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Reports of the Scientific Committee for Food

(Thirty-eighth series)

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food science and techniques

Reports of the Scientific Committee for Food

(38th series)

Opinions of the Scientific Committee for Food on:

Nitrates and nitrite

Draft Commission directive laying down specific purity criteria on food additives other than colours and sweeteners

Cyclamic acid and its sodium and calcium salts

The safety in use of 1,1,1,2-tetrafluoroethane as a solvent for flavour extraction

Bovine spongiform encephalopathy (BSE)

Directorate-General for Industry
1997

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OPINION ON NITRATE AND NITRITE
(expressed on 22 September 1995)

1. Terms of reference

1. To review from a public health standpoint the presence of nitrate in foodstuffs in general and vegetables in particular and to consider:

   A) Whether:
   - the ADI for nitrate established by the Committee in 1990, and associated safety factor of 500, is still valid taking into account any new data;
   - there are special considerations concerning the presence of small and possibly unavoidable amounts of nitrate in foodstuffs intended for babies.

   B) The influence of maximum limits for nitrate in specified foodstuffs on the total intake of the population of the EU.

In formulating its opinion the SCF is asked to consider:

- whether the presence of nitrate as a natural component of vegetables or as a contaminant as opposed to its deliberate and controlled use as a food additive influences the risk evaluation procedure and the ultimate derivation of maximum limits;

- the situation in the Community as a whole, individual Member States, and for particular subsets of the population with higher potential exposure;

- the range (mean and extreme) of nitrate intake;

- the problem of reducing nitrate intake whilst maintaining adequate intake of vegetables.

2. To review the safety in use of nitrates and nitrites (in both cases, the sodium and potassium salts) as food additives in the context of the conditions of use set out in the European Parliament and Council Directive 95/2/EC on food additives other than colours and sweeteners.

2. Background

The Scientific Committee for Food last evaluated nitrates and nitrites in 1990 (Commission of the European Communities, 1992) when they were reviewed in the context of their use as food additives. The summary and conclusions of that evaluation were as follows:

" The Committee has considered the use of nitrates and nitrites as food additives, in context with their intake from other sources. In the latter regard, pollution of the environment with nitrates represents a major public health problem. The Committee has also considered the formation of N-nitrosocompounds in foods containing nitrates and nitrites, in context with the formation of nitrosamines in the human gastro-intestinal tract.

Intake data for nitrates, nitrites and N-nitrosocompounds are not available for the Community as a whole, but in all areas studied it seems clear that:

(a) the use of nitrate as food additives makes a relatively small contribution to the total intake, the majority coming from vegetables and drinking water;

(b) intakes of nitrate and nitrite from food are generally well within the ADI's, except in areas where levels of nitrate in vegetables are high and levels in drinking water exceed Community standards;

(c) no direct toxic effects are therefore expected from food additive uses of nitrates and nitrites when used within the levels indicated in this report.

The situation is less clear-cut in the case of N-nitrosocompounds. There are considerable problems in identifying and measuring the amounts of such substances in foods, except in the case of a few well-characterised volatile nitrosamines. The data available on those nitrosamines whose carcinogenic potential is known suggest that, at the level at which they have been detected in dietary studies, any adverse health effects are likely to be small. However, the Committee is not in a position to make a quantitative assessment of risks from all N-nitrosocompounds present in foods as eaten or formed by nitrosation in the human gastro-intestinal tract. The Committee therefore **recommends** that priority should be given to research on analytical methods, assessment of carcinogenic potency and of *in vivo* nitrosation which will permit a better assessment of the risks from N-nitrosocompounds in food.

It would be prudent to reduce the levels of pre-formed nitroso compounds in the diet as far as possible. The Committee therefore **recommends** that exposure to preformed nitrosamines in food should be minimized by appropriate technological practices such as lowering levels of nitrite and nitrate added to foods to the minimum required to achieve the necessary preservative effect and to ensure micro biological safety. These levels of nitrite and nitrate should be the lowest achievable in accordance with the information provided to the Committee during the course of the present review

The Committee **recommends** that further research should be carried out on the possibility of developing alternative preservatives and in the meantime, on methods of inhibiting the nitrosation reaction in foods.

The Committee underlines the importance of only using nitrite mixed with salt in meat production as this would automatically limit the amount of nitrite which can be added and prevent accidental poisoning through the addition of excessive quantities to foods.

The Committee has established an Acceptable Daily intake (ADI) of 0 - 5 mg of **nitrate** per kg b.w. (expressed as sodium nitrate) and a Temporary Acceptable Daily Intake of 0 - 0.1 mg of **nitrite** per kg b.w. (expressed as sodium nitrite). The ADI's for nitrate and nitrite include human intake from all sources. Pollution of the environment with nitrate is a major public health problem, and this problem should remain on the agenda of the Committee as far as it relates to food ".

Since the opinion was formulated in 1990:

- several Member States notified the Commission of their intention to introduce national limits for nitrate in certain vegetables such as lettuce and spinach and this led the Commission to give consideration to introducing maximum limits for these products.

- In addition, the European Parliament and the Council adopted Directive 95/2/EC[1] on food additives other than colours and sweeteners on 20 February 1995. This Directive establishes the conditions for the use of nitrites and nitrates as food additives at Community level (Table 1):

[1]European Parliament and Council Directive 95/2/EC O J L 61 p 1 of 18 March 95

Table 1: Indicative ingoing amount and residual amount of nitrates and nitrites according to Directive 95/2/EC

E No.	Name	Foodstuff	Indicative ingoing amount	Residual amount
			(mg/kg)	
E 249	Potassium nitrite (1)	non-heat treated, cured, dried meat products	150 (2)	50 (3)
E 250	Sodium nitrite (1)	other cured meat products canned meat products *foie gras, foie gras entier, blocs de foie gras*	150(2)	100 (3)
		cured bacon		175 (3)
E 251	Sodium nitrate	cured meat products canned meat products	300	250 (4)
E 252	Potassium nitrate	hard, semi-hard and semi-soft cheese dairy-based cheese analogue		50 (4)
		pickled herring and sprat		200 (5)

(1) *When labelled 'for food use', nitrite may only be sold in a mixture with salt or a salt substitute*

(2) *Expressed as* $NaNO_2$

(3) *Residual amount at the point of sale to the final consumer, expressed as* $NaNO_2$

(4) *Expressed as* $NaNO_3$

(5) *Residual amount, nitrite formed from nitrate included, expressed as* $NaNO_2$

During the course of the adoption of this directive, the Commission declared that it would review the safety in use of nitrates and nitrites as food additives.

The present opinion updates and extends the previous report as appropriate to respond to the above terms of reference.

Current Review

DIETARY EXPOSURE

Analysis of foods for nitrate and nitrite ion remains somewhat problematic. Various methods have been applied in different laboratories and there are no reference materials available with the contents of nitrate and nitrite certified in a relevant matrix which could be used to check recoveries and analytical performance. A number of published studies have not given details of their intra- and inter laboratory analytical quality control procedures and this hinders comparison of data obtained in different laboratories. There are wide disparities in published results and it is clear that comparisons of levels of nitrate in vegetables in different commodities or in different Member States must be conducted with some circumspection. It should also be noted that differences in methodologies for reporting estimates of dietary intake hampers direct comparison between countries.

Nitrate

Factors affecting nitrate levels in vegetables

Nitrate levels in vegetables are influenced by genetic and environmental factors including species, variety and the inter-related geographic and seasonal factors of light intensity, temperature, extent of fertiliser use and whether or not the crop is grown under glass.

Varietal differences in nitrate levels have been *most* extensively studied in relation to lettuce where open leaf varieties generally have higher nitrate concentrations than tight headed varieties such as iceberg (see Table 2 below and related references).

Variations in nitrate levels with geographic location and climatic conditions are marked in some commodities e.g. lettuce, and tend to be higher in samples from northern Europe than those from Mediterranean countries (International Consumer Research and Testing, 1993; U.K. Ministry of Agriculture, Fisheries and Food, 1992), however, geographical differences are less marked with crops such as spinach, potatoes and carrots.

Generally, in particular vegetables, higher nitrate levels are found in winter than in summer.

The nitrate content in vegetables grown under glass is usually considerably higher than in those grown outdoors in the same seasons where both methods of production are feasible in the same region (Ansorena and Merino, 1992).

The application of artificial fertilisers may also lead to increases in the levels of nitrate in crops but the relationship between levels of application and accumulation is not simple. In beetroot and cabbage, the increase in nitrate concentration consequent on fertilisation was most marked at the immature stage and fell with increasing maturity. However, with the other crops (carrot, leek, onion, potato) optimal fertiliser application had no significant effect on accumulation at the late harvesting stage (U.K. Ministry of Agriculture, Fisheries and Food, 1992).

Nitrate levels in vegetables

Mean levels of nitrate abstracted from recent literature are shown in Table 2. These data represent the range of mean values obtained in recent studies; a single datum indicates that this vegetable was analysed in only one of these recent surveys. In view of concerns over higher levels of nitrate accumulating in crops produced in winter, some of the surveys have concentrated sampling in this season and therefore the mean values cited are biased towards these higher values and the maximum values recorded for some crops are much higher than the mean. For example, the maximum nitrate levels recorded in individual samples of lettuce were between 5820 and 6834 mg/kg in the UK, the Netherlands and Germany. For spinach, maximum levels of 6300 and 7050 mg/kg were reported in the Netherlands and Greece respectively, for celery levels up to about 7000 mg/kg were found in samples in Belgium and similarly high maximum levels of over 6500 mg/kg were found in beetroots.

Table 2: Average concentrations of nitrate in vegetables

Vegetable	Nitrate (NO_3^-) mg/kg	Vegetable	Nitrate (NO_3^-) mg/kg
Asparagus	13	Fennel	2000
Aubergine	215-460	Leek	240-570
Beans: green	195-450	Lettuce: open leaf	907-4674
broad	21	iceberg	140-1750
Beetroot	1560-2588	Mushroom	70
Broccoli	125-471	Onion	80-210
Brussels sprouts	7-12	Parsnip	81
Cabbage: green	150-1600	Peas	15-57
white	93-530*		
Calabrese	220	Peppers	10-78
Carrot	115-271	Potatoes	35-200*
Cauliflower	37-715	Pumpkin	410
Celery: white	870-3700	Radish	1100-1510
green	3741		
Chard	2076	Spinach	390-3383
Chicory	9	Tomato	4-53*
Courgette	600-810	Turnip	970-2870
Cucumber	23-242		

*Analytical outliers not included.

Data derived from Anon (1993); Ansorena & Merino (1992); Bonell (1995); Burt (1993; 1994); Conseil Supérieur d'Hygiène Publique de France, (1992); Cornee et al. (1992); Dejonckheere et al. (1994); International Consumer Research and Testing (1993); Mortensen & Larsen (1989); National Food Agency of Denmark (1990); U.K. Ministry of Agriculture, Fisheries and Food (1992); Weigert et al. (1991)

Nitrates levels in other foods

Nitrate levels in cured products have shown a steady decline over the years with improvements in manufacturing practice, and most recent data indicate that mean levels in these products is generally between 10-30 mg/kg.

Foodstuffs other than vegetables and cured meats usually contribute relatively little to intakes of nitrates.

Nitrate intakes

Estimates of intakes of nitrate published since the previous review have been obtained using three different techniques, viz:

a) the duplicate diet technique;

b) analysis of components of the diet and calculation of intakes from information provided by subjects on their food consumption;

c) calculation of intake from published data on mean values for dietary components and on food consumption.

Table 3: Estimates of daily dietary nitrate intake in various EU countries

Country	Mean Nitrate Intake (mg/day)	Notes	% Intake from vegetables
Belgium	154	By calculation from fruit, vegetables & water	82 %
Denmark	54	13,3 mg from potatoes; includes drinking water	75 %
Finland	54 (46,4 at age 9 to 62, 3 at age 24)	24 hour recall method; includes drinking water	84 %
France	121	By calculation; less than half the estimated intake in 1982; includes drinking water	85 %
Germany	89 male 65 female 156 male 115 female 420 male 310 female	by market basket calculation: ; includes drinking water - based on median concentration - based on mean concentration - based on 90th percentile concentration Estimated intakes at the highest assumed concentration exceed the ADI	
The Netherlands	143 52 95-108 men (median) 96-120 woman (median)	By calculation; includes drinking water Duplicate portion method; Includes drinking water By calculation (highest intake on a body weight basis in children 1 - 10 years of age)	91%
United Kingdom	54 170 - 180 157 94	185 - 195 mg/d in vegetarians. Duplicate portion technique but using distilled water. 97,5th percentile lettuce consumers* 97,5th percentile spinach consumers* 97,5th percentile potato consumers* * includes drinking water	75 %

Data derived from: Dejonckheere et al. (1994); Mortensen & Larsen (1989); National Food Agency (1990), Laitinen et al. (1993);van Duijvenbooden & Matthijsen (1989); Ellen et al. (1990); unpublished data presented at EERO meeting (1993); MAFF(1992); Burt 1993; 1994); Cornée et al. (1992); Anon. (1994)

Quite generally, vegetables have been found to contribute about 70 to 90 % of total nitrate intakes. However, in some studies emphasis tends to have been placed on those with high nitrate concentrations whereas these may not be the major contributors to dietary intakes because of differences in normal portion sizes and levels of plate waste. Data from various member states are presented in Annex I and are summarised in Table 3.

Most recent intake estimates for nitrates are generally similar to earlier estimates and mean intakes range from 52 to 156 mg/day in various European countries. Vegetarians have a rather higher nitrate intake and in the U.K. the mean intakes in this group were 185-195 mg/day, some 4 times the national average, but still within the ADI for nitrate. A number of studies suggest that the ADI is occasionally exceeded by a small number of consumers. Estimates of intakes by calculation using mean values for consumption and for nitrate concentrations of food commodities systematically overestimate intakes compared with duplicate diet studies.

In its opinion of 1990, the Committee concluded that the use of nitrates as food additives make only a minor contribution to total nitrate exposure. The data presently available to the Committee indicate that this is still the case.

As also noted in the previous report, in some areas drinking water may make a major contribution to dietary intake of nitrate. This will be of particular significance for bottle-fed infants where formula is reconstituted with drinking water.

Nitrite

Recent data on nitrite contents of vegetables and of cured meats which have been reported since the previous review are presented in Annex I and are summarised in Table 4.

Table 4: Nitrite levels in cured meats and vegetables

Meat Product	Nitrite content (mg/kg)	Vegetable	Nitrite content (mg/kg)
Bacon	24 (n.d.-76)	Beans (Green)	n.d.
Ham	26 (n.d. - 110)	Beetroot	n.d.-8
Chopped ham & pork	4 (n.d.-15)	Broccoli	2-4
Tongue	17 (1-71)	Cabbage (Green)	n.d.-2
Corned beef	5 (2 - 8)	Carrot	n.d.-2
Luncheon meat	24 (1 - 130)	Celery (White)	n.d.-8
Cured pork shoulder	5 (1 - 13)	Cucumber	3
Chicken pâté	4 (n.d. - 11)	Lettuce: Open leaf	n.d.- 11
Liver sausage	4 (n.d. - 11)	Iceberg	n.d. - 1
Liver pâté	7 (3 - 10)	Potatoes	n.d.-19
Cured beef	11 (7 - 15)	Spinach	n.d.-26
Cured turkey	54 (1-84)	Tomato	n.d.-4

Data derived from Anon (1993); Ansorena & Merino (1992); Burt (1993; 1994); Conseil Supérieur d' Hygiène Publique de France (1992); Cornee et al. (1992); International Consumer Research and Testing (1993); Meah et al. (1994); Mortensen & Larsen (1989); National Food Agency of Denmark (1990); U.K. Ministry of Agriculture, Fisheries and Food (1992); Weigert et al. 1991). *n.d. = not detected*

Foods other than cured meats and vegetables contribute little to dietary intakes of nitrite and the mean nitrite levels in other food classes in the U.K. were (U.K., Ministry of Agriculture, Fisheries and Food, 1992):

Bread & Cereals	1.4 (< 1 - 3.0) mg/kg
Fish	1.2 (< 1 - 5.0) mg/kg
Other food groups	≤ 1.0 mg/kg

Estimates of intakes of nitrite in a number of European countries ranges from 0.7 - 4.2 mg/d (equivalent to 0.01 - 0.07 mg/kg b.w. for a 60 kg adult).

The higher value is recognised to be an overestimate as a result of the methodology used and the assumptions made in the calculation.

However, if the levels of residual levels of nitrite were as high as permitted in Directive 95/2/EC (Table 1), then this could easily lead to the ADI being exceeded.

N-nitrosocompounds (NOC)

The principal dietary sources of N-nitrosocompounds are cured meat, beer and fish.
There is a clear correlation between nitrite added for the curing of meat and the formation of volatile nitrosamines (Sen *et al* 1974; Birdsall, 1976; Gray, 1976; NAS, 1982; Gry *et al*, 1983).

The mean dietary intake of nitrosodimethylamine was estimated to be between 0.1- 1.1µg/day in the last decade as indicated in the data in Table 5 below which are extracted from Gangolli *et al* (1994).

The dietary levels of carcinogenic NOC found in recent studies are in the range of 0.3-0.5µg/day. These low levels reflect the considerable effort made in the last 20 years to detect sources of contamination and to achieve prevention by developing and ensuring GMP in vulnerable areas of food production.

There are no quantitative data available with respect to the endogenously formed carcinogenic NOC at normal dietary intakes of precursors.

It is questioned whether the occurrence of the non-carcinogenic N-nitrosoproline in urine is a good indicator for the potential endogenously formed carcinogenic NOC or merely reflects an effective mechanism for scavenging nitrite by salivary proline-rich proteins which comprise about 70 % of total proteins in the saliva (Carlson 1993).

Table 5: Daily nitrosodimethylamine (NDMA) intake in EU Member States according to dietary surveys published in 1978-1991

COUNTRY	NDMA[a] (µg/day)	Major NDMA source (% contribution)	Reference
UK[b]	0.53	Cured meats (81%)	Gough *et al.* (1978)
UK	0.60	Beer, cured meats	MAFF (1987)
Netherlands	0.38	Beer (71%)	Stephany and Schuller (1980)
Netherlands[c]	0.10	Not evaluated	Ellen *et al.* (1990)
FRG (1979-1980)	1.10 (0.57)	Beer (65%), cured meats (10%)	Spiegelhalder *et al.* (1980)
FRG (1981)	0.53 (0.35)	Beer (40%), cured meats (18%)	Spiegelhalder (1983)
FRG (1989-1990)	0.28 (0.17)	Beer (31%), cured meats (36%)	Tricker *et al.* (1991)
Sweden	0.12	Beer (32%), cured meats (61%)	Österdahl (1988)
Finland[d]	0.08	Beer (75%), smoked fish (25%)	Penttilla *et al.* (1990)
Italy	No data	Cured meats	Gavinelli *et al.* (1988)

[a] Data in brackets relate to women

[b] Beer not included in the survey

[c] Determined by 24 h duplicate diet analysis

[d] Based on limited data

TOXICOLOGICAL CONSIDERATIONS

The Committee reviewed the literature on the toxicology of nitrate, nitrite and NOCs and drew on the information presented at two international conferences: the EERO meeting (Gangolli *et al.*, 1994) and an International Workshop on Health Aspects of Nitrate and its Metabolites (particularly nitrite), organized by the Council of Europe in co-operation with the Ministry of Health, Welfare and Sports and the National Institute of Public Health and Environmental Protection of the Netherlands (Council of Europe, 1995)

Toxicity of nitrate

Further data on nitrate toxicity from epidemiological studies and human volunteer studies have become available since the previous review by the SCF and have been considered by the Committee in forming the current opinion.

Metabolism and pharmacokinetics

A recent pharmacokinetic study of nitrate in human volunteers found the plasma nitrate half-life to be approximately 6,5 hours (Kortboyer *et al.*, 1995). Plasma nitrite levels did not change after ingestion of sodium nitrate. The salivary nitrate levels exceeded the plasma nitrate levels more than 20 times. Salivary nitrate and nitrite levels showed great interindividual variability. The cumulative salivary nitrate secretion, over 24 hours expressed as percentage of the ingested dose, was 28 %. The average amount of nitrite formed in the saliva expressed as percentage of the nitrate dose ingested was 8 %. Omeprazole (a pH elevating drug) treatment, had no effect on the number of nitrate-reducing bacteria. Nevertheless, the nitrite concentration in gastric juice was approximately 6-fold increased after administration of sodium nitrate and omeprazole treatment.

Nitrate is also endogenously formed (Wishnok *et al.*, 1995). A relatively constant daily production of about 1 mmole nitrate was established. A major pathway for endogenous nitrate production is conversion of arginine by macrophages to nitric oxide and citrulline followed by oxidation of the nitric oxide to N_2O_3 and then reaction of N_2O_3 with water to yield nitrite. Nitrite is rapidly oxidized, through reaction with haemoglobin, to nitrate. In addition to macrophages, many cell types can form nitric oxide, generally from arginine. Nitric oxide is mutagenic toward bacteria and human cells in culture, it causes DNA strandbreaks, deamination, oxidative damage, and it can activate cellular defence mechanisms. In virtually all of these cases, the biological response was proportional to the final nitrate levels. (Wishnok *et al.*, 1995).

Toxicological and epidemiological studies

From the available toxicity and epidemiological data it can be concluded that nitrate *per se* is of relatively low toxicity. However, in the human body the more toxic nitrite is formed by reduction of nitrate. This reduction in humans occurs mainly in the saliva (5-20 % of the total nitrate intake; Walker, 1995; Speijers, 1995; Vittozzi, 1993). Potentially, N-nitrosocompounds can also be formed from nitrite and concurrent N-nitrosatable precursors under certain conditions (Shephard, 1995; Janzowski and Eisenbrand, 1995). Thus the assessment of the health risk of nitrate to humans should encompass the toxic effects of nitrite and N-nitrosocompounds. This implies that the animal species used for safety evaluation should be closely related to humans with respect to the toxicokinetics of nitrate and the conversion of nitrate to nitrite. It also implies that the toxicological data of nitrate should be considered in conjunction with that of nitrite and endogenously formed N-nitrosocompounds.

On the basis of limited data it is concluded that in the rat and probably the mouse the reduction of nitrate to nitrite in the saliva is negligible (Walker, 1995; Speijers, 1995; Vittozzi, 1993; Til *et al.*, 1988). Although the rat converts relatively more nitrate to nitrite in the lower part of the gastrointestinal tract than humans, the absorption of the nitrite from this location will be less efficient, because the major absorption of nitrite take place in the stomach (Groen, 1995; Speijers, 1995). Although as an exception the thyroid effects seems to be directly related to nitrate and not to nitrite, this effect does not lead to the lowest NOEL (Gangolli *et al.*, 1994).

As the toxicity of nitrate is encompassed by its conversion to nitrite and the possible endogenous formation of N-nitrosocompounds (Speijers *et al.*, 1987; Gangolli *et al.*, 1994; Speijers, 1995), and the toxicokinetics and biotransformation of nitrate in rat is different from human (Walker, 1995) , the rat could be considered less suitable to extrapolate directly the toxicity of nitrate adequately to man (Speijers *et al.*, 1987; Til *et al.*, 1988; Vittozzi, 1993; Speijers. 1995). Although the rabbit, dog and pig seem to be more comparable to humans, the toxicological data are too limited to allow for a safety evaluation on the basis of these species (Speijers, 1995). For these reasons the safety evaluation of nitrate should be carried out in conjunction with that of nitrite, in other words both 1); the toxicity studies on nitrate and 2); the toxicity studies on nitrite in combination with conversion figures of nitrate to nitrite in human should be considered.

The possible endogenous formation of N-nitrosocompounds which in fact starts with nitrite and N-nitrosatable compounds as precursors, is discussed by Gangolli *et al.*, 1994; Shephard, 1995; Møller, 1995; Janzowski *et al.*, 1995; and Brüning-Fann and Kaneene, 1993; Speijers *et al.*, 1987. However, there is no quantitative evidence for the endogenous formation of carcinogenic N-nitrosocompounds after exposure to realistic levels of nitrate and N-nitrosatable precursors. (Gangolli *et al.*, 1994; Speijers, 1995).

Epidemiological studies thus far have failed to provide evidence of a causal association between nitrate exposure and human cancer risk (Gangolli *et al.*, 1994). Similarly, intense efforts directed at establishing a causal link between N-nitrosocompounds, preformed in the diet or endogenously synthesized, and the incidence of human cancers have so far been unsuccessful in generating clear and unequivocal evidence (Gangolli *et al.*, 1994). On the other hand, there is convincing evidence showing that the consumption of vegetables is associated with a reduced cancer risk in humans (Block *et al*, 1992; Gangolli *et al.*, 1994).

Epidemiological studies of nitrate intake and gastric cancer risk are inconsistent; the more reliable case-control and cohort studies, in particular, do not suggest any association. Case-control studies based on food frequency questionnaires tend to show a protective effect of the estimated nitrate intake on gastric cancer risk. This is probably due to the known strong protective effect of vegetables and fruits on the risk of gastric cancer. Studies which have

assessed the effect of nitrate from sources other than vegetables, such as drinking water or occupational exposure to nitrate dusts, have not shown positive associations with gastric cancer risk (Møller, 1995).

The Committee acknowledges several in depth epidemiological studies demonstrating the health promoting effects of fruits and vegetables, but recognises at the same time the technical limitations of present epidemiological studies to pick up potential effects of specific exposures, such as preformed NOCs in the diet. Studies applying modern biological markers might facilitate this in the future.

Considering the uncertainties still existing with respect to the possible endogenous formation of N-nitrosocompounds after nitrate exposure, and the fact that quantitative evidence of this endogenous nitrosation for the actual dietary nitrate exposure is lacking, at present the realistic approach seems to be to allocate an ADI based on the most sensitive toxicity criteria and the toxicokinetics of nitrate.

Toxicity of Nitrite

Further data on nitrite toxicity from rodent short-term studies have become available since the previous review by the SCF and have been considered by the Committee in forming the current opinion.

Metabolism and pharmacokinetics

Since the previous review by the Scientific Committee for Food, few significant studies on metabolism and pharmacokinetics have been reported (see review by Groen, 1995). However, related studies *in vitro* have shown that proteins and protein digests at similar concentrations to those found in the gastrointestinal tract scavenged nitrite and inhibited the formation of mutagens (Kato & Kikugawa, 1992). The principal products were nitrogen (from the van Slyke reaction), non-mutagenic N-nitrosoproline, S-nitrosocysteine and diazotyrosine; tryptophan gave rise to weakly mutagenic nitrosotryptophan. Proteins, trypsin digests and free amino acids inhibited nitrosation of dimethylamine by nitrite by 50-100%.

In addition to ascorbic acid and tocopherols previously considered, other dietary components which inhibit nitrosation include plant polyphenols and tannins (Leaf *et al.* 1989; Kyrtopoulos *et al.* 1991; Bartsch *et al.* 1990; Wang & Wu 1991). Thus plant sources of nitrite (directly or through salivary reduction of nitrate) may form less N-nitrosocompounds than might otherwise be predicted.

Toxicological studies

No new experimental data on the acute toxicity of nitrite have been published since the previous review. The acute effects include relaxation of smooth muscle, vasodilation and lowering of blood pressure, and methaemoglobinaemia. The LD_{50} in animals is generally about 100-200 mg/kg b.w. and cases of accidental human intoxication indicate that humans are similarly sensitive except for infants below the age of three months who are thought to be more susceptible to methaemoglobinaemia.

The effects of continuous administration of nitrite to experimental animals in drinking water include vasodilation and sedation, methaemoglobinaemia and histopathological changes in cardiac muscle, lung, liver, spleen, kidney and adrenals. Hypertrophy of the adrenal *zona glomerulosa* is the most sensitive indicator in the rat and since the previous review additional studies have been conducted to clarify the possible role of the potassium ion and to establish a clear NOEL (Til *et al.* 1990; Til & Kuper, 1995). In the most sensitive strain of Wistar rat, no significant changes were observed at doses of 10 mg KNO_2 /kg b.w. while in a different Wistar strain adrenal changes were only seen at three times higher doses. Similar effects were seen with $NaNO_2$ but with reduced incidence and severity. The mechanism by which nitrite affects the adrenal remains unclear but it has been suggested that it is related to the appearance of methaemoglobinaemia and subsequent adaptation, possibly due to competition for NADPH between methaemoglobin reductase and hydroxylases involved in corticosteroid synthesis (Boink *et al.* 1995). Together with effects of nitrite on urinary steroids (Violanthe *et al.* 1973) this suggests an adaptive change with time and explains why adrenal effects were not observed in long-term studies.

Further studies have confirmed that nitrite is genotoxic in *in vitro* bacterial assays without metabolic activation, probably due to deamination of DNA bases at the high concentrations used. *In vivo* assays are equivocal.

As previously concluded, nitrite *per se* is not carcinogenic in mice or rats. Further evidence was reported (Grant & Butler, 1989) in a 115-week study in rats in which there was a dose-related **decrease** in the spontaneous incidence and time of onset of lymphomas, leukaemias and testicular tumours. In addition, in a recent study investigating the hypothesis that nitrite might be involved in the aetiology of cerebral glioma (Hawkes *et al.* 1992), no increases in tumours of the nervous system nor at other sites occurred in VM mice given 0.2% sodium nitrite in drinking water.

The NOEL in sub-chronic studies, based on effects on the adrenal in the most sensitive sub-strain of Wistar rat, is 10 mg KNO_2 /kg b.w., equivalent to 5.4 mg NO_2^- /kg b.w. (Til *et al*; 1988, 1990) The NOEL in chronic, two-year studies in rats was 10 mg $NaNO_2$ /kg b.w., equivalent to 6.7 mg NO_2^- /kg b.w., based on histological changes in lung (bronchodilation, hyperinflation and lymphocyte infiltration) and heart (focal degeneration and fibrosis at high doses)(Shuval & Gruener, 1972).

Toxicity of Nitrosamines and Nitrosamides

The acute toxicity of N-nitrosocompounds (NOC) varies within wide limits. No correlation is detectable between their acute toxicity and carcinogenicity. The signs of acute intoxication induced by many nitrosamines are quite similar, the liver being the primary target organ, often accompanied by haemorrhagic lung oedema. On the other hand N-nitrosamides predominantly induce damage to the bone marrow and lymphatic tissue.

Many nitrosamines and nitrosamides have been shown to be potent carcinogens. A wide spectrum of animal species, more than 40 species including 5 primate species, has been exposed experimentally to these carcinogens and none has been found resistant. Tumours are included in a great variety of organs and tissues, in a dose dependent manner, both after single and repeated exposure. For some compounds transplacental carcinogenic activity has been found.

Distinct organ specificity (organotropism) is an important characteristic of NOC which is supposed to be due to structure dependent differences in metabolic activation, formation of conjugates, repair capacities in target tissues and other parameters.

There is strong epidemiological evidence for a relationship between exposure to specific NOC (i.e. cytostatic drugs and those associated with smokeless tobacco) and the incidence of human cancer in specifically exposed human populations.

Nitrosamines have been found positive, after metabolic activation, in a variety of genotoxicity tests, covering several endpoints such as gene mutations, chromosomal aberrations, DNA-interaction and DNA-repair.

Furthermore metabolic activation of nitrosamines to carcinogenic/mutagenic electrophiles occurs in human tissues in a manner similar to that observed in experimental animals and the ensuing genotoxic events such as DNA-damage, DNA adduct formation or mutagenic effects are comparable. Symptoms of toxicity, preneoplastic morphological alterations and genotoxic effects similar to those found in animal experiments have been documented in cases of accidental human exposure and poisoning.

It is generally accepted that NOC that are carcinogenic in animals can also be considered as human carcinogens. Taking into account their genotoxic mechanism of action, no safe level can be determined. It is generally assumed that for carcinogens that are also genotoxic, there is no threshold dose below which no tumour formation would occur.

The formation, occurrence and carcinogenic potential of non-volatile nitrosamines in the diet have not been explored to any great extent (U.K. Ministry of Agriculture, Fisheries and Food, 1992)

It was noted however, that there is no direct epidemiological evidence that current dietary levels of pre-formed NOC in the European Union carries a risk to human health. Moreover, whilst the potential for endogenous NOC formation in the gastrointestinal tract is generally accepted, its importance in comparison to exogenous NOC exposure is far from being settled.

RISK ASSESSMENT

In line with current international trends in procedures and terminology used for the assessment of risks, the process employed by the Committee involved a characterisation of the risk associated with the agents in question based on hazard identification and characterisation (qualitative and quantitative toxicological evaluation) and exposure assessment.

1 Hazard identification and characterisation

1.1 Toxicity and ADI of nitrate

In its previous review of nitrate the Committee derived the ADI for nitrate (expressed as sodium nitrate) of 0-5 mg/kg b.w. from the NOEL in a long term rat study to which a safety factor of 500 was applied (Commission of the European Communities, 1992). The enlarged safety factor was applied because the rat was not considered a good surrogate for man in relation to salivary secretion of nitrate and its oral conversion to nitrite.

The present Committee reiterated this view and considered that the safety evaluation of nitrate should encompass its conversion to nitrite (see Annex II). It therefore took into account data on the extent of salivary secretion and conversion to nitrite in man, together with the new toxicological data on nitrite and the ADI derived from these.

The Committee concluded that long-term animal studies did not indicate that nitrite or nitrate *per se* are carcinogenic and that there was no quantitative evidence for the endogenous formation of carcinogenic N-nitrosocompounds after exposure to realistic levels of nitrate and N-nitrosatable precursors. In addition, the committee concluded that, overall, extensive epidemiological studies on nitrate have failed to demonstrate an association with cancer risk in man. The Committee therefore felt it appropriate to derive an ADI. The Committee considered that the evidence from human metabolism studies on nitrate taken in conjunction

with the toxicity of nitrite provides confirmation of the ADI for nitrate established by the Committee in its previous review.

The Committee concludes that the ADI of 0 - 3.7 mg/kg b.w. for the nitrate ion (equivalent to 0 - 5 mg/kg b.w. for sodium nitrate) should be retained.

The Committee confirms that this ADI is applicable to all sources of dietary exposure

1.2 Toxicity and ADI of nitrite

In its previous review the Committee derived a temporary ADI for sodium nitrite of 0 to 0.1 mg /kg b.w. from the NOEL in a two year rat study and from the NOEL in humans applying a safety factor of 100 (Commission of the European Communities, 1992).

In the chronic two year study on rats, a NOEL of 10 mg sodium nitrite /kg/ b.w. (equivalent to 6.7 mg NO_2^- / kg b.w.) was established based on the histological changes in lung and heart.

The present Committee reviewed the available toxicological data on nitrite and concluded that the most sensitive index of toxicity appears to be the hypertrophy of the adrenal *zona glomerulosa* in the rat. The NOEL for this effect in the most sensitive rat strain is 10 mg potassium nitrite /kg b.w. (equivalent to 5.4 mg NO_2^- / kg.b.w.)

The Committee considers that, in relation to these biological endpoints, it could derive a single ADI for nitrite ion *per se* from the data on both the sodium and potassium salts.

The NOELs for the endpoints are, within the limits of uncertainty of biological assay, the same. Consequently, taking the endpoints and the rounding of the numerical equivalents expressed as nitrite ion into account and applying a safety factor of 100, **the Committee has established a full ADI for nitrite ion of 0 - 0.06 mg/kg b.w.**

The Committee confirms that this ADI is applicable to all sources of dietary exposure

1.3 Toxicity of N-nitrosocompounds

NOC that are carcinogenic in animals can also be considered as human carcinogens. Taking into account their genotoxic mechanism of action, no safe level can be determined. It is generally assumed that for carcinogens that are also genotoxic, there is no threshold dose below which no tumour formation would occur.

2 Exposure assessment and risk characterisation

2.1 Nitrate and vegetables

The data available to the Committee describing the range of levels of nitrate in vegetables in the Member States and the exposure of consumers to various dietary sources are incomplete and difficult to compare. They confirm, however, that vegetables contribute 70 to 90 % of the total nitrate intake and indicate that for the average consumer in the EU, dietary intake probably does not exceed 50 % of the ADI. The data suggest that a small proportion of the population of the EU may exceed the ADI on an occasional basis. As mentioned in the previous report, drinking water may make a major contribution in some areas.

In view of the above observations, the Committee recommends continuation of efforts to reduce exposure to nitrates via food and water.

However, the Committee has currently insufficient information on the consumption of those vegetables which are the primary sources of nitrate in individual EU Member States to judge whether setting maximum limits on nitrate levels in certain vegetables would have a significant impact on overall intakes. The Committee is aware of the current survey of dietary nitrate intake in the framework of Scientific Co-operation on questions related to food which may provide a more comprehensive description of the situation in the EU.

In the meantime the Committee would urge that good agricultural practices are adopted to ensure nitrate levels are as low as possible

The Committee wishes to emphasise that concern over the presence of nitrate should not, however, discourage increase in the consumption of vegetables, a class of foodstuffs which is recognised as providing a unique and essential nutritional source and is accepted as playing a major role in health protection including possibly a reduction in the risk of cancer. Epidemiological evidence from a large number of studies shows that people who consume large quantities of fruit and vegetables have a lower relative risk for most types of cancers.

2.2 Nitrate and Nitrite as Food additives

In its opinion of 1990, the Committee concluded that the use of nitrates as food additives make only a minor contribution to total nitrate exposure. The data presently available to the Committee indicate that this is still the case.

The Committee noted that the residual amount of nitrites permitted by Directive 95/2/EC (Table 1) are much higher than those to be expected from the maximum levels of added nitrates and nitrites which the Committee was informed in its previous review are justifiable on technological grounds. It endorsed the opinions expressed in the previous report the conclusions of which are given under the section Terms of Reference of the present report.

2.3 N-nitrosocompounds

Dietary exposure to N-nitrosocompounds is very low. However, in view of the genotoxic and carcinogenic potential of some of these substances, efforts should continue to reduce dietary exposure.

Therefore, the Committee reiterates its previous opinion, that exposure to preformed nitrosamines in food should be minimised by appropriate technological practices such as the lowering of levels of nitrate and nitrite added to foods to the minimum required to achieve the necessary preservative effect and to ensure micro biological safety.

2.4 Special Consideration Concerning Baby Foods

The Committee endorses the widely held view that solid foods such as cereals, vegetables and fruit etc. should not be introduced into the infant diet before the age of four months (Commission of the European Communities, 1983 and 1991).

The Committee recalls the principles that it has always followed in respect of food for infants and young children i.e. that the use of technological additives should be limited as far as possible in foods intended for them (Commission of the European Communities, 1994). Moreover, in its previous opinion (Commission of the European Communities, 1992) on nitrite and nitrate, the Committee already stated that nitrate should not be used as an additive in infant foods.

As concerns the present question, the Committee advises that whilst recognising that nitrate is a natural and unavoidable component of certain vegetables, levels of nitrate in baby food, whether prepared commercially or in the home, should be kept to the minimum and should be sufficiently low to ensure that the ADI is not exceeded taking into account the higher food consumption to body weight ratio of children in this age group.

In the 1993 official control programme the nitrate content of over 2000 samples of baby food from EU Member States was determined. The highest mean nitrate content for any Member State was 120 mg/kg and the committee notes, in the absence of a more recent relevant

systematic European review, that this value compares favourably with the upper limit of 250 mg/kg proposed in 1981 by the European Society of Paediatric Gastroenterology and Nutrition.

RECOMMENDATIONS FOR FURTHER WORK

During the course of the present review the Committee noted a number of areas calling for further research in order to strengthen the risk assessment in this complex area. In particular, further work is needed concerning

- the bioavialability of nitrate from food matrices, notably from vegetables, and its subsequent secretion in saliva and reduction to nitrite *in vivo* (work in this area should include human data where possible).
- the significance of salivary proline-rich proteins in scavenging nitrite and as a consequent source of N-nitrosoproline (to assess the validity of using urinary N-nitrosoproline as an indicator of potential formation of carcinogenic amines).
- the possible endogenous formation of N-nitrosocompounds after exposure, including "pulse exposure", to realistic dietary levels of nitrate.
- to assess the qualitative and quantitative risks from volatile and non-volatile preformed nitrosamines
- the application of HACCP principles to reduce the levels of added preservatives including nitrites and nitrates
- the hypothesis that oral reduction of nitrate to nitrite might act as a physiological protective mechanism against oral ingress of microbial pathogens.

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Annex I

Intake of nitrate and nitrite in various Member States

Nitrate

Denmark

In Denmark, intakes from fruit and vegetables were about 40 mg/day, 75% of total dietary intakes; water, cereals and dairy produce accounted for most of the remainder. On average, potatoes were the largest contributor (13.3 mg/day; 33 %) followed by winter lettuce, summer lettuce, beetroot, white and red cabbage and finally leek (Mortensen & Larsen, 1989; National Food Agency of Denmark. 1990).

Belgium

Nitrate intakes in Belgium were estimated from 18 different vegetables, 9 different fruits and tap water by calculation. Based on average levels of nitrate in the different commodities it was estimated that the mean intake was 154 mg/day, about 70 % of the ADI, of which 126 mg/day (82%) came from vegetables (Dejonckheere *et al.* 1994).

Finland

Dietary intakes of nitrate of 1212 young Finns aged 9, 12, 18, 21 and 24 years were estimated using the 24 hr recall method. The mean daily intake was 54 mg/day; the mean intake of nitrate rose from 46.4 mg at age 9 to 62.3 mg at age 24. Vegetables (including potatoes) contributed 86% of nitrate intake (Laitinen *et al.* 1993).

France

Based on national average food consumption data and new food composition tables, average nitrate intakes in France were estimated to be 121 mg/day of which 85 % was derived from vegetables, and 5% each from cured meats and cereal products. These estimates were less than half those made in 1982 (Cornee *et al.* 1992).

Estimates of nitrate intakes from individual meals in four situations: school meals, works canteen, hospital and old people's home indicated that total nitrate intake from the meal (solid food and drink) ranged from 42 mg for children to 93 mg in hospital meals but it is not specified whether corrections were made for plate waste. (Conseil Supérieur d'Hygiene Publique de France 1992)

Germany

Estimated intakes of nitrate from food and drinking water were estimated by calculation for males and females based on a market basket survey, assumed food intakes of 3.3 kg and 2.1 kg for males and females respectively, and using median, arithmetic mean and 90th percentile values for the nitrate concentration of all the commodities making up the market. basket. Calculated daily intakes for males were 89, 156 and 422 mg nitrate/day and for

females 65, 114 and 310 mg/day respectively using these assumed concentrations The highest estimate would lead to males and females consuming 165 % and 147 % of the ADI respectively (Anon 1994) However, it must be noted that calculated intakes consistently give overestimates compared with duplicate diet analyses and the worst-case assumption that all commodities contained the 90th percentile nitrate concentration is a statistically highly unlikely postulate.

The Netherlands

The estimated daily intake of nitrate from food in the Netherlands was reported to be about 143 mg of which vegetables (excluding potatoes) contributed about 84 % and potatoes a further 7% This contrasts markedly with the data from Denmark. The intake of nitrate from drinking water averaged 20 mg or less (van Duijvenbooden & Matthijsen, 1989).

In a survey using the duplicate portion method, the average nitrate intake was found to be 52 mg/day (Ellen *et al.* 1990).

In a more recent. study on nitrate consumption in a representative sample of the population in the Netherlands, food intake data were collected using a 48-hour record distributed over a whole year from April 1987 to March 1988. The median daily nitrate intake was calculated from data bank information on mean nitrate levels in products from 1986-89 and from nitrate levels in drinking water. Losses due to preparation were taken into account. Median daily nitrate intakes were estimated to vary over a 12 month period from 95 and 108 mg for males and from 96 to 120 mg for females assuming in both cases a body weight of 70 kg.

The median nitrate intake on a body weight basis was found to be highest among children aged 1 to 10 years, reflecting the higher food intake as a proportion of body weight. The highest median intake levels were observed in April-June when the consumption of nitrate-rich vegetables, like lettuce and spinach, increases while the nitrate levels are still higher than those later in the summer (unpublished data presented at EERO meeting, Wageningen, December 1993).

United Kingdom

In 1985, the estimated mean dietary intake of nitrate using the Total Diet Study was 54 mg/day (52 mg from food and 2 mg from beverages) when the diets were made up with distilled water, little changed from a survey conducted in 1979. In the general population, vegetables contributed about 75 % of total daily nitrate intake. The mean intakes of nitrate were higher in vegetarian groups (185-194 mg/day) (U.K. MAFF 1992).

In a recent analysis of nitrate intakes by extreme consumers of high-nitrate vegetables (Burt 1993; 1994) estimates were made of total dietary intakes among the top 2.5% of consumers of lettuce, spinach and potatoes respectively and their variation by season. The high consumers of lettuce were estimated to have total dietary intakes of 170-180 mg/day. Although extremely high individual concentrations were found in some samples of lettuce, because of

the extent of consumption, it was estimated that potatoes may make a similar contribution to total intakes. High consumers of spinach were estimated to have a mean total dietary intake of 157 mg/day while the high potato group had an estimated intake of 94 mg/day.

Nitrite

Finland

Mean daily dietary intakes of nitrite have been estimated in young Finns in age groups averaging 9, 12, 18, 21 and 24 years using the 48 hour dietary recall method. The mean intakes of nitrite rose from 1.2 mg/day at age 9 to 1.74 mg/day at age 24. Meat and meat products contributed 69% of nitrite intakes (Laitinen *et al.* 1993)

France

Based on national average food consumption data and new food composition tables, average nitrite intake was 1.88 mg/day, 43% from vegetables; 28% from cured meats and 5 % from cereal products (Cornee *et al.* 1992)

The Netherlands

In a duplicate portion study, the highest recorded nitrite intake was 0.7 mg/day (Ellen *et a!.* 1990).

United Kingdom

In 1985 the estimated mean dietary nitrite intake using the Total Diet Study ranged from 2.4-4.2 mg/day. However, this is recognised to be an overestimate as all samples containing undetectable levels of nitrite were assumed to contain nitrite at the limit of detection of the method used
(1 mg/kg). In an earlier study in 1979, the estimated mean dietary nitrite concentration was 0.87 mg/day using methods with a lower limit of detection of 0.2 or 0.4 mg/kg. Intake of nitrite from cured meats was estimated by calculation using mean nitrite levels in cured meats to be 0.4, 0.5 and 1.8 mg/day for the population, consumers only and extreme consumers of cured meats (97.5^{th} percentile) respectively. Calculations using the maximum nitrite concentration in cured meats (a "worst case scenario") gave estimates of 1.5, 1.8 and 6.0 mg/day respectively (U.K. Ministry of Agriculture, Fisheries and Food, 1992).

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Annex II

Comparison of the nitrate ADI with the nitrate NOEL deduced from nitrite data

The following hazard characterisation of nitrate is based on the toxicokinetics of nitrate in humans and the toxicity of nitrite. It assumes a conversion of nitrate to nitrite of 5 % for normally responding individuals and 20 % for individuals at risk (i.e. young infants, high converters). Taking the NOAEL for nitrite as 10 mg/kg b.w. (based on rat studies and expressed as potassium nitrite), the "transposed" NOAEL for nitrate for the two population groups would be 146 and 36.5 mg/kg b.w. respectively (expressed as nitrate ion). This means that for the general population (low converters) and for the human population most at risk (i.e. young infants, high converters), factors of 40 and 10 respectively lie between the "transposed" NOAEL and the present ADI for nitrate (3.65 mg/kg b.w). This is considered to be acceptable since human data are included.

The calculation is as follows:

1. Assuming for the general human population a conversion of 5 % of the total nitrate intake to nitrite means that 20 mmol of nitrate (ion) is converted to 1mmol nitrite (ion).

2. The NOEL for nitrite is 10 mg/kg b.w., expressed as potassium nitrite. This equals:

   $10 \times \frac{mwNO_2}{mwKNO_2} = 10 \times 0.54 = 5.4$ mg/kg expressed as nitrite (ion)

3. This NOEL corresponds to:

   $$\frac{5.4}{46\ (mw.\ NO_2^-)} \quad \text{mmol/kg b.w.}$$

4. From this figure, based on the conversion figure of 20, the NOEL corresponds to:

   $$\frac{5.4}{46} \times 20 \text{ mmol } (N0_3^-)/\text{ kg b.w.}$$

5. This "transposed" NOEL is expressed on a weight base of nitrate ion (mg)

   $$\frac{5.4 \times 20 \times 62}{46} (mw.\ NO_3^-) \text{ mg/kg b.w.}$$

   i. e. $5.4 \times 20 \times 1.35 = 146$ mg/kg b.w. (expressed as nitrate ion).

6. The previously established ADI of 5 mg/kg b.w., expressed as sodium nitrate, corresponds to:

   $$5 \times \frac{mw\ NO_3^-}{mw\ NaNO_3} = \frac{5 \times 62}{85} = 3.65 \text{ mg/kg b.w., expressed as nitrate ion}$$

7. If this established ADI (6) of 3.65 mg/kg b.w. expressed as nitrate ion is compared with the "transposed" NOEL (5) of 146 mg/kg b.w. expressed as nitrate ion, a factor of

   $$\frac{146}{3.65} = 40 \text{ is found.}$$

As for the conversion, human data were applied and the absorption of nitrite was assumed to be 100 %, this factor is considered to be satisfactory. Moreover, it strongly supports the previously established ADI.

N.B. For a 4-fold higher conversion (high converters) which is quite cautious, the factor between the ADI and the "transposed " NOEL will be 10.

As intra-species differences in humans are also taken into account, this factor is also considered to be satisfactory

OPINION ON DRAFT COMMISSION DIRECTIVE LAYING DOWN SPECIFIC PURITY CRITERIA ON FOOD ADDITIVES OTHER THAN COLOURS AND SWEETENERS
(expressed on 14 December 1995)

Terms of reference

The Committee was asked for its opinion on the draft specific purity criteria for food additives other than colours and sweeteners.

Background

The European Parliament and Council Directive 95/2/EC of 20 February 1995 lays down those additives other than colours and sweeteners which may be used [1]. Under Article 3(3) of Directive 89/107/EEC the Commission is obliged to adopt purity criteria for food additives [2]. The Commission is therefore intending to adopt a further Commission Directive on purity criteria for additives other than colours and sweeteners. The Commission has already adopted Directives on purity criteria for colours and for sweeteners, taking into account the Scientific Committee for Food's (SCF) general opinion on purity criteria and its particular remarks on colours and sweeteners, expressed on 25 February 1994 [3].

Comments on the draft purity criteria

The Committee noted that the Commission's proposal for purity criteria was in draft form and that further revisions might be made. This opinion is based on the document available to the SCF, 5112/III/95 EN - Rev 1, dated August 1995. It was noted that in this draft there were a number of inconsistencies which posed problems to the Committee in reaching a conclusive view on their adequacy with respect to health issues.

The Committee considered that the remarks made in its earlier opinion on specifications for food additives [3], concerning the value of specifications, the type of information which should be included in specifications, consideration of additives derived from natural sources, and limits for heavy metals and solvent residues, were also applicable to additives other than colours and sweeteners.

The Committee pointed out that it was not possible to fully assess the adequacy of the draft purity criteria from the point of view of protection of public health without information on the method of manufacture of each additive. In the absence of such information, it was unable to assess the draft purity criteria with respect to any particular impurities which

might be present due to the method of production. The Committee urged that the Commission address this aspect before finalising the purity criteria.

The Committee noted that the parameters covered in some of the draft purity criteria were very detailed (e.g. for the benzoates) whereas others were not. It noted there were inconsistencies between specifications in matters of detail, which were not explicable in terms of protection of public health. The Committee therefore suggested that the Commission should consult appropriate experts to ensure that these anomalies are reviewed. It also noted that some parameters arguably of trivial significance for protection of public health were included (e.g. limits for sodium and chloride in potassium bisulphite), though it recognised that their inclusion might be for other commercial and technical reasons.

The Committee noted that the proposed limits for arsenic, lead and mercury for many of these additives were the same, irrespective of levels of use of each additive. It reiterated its earlier view that in order to protect public health the heavy metal limits should reflect, where necessary, the level of addition of the additive to food, whilst recognising that in other cases this would not be necessary if the level of dilution of an additive in food is such that its contribution to overall heavy metal intakes is negligible compared to other sources.

In relation to the specification for nisin, the Comittee noted that, exceptionally, the limits for arsenic, lead, mercury and total heavy metals were quoted to a first decimal place and questioned whether this was intended.

In relation to the specification for biphenyl, the Committee noted that the purity criterion for polycyclic aromatic hydrocarbons stated only "Absent". The Committee would wish to see this criterion expressed more precisely in analytical terms.

References

1. Official Journal NoL 61, 18.03.1995, p27.

2. Official Journal NoL 40, 11.02.1989, p1.

3. Reports of the Scientific Committee for Food (Thirty-fifth Series). Opinion on Specifications for Food Additives, expressed on 25 February 1994. Commission of the European Communities, Luxembourg , p13 (in press).

OPINION ON CYCLAMIC ACID AND ITS SODIUM AND CALCIUM SALTS.
(expressed on 14 December 1995)

Terms of reference

To review the safety in use of the sweetener cyclamic acid and its sodium and calcium salts.

Background

Cyclamates, cyclohexylamine and dicyclohexylamine were reviewed by the Scientific Committee for Food (SCF) in 1985[1]. The Committee then established a temporary ADI of 0-11 mg/kg bodyweight, expressed as cyclamic acid, for cyclamic acid and its sodium and calcium salts. The ADI was based on a no-observed-adverse-effect-level (NOAEL) of 100 mg/kg b.w. for testicular toxicity of cyclohexylamine (CHA), the metabolite of cyclamate which is produced by microbial fermentation of unabsorbed cyclamate in the lower gut. The ADI was derived from this NOAEL taking into account an estimate for the fraction of ingested cyclamate which was unabsorbed and an estimate of the conversion rate of unabsorbed cyclamate to CHA (as shown in Annex 1). However, the ADI received a temporary status because of "the existing areas of uncertainty relating to the relevance for man of the testicular damage found in rats fed cyclohexylamine".

The Committee reviewed cyclamate again in 1988[2]. The Committee was informed about new studies which confirmed that CHA was metabolised similarly by rat and man and that further studies on the comparative pharmacokinetics of cyclamate in rat and man were ongoing. In the light of that information the Committee did not consider it necessary to change its earlier 1985 assessment of a t-ADI for cyclamate.

In 1991 the Commission of the European Communities again sought the view of the Committee, following the decision of the UK Committee on Toxicity to allocate a new temporary ADI to cyclamate of 0-1.5 mg/kg b.w. The SCF confirmed its existing t-ADI of 0-11 mg/kg b.w. for cyclamate[3]. It was noted that the difference in the ADIs recommended by the two committees stemmed from the different choices made concerning the extent of conversion of cyclamate to CHA in the human population and the safety factor to be applied to the NOAEL. Because uncertainties still existed, the SCF requested in 1991 that before a full ADI could be established:

i) Studies should be carried out to define with greater precision the range of extent of conversion of cyclamate to CHA in humans with respect to variation

with time in the individual and that such studies should pay particular attention to the high converters in the population.

ii) *In vitro* studies on testicular tissue should be carried out to determine the sensitivity of the testis in man relative to species for which data already existed.

In response to this request the industry submitted further information concerning the intra-individual variability in cyclamate conversion in humans with time and the testicular toxicity of CHA. At its 82nd meeting, the SCF decided that this document did not answer its questions of concern. Furthermore, it concluded that "the full reports of the NCI monkey study, together with the relevant histological slides and the Wills' human study were required to enable the testicular toxicity of cyclamate per se to be evaluated further. If adequate and satisfactory study reports and histological specimens were not available, industry should be asked to carry out a short-term study with cyclohexylamine in monkeys to provide more data on the sensitivity of the testis to cyclohexylamine in a species closer to man. The question of whether more needed to be done to investigate intra-individual variability in conversion rates would depend on the outcome of the further assessment of the testicular toxicity of cyclamate/cyclohexylamine."[4]

As no further data were available from the Wills study the SCF decided that the further information from the NCI study alone would not answer the questions raised in relation to the temporary nature of the ADI and that a short-term study with CHA in monkeys was necessary[5].

Two studies in Cynomolgus monkeys have now been submitted to the Committee[6-9]. A document discussing the various uncertainty factors which should be considered when deriving an overall safety factor for cyclamate was also submitted[10]. These reports have now been considered by the Committee.

The Committee noted further information submitted by the industry concerning preliminary data from three different epidemiology studies currently underway in Spain[11]. These studies are examining the testicular and reproductive function of workers involved in the production of cyclamate from CHA and therefore exposed directly to CHA, the intake of cyclamate and excretion of CHA in a sample of the general population in Spain, and the intake of cyclamate and excretion of CHA in male patients and controls attending an infertility clinic. The full results of these studies when available will be of interest to the Committee.

Review of new monkey data

The monkey studies on CHA were carried out in response to the SCF's request for a short-term (90 day) study in monkeys. In the first study, a single test group of 5 male monkeys received CHA in a sustained release preparation administered twice daily via gelatin capsules. The dose administered was built up gradually by giving 2 x 17 mg/kg/day for 1 week, followed by 2 x 34 mg/kg/day for 1 week, then 2 x 50 mg/kg/day for 5 weeks. A control group of 5 males was given capsules containing placebo. In a second follow-up study, the 5 control animals used in the first study were dosed with 2 x 17 mg/kg/day for 4 weeks. There were no concurrent controls.

It was noted that the studies had not been carried out to the duration requested by the SCF and that the design of the first study had been compromised in response to the circumstances encountered of poor oral tolerance to CHA. The second study, which had been carried out because of the problems encountered in the first study, was designed to identify any testicular effects at a lower oral dose which was tolerated by the monkeys. It was noted that it had been terminated after 4 weeks. The Committee considered however that the two monkey studies did provide data which, subject to cautious interpretation, give additional insight into the toxicity of CHA.

The Committee first considered whether the effects observed on the testis in the first monkey study were attributable to CHA or were a non-specific effect. The Committee concluded that the testicular damage seen would not normally be expected from the level of reduction in food and water intake which was observed and that it was not possible to exclude that the effects were CHA mediated. The Committee further noted that the effects were qualitatively consistent with those seen in other species and considered that the balance of evidence suggested the testicular effects probably were directly due to CHA.

The Committee then went on to consider whether the second monkey study demonstrated a clear NOAEL. The study authors considered that the minimal effects seen on spermatogenesis in 2 of the 5 monkeys were consistent with the background pathology of Cynomolgus monkeys. The Committee commented that since there were no controls for this experiment (nor any historical background control data provided) it was not possible to verify this statement. Moreover, the group size was small and the experiment had been terminated after only 4 weeks of dosing. Taking all this into account, the Committee concluded that the possibility could not be ruled out that the lowest level tested of 34 mg/kg b.w./day was a minimal effect level and that it was not possible to establish a clear NOAEL from this study.

It was noted that, due to differences in clearance rate for CHA between monkey and rat, an oral dose of 34 mg/kg b.w./day in the monkey produced similar plasma levels to an oral dose of 200 mg/kg b.w./day in the rat when calculated as the area under the plasma concentration versus time curve (AUC). In earlier experiments in the rat 100 mg/kg b.w./day was a clear NOAEL and 200 mg/kg b.w./day caused slight testicular damage[12]. These data are thus consistent with the possibility that 34 mg/kg b.w./day may be a minimal effect level in the monkey, with the onset of effects occuring at similar plasma levels in rat and monkey (on an AUC basis), but with the effects in the rat becoming more severe than in the monkey, as plasma CHA levels increase.

While regretting the deficiencies in the monkey studies, the Committee did not think that further monkey studies would provide critical new useful data.

Review of the basis for the ADI

The Committee then considered the data available on the variability among the human population in rates of conversion of cyclamate to CHA. The Committee's opinion of 1992 had specifically requested further data on this aspect. The t-ADI for cyclamate of 0-11 mg/kg bw first set in 1985, was based on the assumption that 63% of cyclamate ingested remains unabsorbed and is potentially available in the lower gut for conversion to CHA. Of this the SCF assumed that 30% is actually converted, giving an overall conversion rate of 18.9%. The Committee noted that the available human data, some of which were published since the Committee's last opinion was expressed, but which have been seen since by the Committee[13], indicated variations in the fraction remaining unabsorbed and very wide individual variations in the unabsorbed fraction undergoing conversion, such that conversion rates ranged from negligible to as high as 100% of the daily ingested dose in a few individuals when gut transit times were extended. However, when the t-ADI was set the data available had not allowed a conversion factor to be chosen with any great precision and the Committee considers there are still uncertainties in this critical aspect of setting the ADI. The Committee therefore recommends that the existing conversion factor of 18.9% be maintained *pending the results of further studies. However, since this figure* is at the lower end of the possible range among significant converters, further conversion rate data should be provided within a specified time limit. The figure chosen for overall conversion rate may need to be revised once these further data are available.

The Committee also noted some residual uncertainties concerning the differing relative plasma clearance rates for CHA in rat, monkey and man and a lack of information on

relative sensitivity of testicular tissue to CHA between species. The *in vitro* work requested by the SCF in 1991 had not been carried out because industry considered that the *in vivo* monkey studies would provide better data.

In view of the remaining uncertainties, the Committee did not consider there was sufficent information to establish a full ADI. In view of the lack of a clear NOAEL in monkeys, the Committee considered that the NOAEL for CHA in the rat of 100 mg/kg b.w./day should be maintained as the basis for the t-ADI. This NOAEL is derived from a number of 90-day studies and has been confirmed in a 2-year study which showed a similar dose-response relationship to that seen in the 90-day studies, suggesting that the testicular lesions develop relatively early and do not become progressively more severe with continued treatment[12].

The Committee considered the safety factors to be applied, taking into account the points put forward in the paper by Renwick[10]. It was noted that the approach set out by Renwick, which breaks down a composite safety factor into components for toxicodynamic and toxicokinetic factors, resulted in a figure for the ADI of 0-13 mg/kg b.w.. This was compatible with the original SCF t-ADI. However, the Committee did not consider that the various toxicodynamic and toxicokinetic factors were sufficiently precisely characterised to justify their use as the primary means of establishing the ADI and did not agree that it could be safely assumed man would be no more sensitive than other species and thus a factor of only 1 could be used for inter-species toxicodynamics. The Committee considered it was appropriate to still use a conventional overall safety factor of 100 as before, comprising 10 for inter-species differences and 10 for inter-individual human differences.

Taking a NOAEL of 100 mg/kg b.w. for CHA, allowing for the differences in molecular weight between cyclamate and CHA, using an 18.9% overall conversion rate for ingested cyclamate, and applying a 100-fold safety factor gives a temporary ADI of 0-11 mg/kg b.w. for cyclamate, as before.

Conclusions

The Committee wishes to maintain its present temporary ADI of 0 -11 mg/kg b.w. for cyclamic acid and its sodium and calcium salts, pending submission of further data. The further data required are as follows:-

i) Given the consumption of cyclamate is likely to be on a regular daily basis among users, the following information is required on conversion rates in

humans, using reasonable sample sizes, to be submitted within 2 years of publicising this opinion in the SCF Minutes,

a) to establish what proportion of initially low/negligible converters can become high converters over time with repeated exposure for a few weeks to cyclamate,

b) for those who do convert, to establish within individuals the range of conversion rates which occur over time following repeated exposure to cyclamate, paying particular attention to inter-individual differences in the time course of induction and the persistence and magnitude of the capacity to convert in high converters.

ii) If feasible, *in vitro* studies to compare the relative sensitivity of human, monkey and rat testicular tissue to CHA.

iii) The completed reports of the three Spanish epidemiology studies.

The Committee will re-examine this issue in 3 years' time.

References

1. Reports of the Scientific Committee for Food (Sixteenth Series) (1985). Report of the Scientific Committee for Food on Sweeteners (Opinion expressed 14 September 1984). Commission of the European Communities, Luxembourg, EUR 10210 EN.

2. Reports of the Scientific Committee for Food (Twenty-first Series) (1989). Report of the Scientific Committee for Food on Sweeteners (Opinion expressed 11 December 1987 and 10 November 1988). Commission of the European Communities, Luxembourg, EUR 11617 EN.

3. Reports of the Scientific Committee for Food (Twenty-seventh Series) (1992). Recommendation on cyclamates (Opinion expressed 21 June 1991). Commission of the European Communities, Luxembourg, EUR 14181 EN.

4. Minutes of the 82nd Meeting of the Scientific Committee for Food held on 21 February 1992 in Brussels. Commission of the European Communities, Brussels, III/3160/92-EN

5. Minutes of 83rd Meeting of the Scientific Committee for Food held on 10 April 1992 in Brussels. Commission of the European Communities, Brussels, III/3280/92 EN

6. Progress report on primate study and request for review of the SCF requirements. International Sweeteners Association, Brussels, July 1994.

7. Executive summary of research on the testicular toxicity and toxicokinetics of cyclohexylamine in primates. Submitted by the International Sweeteners Association, Brussels, October 1994

8. Hazleton Deutschland GmbH. Final Report : Cyclohexylamine repeated dose oral (capsule) tolerability study in the male cynomolgus monkey. HD Report No.1193-1117-005A, July 1995. Submitted by the International Sweetners Association, Brussels.

9. Hazleton Deutschland GmbH. Final Report : Cyclohexylamine repeated dose oral (capsule) tolerability study in the male cynomolgus monkey. HD Report No.1193-1117-005B, July 1995. Submitted by the International Sweetners Association, Brussels.

10. A data-derived uncertainty factor for cyclamate by AG Renwick. Submitted by the International Sweeteners Association, Brussels, February 1995.

11. Letter from International Sweetners Association to Commission, October 10, 1995.

12. Bopp BA, Sonders RC and Kesterson JW (1986). Toxicological aspects of cyclamate and cyclohexylamine. CRC Critical Reviews of Toxicology, 16, 213-306.

13. Buss NE, Renwick AG, Donaldson KM and George CF (1992). The metabolism of cyclamate to cyclohexylamine and its cardiovascular consequences in human volunteers. Toxicology & Applied Pharmacology, 115, 199-210.

ANNEX 1

Derivation of SCF t-ADI

Assumptions used

Approximately 37% of ingested cyclamate is absorbed as cyclamate and is not further metabolised in man.

This leaves approximately 63% for conversion to cyclohexylamine by intestinal flora.

Conversion rate for non-absorbed cyclamate taken as 30%.

Overall conversion rate for ingested cyclamate is therefore 18.9% (30% of 63%).

NOAEL for cyclohexylamine is 100 mg/kg b.w./day.

Allowance for difference in molecular weights between cyclamate and cyclohexylamine:-

$$\frac{\text{MW cyclamate}}{\text{MW CHA}} = \sim 2$$

NOAEL for ingested cyclamate:-

$$= \frac{\text{NOAEL for CHA} \times 2}{\text{Proportion available for conversion} \times \text{Conversion rate}}$$

$$= \frac{100 \times 2}{0.63 \times 0.30}$$

$$= 1058$$

$$\text{t-ADI for cyclamate} = \frac{\text{NOAEL}}{\text{Safety factor}}$$

Using a safety factor of 100:-

$$\text{t-ADI} = \frac{1058}{100}$$

$$= \sim 11 \text{ mg/kg b.w.}$$

OPINION ON THE SAFETY IN USE OF 1,1,1,2-TETRAFLUORETHANE AS A SOLVENT FOR FLAVOUR EXRACTION (expressed on 14 December 1995)

Terms of reference

The Committee was asked for its opinion on the safety in use of 1,1,1,2-tetrafluoroethane as a solvent for flavour extraction

Background

Tetrafluoroethane is a non-flammable, colourless gas with a faint ethereal odour. It has been developed as a substitute for fully halogenated chlorofluorocarbons and for partially halogenated hydrochlorofluorocarbons. Its main use is in refrigeration and air conditioning, either alone or as a component of blends. When released into the environment, it partitions almost entirely into the atmosphere. Tetrafluoroethane degradation occurs mainly in the troposphere. The overall atmosphere lifetime is measured in years. Tetrafluoroethane has a Global Warming Potential of 0.3 relative to a reference value of 1.0 for trichlorofluoromethane (CFC-11).

The present opinion refers to the application for use of R134A or HFC 134a (1,1,1,2-tetrafluoroethane) as a solvent for flavour extraction.

Distribution and Metabolism

Tetrafluoroethane distributes uniformly in the body and does not accumulate in any organ or tissue including fat. Two thirds of the inhaled dose are exhaled unmodified while the remaining part is metabolized starting with P-450 catalyzed defluorination, yielding trifluoroethanol which is further oxidized to trifluoroacetic acid. The latter is partly excreted in the urine and partly further defluorinated and oxidized with formation of inorganic fluoride (found in the urine) and carbon dioxide (exhaled).
The absence of trifluoracetylated proteins in rats inhaling tetrafluoroethane at a level of 10,000 ppm for 6 hours, indicates that its metabolism does not form radicals or other reactive intermediates.

Repeated Dose Toxicity

1. Haskell Lab., Report No. 228-79,1979; in ECETOC 1995.

In a 14-day inhalation study, two groups of 10 male rats were exposed to HFC-134a at levels of 0 and 100,000 ppm (6 hours/day, 5 days/week). No compound-related effects were observed apart from increased respiratory rate and higher fluoride excretion in the urine.

2. ICI CTL, 1979, in: ECETOC 31.

In a 28-day inhalation study, four groups of SD rats (n = 16/sex/group) were exposed to HFC-134a at levels of 0; 1,000; 10,000 and 50,000 ppm (6 hours/day, total days of exposure = 20). Evidence of systemic absorption was obtained. Treatment related changes were observed only in males. Increased liver weight, without concurrent pathological alterations, was observed at exposure levels of 10,000 ppm and above. Increased kidney weight, reduced testicular weight and slight focal interstitial pneumonia were detected at the highest exposure level.

3. Ninety-Day Inhalation Toxicity Study in the Rat (ICI, CTL/P/2466, 11/15/1989).

Four groups of Alpk/APfSD rats (n = 20/sex/group) were exposed during 13 weeks by a whole-body system for 6 hours/day, 5 days/week to HFC-134a (purity > 99.5%) at levels of 0; 2,000; 10,000; 49,500 ppm (actual concentration). Ten rats/sex/group were killed at the end of the exposure period, while the rest were killed following a 4-week recovery period.

After 12-weeks, a slightly decreased total haemoglobin value was observed in males exposed at a level of 49,500 ppm, while plasma glucose was reduced in males from all HFA-134C treated groups, without a clear dose-effect relationship; ASAT activity was significantly increased in females exposed at a level of 49,500 ppm. Urine pH was significantly increased in males exposed at a level of 49,500 ppm at the 12-week examination. In the satellite group, decreased urine gravity and increased urine volume and protein content were detected at levels of exposure equal to or greater than 10,000 ppm with a dose-related pattern. No gross or microscopic pathological findings were detected apart from a slight increase in minimal myocarditis incidence in males exposed at a level of 49,500 ppm.

Chronic Toxicity and Carcinogenicity

1. Longstaff et al., Toxicol. Appl. Pharmacol., 1984, 72: 15-31 in: ECETOC 31.

A limited oral study was carried out in Alpk/APfSD Wistar rats. Groups of 36 animals per sex were treated with 0 and 300 mg/kg b.w. tetrafluoroethane in corn oil by gavage (5days/week) for 52 weeks and maintained for life. The study was terminated after 125

weeks. No tumorigenic effect was found. The only possible treatment-related effect was the increased mortality rate in males from week 104 onwards.

2. Two-Year Inhalation Toxicity Study in the Rat (Zeneca CTL/P/3841, 3/19/1993).

A combined chronic toxicity/carcinogenicity study was conducted by whole body inhalation in Alpk/APfSD Wistar rats. Groups of 85 animals of each sex were treated at concentrations of 0; 10,655; 45,200 or 212,000 mg/m^3 (0; 2,500; 10,000 or 50,000 ppm).

At levels of exposure greater or equal to 10,000 ppm there was a slight decrease of body weight gain in males up to 8 months of treatment, reversed thereafter. Food consumption was also slightly decreased in males from all the HFA-134C treated groups up to 4 months of treatment, with no effect on food utilization. A slight decrease in the total haemoglobin and total RBC values was observed in the males at levels of exposure greater than or equal to 2,500 ppm up to the terminal kill; statistically significant differences were only observed at 14 and 27 weeks and there was no clear dose-effect relationship. Also in males treated at 50,000 ppm, the total WBC count was significantly lower than controls at 14 weeks and was consistently, though not significantly, higher at the following examinations up to and including the terminal kill. ALAT and ASAT activities increased at levels of exposure greater than or equal to 10,000 ppm from week 79 up to the terminal kill. A small increase in urinary fluoride was seen at a level of 50,000 ppm from week 13 onward.

The main treatment related effect of toxicological significance was confined to the testes of male rats, with an increased incidence of Leydig cell hyperplasia and benign Leydig cell tumours. The NOEL for such effects was considered to be 10,000 ppm tetrafluoroethane. (Hext and Parr-Dobrzanski, 1993). In view of the non-genotoxicity of tetrafluoroethane in a comprehensive battery of test systems at gene and chromosomal level, these changes are considered to be non-genotoxic. This is in line with effects frequently shown by non-genotoxic hydrophobic agents capable of membrane modulation and may be attributable to effects on the hormonal balance.

Genotoxicity

Tetrafluoroethane has been tested in a large battery of assays including all important genetic end-points. It did not induce gene mutations in four separate Ames tests in different strains of S.typhimurium (TA1535, TA1537, TA1538, TA98 and TA100) and E.coli WP2 UvrA, with or without S9 mix. (Brusick, 1976; Longstaff et al., 1984; Callander and Priestley, 1990; Araki, 1991).

It was not genotoxic in S.cerevisiae strain D4 with or without S9 mix (Brusick, 1976). It did not induce chromosomal aberrations in cultured human lymphocytes (Mackay, 1990) or in Chinese hamster lung cells (CHL) (Asakura, 1991) with or without S9 mix. In vivo inhalation did not induce micronuclei in NMRI mice at levels of up to 500,000 ppm (6 hours) (Müller and Hofman, 1989) nor chromosome aberrations in Alpk/APfSD Wistar-derived rats up to 50,000 ppm (6 hours x 5 days) (Anderson and Richardson, 1979), unscheduled DNA synthesis (UDS) in Alpk/APfSD Wistar-derived rats up to 100,000 ppm (6 hours) (Trueman, 1990) and dominant lethals in CD1 mice at levels of up to 50,000 ppm (6 hour x 5 days) (Hodge et al., 1979).

Teratogenicity

1. Teratogenicity Study in the Rat (ICI, CTL/P/417, 1/9/1980).

Four groups of pregnant Sprague-Dawley rats (n = 23-29/group) were exposed by a whole-body system for 6 hours/day to tetrafluoroethane (purity > 99.5%) at levels of 0; 1,000; 10,000; 50,000 ppm of on gestational days 6-15. As an additional end point of maternal toxicity, lung histopathology was assessed in 10 females/group. No effects were observed on clinical condition, body weight gain or food consumption of females. However, an increased rate and severity of histopathological alterations in the lungs (i.e., presence of polymorphs and/or bronchiolar irritation) was detected at levels of 10,000 and 50,000 ppm. No developmental effects were produced by the treatment except for slight but statistically significant reductions in fetal weight and ossification and increased prevalence of sternebral anomalies at the highest exposure level.

2. Teratogenicity Study in the Rat (Haskell Lab. 1981 in ECETOC 1995)

Four groups of pregnant SD rats (n = 7/group) were exposed by a whole-body system to tetrafluoroethane at levels of 0; 30,000; 100,000 and 300,000 ppm for 6 hours/day on gestational days 6-15. Reduced maternal weight gain was observed at levels greater than or equal to 100,000 ppm, whereas delayed fetal ossification was reported only at the highest exposure level.

3. Teratogenicity Inhalation Study in the Rabbit (CTL/P/2504, 11/23/1989).

Four groups of pregnant NZW rabbits (n = 18-24/group) were exposed by a wholebody system for 6 hours/day to tetrafluoroethane (purity > 99.5%) at levels of 0; 2,500; 10,000; 40,000 ppm on gestational days 7-19 (day of insemination = gestational day 1). At levels of 10,000 and 40,000 ppm the dams showed significantly reduced body weight gain and food

consumption during the dosing period, which were partly reversed thereafter. At the 40,000 ppm level, slight increases of both pre-implantation losses (20.4 % vs 14.6 % in controls) and post-implantation losses (13.2 % vs. 9.5 % in controls) were observed.

Studies on Specific Effects

Cardiac Sensitization Potential in Dogs (CTL/C/2521, 9/11/1991).

Male beagle dogs were exposed by inhalation to 4-32 % tetrafluoroethane in the atmosphere for 10 minutes. Positive evidence of cardiac sensitization was assessed as the presence of multiple multifocal ectopic beats following adrenaline i.v. administration during treatment, with no or minimal response to the same adrenaline dose without exposure to the test compound. No evidence of positive or borderline response was observed with 4 % tetrafluoroethane; at concentrations of 8 % and 16 % the frequencies of positive responses were 33-40 %, while 100 % of positive or borderline responses was observed at a concentration of 32 %. Tetrafluoroethane blood levels greater than 50 µg/ml were associated with a positive response; however, there was no linear blood level/effect relationship. A known cardiac sensitizer, dichlorodifluoromethane, was used as positive control; this compound induced positive responses at blood levels greater than or equal to 40 µg/ml. The dose-response relationship was much steeper than for tetrafluoroethane with 0 % and 100 % positive responses at 8 % and 16 % atmospheric concentrations, respectively.

Summary

Tetrafluoroethane is rapidly absorbed and equilibrated in rat tissues after inhalation and is eliminated from the blood in expired air with a half life of a few minutes. Metabolism to trifluoroacetic acid occurs only in minor amounts. Tetrafluoroethane has an extremely low order of acute toxicity. Lethal effects occur at concentrations of 700,000 ppm in the inhaled air.

In an adequate inhalatory 90-day rat study, minor effects on urinary parameters were observed at levels greater than or equal to 10,000 ppm; such effects were not apparently reversible upon cessation of treatment. At the highest exposure level (49,500 ppm) minor haematological and biochemical effects and a slight increase of minimal myocarditis were observed. Although the toxicological significance of the findings was doubtful, the NOEL upon 90-day exposure should be conservatively set at 2,000 ppm.

In the inhalatory 2-year rat study, a slight but persistent effect on total haemoglobin and RBC count was observed at all exposure leves, while a minor but persistent increase of WBC count was detected at the highest level, 50,000 ppm. Aminotrasferase activity increased at the end of the exposure period at at levels greater than or equal to 10,000 ppm. Although a clear NOEL could not be set for such effects, their toxicological significance can be considered slight.

Tetrafluoroethane has been found unable to induce genotoxic effects in several *in vitro* and *in vivo* studies including all important genetic endpoints.

As for as carcinogenicity, two studies were conducted. In a limited oral study in rats using only one dose level (300 mg/kg b.w.) no tumorigenic effects were observed, although male rats showed increased mortality at the end of the study. In an adequate two-year inhalation combined chronic toxicity/carcinogenicity study, no neoplastic changes were observed in the females, while in male rats increased incidences of testicular Leydig cell hyperplasia and benign Leydig cell adenomas were observed. The NOEL for these effects was considered to be 10,000 ppm. Based on the non-genotoxicity of this compound, such effects are very likely due to indirect mechanisms, such as those involving hormonal disturbances. Reduced testicular weight was detected at 50,000 ppm with a NOEL of 10,000 ppm in a 28-day inhalation rat study.

Tetrafluoroethane did not show any specific effect on prenatal development in two adequate studies on pregnant rats and rabbits exposed by inhalation. The NOELs for maternal toxicity were 1,000 and 2,500 ppm in the rat and the rabbit respectively.

A fertility study was not provided.

Finally, tetrafluoroethane showed a clear-cut potential for cardiac sensitization in the dog upon acute exposure. The effects were comparable but less severe than those induced by a known cardiac sensitizer, dichlorodifluoromethane and were seen at blood levels in excess of 50 μg/ml.

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As a general conclusion, the lowest NOEL was 1,000 ppm for rat maternal toxicity; from 90-day and 2-year studies the overall NOEL was around 2,000 ppm (8,500 mg/m^3) when given 6 hrs/day for 5 days/week.

Conclusion

In view of the very low levels of solvent residues in essential oils (< 0.02 mg/litre) which are themselves added to foods at low levels, the use of tetrafluoroethane as a solvent for flavour extraction is regarded as acceptable.

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OPINION ON BOVINE SPONGIFORM ENCEPHALOPATHY
(expressed on 8 March 1996)

N.B. This opinion was issued prior to the announcement by the British Government (20th March 96) of new information relating to a possible link between BSE and Creutzfeldt-Jakob-Disease (CJD).

Terms of reference

The Commission, as requested by the German Federal Government, has asked the Committee to give an opinion on the adequacy of the measures in force for controlling the presence of the causative agent of BSE in baby food and infant food in the light of the confirmation of BSE in cattle born after 1.1.1992 (31 cases as of January 31, 1996).

Background and Discussion

The Committee heard evidence from three invited experts (Mr R Bradley, Prof. H Diringer and Prof M Pocchiari) who have participated also in the deliberations of the sub-committee on BSE of the Scientific Veterinary Committee. They addressed various aspects of the problem including the efficiency of the control measures on the incidence of BSE in cattle following the feed ban, the potential exposure of man to BSE-infected tissues, the possibility of transmission of BSE from cattle to man and whether or not there might be a link between BSE and Creutzfeldt-Jakob-Disease (CJD).

The Committee noted the divergence of views among the experts and that many of the arguments were based on assumptions. It further noted that adequate scientific basis necessary for it to evaluate the possible risk to public health arising from the presence of BSE in cattle is still lacking. On the other hand, it is clear to the Committee that measures hitherto taken have failed to result in the cessation of the BSE epidemic. Rather, more than 24.000 animals born after the 18 July 1988 (i.e. the date when the feeding ban of special bovine offal to cattle was introduced) succumbed to date to BSE. Further, approximately 300 new cases are being diagnosed each week. Moreover, human epidemiological data available at present are of questionable value in forecasting the possible impact of BSE on human health, as the time which has elapsed since the emergence of BSE is too short in relation to the latency period for CJD.

Conclusion

In the opinion of the SCF:
- the potential for human exposure to BSE-infected tissues still exists;
- human food safety issues pertaining to BSE are not limited to children or any other groups of the population;
- given the extent of currently available information on the pathogenesis of BSE, a conclusive answer regarding the question raised by the German Federal Government cannot be given at present.

The SCF acknowledges the work already done by the Scientific Veterinary Committee and supports its continuous efforts. It expresses its desire to be informed about progress in this endeavour. The Committee also urges the Commission to observe the development in the epidemic as well as the development in the scientific knowledge regarding BSE and human risk and to take any measures it deems necessary.

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

Responsibility for the secretariat of the Scientific Committee for Food was transferred from Directorate-General III 'Industry' to Directorate-General XXIV 'Consumer Policy and Consumer Health Protection' with effect from 1 April 1997.

The present report deals with opinions on:

- nitrates and nitrite;
- draft Commission directive laying down specific purity criteria on food additives other than colours and sweeteners;
- cyclamic acid and its sodium and calcium salts;
- the safety in use of 1,1,1,2-tetrafluoroethane as a solvent for flavour extraction;
- bovine spongiform encephalopathy (BSE).

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