FOOD — SCIENCE AND TECHNIQUES

# Reports of the Scientific Committee for Food
## (Thirty-fourth series)

EUROPEAN COMMISSION

European Commission

food science
and
techniques

## Reports of
## the Scientific Committee for Food

### (Thirty-fourth series)

**Reports of the Scientific Committee for Food on:**
*Smoke flavourings*
*Essential requirements for infant formulas and follow-on formulas*

**Opinions of the Scientific Committee for Food on:**
*Microcrystalline cellulose*
*Polyoxyethylene (20) sorbitan mono-oleate (polysorbate 80)*
*Dimethylterephthalate recovered from PET bottles*
*Three chymosins from genetically modified organisms*

**LEGAL NOTICE**

Cataloguing data can be found at the end of this publication.

*Printed in Spain*

# TABLE OF CONTENTS

## Present membership of the Scientific Committee for Food

S. BARLOW

A. CARERE

A. FERRO-LUZZI (Vice-Chairman)

M. GIBNEY

C. GOMEZ CANDELA

W. HAMMES

A. KNAAP

P. JAMES

I. KNUDSEN (Vice-Chairman)

A. NOIRFALISE

M. NUÑEZ

G. PASCAL (Chairman)

J. REY

M. RIBEIRO

A. SOMOGYI

A. TRICHOPOULOU

R. WENNIG


## Consultores emeriti

P. ELIAS

A. LAFONTAINE

E. POULSEN

R. TRUHAUT

*For their valuable and kind assistance to the following studies,*
*the Scientific Committee for Food wishes to thank:*

---

1. Opinion on microcrystalline cellulose

    W.    GRUNOW

    K.    NETTER

---

2. Opinion on polyoxyethylene (20) sorbitan mono-oleate (polysorbate 80)

    W.    GRUNOW

---

3. Report on smoke flavourings

    W.    GRUNOW

    J.    GRY

---

4. Report on the essential requirements for infant formulae and follow-on formulae

    M.      GIOVANNINI

    A       JACKSON

    H.K.A.  VISSER

    B.      KOLETZKO

    J.      SENTERRE

## 1. Foreword

The EEC "framework" Directive on Flavourings (1) contains a category of flavourings entitled "Smoke Flavourings". It also stipulates that further appropriate provisions should be adopted to ensure the safety of flavourings used or intended for use in or on foodstuffs to impart odour and/or taste.

In the preliminary opinion of the Scientific Committee for Food given at its 73rd meeting on 18 May 1990 it was stated:

> "The Committee (also) noted the absence of inventories on smoke flavours.
>
> The Committee concluded that a safety evaluation of flavours should be performed and that an inventory of flavours in use together with information on usage would be needed for this purpose".

As stated in its GUIDELINES FOR THE EVALUATION OF FLAVOURINGS FOR USE IN FOODSTUFFS: 1. CHEMICALLY DEFINED FLAVOURING SUBSTANCES (2) adopted at the 81st Plenary Meeting 9-10 December 1991, the Committee emphasises that before an additive is accepted for use in food it should have been subjected to an adequate toxicological evaluation. In that context the Committee intends among other tasks to address the issues of the evaluation of flavourings categorised as smoke flavourings. As the Directive does not cover the process of smoking only general technical aspects of it will be addressed in this report.

In elaborating the present document the Committee has drawn extensively on the Council of Europe documents (3,4) and on the assessment of some smoke flavourings by JECFA (5).

## 2. General considerations

**Smoking** is next to drying and salting of food perhaps the oldest process for preserving and flavouring food. It has been probably in use since man knew fire for about 90 000 years and possibly longer. The original primary objective to preserve food is achieved by dehydration and by diminishing the number of surface bacteria through the action of certain components of the smoke and thus to enhance its keeping qualities. The secondary objectives of imparting desirable structural and sensory alterations such as smoke colour and smoke flavour to the final product serve to make the smoked food attractive to the consumer. Today smoking is primarily used for organoleptic purposes.

Hard woods are commonly used in the traditional smoking process occasionally with added aromatic herbs, spices and twigs of certain plants. Food is then exposed directly or indirectly to the generated smoke.

Smoke flavour can be imparted to food also by the use of **smoke flavourings** with acceptable organoleptic properties and processed to eliminate undesirable components such as PAHs. These smoke flavourings are intended for direct use in or on food and are therefore regarded as food additives.

## 3. Technological considerations

Although not within the ambit of the Directive, it is considered useful to include a brief discussion of the process of smoking in this section.

### 3.1 Smoking with freshly generated smoke from pyrolysed wood or moist wood chips

In the direct conventional smoking of food the components of the smoke attach to the surface of the exposed food by chemical and physical processes, e.g. condensation, adsorption, adhesion. The deposited smoke components subsequently penetrate into the treated food to a variable depth, depending on the nature of the food. As a result the smoked food becomes contaminated, albeit in small amounts, with components of the smoke, such as polycyclic aromatic hydrocarbons (PAH), phenols and formaldehyde, which may be hazardous to health. As smoking is difficult to control and standardize, it is necessary to develop smoking techniques which optimise the desirable effects of smoking and avoid, as far as possible, any of its adverse effects.

Commercial smoking processes use various types of smoke generators in which a controlled combustion of wood takes place. Wood is either pyrolysed yielding fumes which on oxidation form smoke, or moist wood chips are heated to generate smoke directly. Hard woods are commonly used in the traditional smoking process but it is essential that only specified, acceptable types of natural wood are used. The following table sets out some examples and is not meant to be exhaustive:

**List of examples of untreated wood, bark and twigs for the generation of smoke**

| | |
|---|---|
| Acer negundo L. | Maple tree |
| Betula pendula Roth. | White birch |
| B. alba L. and B. verrucosa Ehrh. | |
| Betula pubescens Ehrh. | European birch |
| Carpinus betulus L. | Hornbeam |
| Carya ovata (Mill.) Koch | Hickory |
| Castanea sativa Mill. | Chestnut tree |
| Eucalyptus sp. | Eucalyptus |
| Fagus grandifolia Ehrh. | Beech |
| Fagus silvatica L. | Beech |
| Fraxinus excelsior L. | Common ash |
| Juglans regia L. | Walnut tree |
| Malus pumila Mill. | Apple |
| Prosopis juliflora DC. | Mesquite wood |
| Prunus avium L. | Cherry tree |
| Quercus alba L. | White oak |

| Quercus ilex L. | Holm oak |
|---|---|
| Ouercus robur L. | Common red oak |
| Rhamnus frangula L. | Alder Buckthorn |
| Robinia pseudoacacia | Black locust |
| Ulmus fulva Michx. | Sweet elm |
| Ulmus rubra Mühlenb. | Elm |

Herbs and spices may also be added, as well as twigs of Juniper and twigs, needles and cones of Picea.

To minimize the presence, particularly of PAHs, in traditionally smoked foods the direct contact of the food with smoke can be avoided by the introduction of indirect smoking processes. Other measures to reduce the transfer of toxic components to the food being smoked include the interposition of cooling traps, washing filters and spatial separation from the smoke source. Every one of these modifications can however affect adversely the organoleptic results. PAH production can be reduced by keeping the smoke generation temperatures below 400°C, by filtering off the particulates, by wrapping products in casings impermeable to PAH, and by reducing the duration of smoking if the surface/weight ratio is high. However the smoking time depends largely on the product to be treated and the flavour to be achieved. The introduction of smoke flavourings now permits the reduction of contamination with hazardous compounds and enables accurate control of flavour intensity in the final product.

## 3.2 Smoke flavourings

Smoke flavourings may be divided into the following three types: Smoke condensates, smoke flavours prepared by mixing chemically defined flavouring substances, and smoke flavouring preparations. The latter are based on smoke condensates or on smoke flavours prepared by mixing chemically defined flavouring substances with the addition of other substances.

Smoke condensates are traditionally based on three ways of fixing smoke:

*Firstly*, smoke is trapped in water or possibly other liquids, e.g. alcohol/water mixtures or oil. These condensates are usually further processed, e.g. fractionated by extraction. The aqueous smoke condensates, from which the tarry organic phase containing the PAHs has been removed, are the commercially most important products.

*Secondly*, smoke may be condensed to yield whole smoke condensates. They are obtained as pyroligneous acid products by the dry distillation of certain hard woods at 200-900 °C in the absence of air or in the presence of limited air, or from steam distillation at 100-400 °C with or without pyrolysis. These whole smoke condensates may be further processed e.g. by dilution with water to separate off the tarry phase combined with adjustment of the phenolic content or by the fractionally distilling the tarry part and recombining the purified acid, phenolic and neutral fractions in various patented proportions. These types of products are however of little commercial interest.

*Thirdly,* smoke is condensed on solids, e.g. sugar, salt, maltodextrins, meat, or bacon rind, which may be subsequently extracted with certain solvents.

Smoke condensates are complex mixtures of variable composition and contain different amounts of a spectrum of compounds, e.g. carboxylic acids, ketones, furfural derivatives, lactones, and phenols (7,8). The likely most hazardous constituents are the PAHs, the total amount of potentially harmful PAHs other than benzo(a)pyrene being 5 to 10 times the amount of benzo(a)pyrene found (6).

Smoke condensates prepared from smoke trapped in liquids, usually water, are separated from the tarry phase. The aqueous phase is then usually purified by filtration through charcoal and concentrated by distillation for the preparation of smoke flavours.

Smoke condensed on solids, e.g. sugar, salt, meat or bacon rind, may be used as such or extracted with alcohol, methanol or other solvents and then purified by charcoal filtration and concentrated by distillation. Those products are not widely used and are therefore of minor commercial importance.

The final smoke flavour preparations are usually formulated by adsorption of the condensate on a carrier, or by spray drying, or by incorporation into solvents or emulsions. They are applied to the surface of or mixed into food products and are not normally incorporated into curing salt preparations. Smoke flavouring preparations may be incorporated into food usually at concentrations from 0.1% to 1%.

# 4. Toxicological considerations

As previously pointed out, the process of smoking is difficult to standardize and to control. As it is not covered by the Directive, no specific toxicological considerations have been developed for the evaluation of the safety of the process of smoking.

## 4.1 Contaminants

It is essential that woods treated with paint or impregnated with tar or wood preservatives including pesticides, e.g. arsenicals, chlorinated phenols, must be excluded as raw materials for smoke generation. Herbs, spices and twigs of certain plants may, however, be added for special flavouring properties.

Furthermore, because smoke flavourings are intended to be added deliberately to the food, they must comply with the following maximum limits for contaminants[*] :

Benzo(a)pyrene            10 µg/kg condensate (solvent/water free) giving rise to less than 0.03 µg/kg final foodstuff as consumed

Benzo(a)anthracene        20 µg/kg condensate (solvent/water free) giving rise to less than 0.06 µg/kg final foodstuff as consumed

As 3mg/kg, Hg 1mg/kg, Cd 1mg/kg, Pb 10mg/kg smoke condensate.

## 4.2  Principles of toxicological evaluation

Smoke flavourings need to be evaluated as any other food additive.

Smoke flavouring preparations, *based on mixtures of chemically defined substances*, require no further assessment of their safety, provided the individual components have been evaluated previously and accepted as flavouring substances for foodstuffs. However, any components, which have not been evaluated previously, require the usual establishment of their safety to health as flavouring substances prior to their use (see Guidelines for the safety evaluation of flavouring substances (2)).

Because of the wide physical and chemical differences in the preparations used for imparting a smoke flavour to food, it is not possible to design a common approach to the safety assessment of smoke flavourings. However, the existing multitude of individualised smoke flavouring preparations is based on only a limited number of commercially available smoke condensates mainly of the aqueous type.

The SCF considered, as did JECFA (5), that ADIs cannot be allocated to such a complex group of substances and that other ways of establishing their safety have to be chosen. Only some well specified aqueous condensates and one tarry extract of a smoke condensate, prepared from a specific wood, have been tested in standard toxicological studies and evaluated by JECFA as provisionally acceptable for use as a flavouring in foods otherwise traditionally treated by smoking.

In the opinion of the SCF the toxicological evaluation should therefore concentrate on the safety of smoke condensates on a case by case basis.

---

[*]  The maximum limits for 3,4-benzpyrene of 0.03 µg/kg foodstuff and 0.03 µg/kg beverage are set out in Council Directive 88/388/EEC (1).

The following technological information is a prerequisite for the safety evaluation of smoke flavourings:

- information on the production method of the smoke condensate, the further processing, and the final steps in the production of the flavouring preparation;

- approximate qualitative and quantitative composition of the smoke condensate as well as the concentration used and similar data on the final smoke flavouring preparation;

- information on the use levels and the kind of foods for which the smoke flavouring is intended;

The core set of toxicological data on the smoke condensate or the individual derived smoke flavouring preparation should comprise:

- an *in vitro* mutagenicity test in a prokaryotic system;

- an *in vitro* gene mutation test in cultured mammalian cells;

- an *in vitro* test for chromosomal damage in cultured mammalian cells;

- a 90-day feeding study in laboratory animals.

Additional toxicological data may be required for assessment of the safety of the smoke flavourings as necessary. Here a stepwise approach may be used, depending among other things on the potential intake and the results of the core set of toxicological data.

# References

1.  Directive of 22 June 1988 on the approximation of the laws of the Member States relating to flavourings for use in foodstuffs and to source materials for their production (88/388/EEC, OJ L184 of 15.07.1988).

2.  Guidelines for the evaluation of flavourings for use in foodstuffs. Chemically defined flavouring substances. Opinion of the Scientific Committee for Food (9-10 December 1991) - 29th series.

3.  Council of Europe guidelines concerning the transmission of flavour of smoke to food (Council of Europe Press - 1992).

4.  Health aspect of using smoke flavours as food ingredients (Council of Europe Press - 1992).

5.  Evaluation of certain food additives and contaminants. Thirtyfirst meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additives series, no. 22—1988 (Distributed by Cambridge University Press).

6.  Phenole im Räucherrauch - Nachweis und Identifizierung by Reiner Wittkowski. Deutsche Forschungsgemeinschaft - Wissenschaftliche Arbeitspapiere, 1985.

7.  The flavour chemistry of wood smoke by Joseph A. Maga, Department of Food Science and Human Nutrition, Colorado State University. Food Reviews International, 3 (1&2), 139-183 (1987).

8.  Analysis of Liquid Smoke and Smoked Meat Volatiles by Headspace Gas Chromatography by R. Wittkowski, W. Baltes, W. G. Jennings - Food Chemistry 37 (1990) 135-144.

REPORT ON THE ESSENTIAL REQUIREMENTS

FOR INFANT FORMULAE AND FOLLOW-ON FORMULAE

OPINION EXPRESSED ON 17 SEPTEMBER 1993

## 1. Terms of reference

To review certain essential characteristics of infant formulae and follow-on formulae.

## 2. Introduction

In 1983 the Committee adopted an Opinion on the minimum characteristics which should be required for infant formulae and follow-up milks made from cow's milk proteins (1). A second Opinion, delivered in 1988, specified the requisite characteristics of soya-based formulae (2), but the first report has, so far, been subject only to *ad hoc* amendments concerning the minimum levels of vitamin D in such products and the list of nutrients which may be added to them (3, 4).

The Annexes to the three first opinions referred to above make up the body of Commission Directive 91/321/EEC of 14 May 1991 on infant formulae and follow-on formulae.

The Commission must ensure that the existing Community legislation is updated whenever necessary. A request for a review was recently sent to the Commission and, accordingly, the Committee was asked to review the provisions of Directive 91/321/EEC likely to have an effect on public health, as it is nearly 10 years since the first report was adopted. The review will apply only to those fields in relation to which there is new information likely to alter the Committee's previous opinion.

## 3. Essential requirements for infant formulae

### 3.1 Proteins

The protein requirement for growth has been directly related to nitrogen composition of human milk (1,2). The protein content is estimated to be about 1.2 g protein/100 ml (determined as N x 6,38). However, the true protein content is more likely to be around 0.9 g/100 ml, but available protein is only in the region of 0.7 g/100 ml when account is taken of the limited availability of IgA, lactoferrin and lysozyme, which are stable at low pH and relatively resistant to digestion (5).

Since 1983 a number of studies have suggested that there may be adverse effects associated with the ingestion of high levels of protein and that, if the intake of protein from formula is reduced to a level more similar to that in human milk, the infants grow well and thrive. Further, infants between 4 and 6 months of age given a level of protein reduced from that in conventional formula (from 2 g/100 ml to 1.3 - 1.8 g/100 ml) achieve satisfactory rates of growth (6). On the other hand a whole series of studies published after the preparation of the Community Directive did not confirm, in term infants, that products with alteration in the casein/whey protein ratio were superior to the products made from unmodified cows milk (7 -15).

Therefore, it is questionable whether different criteria for the essential composition of these two types of formulae should be maintained or not.

The quality of a protein is determined by the pattern of amino acids in the protein relative to the pattern of amino acids required by the metabolic demand in the body. Classically protein quality has been perceived in relation to the requirements for essential amino acids, but the evidence is increasing that, in infancy especially, attention has to be given to satisfying the metabolic demand for conditionally essential amino acids also (16-18). Moreover both human and bovine milk contain casein and whey proteins, but in different proportions, and the protein constituent of each major class of proteins is different. The whey proteins in bovine milk differ particularly in function and composition from those in human milk. In addition human milk contains quantitatively small amounts of other proteins (enzymes, growth modulators, hormones) which may be of considerable functional significance (19-22).

In the past the growth and nitrogen balance of a breast fed infant and the composition of human milk have been used as the reference. More recently the metabolic response was assessed, using quantitative and qualitative biochemical indices or markers : for example either fasting plasma concentrations of amino acids and urea or the post prandial response in the concentrations of plasma proteins with variable turnover times and functions have been measured, e.g. albumin, prealbumin, retinol binding protein, fibronectin, etc.

The plasma concentration urea during the first six months of life in infants taking human milk (2-3 mmol/l) is lower than in infants taking conventional formula (4-6 mmol/l) and the urinary excretion of urea for infants on human milk (81 mg/kg/d) is less than on conventional formula (140 mg/kg/d). When the protein content of the formula is reduced (1.8 g/100 kcal) the urea concentration in plasma decreases to levels similar to those in infants on human milk. An understanding of the kinetics of urea metabolism in infancy should include an appreciation of the rate at which urea nitrogen is salvaged by the colonic microflora for further metabolic interaction (18). The possibility that salvaged urea nitrogen might contribute in significant amounts to the availability of nonessential (and possibly essential) amino acids cannot be excluded at this time (16,18).

The plasma concentration of an individual amino acid is the resultant of a number of factors which do not necessarily vary together and include the protein intake, protein quality, pattern of synthesis and degradation of endogenous proteins and the demand for specific metabolic pathways other than protein synthesis. For conventional formula in the fasted state the amino acid profiles differ greatly from those seen on human milk, and also vary with the relative proportions of whey/casein (casein : higher concentrations of tyrosine, phenylalanine, methionine ; whey : higher concentrations of threonine) (5, 7, 8, 14, 23). In any case neither casein or whey dominant formula gives a pattern of amino acids which is the same as human milk at any level of protein intake (9, 10, 12, 24).

The differences in plasma amino acids postprandially are even more marked than in fasting, although the difference is less marked in term than in preterm infants (23). Whereas in the adult the liver exerts a significant modulating effect upon the pattern of amino acids measured in peripheral blood relative to the dietary intake, this modulating effect is not seen extensively in the infant. The result is that large variations in the amino acid pattern of proteins ingested are transferred almost directly to peripheral blood.

Thus there are large excursions in the circulating concentrations of most of the essential amino acids which suggest that the ability of the infant to handle the metabolic load adequately is exceeded and that the dietary intake of essential amino acids exceeds the combined capacity of the needs of the infants for tissue protein synthesis and the catabolic capability (25). Finally compared with the plasma protein concentrations seen with human milk, low protein formula give similar concentrations of albumin and prealbumin and not dissimilar to higher protein concentration formula.

Under these conditions, the Committee considered that **there is no evidence to justify having different minimal values for the protein content of formula which vary in the ratio of bovine whey to casein proteins. It is considered safe to have a minimum level of protein in formula made from bovine milk proteins of 1.8 g/100 kcal. For an equal energy value, the formula must contain an available quantity of each essential and semi-essential amino acid at least equal to that contained in the reference protein (breast milk, as defined in Annex V of the Directive), allowing methionine and cystine to be added together.**

## 3.2 Fats

The Committee had proposed that minimum and maximum fat levels in infant formulae should be between 3.3 and 6.5 g/100 kcal. This range now appears rather too wide: fats are the main source of energy in mother's milk (some 40 to 55%) and if fat levels in infant formulae are to be considerably reduced (3.3 g/100 kcal accounts for only 30% of the energy content) this must be compensated by an increase in the protein and/or carbohydrate content. This would necessarily lead to a hyperosmolarity in the foodstuff and an increased renal osmotic charge which might exceed the infant's metabolic capacity. Furthermore, during the first six months of life, fats provide some 90% of the energy used for growth and it is known that fat synthesis from carbohydrates constitutes an energetic disadvantage. Fat synthesis from carbohydrates consumes about 25% of the energy which they provide, whereas the deposition of fat derived from fats present in the food consumes only about 1% of the energy provided. The endogenous synthesis of fats produces only non-essential fatty acids, which may unfavourably alter the ratio of unsaturated to saturated fatty acids in the tissues (26). **Accordingly, the Committee concluded that the fat content of infant formulae should amount to at least 40% of the energy content, which is equivalent to 1.05 g/100 kJ (4.4 g/100 kcal).**

**The use of sesame seed oil and cotton seed oil in infant formulae and follow-on formulae should continue to be prohibited.** Sesame seed oil can induce allergies to certain unsaponifiable ingredients (27) and cotton-seed oil may contain cyclopentenic fatty acids which may have negative effects on the desaturation of fatty acids (28).

Animal experiment in the perinatal period reveal a number of untoward effects of *trans* fatty acids, including a disturbed conversion of precursor essential fatty acids into long-chain polyunsaturated metabolites and impairment of prenatal and postnatal growth. In human premature infants inverse correlations between the exposure to *trans* fatty acids and body weight as well as essential fatty acid conversion were observed (29), indicating the potential occurrence of similar and serious side effects in man as observed in animals. The Committee therefore considered that the *trans* fatty acid content of formulae should be as low as practically feasible.

Accordingly, it decided to introduce a maximum level for *trans* and positionally isomeric fatty acids in place of the general ban on fats containing more than 8% of trans fatty acids as currently laid down in the Directive. Apart from partially hydrogenated fat, the major source for *trans* fatty acids in infant formulae is cow's milk fat which may contain about 2 to 5% of *trans* fatty acids. Cow's milk fat is only used in fat blends in European formulae and, since it does not exceed 80% of total fat, **an upper limit of trans fatty acid content of 4% of total fat can be set without limiting the current use of cow's milk fat in formula.** This latter value is also similar to the average *trans* fatty acid content in mature human milk in Europe (30).

Even though human milk composition is variable, typical contents of human milk of women consuming European diets are 4.4-7.1% for lauric acid and 6.3-8.2% for myristic acid of total fatty acids . Clearly higher values are found in the milk of women consuming diets with a low fat and very high carbohydrate content, with the highest values reported from poor populations in rural Africa (31, 32). It is conceivable that even higher values of lauric and myristic acids might be found in human milk if lactating women consumed more extreme diets. High levels of lauric and myristic acids in infant formulae are undesirable because of their marked hypercholesterolemic effects that exceed those of all other saturated fatty acids (33, 34). These side effects are considered undesirable even in infancy. The available data do not exclude untoward effects of lauric and myristic acid on lipoprotein metabolism in formula fed infants. Since other studies have documented marked hypercholesterolemic effects of these fatty acids, a cautious approach is appropriate and high formula contents of lauric and myristic acids should be avoided. The Committee therefore considered that there was insufficient reason to alter its position in this regard. **The maximum lauric and myristic acid contents in infant formulae should remain 15% each of the total fat content.**

Side effects of dietary erucic acid intake have been documented in numerous studies. High dietary intakes of erucic acid induce myocardial lipidosis and necrosis as well as structural and functional abnormalities of mitochondria in rats and monkeys. Dietary erucic acid accumulates in large amounts in the rat heart indicating its slow catabolism. In myocardial mitochondria erucic acid reduces ß-oxidation, oxygen consumption and ATP production. High dietary erucic acid intakes are associated with reduced rates of weight gain in chicks and rats and reduced energy utilization in humans as well as with greater mortality in young rats exposed to cold stress (35-37). Young infants with their immature metabolism are often more susceptible to untoward side effects of substances than mature organisms, which may also be the case with respect to erucic acid toxicity. Animal studies suggested that the severity of myocardial lipidosis induced by erucic acid is more severe in newborn piglets than in weaned pigs but below 0.8% of total fatty acids no adverse effects have been detected in newborn piglets (38). Of interest with respect to infant feeding is also the observation that erucic acid, like nervonic acid, inhibits fatty acid elongation in human fibroblasts (39). High erucic content of infant formulae thus could impair the infants' ability to synthesize adequate amounts of the physiologically important long-chain polyunsaturated fatty acids from dietary essential fatty acids. **In the absence of hard data on safe levels of erucic acid intake in human infants, the Committee considered it prudent that, for the present, contents in infant formulae should not exceed 1% of total fatty acids.** The Committee intend to examine this further.

Human milk in Europe contains average linoleic acid levels between 6.9 and 16.4% of fatty acids, with a median of 11% (32). Much lower and higher values have been reported in women consuming various diets. No firm evidence has been published documenting harmful effects in infants consuming formulae with a level of up to 1900 mg linoleic acid/100 kcal, but also no studies were published that investigated closely enough the possibility of possible side effects such as enhanced peroxide formation and immunosuppression in infants fed formulae with very high linoleic acid contents. Side effects of high parenteral intakes of linoleic acid have been reported in newborn infants, including depression of biologically active linoleic and alpha-linolenic acid metabolites in plasma and tissues, alteration of prostaglandin metabolism and increased peroxidation (40, 41). Also, there is no evidence of any benefit of enteral intakes of linoleic acid higher than 1200 mg/100 kcal. Therefore, **the Committee decided that the present upper limit for linoleic acid content in infant formulae (285 mg/100 kJ) should be maintained.**

There is general agreement in the scientific community that both n-6 and n-3 fatty acids are essential nutrients that need to be supplied to the infant for adequate growth and development. Human infants require large amounts of both n-6 and n-3 polyunsaturated fatty acids for deposition in growing tissues, in particular in the brain and retina, and there is evidence that deficiency of n-3 fatty acids during early growth leads to functional impairment of neural tissues (42). In agreement with the report on Population Reference intakes for the Community (43), **the minimum alpha-linolenic acid content in infant formulae should be set at 0.5% of energy (50 mg/100 kcal). In addition, a range for the linoleic/alpha-linolenic acid ratio of 5-15 should be set,** because both fatty acids compete for the same metabolic pathway and extreme ratios may interfere with the synthesis of their long-chain metabolites (26).

The essential fatty acids in structural lipids of neural and other tissues are primarily long-chain polyunsaturated fatty acids (LCP) with 20 and 22 carbon atoms, such as arachidonic (20:4 n-6) and docosahexaenoic (22:6 n-3) acids. Infants with low birth weights appear to be unable to synthesize sufficient amounts of LCP from the dietary precursors linoleic (18:2 n-6) and alphalinolenlc (18:3 n-3) and, therefore, require a dietary supply of LCP for optimal functional development (26). It is not yet known whether healthy infants born at term will also benefit from a dietary supply of preformed LCP. However, the Committee does not object to the possibility of adding them in infant formulae provided that the resulting content in n-3 and n-6 LCP is similar to that present in breast-milk in Europe (32). Therefore if preformed LCP are added the content of n-6 LCPs should not exceed 2 % and that of n-3 LCPs should not exceed 1 % of total fatty acid content ; in addition the eicosapentaenoic acid (20:5 n-3) content should not exceed that of docosahexaenoic acid.

## 3.3 Carbohydrates

It is a well-established fact that infants suffering from lesions of the intestinal mucosa absorb sucrose better than lactose (44, 45), even though saccharasa activity is reduced in these circumstances (46). Whereas lactase activity is the limiting factor for lactose, saccharase is not a limiting factor for sucrose absorption; what limits it is the transfer of glucose and fructose resulting from its hydrolysis (47). The Committee considered, however, that Directive 91/321/EEC relates only to formulae intended for healthy infants in the Community and that consequently it did not have to take account of pathological situations. Since there were no other data to justify amending the Directive in this respect, **the Committee decided to maintain the provisions relating to the carbohydrate content of infant formulae.**

## 3.4 Iron

The acceptable maximum limits for iron in infant formulae are lower in Europe (0.36 mg/100 kJ, i.e. 1.5 mg/100 kcal) as compared with North America. The important argument in the United States to enrich infant formulae with higher iron concentrations is to prevent iron deficiency in high risk populations. It has been reported that because of the participation in the "Special Supplemental Food Program for Women, Infants and Children (WIC)" in the United States the incidence of iron deficiency in such high risk populations has considerably decreased (48). Infants fed formulae in which the concentration of iron is approximately 12 mg/100 kcal rarely present iron deficiency anaemia in the first year of life (49).

Although iron deficiency anaemia during the first year of life is observed in European countries, its prevalence is not a major public health problem. Additional food of good quality (meat, cereals, vegetables and fruits) are introduced in most European countries at 3-5 months of age (50) and the use of follow-on milks with a higher iron content (up to 2 mg/100 kcal) in the age period 6-12 months has been widely accepted. No long term studies are available comparing the effects on iron status of infant formulae enriched with different iron contents. Infants receiving milk formulae with 6 mg iron per litre (0.9 mg/100 kcal) up to 9 months with additional fruits and vegetables from 3 months and cereals (with 40 mg iron/kg) and meat from 5 to 6 months had satisfactory haematological parameters at the age of 9 months (51).

There may be two disadvantages of iron excess in infant formulae : the possible interaction of iron with the absorption of trace elements and the possible increase of the predisposition to infections. Indeed there is evidence for an interaction between iron and manganese (52) and iron and copper (53). Several studies in the interaction between iron and zinc demonstrate conflicting results (54-56). On the other hand, it has been reported that infants fed iron-fortified formulae (5 mg/l) had high counts of E. coli and low counts of bifidobacteria in the faecal flora. Infants fed unfortified formula had an intestinal flora more comparable to breast milk (57). Studying the effect of iron supplementation to whey and casein based infant formulae, both the type of protein and iron content had an effect on faecal flora (58). No convincing evidence of an effect of iron fortification on the prevalence of infections has been shown. It would require a very large study population to demonstrate such an effect in a comparative study.

In view of the possible interaction between iron absorption and the absorption of some trace elements, the possible effects of iron excess on the prevalence of infections and since there is no evidence that an iron content falling within the limits currently authorised results in a worse iron status, the **Committee has decided that there is no reason to change the present day accepted limits for iron in infant formulae in Europe.**

## 3.5 Trace elements

Selenium (Se) is an element of the active site of glutathione peroxydase (GSH-Px), an enzyme which catalyses the reduction of hydroperoxydes resulting from the oxidative metabolism of polyunsaturated membrane phospholipids and xenobiotics (59). The deiodinase I enzyme responsible for triiodothyronine production is also a selenoenzyme and the deiodinases I and II are responsive to selenium intake (60). In humans, apart from cardiomyopathy of Keshan and chondrodystrophy of Kaschin-Beck described in endemically Se-deficient areas of China, clear evidence of selenium deficiency has been observed only in protein-energy malnutrition, long term total parenteral nutrition (TPN) and in certain inborn errors of metabolism (61).

The selenium intake of breast-fed babies and infants receiving formulae based on cow's milk or soya protein, with a selenium content of more than 10-15 µg/l, is certainly enough to cover their requirements (61). In a number of European countries the selenium content of infant formulae is nonetheless under 10 µg/l, and even as low as 3-5 µg/l, meaning that the "minimum reference intake" of 4 to 6 µg/day (62) could be not covered (63). Therefore, where the selenium content would be naturally lower than 0.25 µg/100 kJ (1 µg/100 kcal), **selenium may be added to infant formulae.** Sodium selenate and selenite, selenomethionine or selenium enriched yeasts, provided that the selenomethionine concentration of these yeasts is well standardized, should be permissible to this effect. In order to maintain a sufficient margin of security, **the maximum content of selenium, if added, should not exceed 0.7 µg/100 kJ (3 µg/100 kcal).**

Chromium (Cr) in its cationic trivalent form is a biologically active element essential for animal and human beings (64). Until recently, human milk was thought to contain between 40 and 80 µg of chromium per litre and it was generally accepted that cow's milk had a lower chromium content than human milk. Once the technical problems concerning measurement and sample contamination had been eliminated, it emerged that the real chromium concentration in human milk was on average of 0.3-0.4 µg/l (65, 66). Consequently the estimated average chromium intake of breastfed infants does not exceed 0.3 µg/day (0.05 µg/kg). Since chromium was consistently found in high concentrations in human tissues during the first few weeks of life, it is highly unlikely that the chromium in mother's milk plays any significant role in the nutrition of young infants. Indeed no signs of chromium deficiency have been detected in young infants, except for glucose tolerance disorders responding to chromium supplementation in certain cases of protein-energy malnutrition (67). Although no recent data on the chromium content of infant formulae are available, there is **nothing to suggest that chromium supplementation of such formulae is either necessary or desirable.**

Molybdenum (Mo) is one of the elements which make up various enzymes, including sulphite oxydase, which catalyse oxydation reduction reactions (68). The adequate and safe intake has been estimated at 0.5-1.5 µg/kg/day in infants, children and adolescents (62). Breast milk contains 1-2 µg/l at 1 month lactation (69), whereas the molybdenum content of cow's milk is 30-70 µg/l (70). No clinical or biochemical anomaly which can be attributed to a molybdenum deficiency has so far been described in infants or children. Only hereditary deficiencies in sulphite oxydase enable us to affirm the essential role of molybdenum for the development of the foetus and the child (68). **The Committee can thus find no justification for supplementing infant formulae or follow-on formulae with molybdenum.**

## 3.6  Vitamins

The new data which have become available regarding the young child's vitamin requirements do not justify amending Directive 91/321/EEC with regard to the essential composition criteria for infant formulae. It should be noted, however, that there was an error in the Committee report adopted in 1983: the figures given for nicotinamide (60 µg/100 kJ or 250 µg/100 kcal) are the minimum values expressed in terms of nicotinamide and not in niacin equivalents (NE). **They should therefore be replaced by 0.2 mg-NE/100kJ and 0.8 mg-NE/100kcal.**

# 4.  Essential requirements for follow-on formulae

## 4.1  Fats

The provisions relating to the minimum and maximum fat content of follow-on formulae remain unchanged: the minimum value of 3.3 g/100 kcal is acceptable in the context of an already varied diet, even though ESPGAN recently expressed a preference for 4 g/100 kcal rather than the previously accepted figure of 3 g/100 kcal (71).

The prohibition on the use of sesame seed oil and cotton seed oil is maintained (cf § 13). The same upper limit for *trans* fatty acid content as that proposed for infant formulae is suggested (cf § 14). The maximum lauric and myristic acid contents in follow-on formulae should remain 15% each of the total fat content (cf § 15). Moreover erucic acid content of follow-on formulae should not exceed 1% of total fatty acids (cf § 16). There is no change to the specifications relating to polyunsaturated fatty acids.

## 4.2  Other composition criteria

The provisions relating to the energy, protein, carbohydrate, mineral and vitamin contents given in Annex 11 to Directive 91/321/EEC remain unchanged. The Committee were concerned that the existing provision for the use of 20% of total carbohydrate as sucrose in follow-on infant formulae may contribute to dental caries in infants and young children. Therefore the Committee propose to examine this issue in the future.

## 5. Use of follow-on formulae

In its report of 27 April 1983 the Committee had considered that follow-on formulae could be used by infants over the age of 4 months and young children. This position was in agreement with the recommendations of ESPGAN and is included in the relevant Codex Alimentarius Standard (CODEX STAN 156-1987). However, Directive 91/321/EEC restricts the term "follow-on foods"' to "foodstuffs intended for particular nutritional use by infants aged over four months". There is a legal vacuum therefore for the essential requirements of formulae intended specially for young children (aged between 1 and 3 years) and which are beginning to be marketed in some Member States. The use of follow-on formulae of inappropriate composition in this age group is of concern. If a formula is to be used by 1-3 year old children it should have the same composition as the follow-on formula made for infants.

# References

1.  First Report of the Committee for Food on the Essential Requirements of Infant Formulae and Follow-up Milks based on Cow's Milk Proteins (1983). *Food-Science and Techniques.* Commission of the European Communities, Luxembourg: 14th series, EUR 8752.

2.  First Report of the Scientific Committee for Food on the Minimum Requirements for Soya-based Infant Formulae and Follow-up Milks (1989). *Food-Science and Techniques.* Commission of the European Communities, Luxembourg: 23th series, EUR 12536.

3.  First Addendum to the Reports of the Scientific Committee for Food concerning the Essential Requirements of Infant Formulae and Follow-up Milks based on Cow's milk Proteins and the Minimum Requirements for Soya-based Infant Formulae and Follow-up Milks (1991). *Food-Science and Techniques.* Commission of the European Communities, Luxembourg 24th series, EUR 13140.

4.  Second Addendum to the Reports of the Scientific Committee for Food concerning the Essential Requirements of Infant Formulae and Follow-up Milks based on Cow's milk Proteins and the Minimum Requirements for Soya-based Infant Formulae and Follow-up Milks. *Food-Science and Techniques.* Commission of the European Communities, Luxembourg, 28th series.

5.  Räihä NCR. Milk protein quantity and quality and protein requirements during development. *Adv Pediatr* 1989; 36: 347-68.

6.  Räihä NCR. New developments related to nutritional aspects of protein in human milk. In: Biology of Human Milk. Hanson LA, ed. *Nestlé Nutrition Workshop Series,* Vol 15. New York: Raven Press, 1988: 1-5.

7.  Järvenpää AL, Räihä NCR, Rassin DK, Gaull GE. Milk protein quantity and quality in the term infant: I. Metabolic responses and effects on growth. *Pediatrics* 1982; 70: 214-20.

8.  Järvenpää AL, Rassin DK, Räihä NCR, Gaull GE. Milk protein quantity and quality in the term infant: II. Effects on acidic and neutral amino acids. *Pediatrics* 1982; 70: 221-30.

9.  Tikanoja T. Plasma amino acids in term neonates after a feed of human milk or fomula: I. Total amino acids and glycine/valine ratios as reflectors of protein intake. *Acta Paediatr Scand* 1982; 71: 385-9.

10. Tikanoja T, Simell O, Viikari M, Järvenpää L. Plasma amino acids in term neonates after a feed of human milk or formula: II. Characteristic changes in individual amino acids. *Acta Paediatr Scand* 1982; 71: 391-7.

11. Volz VR, Book LS, Churella HR. Growth and plasma amino acid concentrations in term infants fed either whey-predominant formula or human milk. *J Pediatr* 1983; 102: 27-31.

12. Räihä N, Minoli I, Moro G, Bremer HJ. Milk protein intake in the term infant: II. Effects on plasma amino acid concentrations. *Acta Paediatr Scand 1986;* 75: 887-92.

13. Janas LM, Picciano MF, Hatch TF. Indices of protein metabolism in term infants fed either human milk or fomulas with reduced protein concentrations and various whey/casein ratios. *J Pediatr* 1987: 110: 838-48

14. Scott PH, Sandham S, Balmer SE, Wharton BA. Diet-related reference values for plasma amino acids in newborns measured by reversed-phase HPLC. *Clin Chem* 1990; 36: 1922-7.

15. Lindberg T, Borulf S, Jakobsson I. Digestion of milk protein in infancy. *Acta Paediatr Scand* 1989; 351: 29-33.

16. Jackson AA. Amino acids: essential and non-essential ? *Lancet* 1983; i: 1034-7.

17. Laidlaw SA, Kopple JD. Newer concepts of indispensable amino acids. Am *J Clin Nutr* 1987; 46: 593-605.

18. Jackson AA. Optimizing amino acid and protein supply and utilization in the newborn. *Proc Nutr Soc* 1989; 48: 293-301.

19. Carlson SE. Human milk non-protein nitrogen: occurence and possible functions. *Adv Paediatr* 1985; 32: 43-70.

20. Donovan SM, Lonnerdal BO. Non-protein nitrogen and true protein in infant formulas. *Acta Paediatr Scand* 1989; 78: 497-504.

21. Janas LM, Picciano MF. Quantities of amino acids ingested by human milk fed infants. J *Pediatr* 1986; 109: 802-7.

22. Lönnerdal B, Woodhouse LR, Glazier C. Compartmentalization and quantitation of protein in human milk. *J Nutr* 1987; 117: 1385-95.

23. Janas LM, Picciano MF, Hatch TF. Indices of proteins metabolism in term infants fed human milk, whey-predominant formula, or cow's milk formula. *Pediatrics* 1985; 75: 775-84.

24. Räihä CR, Minoli I, Moro G. Milk protein intake in term infants: I. Metabolic responses and effects on growth. *Acta Paediatr Scand* 1986; 75: 881-6.

25. Heird WC, Kashyap S. Protein and energy requirements of low birth weight infants. *Acta Paediatr Scand* 1989; 351: 13-23.

26. ESPGAN Committee on Nutrition. Comment on the content and composition of lipids in infant formulas. *Acta Paediatr Scand* 1991; 80: 887-96.

27. Hayakawa R, Matsunuga K, Suzuki M et al. Is sesamol present in sesame oil ? *Contact Dermatitis* 1987; 17: 133-5.

28. Phelps RA, Shenstone FS, Kemmerer R, Evans RJ. A review of cyclopropenoid compounds: biological effects of some derivates. *Poult Sci* 1965; 44: 358-94.

29. Koletzko B. Trans fatty acids may impair biosynthesis of long-chain polyunsaturates and growth in man. *Acta Paediatr* 1992; 81: 302-6.

30. Koletzko B, Mrotzek M, Bremer HJ. Fatty acid composition of mature human milk in Germany. *Am J Clin Nutr* 1988; 47: 954-9.

31. Jensen RG, Ferris AM, Lammi-Keefe CJ. Lipids in human milk and infant formulas. *Annu Rev Nutr* 1992; 12: 417-41.

32. Koletzko B, Thiel I, Abiodun PO. The fatty acid composition of human milk in Europe and Africa. *J Pediatr 1992*; 120: S62-70.

33. Grundy SM, Vega GL. Plasma cholesterol responsiveness to saturated fatty acids. *Am J Clin Nutr* 1988; 47: 822-4.

34. Hayes KC, Pronczuk A, Lindsey S, Diersen-Schade D. Dietary saturated fatty acids (12:0, 14:0, 16:0) differ in their impact on plasma cholesterol and lipoproteins in nonhuman primates. *Am J Clin Nutr* 1991; 53: 491-8.

35. Dept. of Health and Human Services. Food and Drug Administration. 21 CFR Part 184. Direct Food Substances Affirmed as Generally Recognized as Safe; Low Erucic Acid Rapeseed Oil. *Federal Register* 1985; 50: 3745-55

36. Kramer KJG, Farnworth ER, Thompson BK, Corner AH. Testing a short-term feeding trial to assess compositional and histopathological changes in hearts of rats fed vegetable oils. *Lipids* 1988; 23: 199-206.

37. Dupont J, White PJ, Johnston KM et al. Food safety and health effects of canola oil. *Am J Coll Nutr* 1989; 8: 360-75.

38. Kramer JKG, Farnworth ER, Johnston KM et al. Myocardial changes in newborn piglets fed sow milk or milk replacer diets containing different levels of erucic acid. *Lipids* 1990; 25: 729-37.

39. Koike R, Tsuji S, Ohno T et al. Physiological significance of fatty acid elongation system in adrenoleukodystrophy. *J Neurol Sci* 1991 ; 103: 188-94.

40. Pitkänen O, Hallmann M, Andersson S. Generation of free radicals in lipid emulsion used in parenteral nutrition. *Pediatr Res* 1990; 29: 56-9.

41. Martinez M. Tissue levels of polyunsaturated fatty acids during early human development. *J Pediatr* 1992; 120: S129-38.

42. Uauy R, Birch E, Birch D, Peirano P. Visual and brain function measurements in studies of n-3 fatty acid requirements of infants. *J Pediatr* 1992; 120: S168-80.

43. Scientific Committee for Food. Report of the Scientific Committee for Food on Nutrient and Energy Intakes for the European Community. Opinion expressed on 11 December 1992, 31st series.

44. Santosham M, Foster S, Reid R et al. Role of soy-based, lactose-free formula during treatment of acute diarrhea. *Pediatrics* 1985; 76: 292-8.

45. Mann MD, Hill ID, Bowie MD. Absorption and retention in acute diarrhea. *Eur J Clin Nutr* 1990; 44: 629-35.

46. Lebenthal E, Lee PC. Glucoamylase and disaccharidase activities in normal subjects and in patients with mucosal injury of the small intestine. *J Pediatr* 1980; 97: 389-93.

47. Schmitz J. Digestion and absorption of sugars. In: Paediatric Gastroenterology. Navarro J, Schmitz J, eds. Oxford/New York/Tokyo: Oxford University Press, 1991: 16-25.

48. Dallman PR, Yip R. Changing characteristics of childhood anemia. *J Pediatr* 1989; 114: 161-4.

49. Dallman PR, Siimes MA, Stekel A. Iron deficiency in infancy and childhood. *Am J Clin Nutr* 1980; 33: 86-118.

50. Ballabriga A, Schmidt E. Actual trends of the diversification of infant feeding in industrialized countries in Europe. In: Weaning: Why, What and When ? Ballabriga A, Rey J, eds. *Nestle Nutrition Workshop Series,* vol 10. New York: Raven Press, 1987: 129-46.

51. Siimes MA, Salmenpera L, Perheentupa J. Exclusive breast-feeding for 9 months: risk of iron deficiency. *J Pediatr* 1984; 104: 196-9.

52. Davidson L et al. Manganese absorption from human milk, cow's milk and infant formulas. In: Hurley LS et al., eds. Trace elements in man and animals. New York: Plenum Press, 1988: 511-2.

53. Haschke F, Ziegler EE, Edwards BB, Fomon SJ. Effect of iron fortification of infant formula on trace mineral absorption. *J Pediatr Gastroenterol Nutr* 1986; 5: 768-73.

54. Salvioli GP, Faldella G, Alessandroni R et al. Plasma zinc concentrations in iron supplemented low birthweight infants. *Arch Dis Child* 1986; 61: 346-8.

55. Hallberg L et al. Inhibition of iron absorption in man by manganese and zinc. In: Hurley LS et al, eds. Trace elements in man and animals. New York: Plenum Press, 1988: 233-4.

56. Craig WJ et al. Plasma zinc and copper levels of infants fed different milk formulas. *J Am Coll Nutr* 1989; 3: 183-6.

57. Mevissen-Verhage et al..

58. Balmer SE, Wharton BA. Diet and faecal flora in the newborn: iron. *Arch Dis Child* 1991, 66: 1390-4.

59. Nève J. Physiological and nutritional importance of selenium. *Experientia* 1991; 47: 187-93.

60. Arthur JR, Nicol F, Beckett GJ. Selenium deficiency, thyroid hormone metabolism, and thyroid hormone deiodinases. *Am J Clin Nutr* 1993; 57: S263-9.

61. Litov RE, Combs GF Jr. Selenium in pediatric nutrition. *Pediatrics* 1991; 87: 339-51

62. Committee on Medical Aspects of Food Policy. Dietary reference values for food energy and nutrients for the United Kingdom. *Report of Health and Social Subjects* n°41. London: HMSO, 1991 .

63. Kumpulainen J, Salmenperä L, Siimes MA et al. Formula feeding results in lower selenium status than breast-feeding or selenium supplemented formula feeding: a longitudinal study. *Am J Clin Nutr* 1987; 45: 49-53.

64. Offenbacher EG, Pi-Sunyer FX. Chromium in human nutrition. *Annu Rev Nutr* 1988; 8: 543-63.

65. Kumpulainen J, Vuori E. Longitudinal study of chromium in human milk. *Am J Clin Nutr* 1980; 33: 2299-302.

66. Casey CE, Hambidge KM. Chromium in human milk from American mothers. *Br J Nutr* 1986; 52: 73-7.

67. Gürson CT, Saner G. Effects of chromium supplementation on growth in marasmic protein-calorie malnutrition. *Am J Clin Nutr* 1973; 26: 988-91

68. Ralagopalan KV. Molybdenum - An essential trace element. *Nutr Rev* 1987; 45: 321-8.

69. Casey CE, Neville MC. Studies in human lactation 3: molybdenum and nickel in human milk during the first month of lactation. *Am J Clin Nutr* 1987; 45: 921-6.

70. Tsongas TA, Meglen RR, Walravens PA, Chappell WR. Molybdenum in the diet: an estimate of average daily intake in the United States. *Am J Clin Nutr* 1980; 33: 1103-7.

71. ESPGAN Committee on Nutrition. Comment on the composition of soy protein based infant and follow-up formulas. *Acta Paediatr Scand* 1990; 79: 1001-5.

# OPINION ON MICROCRYSTALLINE CELLULOSE

## ADOPTED ON 17 SEPTEMBER 1993

## 1. Terms of reference

The Committee was asked to consider the safety in use of microcrystalline cellulose in relation to its particle size, particularly with respect to use in weaning and infant foods.

## 2. Background and discussion

Microcrystalline cellulose has been considered previously by the Scientific Committee for Food in its Fifth (1978), Seventh (1978) and Twenty-fourth (EUR 13140, 1991) Series of Reports. In the Fifth Series of Reports it was considered in the context of its use in fine bakers' wares and in the Seventh Series, in the broader context of its use in food in general. In both cases, the Committee endorsed the ADI "not specified" established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). In the case of its Seventh Series of Reports however, the Scientific Committee for Food noted that it wished to be informed of any further work elucidating the problem of persorption and indicated that it would re-assess the scientific evidence if any new findings became available. In its Twenty-fourth Series of Reports the Committee considered microcrystalline cellulose in the context of its use in weaning foods when it approved its use, in conjunction with other thickeners, at levels up to 1g/100g in all weaning foods and at levels up to 2g/100g in gluten-free cereal-based foods.

Microcrystalline cellulose is prepared by partial depolymerisation of alpha-cellulose with mineral acid. This process separates the hydrophilic and amorphous parts of the native cellulose molecule from the original starting material leaving material which is crystalline in nature. The article of commerce is then milled to give a product with a particle size appropriate for the intended use, in the case under consideration, as a food additive. The possibility exists that intact particles of microcrystalline cellulose may be persorbed across the wall of the gastro-intestinal tract with uncertain consequences for the health of consumers and this possibility may be increased where particles of small size are used. The Committee was asked to consider this question particularly in relation to weaning foods and infants foods because the permeability of the gastro-intestinal tract is known to be increased in babies and young children. The available toxicological studies with microcrystalline cellulose have been carried out only with materials having particle sizes in the range of 5 - 150 µm. Developments in milling technology and the demand for smaller particle sizes having improved suspension and "mouth feel" properties have led to the availability of materials with particle sizes as small as 0.15 µm.

## 3. Conclusion

With due regard to the increased intestinal permeability in infants and in older children and adults with compromised gut function, the Committee adopted the following opinion:

- microcrystalline cellulose of any particle size should not be permitted in any gluten-free foods specially prepared for infants and young children. In reaching this conclusion the Committee acknowledges and confirms that this represents a withdrawal of the acceptance previously given to this usage as recorded in its Twenty-fourth Series of Reports (EUR 13140, 1991);

- in the absence of toxicity data relevant to microcrystalline cellulose with a particle size less than 5 µm, the Committee is unable to give any opinion on the safety in use of such material;

- to ensure that only microcrystalline cellulose for which adequate toxicity data exist be permitted for food use, a minimum particle size limit of 5 µm should be introduced into the statutory specification;

- should approval for any food uses of microcrystalline cellulose with a particle size of less than 5 µm be requested, information should be provided on the lowest particle size required for the technological purpose intended; the foods in which such material would be used; the levels of use; and toxicity data to support the safety in use of material of the particle size in question;

- the Committee continues to be pre-occupied with the use of microcrystalline cellulose of particle size greater than 5 µm in infant foods generally as well as in foods for individuals of any age suffering from increased gut permeability and will give this matter and its nutritional implications further consideration.

# Opinion on Polyoxyethylene (20) Sorbitan Mono-oleate (Polysorbate 80)

## Expressed on 17 September 1993

## 1. Background and discussion

The Committee was asked to review the acceptability of polyoxyethylene(20)sorbitan mono-oleate (polysorbate 80) because a recent NTP study had raised the possibility that the substance was carcinogenic in F344/N rats. Polysorbate 80 has previously been evaluated by JECFA and the SCF. In 1983 the SCF allocated to it, along with the polyoxyethylene sorbitan mono-esters of lauric and palmitic acids and the mono- and the tri-ester of stearic acid, a group ADI of 10 mg/kg bodyweight .

In reviewing the data from the NTP study, the Committee noted that the effect (an increased incidence of neoplastic and non-neoplastic lesions of the adrenal medulla, spleen and haemopoetic system) was seen in the high dose male rats but not in female rats nor in male and female B6C3F$_1$ mice. Ames test results in four strains of *S. typhimurium*, with and without metabolic activation, were negative. Several mutagenicity studies carried out by other authors (gene mutations in bacterial cells, chromosome aberrations (CA) and sister chromatid exchanges (SCE) in cultured mammalian cells, CA and micronuclei in rodent bone marrow cells *in vivo*) had shown that polysorbate 80 is non-genotoxic.

## 2. Conclusion

The data available are adequate to provide assurance of the absence of genotoxicity. The data on carcinogenicity are equivocal, effects being observed at high doses and in male rats only. Lesions of the type observed in the adrenal medullar, phaeochromocytomas, have also previously been associated with poorly metabolised food additives given to animals at high doses and have been regarded as of no significance for humans. In this instance the effects were seen at such high doses that there is an adequate safety margin between the no observed effect level and the present ADI. In the view of the Committee there are no grounds for requesting further studies nor for changing the existing acceptable status of polysorbate 80.

## OPINION ON DIMETHYLTEREPHTHALATE RECOVERED FROM PET BOTTLES

### EXPRESSED ON 17 SEPTEMBER 1993

## 1. Terms of reference

To advise on the use of dimethylterephthalate (DMT) obtained by depolymerization of PET bottles already used in contact with drinks.

## 2. Discussion

The data submitted refer essentially to the regeneration of purified dimethylterephthalate (DMT) from scrap polyethylene terephthalate (PET) derived entirely from PET bottles used for drinks.

The regeneration of DMT as described by Coca Cola International involves the thermal depolymerization of chopped PET scrap by glycolysis with ethylene glycol. The resulting intermediate mono (hydroxyethyl) terephthalate is converted to the bis (hydroxyethyl) terephthalate and then by ester exchange in the presence of methanol and a catalyst to DMT. This DMT is then purified by several steps including washing, recrystallization and vacuum-distillation and the pure material blended in a ratio of 1 + 3 with DMT produced by another industrial process from p-xylene. The blend is then repolymerised in the presence of ethylene glycol into PET resin, from which new PET bottles are produced by extrusion and stretch-blowing.

The data supplied give chromatographic evidence of purity of DMT and analytical evidence of absence of any carry-over of 4 chemical contaminants. The absence of microbial contaminants or microbial metabolites is not shown by any test.

From the toxicological point of view the starting monomer DMT is in the SCF list 2 (substances for which a TDI or a t-TDI has been established by this Committee) with a TDI of 1 mg/kg b.w., based on 90-day oral mouse and rat studies and long-term studies in mice and rats which do not indicate any tumorigenic potential.

## 3. Conclusion

The Committee considered that there is no special requirement for dealing with DMT recovered from PET bottles already used in contact with drinks. It recalled that the criteria for approval of plastic materials were already set out in EC Directives and, for the special case of recovering monomers by depolymerization of finished plastic articles already used, in the Guidelines on the Community Positive Lists for Plastic (Practical Guide NI, CS/PM/2024 page 20, para 6).

On the basis of the data presented the monomer complies with the above criteria. Therefore there is no reason why this DMT recovered from PET bottles already used in contact with drinks should not be used. It should be emphasized that the final responsibility for compliance of the plastic materials with EC Directives, including the absence of contaminants, still rests with the manufacturer.

# 1. Background

Chymosin (chymase, rennase or rennin) is the principal milk-clotting agent in rennet, which is commercially prepared by aqueous extraction of the dried fourth stomach of unweaned calves. Rennet has a long history of safe use in making cheese. The aqueous extract contains a chymosin precursor, prochymosin, which is subsequently converted to enzymatically active chymosin. Calf rennet contains different forms of chymosin, viz. A and B at concentrations of ca. 22% and ca. 55% respectively (derived from prochymosin A and B), chymosin C2, which is a degradation product of chymosin A, and chymosin C, which is assumed to be a third genetic variety. In addition bovine rennet contains pepsin (ca. 15%).

Biochemically, chymosin is a protein consisting of a single polypeptide chain of 323 amino acids with intramolecular disulfide linkages. Chymosin A and B have been shown to differ only by one amino acid in the polypeptide chain. Chymosin A has an aspartic acid residue at position 286, whereas chymosin B has a glycine residue at the same position.

The Committee was informed that due to a decrease in the amount of calves slaughtered and an increase in cheese production there is a shortfall in the rennet required for cheesemaking. To compensate this shortfall, microbial cheese rennets produced as extracellular enzymes of fungi are available and, to widen the choice still further, recombinant calf rennets have been developed. Recombinant DNA technology has made it possible to obtain calf chymosin as a fermentation product from strains of bacteria, yeasts or filamentous fungi which have been transformed with a vector including a sequence of calf DNA coding for the chymosin precursor.

After isolation and purification of preprochymosin mRNA from the fourth stomach of the calf, purified mRNA serves as a template for the synthesis of a complementary DNA strand (cDNA) using the enzyme reverse transcriptase. This cDNA is subsequently converted to double-stranded DNA with the use of DNA polymerase. The double stranded DNA is then ligated into an expression vector, which can be a plasmid or a virus, and the resultant recombinant DNA material is introduced into the host cells which serve as the source organism for enzyme production, using standard transformation techniques.

The Committee was asked to evaluate chymosins obtained from three different microorganisms modified by recombinant DNA techniques:

1. Chymosin A produced by *Escherichia coli* containing the bovine prochymosin A gene.

2. Chymosin B produced by *Kluyveromyces lactis* containing the bovine prochymosin B gene.

3. Chymosin B produced by *Aspergillus niger* var. Awamori containing the bovine prochymosin B gene.

## 2. Toxicological evaluations and conclusions

In its 27th series of reports (EUR 14181, 1992) the Committee set out the factors which it considered must be taken into account in the evaluation of enzyme preparations, including those obtained from genetically modified organisms. In evaluating the three chymosin products mentioned above, the Committee has applied the principles enunciated in its 27th series of reports. In accordance with these principles, the Committee draws particular attention to the fact that the evaluations which follow are confined to the products described in the dossiers submitted and cannot be considered to cover preparations of the same enzyme prepared from other sources or by other processes.

### 2.1  Chymosin A produced by Escherichia coli K12 containing the calf prochymosin A gene

The product evaluated by the Committee is derived from a genetically modified strain of *Escherichia coli* K12 GE81. The Committee was provided with information on the identity of the parent strain and the construction of the plasmid and method used to introduce the calf prochymosin A gene. Information was also provided to demonstrate that the modified organism is genetically stable. Intact plasmid is not detectable in the final product and acid treatment which is necessary for the purification procedure ensures that any residual DNA fragments present are smaller than 200 base-pairs.

Available toxicity data include the results of a 1-month oral gavage study in the rat, a 90-day feeding study in the rat and mutagenicity assays covering different genotoxic endpoints. In addition, a study in dogs and *in vitro* cytotoxicity assays showed no evidence of the production of any "shiga-like" toxin that might have been potentiated by the modification of the *E. Coli* genome. The results of one passive cutaneous anaphylaxis study in guinea-pigs were equivocal but two further studies provide adequate assurance that allergenic potential is negligible.

The Committee agreed that the use of the product is acceptable for use in the manufacture of cheese.

### 2.2  Chymosin B produced by Kluyveromyces lactis containing the calf prochymosin B gene

The product evaluated by the Committee is derived from a genetically modified strain of *Kluyveromyces lactis* (Dombrowski) van der Walt SL56. The Committee was provided with information on the parent strain and the construction of the plasmid and method used to introduce the calf prochymosin B gene. Evidence indicating the genetic stability of the producing organism over 60 generations was also provided. The use of an acid treatment to activate the enzyme extracellularly makes it unlikely that high molecular weight fragments of DNA will persist and no plasmid-derived DNA was detectable in the final product.

Available toxicity data include the results of an *in vitro* cytotoxicity test, acute toxicity and 28 and 90-day studies in rats, a 90-day study in which rats were fed cheese prepared using the product, a passive cutaneous anaphylaxis assay in guinea-pigs and bacterial and mammallian cell mutagenicity assays.

In the 90-day rat study a slight but significant increase in relative liver weight was observed but this was not repeated in the 28-day study which was undertaken with special reference to hepatic function and histopathology. A slight increase in revertants was noted at high dose levels in some of the strains of *Salmonella* tested in the bacterial mutagenicity assays. No mutagenic activity was seen in several mammalian cell assays, neither *in vitro* nor *in vivo*. Judging the potential for genotoxicity on the entirety of the mutagenicity data, the Committee concluded that the positive reports were of no significance for the safety of the product.

The Committee agreed that the use of this product is acceptable for use in the manufacture of cheese.

## 2.3    Chymosin B produced by Aspergillus niger var. Awamori containing the calf prochymosin B gene

The product evaluated by the Committee is derived from a genetically modified strain of *Aspergillus niger* var. *awamori* designated GC1HF1-3, dgr246. The Committee was provided with information on the identity of the parent strain of *A. Niger*, the identity of the bacterial intermediate host which was the primary recipient of the calf prochymosin B gene and the construction of the plasmid which served as the means of transferring the calf gene to the final host. Information was also provided to demonstrate that the plasmid was integrated into the final host in a stable fashion and that neither plasmid-derived DNA nor RNA are detectable in the final product.

Available toxicity data include the results of dermal and eye irritation studies in rabbits, a 50-day feeding study in rats, a sensitisation test in guinea-pigs and mutagenicity assays covering different genotoxic endpoints. In addition, the product was subjected to a cytotoxicity assay using cultured human lymphoblasts and live recombinant and host organisms were administered to mice by gavage with no evidence of toxicity.

The Committee agreed that the use of this product is acceptable for use in the manufacture of cheese.

# References

## Chymosin A from E. Coli K12

Bachmann, B.J. (1972)
Pedigrees of some mutant strains of Escherichia coli K-12. Bacteriological Reviews 36, 525-557.

Bolivar, F. Et al. (1977)
Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene 2, 95-113.

Buehler E.V. (1989)
Hill Top Biolabs Project No. 89-3729-21 submitted by Pfizer
Passive Cutaneous Anaphlaxis Study in Guinea Pigs of: CHYMOSIN SOLUTION for Pfizer Inc.
Dated 15.8.1989

Buehler E.V. (1990a)
Hill Top Biolabs Project No. 89-3910-21 submitted by Pfizer
Passive Cutaneous Anaphlaxis Study in Guinea Pigs of: CHYMOSIN SOLUTION LOT 17032-15-6, MAXIREN 15 LOT 1159, AND LEBSTREMSEL for Pfizer Inc.
Dated 1.2.1990

Buehler E.V. (1990b)
Hill Top Biolabs Project No. 89-3920-21 submitted by Pfizer
Passive Cutaneous Anaphlaxis Study In Guinea Pigs of: CHYMOSIN SOLUTION LOT 17032-15-6, MAXIREN 15 LOT 1159, AND LEBSTREMSEL for Pfizer Inc.
Dated 22.1.1990

King T.O. (1990)
Department of Drug Safety Evaluation Pfizer Central Research Groton, Connecticut
Genetic Toxicology Report CP-72,962 (Chymosin) Milk-Clotting Agent
Protocol No. 89-605-03 Microbial Reverse Mutation Assay
Dated 6.7.1990

King T.O. (1991)
Department of Drug Safety Evaluation Pfizer Central Research Groton, Connecticut
Genetic Toxicology Report Addendum I CP-72,962 (Chymosin) Milk-clotting Agent
Protocol Nos. 89-605-04 and 89-605-05
Mammalian Cell Gene Mutation Assays
In vitro Cytogenetic Assays
In vivo Cytogenetic Assay
Dated 24.1.1991

Pfizer (1990)
Unpublished report from Fraunhofer Institut für Toxikologie und Aerosolforschung Hannover. 90-Day Oral Toxicity Study of CP-72,962 in Rats (89/7). Final Report
Dated August 1990

Pfizer (1991)
Submission to Commission of the European Communities, prepared by Pfizer Central Research, 235 East 42nd Street, New York, NY 10017
Dated December 1991


## Chymosin B from A. Niger var. Awamori

Berka *et al* (1990)
Molecular cloning and deletion of the gene encoding aspergillopepsin A from *Aspergillus awamori*. Gene, 86 (1990), pp. 153–162.

David, R.M. (1989)
Microbiological Associate Inc. for Genencor Inc.
MBA Study G-7209.221 Acute oral toxicity study of *Aspergillus niger* var. *awamori* in mice.
Dated 6.6.1989

Hansen's (1990)
Chymosin manufactured by recombinant DNA technology. Unpublished reports from Chr. Hansen's laboratorium A/S
Dated 21.6.1990 and supplemented 8.1.1993 and 22.6.1993

Hill Top (1988)
Biolabs Project No. 88-3198-21 Delayed Contact Hypersensitivity Study in Guinea Pigs of: Chymosin for: Genencor, Inc.
Dated 13.10.1988

IRDC (1988a)
International Research and Development Corporation
13 Week dietary toxicity study in rats. Sponsor Genencor Incorporated.
Dated 5.12.1988

IRDC (1988b)
International Research and Development Corporation, Protease: Eye irritation study in rabbits (Low Volume Procedure) Sponsor Genencor Inc.
Dated 25.10.1988

IRDC (1988c)
International Research and Development Corporation
Protease. Primary dermal irritation test in rabbits. Sponsor Genencor Inc.
Dated 25.10.1988

Lawlor, T.E. (1989)
Hazelton Laboratories Inc.
Mutagenicity test on Chymosin Lot P88032FP in the Ames salmonella/microsome reverse mutation assay. Final report. HLA Study No.: 10678-0-401. Submitted to Genencor Inc.
Dated 30.5.1989

Valentine, D.C. and Lawlor, T.E. (1989b)
Hazleton Laboratories Inc.
Mutagenicity test on Chymosin Lot P88040FP in the Ames Salmonella/microsome reverse mutation assay. Final report. HLA Study No.: 10715-0-401. Submitted to Genencor Inc.
Dated 2.6.1989

Ward *et al* (1990)
Improved production of chymosin in *Aspergillus* by expression as a glucoamylase-chymosin fusion.
Bio/Technology, Vol. 8, No. 5 (May 1990), pp. 435-440.

Young, R.R. (1989a)
Hazleton Laboratories Inc.
Mutagenicity test on Chymosin Lot Number P88035FP in the mouse lymphoma forward mutation assay. Final report. HLA Study No.: 10714-0-431. Submitted to Genencor Inc
Dated 5.6.1989

Young, R.R. (1989b)
Hazleton Laboratorles Inc.
Mutagenicity test on Chymosin Lot Number P88040FP in the mouse lymphoma forward mutation assay. Final report. HLA Study No.: 10715-0-431.
Submitted to Genencor Inc.
Dated 5.6.1989

## Chymosin B from K. Lactis

Anonymous (1988a)
Laboratory of Pharmacology and Toxicology, Hamburg, FRG
Prüfung von Maxiren 15 TL, LOT No. 151/8/LRE im Zellkulturtest auf cytotoxische Eigenschaften.
Unpublished report provided by Gist-Brocades.
Dated 22.11.1988

Anonymous (1988b)
Laboratory of Pharmacology and Toxicology, Hamburg, FRG
Prüfung von Animal Rennet, LOT No. F H3 im Zellkulturtest auf cytotoxische Eigenschaften.
Unpublished report provided by Gist-Brocades.
Dated November 1988

Berg, J.A. v.d. (1990)
Bio/technology *8*, 135.

Eeken, C.J.v., (1986)
An Investigation in the rat of the acute oral toxicity of Chymosin.
Protocol number: 8605. Unpublished report provided by Gist-Brocades.
Dated 31.10.1986

Eeken, C.J.v., (1988a)
81 Day toxicity study after oral administration of Chymosin to Wistar rats. Protocol Number: 8711.
Unpublished report provided by Gist-Brocades.
Dated 16.5.1988

Eeken, C.J.v., (1988b)
91 Day feeding study in Wistar rats after addition of Chymosin prepared cheese to the feed. Protocol
number: 8706. Unpublished report provided by Gist-Brocades.
Dated 4.5.1988

Hoorn, A.J.W. (1987)
Hazleton Biotechnologies laboratory Project ID:
HBC Study No.: E-9792-0-401. Mutagenicity test on chymosin in the Ames
Salmonella/microsome reverse mutation assay.
Unpublished report provided by Gist-Brocades.
Dated 18.12.1987

Lambalgen, R.v., (Undated a)
Allergenicity of chymosine tested by passive cutaneous anaphylaxis assay. Unpublished data
provided by Gist-Brocades.

Lambalgen, R.v., (Undated b)
Pathogenicity of Kluyveromyces lactis CHY-1
Unpublished data provided by Gist-Brocades.

Reijenders, J.B.J. (1990)
Draft Report of RCC N0TOX B.V. No. 038791
Comparative subacute 28-day oral toxicity with
a)  Maxiren, active/RER 001-CHYM1 LY01
b)  Maxiren, inactive/RER 001-CHYM LYO CT1
c)  Calfrennet, active/P.A. (J1+J2) LY0
d)  Calfrennet, inactive/P.A. (J1+J2) CT LYO
(RCC NOTOX Substance 11169) by daily gavage in the rat.
Unpublished data provided by Gist-Brocades.
Dated 30.10.1990

Reiss, B. et al. (1984)
EMB0 J. 3, 3317

Chromosome aberrations test in cultured peripheral human lymphocytes, performed by RCC Notox.
Completion date: 27.10.1989

*In vitro* mammalian cell gene mutation test with V79 Chinese hamster cells (with independent repeat), performed by RCC Notox. Completion date: 28.11.1989

AMES Salmonella/microsome test (with independent repeat) in the strains TA 98 and 100 (+S9), performed by RCC Notox. Completion date 10.12.1991

AMES Salmonella/microsome test in the strains TA 98 and 100 (+S9), performed by Institut Pasteur. Completion date: 28.1.1992

a) AMES Salmonolla/Microsome screening test on cymosin from *K. lactis* and
b) AMES Salmonella/Microsome screening test on kalfstremsel (calf rennet), performed by RCC Notox. Completion date: 18.2.1992

Micronucleus test in bone marrow cells of the mouse (intravenous), performed by RCC Notox. Completion date 16.3.1992

AMES Salmonella/Microsome treat and plate test (with independent repeat) in the strains TA 98 and TA 100 (+S9), performed by RCC Notox. Completion date: 16.6.1992.

The Scientific Committee for Food was established by Commission Decision 74/234/EEC of 16 April 1974 (OJ L 136, 20.5.1974, p. 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 contaminants.

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

The secretariat of the Committee is provided by the Directorate-General for Industry of the Commission. Recent Council directives require the Commission to consult the Committee on provisions which may have an effect on public health falling within the scope of these directives.

The present report deals with:

Smoke flavourings
Essential requirements for infant formulas and follow-on formulas
Microcrystalline cellulose
Polyoxyethylene (20) sorbitan mono-oleate (polysorbate 80)
Dimethylterephthalate recovered from PET bottles
Three chymosins from genetically modified organisms