16. The Committee was asked to give special attention to the question of so-called "aluminium lakes" through the use of alumina. It was emphasized to the Committee that the alumina itself is an integral part of the aluminium lakes (e.g. of the sulphonated organic food colouring matters and erythrosine) and because of this it cannot be looked upon simply as a diluent.

17. An aluminium lake of food colouring matter can be described chemically as the aluminium salt of the food colouring matter absorbed upon a substrate of alumina. These have considerable technological use because, having the character of insoluble pigments, they can readily be dispersed into oily, fatty or dry foods to impart the desired colour. In the presence of water and outside the pH range 4-9 food colouring lakes revert to the water soluble dye and a salt of aluminium. Food colour lakes as sold usually have dye contents in the range of 10-40%.

18. Depending on the reactants selected, the method of mixing, the temperature and the final pH so the chemical composition of the final "alumina" will vary somewhat. In most cases it is not represented entirely by the formula $Al(OH)_3$, but consequent upon the details of its preparation, will contain certain amounts of other ions, e.g. sulphate and/or carbonate.

19. Because of the route of administration and the levels of aluminium concerned, the Committee saw no toxicological reason which would suggest that aluminium lakes of food colouring matters were unacceptable from the point of view of health of the consumer.

20. The problem of sensitivity of individuals to particular colouring matters has not been especially studied during the present review as the subject has already been comprehensively covered in 1982 in the 12th Series of Reports of the Committee . Nevertheless the Committee continues to be concerned by the problems raised, and reminds those interested of the conclusions in its 1982 Report, especially in relation to labelling and a more rigorous appraisal of the technological need for colouring matters.

ANNEX 1

Colouring matters submitted to the Committee for which the data are insufficient to assess the safety of the substance, or for which the data show cause for concern

Alkanet	(a)
Antheraxanthin oleoresin	(b)
Black 7984	(a)
Burnt Umber	(a)
Chocolate Brown FB	(a)
Chrysoine S	(a)
Fast Red E	(a)
Fast Yellow AB	(a)
Indanthrene Blue RS	(a)
Methyl Violet	(b)
Orange G	(a)
Orange GGN	(a)
Orange RN	(a)
Orchil and Orcein	(a)
Ponceau 6R	(a)
Red 10B	(b)
Scarlet GN	(a)
Ultramarine	(b)
Violet BNP	(b)
Violet 6B	(a)
Yellow 2G	(a)(c)

(a) 1st Series 1975
(b) 4th Series 1977
(c) 8th Series 1979

ANNEX 2

Summary of the evaluations of the Committee for colouring matters for which data were supplied during the current review

Allura Red AC		ADI	0 - 7	mg/kg b.w.
Amaranth		ADI	0 - 0.8	mg/kg b.w.
Brilliant Black PN		ADI	0 - 5	mg/kg b.w.
Brilliant Blue FCF		ADI	0 - 10	mg/kg b.w.
Brown FK		ADI	0 - 0.15	mg/kg b.w.
Brown HT		ADI	0 - 3	mg/kg b.w.
Caramels: burnt sugar, plain (spirit)	acceptable			
caustic sulphite, ammonia, ammonia sulphite	temporarily acceptable			
Carmoisine (azorubine)		ADI	0 - 4	mg/kg b.w.
Cochineal (carmines) (opinion given 23 October 1981)		ADI	0 - 5	mg/kg b.w.
Erythrosine	temporary	ADI	0 - 1.25	mg/kg b.w.
Green S		ADI	0 - 5	mg/kg b.w.
Indigotine		ADI	0 - 5	mg/kg b.w.
Lithol Rubine BK		ADI	0 - 1.5	mg/kg b.w.
Patent Blue V		ADI	0 - 15	mg/kg b.w.
Ponceau 4R		ADI	0 - 4	mg/kg b.w.
Quinoline Yellow		ADI	0 - 10	mg/kg b.w.
Sunset Yellow FCF		ADI	0 - 2.5	mg/kg b.w.
Tartrazine		ADI	0 - 7.5	mg/kg b.w.

ASSESSMENT OF INDIVIDUAL COLOURING MATTERS

Allura Red AC

The available toxicological data relate to the metabolism, including identification of cresidine sulphonic acid as the major metabolite, reproduction and teratogenicity, *in vivo* and *in vitro* mutagenicity, acute toxicity including skin sensitisation, short-term studies in rats, dogs and pigs as well as several long-term studies in rats and mice. The question of the increase in the incidence of lymphomas in the first long-term mouse study was not confirmed in the second long-term mouse study.

Orally administered allura red undergoes partial azo reduction prior to absorption. Gastrointestinal absorption is poor, most dye being excreted in the faeces. The "no-adverse-effect" level for reproductive function in the rat was 1.39% in the diet. Several teratogenic studies revealed no embryotoxic nor teratogenic potential. The available mutagenicity studies did not show any genotoxic activity. The acute, short-term and dermal toxicity studies on several species indicated no colour-induced toxic responses.

The long-term studies in mice and rats did not reveal any carcinogenic potential. The Committee therefore established an ADI of 0-7 mg/kg b.w., agreeing with the evaluation of JECFA at its 25th meeting.

References:

SCF (1975) First Series of Reports, 31.12.1975, p. 28.

Hazelton Lab. Inc. (1970, 1977, 1978, 1979): Unpublished Reports submitted by the EEC Colours Group.

Collins, T.F.X. and Black, T.N. (1980) Fd. Cosmet. Toxicol., 18, 561.

WHO (1980) Food Add. Series No. 15, 5-17.

Amaranth

The available toxicological data on this colour comprise numerous biochemical, acute toxicity, short-term toxicity, mutagenicity, reproduction, teratogenicity and long-term studies. Some 10-20% of the colour is absorbed after azoreduction in the gut, 75-85% being excreted in the faeces. Naphthionic acid is the major metabolite in the urine and faeces. The acute and short-term studies in several species indicated no serious toxic response. The *in vitro* and *in vivo* mutagenicity studies revealed no genotoxic activity. Extensive teratogenicity and multigeneration reproduction studies showed no adverse effects on reproductive function with a "no-adverse-effect" level similar to the one observed in the earlier long-term studies. Several long-term studies in mice and numerous studies in rats were available, but the apparent tumourigenic potential reported in two of the studies could not be evaluated in the absence of knowledge of the specification of the material then tested. A recently completed long-term study showed no effect on tumour incidence, but a clear no-effect level could not be established for the renal effects noted. Such chronic renal toxicity had never been observed previously. The possibility existed that the physical effects of large amounts of unabsorbed dye in the gut might contribute to these findings, and that species specific reactions might also be involved. The Committee requested the results of a further 90-day study to establish a no-effect level for the renal effects and clarification of the mechanisms underlying the observed adverse findings. On the basis of this study an ADI of 0 - 0.8 mg/kg b.w. was established.

References:

SCF (1975) First Series of Reports, 31 December 1975, p. 24.

SCF (1976) Second Series of Reports, December 1976, p. 7.

SCF (1979) Eighth Series of Reports, May 1979, p. 11.

Clode, S.A., Butler, W.H. and Conning, D.M. (1981) BIBRA Report 242/1/82, submitted by EEC Colours Group.

BIBRA (1982) Unpublished Report 453/1/82 submitted by EEC Colours Group.

BIBRA (1982) Unpublished Report 452/1/82 submitted by EEC Colours Group.

Phillips, J.C., Mendis, D., Bex, C. and Gaunt, I.F. (1982) BIBRA Report 397/1/82, submitted by EEC Colours Group.

Brown, J.P. et al., (1978) Mutation Res., 56, 249-271.

Garner, R.C. and Nutman, C.A. (1977) Mutation Res., 44, 9-19.

WHO (1978) Food Additive Series No. 13, 4-6.

BIBRA (1983) Unpublished Report 453/2/83 submitted by EEC Colours Group.

Brilliant Black PN

The available toxicological studies cover metabolism, acute toxicity, short-term toxicity in the rat and pig, multigeneration reproduction and teratogenicity in rats, in vitro mutagenicity and long-term toxicity in mice and rats. The metabolism studies show that the dye is poorly absorbed. The gut flora readily degrades the substance into metabolites containing the Cleve's acid moiety. No significant effects were noted on reproductive function nor was there evidence of genotoxicity. The long-term studies revealed no carcinogenic potential, the "no-adverse-effect" level in rats being 500 mg/kg b.w.. The Committee was able to establish an ADI of 0-5 mg/kg b.w./day on the basis of the further studies now available, agreeing with the ADI established by JECFA in 1981.

References:

SCF (1975) First Series of Reports, 31.12.1975, p. 24.

SCF (1979) Eighth Series of Reports, May 1979, p. 11.

Leegwater, D.C. (1980) Report R5875 submitted by EEC Colours Group.

Köeter, H.B.W.M. (1979) Report R6106 submitted by EEC Colours Group.

Köeter, H.B.W.M. and Dreef van der Meulen, H.C. (1977) Report R6417 submitted by EEC Colours Group.

Drake, J.J.F., Butterworth, K.R., Gaunt, I.F. and Grasso, P. (1977) Fd. Cosmet. Toxicol., 15, 503.

WHO (1981) Food Add. Series, No. 16, 45-51.

Brilliant Blue FCF

The available toxicological data include biochemical and metabolic studies, acute toxicity, short-term toxicity in 4 species, reproduction and teratology studies in the rat and several long-term studies in mice and rats including two recent studies in these rodents. The colour is poorly absorbed and almost completely excreted in the faeces following biliary excretion if parenterally administered. The short-term studies include also a 13 weeks rat study on o-sulphobenzaldehyde, one of the components of the colour. These showed no significant toxic effects. The reproductive functions were not affected by the ingestion of the colour. The older long-term studies revealed no carcinogenic potential. The new mouse and rat studies confirmed this but showed a slight reduction in body weight of females at the top dose level. The no-adverse-effect level was 2% in the diet. The Committee had originally established an ADI of 0-12.5 mg/kg b.w. on the basis of the available data. Evaluation of the modern studies allowed the Committee to establish a revised ADI of 0-10 mg/kg b.w..

References:

SCF (1975) First Series of Reports, 31.12.1975, p. 24.

IRDC (1981) Reports dated 31.8.1981 submitted by EEC Colours Group.

Brown, J.P., Dorsky, A., Enderlin, F.E., Hale, R.L., Wright, V.A. and Parkinson T.M. (1980) Fd. Cosmet. Tox. 18, 1-5.

Phillips, J.C., Mendis, D., Eason, C.T. and Gangoli, S.D. (1980) Fd. Cosmet. Toxicol. 18, 7-13.

IARC (1978) Monographs Vol. 16, 171-186.

WHO (1970) Food Add./70.36; 24-27.

International research and development corporation (1981) long-term dietary toxicity/carcinogenicity studies in rats and in mice, unpublished reports, dated 31/8/81 and 11/11/81.

Brown FK

Extensive toxicological data are available including: chemical and biological characterisation of the six major colour components, metabolism, acute toxicity, short-term studies, in vitro mutagenicity studies in S. typhimurium, reproduction and teratology studies, several long-term studies in mice and rats as well as an investigation into the nature of the pigment deposited in tissues of treated animals. The major components are reductively cleaved to sulfanilic acid and the corresponding amine, which are then subsequently absorbed. Very high doses caused pigment deposition in several tissues and were cardiotoxic. No teratological effects were noted. A multigeneration study, using, however, only 1 dose level (15 mg/kg b.w.) about 100 times the calculated human intake, showed no toxic effects on reproductive function and no pigment deposition in the F_{3a} generation. The F_1 generation part of the long-term study used 3 dose levels and also showed no reproductive effects or pigment deposition in F_1 weanlings. The new long-term rat studies showed reduced body weight and tissue pigmentation at the highest test dose but no evidence of carcinogenic potential. The no-adverse-effect level was 15 mg/kg b.w. determined in the multigeneration study. The Committee established an ADI of 0-0.15 mg/kg b.w. on the basis of the further information provided.

References:

SCF (1975) First Series of Reports, 31.12.1975, p. 25.

SCF (1979) Eighth Series of Reports, May 1979, p. 11.

Philp, J.M. (1978) Unilever Research Report, August 1978, submitted to EEC Commission.

Wilson, R. et al. (1983) Report of Unilever ESL dated Jan. 1983 submitted to EEC Commission.

LSR (1982) Report No. 82/URL012/573 submitted by Unilever to EEC Commission.

LSR (1980) Report No. 80/URL072/051 submitted by Unilever to EEC Commission.

Wilson, R., Hague, P.H. and Hardy, W.S. (1983) Report of Unilever ESL dated Jan. 1983 submitted to EEC Commission.

Haveland-Smith, R.B. and Combes, R.D. (1982) Mutation Res., 105, 51-58.

Brown HT

The available toxicological data include studies on metabolism, acute toxicity, short-term toxicity in rats and pigs, multigeneration reproduction and teratogenicity in rats, in vitro mutagenicity studies and long-term studies in the mouse and rat. The composition of the commercially used colour includes about 20% of unidentified subsidiary colour. The metabolic studies showed a small proportion of the colour was observed in the kidney and mesenteric lymphnodes on prolonged ingestion. The acute and short-term studies revealed no significant toxic effects. The multigeneration and teratogenicity studies showed staining of kidney and mesenteric lymphnodes, but no specific toxic effects on reproductive function or evidence of teratologic potential. The mutagenicity tests in prokaryotic systems showed no activity. The long-term studies revealed staining of the tissues at the highest levels tested but no carcinogenic potential. The Committee requested additional information on the nature of the subsidiary dyes and information on the absence of any histological evidence of damage from pigment deposition. These data have now been provided. The Committee established an ADI of 0-3 based on the no-adverse-effect level in the long-term mouse study.

References:

SCF (1975) First Series of Reports, 31.12.1975, p. 25.

SCF (1975) Supplementary Report 14.9.1975.

SCF (1979) Eighth Series of Reports, May 1979, p. 12.

BIBRA (1981) Report No. 294/1/82 submitted by EEC Colours Group.

Gaunt, I.F., Phillips, J.C., Mendis, D. (1981) Research Report 1/1981 submitted by EEC Colours Group.

Haveland-Smith, R.B. and Combes, R.D. (1980) Fd. Cosmet. Toxicol. 18, 215.

WHO (1977) Food Add. Series No. 12, 68-71.

Caramels

When caramels were first considered by JECFA in 1969, they were understood to be a large number of ill-defined and complex products, for which no adequate specifications existed. They were formed from various carbohydrates by heating with any of a wide range of acids, bases and salts, under different conditions of temperature and pressure.

A considerable amount of toxicological data on caramels has been accumulated since then, but the evaluation of these data was hampered by the absence of satisfactory specifications for the materials tests at various times. During the present review, the Committee noted that it is now generally accepted that caramels fall into five classes: burnt sugar caramels, plain (spirit) caramels made with caustic soda, caustic sulfite caramels, ammonia caramels and ammonia sulphite caramels.

Burnt sugar caramels and plain caramels could be considered as natural constituents of the diet as they are only likely to contain compounds which would be expected to arise when sucrose is heated or when certain foods are cooked. In 1969 JECFA considered that degrees of difference could not be delineated for these caramels and that a toxicological discrimination was unwarranted between such caramels produced on cooking or heating sugars and caramels produced commercially by processes not using ammonia or ammonium salts. At the time of its first review of food colours in 1975 the Committee expressed the same opinion as proposed by JECFA already in 1969, when evaluating burnt sugar caramels and plain caramels, and no new information has been received to cause the Committee to change its opinion.

The Committee did not evaluate caustic sulfite caramels as no adequate data were available for assessment.

At the time of its first review of food colours in 1975 the Committee established a temporary ADI of 0-100 mg/kg b.w. for ammonia caramels with a limit of 200 ppm 4-methyl-imidazole per 20 000 EBC units. However, further toxicity testing revealed that the principal adverse effect of ammonia caramels was a depression of circulating lymphocytes. In 1977 JECFA withdrew its temporary ADI for ammonia caramels because a no-effect level and an adequate specification had not been established. The Committee reviewed the Japanese long-term studies in rats and mice and reproduction teratology studies on ammonia caramels as well as the long-term study on 4-methyl-imidazole in 1979, and was informed about further work in progress. The Committee took no final decision at that time but had no objection to the continued use of ammonia caramels while receiving six-monthly reports on the progress of work.

A factor possessing antipyridoxine properties and capable of causing reduction in lymphocytes only in rats on a low-pyridoxine diet has now been identified as 2-acetyl-4(5)-tetrahydroxybutyl imidazole. However, a clear causal relationship between lymphocyte reduction ability and antipyridoxine activity has not been established. Moreover the lymphocyte-depressing activity was still present at levels as low as 0.1 mg/kg b.w.. However, the absence of any observed effects in man from the traditional use of this colouring matter and the presumed adequacy of the normal diet with regard to the supply of pyridoxine would not sustain the expectation of any significant lymphocyte reduction occurring in man as a result of the antipyridoxine activity of ingested ammonia caramels.

Characterization of commercially available ammonia caramels is in progress. An analytical method for determining the antipyridoxine factor remains to be developed. The available toxicological information on the absence of carcinogenic potential is sufficiently reassuring for the Committee to raise no objections to the continued use of this colouring matter until the end of 1987 while further work in progress is being completed. However, in the absence of adequate information on the material finally to be evaluated by the Committee, the Committee cannot exclude the need for further toxicological studies on ammonia caramels.

In evaluating ammonia sulphite caramels in 1975 grouped at that time with ammonia caramels, the Committee established a temporary ADI of 0-100 mg/kg b.w. because of the inadequacy of the available chronic toxicity studies. When reviewing the long-term and reproduction studies on ammonia sulphite caramels in 1979 the Committee maintained the temporary ADI of 0-100 mg/kg b.w. while further work was in progress. Since that time the Committee has continued to receive six-monthly reports on progress. At the time of the present review the Committee noted that the recent mutagenicity studies on ammonia sulphite caramels revealed no genotoxic potential. Subchronic studies in rats including a one generation study showed no haematological effects and yielded a no-effect level of 10% in the diet. Chronic toxicity/carcinogenicity studies in rats and mice are in progress. The absence of any adverse findings justifies continued use until the results of the ongoing long-term tests are to hand and an adequate specification has been prepared. Present efforts at characterizing the internationally available caramels show great qualitative similarities between the various products.

In summary, although the Committee was not able to make final evaluations on caustic sulphite caramels, ammonia caramels and ammonia sulphite caramels, it has no objections to the continued use of these colouring matters while further information on specification and toxicology is generated for all these materials. The Committee wishes to be kept informed at regular intervals of the results of ongoing studies which are expected to be completed by December 1987.

In view of the likely human intake there are methodological difficulties in feeding caramels, particularly ammonia caramels, to test animals at dose levels which would allow the establishment of a classical ADI. This is a general problem which relates not only to caramels but also to other substances ingested in large amounts. The Committee recommends that this should be reviewed.

References:

SCF (1975) First Series of Reports, 31.12.1975, p. 25.

SCF (1979) Eighth Series of Reports, May 1979, p. 11.

Sinkeldam, E.J. (1982) Unpublished Report No. V82.291/221153, submitted by European Technical Caramel Association.

ITCA (1977-1983) Summaries of biological data on caramel colours submitted by European Technical Caramel Association.

WHO (1977) Food Add. Series No. 12, 47-63.

Carmoisine (azorubine)

The available toxicological data comprise metabolic studies in 3 species, acute toxicity including sensitisation, in vitro mutagenicity study, short-term studies in rats and pigs, reproduction and teratogenicity studies in the rat and several long-term studies in the mouse and rat. Metabolism involves azo reduction to naphthionic acid and aminonaphthol sulphonate. Most of the colour is excreted within 24-72 hours without any accumulation and with minimal transplacental passage. Short-term tests revealed no adverse toxic effects. Multigeneration reproduction and teratogenicity studies showed no adverse effects on reproductive function and no teratogenic potential. In vitro mutagenicity tests did not indicate genotoxic activity. The long-term studies produced no evidence for carcinogenic potential. The Committee established an ADI of 0-4 mg/kg b.w. based on the NEL in the long-term rat studies.

References:

SCF (1975) First Series of Reports, 31.12.1975, p. 24.

SCF (1979) Eighth Series of Reports, May 1979, p. 11.

Phillips, J.C., Bex, C.S., Gangoli, S.D. and Gaunt, I.F. (1982). BIBRA Report 303/1/1982 submitted by EEC Colours Group.

BIBRA (1982) Unpublished Report 251/1/82 submitted by EEC Colours Group.

Galli, C.L., Marinovich, M. and Costa, L.G. (1981) Fd. Chem. Toxicol. 20, 351-6.

NTP (1982) Carcinogenesis Bioassay Techn. Rep. Ser. No. 20, NTP-80-67.

WHO (1978) Food Add. Series, No. 13, 7-10.

Cochineal (Carmines)

The Committee already evaluated this substance in 1981. The then available data included short-term studies on 3 species, multigeneration and teratogenicity studies in mice and rats, in vitro mutagenicity studies and a long-term study in rats. No metabolic studies were available.

The short-term studies showed no obvious toxicological effects. The colour did not affect reproductive function nor did it show any teratogenic potential. Mammalian tissue cultures produced no evidence of cytotoxicity and a number of in vitro mutagenicity tests in prokaryotic and eukaryotic systems indicated absence of any genotoxic activity. The long-term study included in-utero exposure and did not show any carcinogenic potential. The Committee established an ADI of 0-5 mg/kg b.w. on the basis of the no-adverse-effect level of 500 mg/kg b.w. in the long-term rat study. JECFA has subsequently evaluated the colour similarly.

References:

SCF (1975) First Series of Reports, 31.12.1975, p. 28.

SCF (1979) Eighth Series of Reports, May 1979, p. 12.

SCF (1981) Opinion given 23.10.1981.

BIBRA (1981) Unpublished Report 230/1/81 submitted by EEC Colours Group.

BIBRA (1979) Unpublished Report 230/1/79 submitted by EEC Colours Group.

WHO (1983) Food Add. Series (in press).

Erythrosine

The available toxicological data include metabolic studies and neurophysiological studies, acute toxicity, short-term studies in rats and pigs, multigeneration reproduction and teratology studies, several long-term studies in mice, rats, gerbils and dogs, as well as in vitro mutagenicity studies. A number of long-term studies in mice and rats were carried out recently in Japan and U.S. as well as a multigeneration reproduction study in rats in the U.S. but only summaries are available. There is some information on metabolism including the contribution to human iodine intake from the ingested colour. The significance to man of the neurophysiological effects related to neuronal transmission observed in vitro and to the activity and behaviour of treated animals is not clear. The short-term studies showed only specific effects due to the iodine released on ingestion, but no other significant toxic effects were seen. The in vitro mutagenicity tests showed no genotoxic activity. The earlier multigeneration study revealed no effects on reproductive function, and the earlier long-term studies in rats and mice showed no carcinogenic potential. The more recent Japanese studies apparently confirmed this. The recent US study apparently showed increased thyroid tumours at the high dose. The study in gerbils and in dogs cannot be evaluated as essential information is missing. The Committee considered that the new information required the withdrawal of the full ADI of 0-2.5 given in 1975 and substitution by a temporary ADI of 0-1.25 mg/kg b.w. based on a no-effect level in the rat of 250 mg/kg b.w. determined in the studies reviewed by the Committee. The Committee requires details of the recent U.S. and Japanese studies, and studies to elucidate the contribution to iodine intake from oral ingestion and clarification of the neurophysiological effects observed.

References:

SCF (1975) First Series of Reports, 31.12.1975, p. 23.

Schaywitz, B.A., Goldenring, J.R. and Wool, R.S. (1979). Neurobeh. Toxicol. Vol. 1, 41-47.

Logan, W.J. and Swanson, J.M. (1979) Science, 206, 363-364.

Sekigawa, S., Shimamura, H., Yamamoto, H., Okuyama, T. and Tsubura, Y. (1978) J. Nara. Med. Assoc. 29 (6), 709-721.

WHO (1975) Food Add. Ser., No. 6, 80-88.

Food Green S

The following toxicological data are available: metabolic studies in 3 species, acute and short-term toxicity studies in rats, multigeneration reproduction studies and teratology studies in rats and long-term studies in mice and rats. No mutagenicity studies are available. The colour is practically unabsorbed from the gut. No toxic effects were noted in the acute and short-term studies. The colour was neither embryotoxic nor teratogenic and did not affect reproductive function. The long-term studies in mouse and rat showed no carcinogenic potential. The Committee established an ADI of 0-5 mg/kg b.w. based on the no-adverse-effect level in the rat of 500 mg/kg b.w.

References:

SCF (1975) First Series of Reports, 31.12.75, pg. 25.

SCF (1979) Eighth Series of Reports, May 1979, pg.11.

BIBRA (1978) Report No. 6/1978 submitted by EEC Colours Group.

BIBRA (1978) Report No. 196/1/1978 submitted by EEC Colours Group.

Clode, S.A. (1979) BIBRA Report No. 3.22.08 submitted by EEC Colours Group.

BIBRA (1982) Long-term study in mice, Multigeneration study in rats submitted by EEC Colours Group.

Indigotine

Toxicological data available on this colour comprise metabolic studies, acute toxicity and short-term studies, including one on the metabolite isatin-5-sulphonic acid, teratology studies and several long-term studies in mice and rats. Summary information also exists on mutagenicity studies and two further long-term studies in the mouse and rat, all performed in the U.S. apparently confirming the findings of the earlier studies. The colour is poorly absorbed from the gut. The earlier short-term studies, including one on the major metabolite, revealed no obvious toxic effects. No evidence of teratogenic potential was found. The earlier long-term studies showed no carcinogenic potential. The Committee therefore agreed to retain the ADI of 0-5 mg/kg b.w. based on the no-adverse-effect level of 500 mg/kg b.w. in the long-term rat study.

References:

SCF (1975) First Series of Reports, 31.12.1975, pg. 23.

WHO (1975) Food Add. Ser. No. 6, 95-99.

FDA (1983) Fed. Reg. 48 (25), 5252-5261.

Lithol Rubine BK

The following studies are available: acute toxicity studies on the calcium salt, dermal toxicity tests, in vitro mutagenicity studies, short-term studies in the rat and dog, reproduction and teratology studies on the calcium and disodium salt as well as long-term studies in the mouse and rat using the disodium salt and long-term studies in rats and dogs with the calcium salt. No metabolic studies are available. The dermal toxicity studies reveal no serious effects. The in vitro mutagenicity studies show no genotoxic potential. The reproductive function is not affected by either salt, but absence of teratological effects was only confirmed for the calcium salt, the only compound tested. The Committee established an ADI of 0-1.5 mg/kg b.w. based on the no-adverse-effect level in the long-term rat study of 150 mg/kg b.w.

References:

SCF (1975) First Series of Reports, 31.12.1975, pg. 28.

SCF (1979) Eighth Series of Reports, May 1979, pg. 12.

IRDC (1981) Unpublished data supplied to EEC Commission.

Muzzall, J.M. and Cook, W.L. (1979) Mutation Res., 67, 1.

Patent blue V

The toxicological data available on this colour include metabolic studies, in vitro mutagenicity studies, acute and short-term toxicity studies, studies on teratology in rats and long-term studies in mice and rats. The latter included a three generation reproduction study. The colour is poorly absorbed by the rat and dog, but the nature of the metabolites was not determined, and only single administration was investigated. The short-term studies in cats and dogs revealed no obvious toxic effects. The reproduction and teratology tests showed no effects on reproductive function or any teratogenic potential. The long-term tests showed no carcinogenic potential. The Committee established an ADI of 0-15 mg/kg b.w. based on the no-adverse level of 1500 mg/kg b.w. in the mouse study.

References:

SCF (1975) First Series of Reports, 31.12.75, pg. 25.

SCF (1979) Eighth Series of Reports, May 1979, pg. 12.

Coquet, B. (1978) IFREB Report No. 806216 submitted by EEC Colours Group.

Coquet, B. (1980) IFREB Report No. 110203 submitted by EEC Colours Group.

Haveland-Smith, R.B. and Combes, R.D. (1980) Fd. Cosmet. Toxicol. 18, 215.

Ponceau 4R

The following toxicological data are available: metabolic studies, acute and short-term toxicity studies, multigeneration reproduction studies, teratology studies in 3 species and several long-term studies in mice and rats. The colour is absorbed from the gut and excreted in urine and faeces without tissue accumulation. The acute and short-term studies showed no significant toxic effects. No adverse effects on reproductive function were noted and no teratogenic potential was detected. The recent long-term studies in rats confirmed the absence of carcinogenic potential, thus offering further assurance. Nepthrotoxicity was seen in the high doses. The Committee established an ADI of 0-4 mg/kg b.w. based on the no-adverse level in the long-term mouse study of 500 mg/kg b.w.

References:

SCF (1975) First Series of Reports, 31.12.75, pg. 25.

SCF (1979) Eighth Series of Reports, May 1979, pg. 12.

BIBRA (1980) Report No. 1/205/80 submitted by EEC Colours Group.

BIBRA (1981) Report No. 293/2/81 submitted by EEC Colours Group.

Phillips, J.C., Bex, C.S. and Gaunt, I.F. (1982) Fd. Cosmet. Toxicol., 20, 499-507.

Meyer, O. and Hansen, E.V. (1975) Toxicology, 5, 201-207.

WHO (1975) Food Add. Series No. 6, 109-112.

Quinoline Yellow

The available toxicological data include: metabolic studies, mutagenicity studies, acute and short-term studies, multigeneration reproduction and teratology studies, long-term studies in the rat and mouse. Although this colour exists in methylated and unmethylated form, the toxicological data on either derivative can be used for the evaluation of the 2:1 mixture of unmethylated and methylated material in actual use. The colour is poorly absorbed and hardly metabolised. The nature of the metabolites has not been established. The in vitro mutagenicity studies showed no genotoxic potential. The acute and short-term tests in rats and dogs produced no obvious toxic effects. Reproductive function was not affected and no teratogenic potential was detected in rats and rabbits. Long-term studies in mice and rats revealed no carcinogenic potential. The Committee established an ADI of 0-10 mg/kg b.w. based on the no-adverse level in the long-term mouse study of 1000 mg/kg b.w.

References:

SCF (1975) First Series of Reports, 31.12.1975, p. 26.

SCF (1979) Eighth Series of Reports, May 1979, p. 12.

IFREB (1978) Unpublished Report No. 110202 submitted by EEC Colours Group.

Pierce, E.C. et al. (1974) Tox. Appl. Pharmacol., 29, 121.

Hollstein, M., Talcot, R. and Wei, E. (1978) J. Natl. Cancer Inst., 60, 405-410.

WHO (1978) Food Add. Ser. Nos. 13, 19.

Sunset Yellow FCF

Toxicological data available on this colour comprised: metabolic studies, in vitro mutagenicity studies, sensitisation tests, acute and short-term toxicity studies, multigeneration and teratology studies and several long-term studies in mice, rats and dogs. The colour is metabolised by azoreduction and some of the breakdown products are absorbed ane excreted in bile and urine. The mutagenicity tests showed no genotoxic activity. Acute and short-term tests showed no obvious toxic effects. Reproductive function was not affected and no teratological potential noted. The long-term studies in rats, mice and dogs revealed no carcinogenic potential. The Committee established an ADI of 0-2.5 mg/kg b.w. based on the long-term dog study, being the most sensitive species, with a no-adverse-effect level of 250 mg/kg b.w..

References:

SCF (1975) First Series of Reports, 31.12.1975, p. 24.

Haveland-Smith, R.B. and Combes, R.D. (1980) Fd. Cosmet. Toxicol., 18, 215-221.

Garner, R.C. and Nutman, C.A. (1977) Mutation Res., 44, 9-19.

NCI (1981) Techn. Rep. Ser. No. 208 of National Toxicology Programme.

WHO (1983) in press.

Tartrazine

The following data were available: metabolic studies, an early in vitro mutagenicity study, acute and short-term toxicity studies, multigeneration reproduction studies and teratology studies, several long-term studies in the mouse and rat and summaries of recent U.S. long-term studies in the mouse and rat. The colour is metabolised by azoreduction and absorption of the breakdown products with subsequent excretion in faeces and urine. The acute and short-term tests revealed no obvious toxic effects. Reproductive function was not affected and no teratogenic potential was noted in rats and rabbits. The available long-term studies reveal no carcinogenic potential and the apparent absence of any adverse effects in the recent U.S. studies offers additional reassurance. The Committee maintained the ADI of 0-7.5 mg/kg b.w. established previously on the basis of the no-adverse-effect level of 750 mg/kg b.w. in the earlier long-term rat study.

References:

SCF (1975) First Series of Reports, 31.12.1975, p. 24.

WHO (1966) Food Add./66.25, 88-92.

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The Scientific Committee for Food was established by Commission Decision 74/234/EEC of 16 April 1974 (OJ L 136, 20.5.1974, page 1) to advise the Commission on any problem relating to the protection of the health and safety of persons arising from the consumption of food, and in particular the composition of food, processes which are liable to modify food, the use of food additives and other processing aids as well as the presence of contaminents.

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

The present series relates to the opinions of the Committee on the evaluation of the potential hazards to human health:

- arising from the migration into food of di-2-ethylhexylphthalate (DEHP) and di-2-ethylhexyladipate present in certain plastic materials and articles intended to come into contact with foodstuffs,
- by the use for fattening purposes in animals of certain anabolic agents,
- by the use of butylated hydroxyanisole (BHA) as a food antioxidant,
- from the consumption of caffeine from food and beverages,
- arising from the use of certain colouring matters, particularly those previously classed by the Committee as temporarily acceptable,

and to advise on the essential requirements of products (infant formulas and follow-up milks) based on cow's milk proteins intended for the feeding of infants and young children.

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