

COMMISSION OF THE EUROPEAN COMMUNITIES

REPORTS  
OF THE SCIENTIFIC COMMITTEE  
FOR FOOD

Fourth series

1977

## SCIENTIFIC COMMITTEE FOR FOOD

The Committee was established by Commission Decision 74/234/EEC of 16 April 1974 (OJ No L 136 of 20.5.1974 page 1) to advise it on any problem relating to the protection of the health and safety of persons arising from the consumption of food, and in particular the composition of food, processes which are liable to modify food, the use of food additives and other processing aids as well as the presence of contaminants.

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

The opinions of the Committee are submitted to the Commission and as a rule are published. The present series relates to opinions given during the period indicated in the individual titles.

**COMMISSION OF THE EUROPEAN COMMUNITIES**

**REPORTS  
OF THE SCIENTIFIC COMMITTEE  
FOR FOOD**

**Fourth series**

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Catalogue number: CB-AH-77-004-EN-C

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## REPORT OF THE SCIENTIFIC COMMITTEE FOR FOOD ON SACCHARIN

(Opinion expressed 24 June 1977)

### TERMS OF REFERENCE

To give an opinion on the toxicological acceptability of the use of saccharin in food.

### CONCLUSIONS

1. The temporary ADI of 0-2.5 mg/kg bw proposed by JEFCA is endorsed<sup>1</sup>.
2. There is a need for a specification, listing detailed purity criteria, which includes appropriate limits for impurities.
3. Saccharin should not be used in food specially prepared for young children (up to 3 years old).
4. The intake of saccharin by children and pregnant women should be limited. To facilitate this limitation the Committee is of the opinion that the consumer should be adequately informed of the presence of saccharin in any food.

### INTRODUCTION

Saccharin was first synthesised by Constantine Fahlberg in 1879. Its potential as a sweetening agent was quickly recognised and it was soon used as a substitute for sugar in sweets and bakery products. It is well known that the material may be manufactured by the Remsen - Fahlberg technique or the Maumee process. The latter is used predominantly in the USA and Canada. Furthermore, the compound may be in the form of a free acid, the sodium or calcium salt. A summary of the two processes is given in Annex I.

Feeding studies conducted at the Wisconsin Alumni Research Foundation (WARF) and the Food and Drug Administration (FDA) indicated some time ago that saccharin at high dosage produced tumours in the bladders of male rats (1,2). However, these findings were not supported by similar studies in rats, mice, hamsters and monkeys in 7 other laboratories (2 to 5).

Quite recently the Health Protection Branch in Canada have carried out a two-generation study in Sprague Dawley rats and it has been found that there is an increased incidence of bladder cancer in the male rat.

In assessing the problem of saccharin, the Committee believed that in the Report it would be useful to outline some of the previous history of the subject, and to assess all available animal and human data of which it was aware. For this purpose the Report is divided into the following headings:

- A. Chemical : 1. Specifications and Impurities
- B. Biological: 2. Metabolism
  - 3. Mutagenicity/Teratogenicity
  - 4. Chronic Studies
- C. Human : 5. Epidemiology

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<sup>1</sup>Some members of the Committee felt that saccharin should not be used in food for general consumption.

## A. CHEMICAL CONSIDERATIONS

### 1. Specifications and Impurities

Because of the findings with respect to the WARF and FDA study, the Canadians decided, as did the Battelle Institute, that they would analyse various samples of saccharin for the impurity ortho-toluene-sulphonamide (OTS). They found that in the Remsen-Fahlberg technique, levels of this impurity were present in the range 6,100 to 118 ppm. With respect to the Maumee process, the levels vary between 25 and less than 1 ppm.

The impurities present in saccharin have been studied by several workers (6,7) some of which have been identified in amounts as low as 0.1 mg/kg. Several of these impurities are listed below.

diphenylsulphone  
tetracosane  
o-sulphamylbenzoic acid  
p-sulphamylbenzoic acid  
o-toluenesulphonamide  
p-toluenesulphonamide  
1,2-benzisothiazole 1,1-dioxide  
1,2-benzisothiazoline 1,1-dioxide  
p,p-ditolylsulphone  
o,o-ditolylsulphone  
o,m-ditolylsulphone  
o,p-ditolylsulphone  
m,p-ditolylsulphone

Unpublished information (Dr. Lafontaine) has shown yet other impurities might be present (e.g. p-toluenesulphonamide as a major impurity of saccharin produced by the Remsen-Fahlberg process; and in saccharin produced by the Maumee process, p-nitrobenzoic acid and certain phthalates - for instance dibutylphthalate have been tentatively identified).

The saccharin used in food should be as pure as is practicable by good technicological practice. The Committee recommends that saccharin for food use should comply with a detailed specification giving limits for the different impurities. These limits should take into consideration the chemical nature and toxicological properties of the impurities.

## B. BIOLOGICAL CONSIDERATIONS

### 2. Metabolism

Several studies have been carried out on the metabolism of saccharin in man and these early studies showed that it was relatively rapidly excreted, mainly in the urine in the unchanged state (8 and 9). These studies as far back as 1905 have recently been repeated by other workers using the monkey, rat and several other species and no worker has been able to detect any bio-transformation products (10 and 12).

Recent work (13) has shown that when C<sub>14</sub> labelled saccharin was administered orally to rats on a normal diet fed both 1% and 5% saccharin for up to 12 months, some 90% of the dose was excreted within 24 hours, the major amount in the urine (up to 80%) and the remaining 20% in the faeces. It has been stressed that no metabolite was detected in the excreta by chromatography or reverse isotope dilution techniques. The same work (13) showed that no metabolite was detected in man after the ingestion of 1 gm of saccharin daily for 21 days.

### 3. Metagenicity and Teratogenicity

It has recently been shown that a particular batch of sodium saccharin does not induce mutations by the Ames assay technique and that 5-chloro-saccharin is also negative. Ashby has shown that all saccharins by examinations of both the Ames and cell transformation tests have given negative results (14).

Professor Lederer using saccharin prepared from the two processes - Remsen-Fahlberg, and Maumee studied the resorption of the foetus of pregnant rats with various levels of the saccharins and with each of the following impurities:

- o-toluene sulphamide (OTS)
- p-toluene sulphonamide
- o-sulphamoylbenzoic acid
- p-sulphamoylbenzoic acid
- o-sulphobenzoic acid
- p-sulphobenzoic acid
- and ammonium o-sulphobenzoic acid

He examined changes in the retina crystalline lens and optic nerve using a teratogenic index based on the gravity of the lesion. The changes seemed to be related solely to the impurities shown to be present in commercial saccharin. (Tables 1 and 2)(15)(16)(17)(18)(19).

### 4. Chronic Studies

After considering the WARF and FDA studies, it was decided in Canada that because these findings were not supported by similar studies using rats, mice and hamsters at other laboratories, it would be useful to carry out a two-generation study involving the exposure of the F<sub>1</sub> generation in utero. They felt that the discrepancies between the results of various studies also suggested that impurities (in particular OTS) in commercial saccharin might be involved in the development of bladder cancer. They therefore decided that they would set up an OTS and 5% sodium saccharin study to elucidate the problem.

A full clinical assessment of these animals has been carried out throughout the time of the study and furthermore all animals have been autopsied and the individual reports on these are available.

The main issue that has emanated from the interim report of the Canadian study is the fact that there is an increased incidence of bladder carcinoma in the male animals of the F<sub>0</sub> and F<sub>1</sub> generations. (Table 3)

The Canadian workers have stressed that there has been a low incidence of kidney stones and they have found no evidence of the bladder parasite *Tricosomoides Crassicauda* or its ova. The Committee was aware that in Canada examination of the bladder sections revealed little evidence of pre-malignant lesions such as hyperplasia and metaplasia. The tumours found had arisen de novo and the criterion for judging malignancy was one of invasion of either the stalk of the papillary lesion seen, the sub mucosa or the muscle wall. The typical transitional cell carcinoma was a rarity as most of the lesions were anaplastic with accompanying invasion.

With respect to saccharin causing malignancy in the male rat urinary bladder, some work has shown that the compound does accumulate in the bladder wall of the male. The defect of this work is that it has not been carried out in both males and females and at the present moment is being investigated in the United Kingdom (14).

Other studies which have been carried out are shown in Table 4 and the majority of these studies in all other species are negative.

On the basis of the animal studies, the Committee endorsed the temporary ADI of 0-2.5 mg/kg/bw/day established by JECFA (April 1977) for total intake of saccharin. However, this will have to be reassessed in the light of new information as it becomes available. The Committee also recognises that the regulatory application of an ADI is complicated by

the sale and use of the substance in tablet form, and by the various types of legislation used to control its use (e.g. as food additive or as drug). The Committee did not believe that its terms of reference would permit it to enter into detailed discussion of this aspect but noted that it would appear that an ADI of this magnitude might require a limitation of the foodstuffs in which saccharin is used. Indeed, some Members of the Committee felt that saccharin should not be used in food for general consumptions (46).

## C. HUMAN CONSIDERATIONS

### 5. Epidemiology

Studies so far published have been carried out to ascertain the relationship between artificial sweetener consumption and bladder cancer risk reveal no correlation. (37)-(45)

However, these studies are not entirely satisfactory from the point of view of sample population and size. At the same time they are not solely related to sweeteners but deal also with other factors such as smoking. More recent epidemiological studies have produced conflicting results. In view of this the Committee recommends that prospective epidemiological studies should be carried out on the incidence of certain chronic state diseases in populations with a high intake of saccharin.

After considering all the negative data presented (i.e. data showing no effects), the positive data in the WARF and FDA animal studies and the latest interim results of the latest animal study in Canada, the Committee believes that it is particularly important that the intake of saccharin by children and pregnant women be limited. To facilitate this limitation the Committee is of the opinion that the consumer should be adequately informed of the presence of saccharin in any food. The Committee recommends that saccharin should not be used in food specially prepared for young children (up to 3 years old).

The Committee was quite aware that a risk/benefit assessment might be made for critical population groups (such as diabetics, and those suffering from diseases originating from obesity). The Committee thought that these individuals should be guided by their physician. The Committee agreed that the situation should be kept under review as further data become available.

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LIST OF TABLES





Table 1 Development of gestation in the rat receiving saccharin or its impurities in the food ration

	no. mothers	no. of embryos				Resorptions %	Mass in mg		Ratio E/P
		total		per mother			Embr. (E)	Placen. (P)	
		9th day	20th day	9th day	20th day				
controls	52	465	417	9.1	8.1	10.3	3 095	470	6.6
saccharin Rensen 3%	35	284	188	8.1	5.4	33.4	3 204	548	5.8
saccharin Rensen 0.3%	13	125	79	9.6	6.1	36.4	3 284	452	7.3
saccharin Maumee 3%	20	192	164	9.6	8.2	14.6	3 209	505	6.3
saccharin Maumee 0.3%	28	271	257	9.7	8.9	8.3	3 161	440	7.2
saccharin Maumee 0.15%	22	202	182	9.2	8.2	10.9	3 142	462	6.8
o-toluenesulphonamide 0.1%	20	184	165	9.2	8.25	10.3	2 969	522	5.1
o-sulphamoylbenzoic acid, 0.1%	24	221	187	9.2	7.8	20.0	3 208	482	6.6
o-sulphobenzoic acid, 0.1%	20	187	158	9.3	7.9	15.1	3 172	470	6.7
ammonium-o-sulphobenzoic acid 0.1%	20	186	141	9.3	7.0	24.1	2 983	517	5.7

Table 2 Teratogenic Index for two kinds of saccharin and their impurities administered by mouth

Treatment	Teratogenic Index
controls	0.75
saccharin Rensen 3%	6.38
saccharin Rensen 0.3%	5.36
saccharin Maumee 3%	0.00
saccharin Maumee 0.3%	2.33
saccharin Maumee 0.15%	0.00
o-toluenesulfonamide 0.1%	2.42
o-sulphamoylbenzoic acid 0.1%	6.42
o-sulphobenzoic acid 0.1%	10.16
ammonium-o-sulphobenzoic acid 0.1%	5.68

Table 3 Incidence of urinary tract tumour

TREATMENT	F <sub>0</sub> GENERATION (1)		F <sub>1</sub> GENERATION (2)	
	BENIGN	MALIGNANT	BENIGN	MALIGNANT
CONTROL	1	0	0	0
2.5 MG/KG/DAY OTS	1	0	0	0
25 MG/KG/DAY OTS	0	0	0	0
250 MG/KG/DAY OTS	1	0	0	0
250 MG/KG/DAY OTS 1% NH <sub>4</sub> Cl	0	0	0	0
5% Na SACCHARIN	5(3)	3	4	9(4)

FEMALES:

CONTROL	0	0	0	0
2.5 MG/KG/DAY OTS	1	0	2	0
25 MG/KG/DAY OTS	0	0	0	0
250 MG/KG/DAY OTS	0	0	0	0
250 MG/KG/DAY OTS 1% NH <sub>4</sub> Cl	0	0	0	0
5% Na SACCHARIN	0	0(5)	0	2

(1) 87 weeks on test before first tumour was observed.

Male - Control - 36 5% Na saccharin - 38  
 Female - Control - 38 5% Na saccharin - 40

(2) 67 weeks on test before first tumour was observed.

Male - Control - 42 5% Na saccharin - 45  
 Female - Control - 47 5% Na saccharin - 49

(3) Four (4) bladder tumours plus one (1) urethral tumour. In addition, there was one malignant lesion of the kidney pelvis not indicated.

(4) Eight (8) bladder tumours plus one (1) urethral tumour.

(5) There were two (2) malignant lesions of the kidney pelvis not indicated.

Table 4 Saccharin animal laboratory studies

Reference	Type and of study	Duration	Comments	Results	
20	Rats	2 yrs	1 & 5%	Slight effect at 5%	
21	Acute: Rats, mice, rabbits : Gestating ♀ mice	5 days -	10:1 cyc/sacc 0:1 & .5%	Negative Negative	
	Subacute: 4 groups rats : 1 group of rats : 3 groups of dogs	38 days 16 days 30 days	10:1 & .5% 1 g/kg & 10:1 0,2,4, g/kg	Negative Negative Negative	
	Chronic: 2 groups of rats : dogs	6 mths 11 mths	2% & 10:1 0.065 g/kg	Negative Negative	
	22	2 groups of 50 ♀ mice	18 mths	5% (pre-treat W/ PEG or PEG & BP)	Negative
	23	4 groups of 80 rats	To 105 wks	0,500,1120,2500 mg/kg daily	+ in 2500 mg grp
24	4 groups ♀ Swiss mice	To 400 days	20% sacc pellets	+	
25 & 26	Tumours in 20+ in each of 2+ trials in same/diff. labs.			+	
27 & 28	4 groups of 50 Wistar rats	To 56 wks	2.0 g/kg <sup>-1</sup> w/or w/out MNU	+ for co-car. effect	
29	Sprague-Dawley rats	Lifetime	100,250 mg/kg or 10:1 cyc/sacc	Negative	
30	Inhibitory effects on AAF fed ♀ rats	40 wks	5%	Negative	
31	Groups of rhesus monkeys	5.4 yrs+	0,20,100,500 mg/ kg daily	Negative	
32	Review of mutation research			+ or -	
33	Syrian Golden hamsters	8 days life	0,0.625,1.25,2.5, 5,10%	Negative	
34	Groups of 60 0/60 ♀ Chas River rats	26 mths	0,90,270,810, 2430 mg/kg	Negative	
35	186 rats and mice	2 yrs	5% & 1%	Negative	
36	Six generation study with mice including long term studies with P. F3 and F4	2 yrs	0.1 & 0.5% sacc	Negative for carcinogenicity embryogenicity and terato- genicity	

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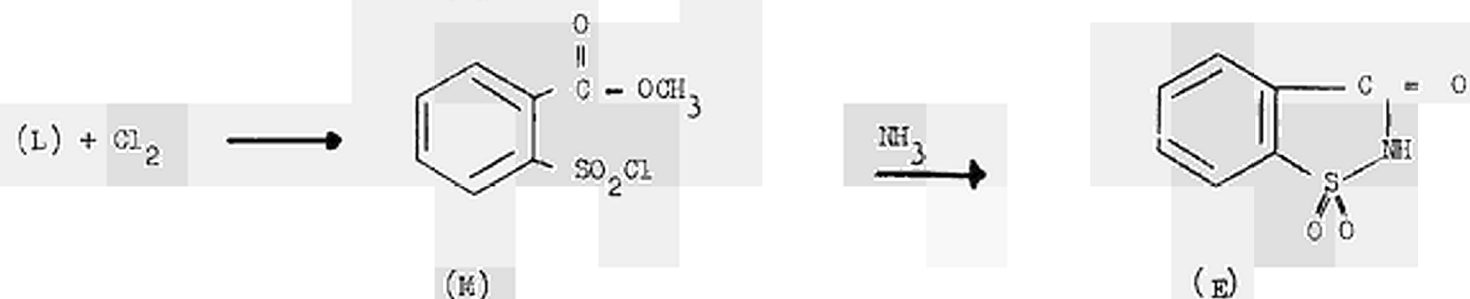
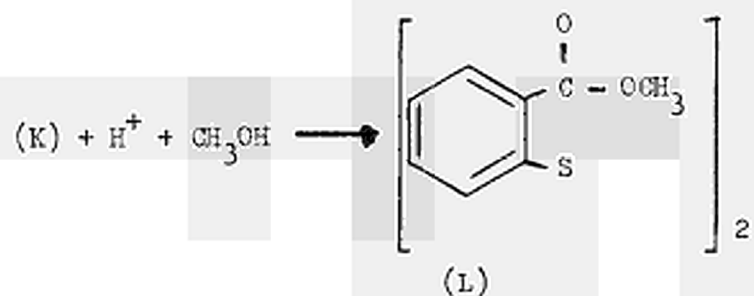
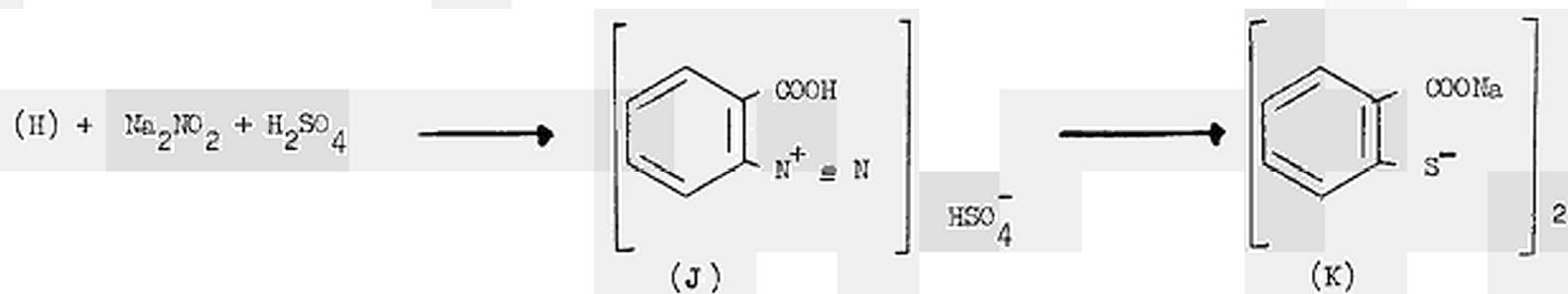
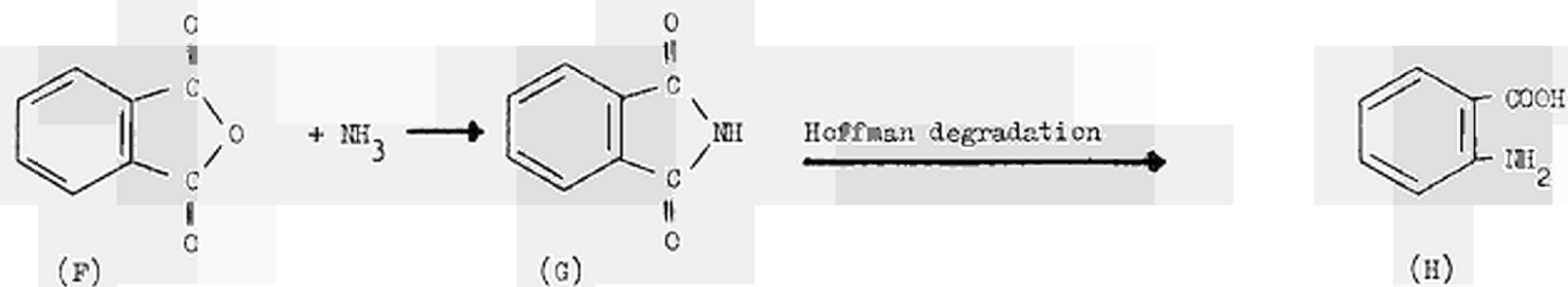
PRODUCTION OF COMMERCIAL SACCHARIN

Summary of Remsen and Fahlberg, and Maumes methods





REISEN AND FAHBERG





KEY TO ABBREVIATIONS USED

- (A) toluene
- (B) o-toluenesulphonylchloride
- (C) o-toluenesulphonamide
- (D) o-sulphamoylbenzoic acid
- (E) saccharin
- (F) phthalic anhydride
- (G) phthalimide
- (H) anthranilic acid
- (J) diazo salt of H
- (K) disulphide salt of H
- (L) disulphide ester of H
- (M) o-carbethoxybenzenesulphonylchloride

Acknowledgement

The Committee is grateful for the assistance given by J. Lederer, Professor of the University of Louvain.



REPORT OF THE SCIENTIFIC COMMITTEE FOR FOOD ON CALCIUM DISODIUM

ETHYLENEDIAMINE TETRA-ACETATE

(Opinion expressed 24 June 1977)

TERMS OF REFERENCE

To give an opinion on whether calcium disodium ethylenediamine tetra-acetate (calcium disodium EDTA) could, from the point of view of safety-in-use, be added to the Community list of antioxidants for use in food.

CONCLUSIONS

1. The Committee endorsed the ADI established by JECFA for calcium disodium EDTA of 0-2.5 mg/kg body weight.
2. Calcium disodium EDTA is acceptable as an additive for use in food within this limit and can, from the point of view of safety-in-use, be added to the Community list of antioxidants for use in food.
3. Calcium disodium EDTA should not be used in food intended for consumption by infants less than 2 years of age.

BACKGROUND

1. The present Directive on the approximation of the laws of the Member States concerning the antioxidants authorised for use in foodstuffs intended for human consumption (70/357/EEC of 13/7/1970)(1) does not provide for the use at Community level of calcium disodium EDTA.
2. The Treaty of Accession (2) and a modification to the Directive (74/412/EEC) of 1/8/1974 (3) permit Member States, until 31 December 1977, to maintain the provisions of their national laws authorising the use of this substance.
3. The Commission is considering whether or not it should propose to the Council that calcium disodium EDTA should be included in the Directive and has asked the Scientific Committee for Food for its opinion.

DISCUSSION

1. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) considered a number of data at its 9th (4) and 17th (5) meetings. JECFA considered that the level causing no toxicological effect in the rat was 0.5% in the diet equivalent to 250 mg/kg bw. JECFA estimated that the acceptable daily intake for man was 0-2.5 mg/kg bw.
2. The Committee examined all of this information and also took into account subsequent short-term studies. It endorsed the ADI established by JECFA of 0-2.5 mg/kg bw.
3. However, the Committee points out that in certain circumstances calcium disodium EDTA is used in human therapy for the treatment of heavy metal intoxication. The Committee was informed that calcium disodium EDTA had only been requested for use in certain well-specified products of low consumption. In these circumstances, the Committee felt that the use of this product could be accepted, but stressed that attention should be paid to ensure that the ADI was not exceeded if new uses were to be envisaged. The Committee would wish to re-evaluate calcium disodium EDTA if the usage of the substance was substantially extended.
4. The Committee continued to be concerned about the usage of additives in foods intended for consumption by infants less than 2 years of age. Calcium disodium EDTA is an effective sequestrant of mineral elements and should not be used in such foods.

#### REFERENCES

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3. O.J.L. 221 of 12/8/1974 p 18.
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REPORT OF THE SCIENTIFIC COMMITTEE FOR FOOD ON COLOURING MATTERS AUTHORIZED  
FOR USE IN FOODSTUFFS INTENDED FOR HUMAN CONSUMPTION

(Opinion expressed 16 September 1977)

TERMS OF REFERENCE

The Committee was asked to review the safety in use of certain colouring matters not included in its first report on colouring matters authorized for use in foodstuffs intended for human consumption (June 1975) and to consider information presented subsequently on colouring matters included in the earlier report.

CONCLUSIONS

1. Colours for which an ADI could be established and which are therefore toxicologically acceptable for use in food within these limits:

citranaxanthin (in feedingstuff for poultry) ADI 0-0.4 mg/kg bw

to be included in the ADI of 0-5 mg/kg bw

for  $\beta$ -carotene

$\beta$ -apo-8'-carotenal

$\beta$ -apo-8'-carotenoic acid methyl and ethyl esters

2. Colours for which an ADI was not established but which could be used in food:

riboflavin-5'-phosphate

vegetable carbon

titanium dioxide

3. Colours for which an ADI could not be established and which are not toxicologically acceptable for use in food:

antheraxanthin oleoresin

methyl violet (★)

red 10 B

ultramarine

violet BNP

BACKGROUND

1. Following the issue of the Committee's Report of June 1975<sup>1</sup> the Commission made enquiries as to whether other information was available which would permit the Committee to modify or change its opinion. As a result of these enquiries the Commission decided that the Committee should be asked for an opinion on the colours not considered in the 1975 Report, and information presented subsequently on colours which were included in that Report.
2. The Committee was provided with information on the specification, uses and toxicological properties of these colours. The Committee wishes to reiterate that its opinion relates, as requested by the Commission, to the evaluation of the safety in use of these colours, and that the reservations of some of its members on the need to use colours continues to exist (see paragraph 7 of the Report of June 1975).

(★) use as a marker should be reconsidered.

<sup>1</sup>Reports of the Scientific Committee for Food (First Series) 31 December 1975 (8801/7290).

3. In giving its opinion the Committee has maintained the approach used in its first Report in which its principles for assessment including specification are described (paragraphs 6-11).

The colours under discussion fall into three of the groupings used in the earlier report:

- colours for which an ADI could be established and which are therefore toxicologically acceptable for use in food within these limits;
- colours for which an ADI was not established but which nevertheless could be used in food;
- colours for which an ADI could not be established and which are not toxicologically acceptable for use in food.

4. The detailed considerations of the Committee are listed below:

Citraxanthin in feedingstuffs for poultry (5', 6'-dihydro-5'-apo-18'-nor- $\beta$ -caroten-6-one)

No clear evidence exists for the natural occurrence of this carotenoid colour. The available metabolic information points to conversion into vitamin A of the greater part of absorbed citraxanthin, the remainder being deposited in various tissues, including the egg yolk of birds.

The long-term study in rats which has recently been completed (using a protocol established some 3 or 4 years ago) revealed no adverse findings at all intake levels tested, the highest being 86 mg/kg bodyweight citraxanthin. The available 6 months dog study and three generation-reproduction study in rats showed no evidence of adverse effects on the parameters studied including reproduction and teratogenicity.

On the basis of these data the Committee established an ADI of 0-0.4 mg/kg bodyweight for man. In addition, in view of the metabolic transformation to vitamin A, this ADI should be included in the ADI of 0-5 mg/kg bodyweight for all carotenoids with provitamin A activity established for  $\beta$ -carotene,  $\beta$ -apo-8'-carotenal and the methyl and ethylesters of  $\beta$ -apo-8'-carotenoic acid (see Committee Report of 25 June 1975).

Taking into account the ADI and the small amounts that would be used, the Committee has no objection on toxicological grounds to the use of this colour as an additive to poultry feed. However, the Committee draws attention to the fact that neither the nutritional quality of a chicken, nor that of an egg, can be judged on the basis of its colour.

The Committee was aware that carotenoids with provitamin A activity introduced into foods may contribute to the total intake from all sources and recommends that these nutritional implications be considered in greater depth at a future date.

The Committee has not been asked to consider the implications of the practice of colouring egg yolks by adding citraxanthin to poultry feed except in terms of safety to the consumer. It is aware that other aspects of this question are being discussed by the Commission Services in conjunction with the Member States Governments, and by the Scientific Committee for Animal Nutrition.

The Opinion of the Committee has already been transmitted to the Scientific Committee for Animal Nutrition.

Riboflavin-5-phosphate and Riboflavin

The Committee was presented with information on riboflavin-5'-phosphate and additional data to that considered earlier in relation to riboflavin. Since riboflavin-5'-phosphate is the normal metabolite of riboflavin, the Committee has no objection to the use of this substance to colour food despite the absence of extensive animal toxicological data. However, the Committee is of the opinion that, because of the photo-chemical instability of this substance, the problem of breakdown products requires investigation and that the use of this substance to colour food should not alter significantly the average daily intake of riboflavin. The Committee also recommends that riboflavin should be maintained in the list of permitted colouring matters, and not excluded from the scope of the Directive as was proposed in the Committee's first report on colouring matters.

### Antheraxanthin Oleoresin

Antheraxanthin (5,6-epoxy-5,6-dihydro- $\beta$ , $\beta$ -carotene-3,3'-diol), is a xanthophyll which occurs naturally in flowers, including the Aztec Marigold. The product intended for use as a food colour is a hexane extract of the flower petals. The product contains antheraxanthin at a level of about 30% in the form of a dipalmitate.

No toxicological information was supplied either on the xanthophylls or on the commercial oleoresin intended to be used as a food colour. The Committee recommends therefore, that antheraxanthin oleoresin should not be used to colour food.

### Methyl violet

Methyl violet is a mixture of the hydrochlorides of the more highly methylated pararosanilines containing principally tetra-, penta, and hexamethyl derivatives. On the basis of the very scanty acute toxicity data available which did not include any results of tests by the oral route the Committee could not evaluate this colour.

The Committee understands that the only current use of methyl violet is as a marker (e.g. for meat, citrus fruit). The Committee recommends that this substance should not be used to colour food and that its use as a marker should be reconsidered.

### Red 10 B (C.I 17200)

The structure of this azodye is closely related to that of Red 2 G, which hydrolyses slowly in acid solution to give Red 10 B. From the analytical point of view therefore all food containing Red 2 G could contain traces of Red 10 B. Significant quantities of this hydrolysis product are likely to be found in products of high acidity and subjected to high temperature during processing. This phenomenon is not unique to these two colours. Red 10 B has also been put forward as a colour in its own right.

Only scanty metabolic information is available on Red 10 B. Information on short- and long-term tests in rats and dogs as well as skin painting studies was supplied only as a summary prepared by the U.S. Food and Drug Administration.

These studies showed that Red 10 B belonged to the class of compounds causing haemolytic anaemia and Heinz body formation. The results of the long-term studies were not available in full and could not be examined by the Committee, but even from the summaries available they appeared inadequate in terms of numbers of animals used and design, when judged by modern standards. No reproduction studies were available but the summary of the teratology studies appeared to show no adverse effects.

On the basis of the data available to it, the Committee was unable to recommend that Red 10 B be used to colour food. In addition Red 2 G should not be used under conditions in which significant hydrolysis to Red 10 B occurs.

### Ultramarine (C.I Pigment Blue 29, C.I Pigment Green 24, etc.)

Ultramarine is a polysulphide of sodium (or potassium lithium, or silver) alumino silicate of unknown constitution which is obtained by grinding mineral lapis lazuli or by fusing together kaolin, sodium carbonate (or sulphate), sulphur and carbon.

No information was available on the metabolism of this colour. The short-term study in rats established a no-effect level but no other animal data were available. The Committee was unable to recommend that ultramarine be used to colour food.

### Violet BNP

The available data on this colour show that there is no adequate specification and that although some long-term studies appear to have been carried out in mice and rats most of the results were not available for evaluation and their adequacy could not be estimated.

The Committee recommends that Violet BNP should not be used to colour food.

### Titanium dioxide

At the time of its earlier assessment of this colouring matter (June 1975), the Committee had been informed that the principal use was to colour sugar confectionary. In the light of the new information presented to it on other potential uses and on specification the Committee felt able to recommend its inclusion in the Directive for food use in general.

### Vegetable carbon

The Committee's earlier advice (June 1975), related to "carbon black" as well as to the vegetable carbon currently permitted by the Directive under E153 (vegetable carbon having the same properties as medicinal carbon). The Committee has been now informed that the only product which complied with its earlier recommendations on limits for polycyclic aromatic hydrocarbons was in fact E153. The Committee has also been informed that there was an application of this colouring matter in giving the necessary tint to the colour of several foodstuffs. The quantities used are not large. In view of its use as a traditional therapeutic agent the Committee was therefore able to recommend the maintenance of the substance in the Directive for food use in general, despite the absence of extensive animal toxicological data.



# REPORT OF THE SCIENTIFIC COMMITTEE FOR FOOD ON FORMALDEHYDE IN "GRANA PADANO" CHEESE

(Opinion expressed 20 October 1977)

## TERMS OF REFERENCE

To give an opinion on the acceptability on a Community basis from the point of view of the health of the consumer, of the use of formaldehyde in the production of "Grana Padano" cheese.

## CONCLUSIONS

The Committee accepts that the provision in the Directive on Preservatives that permits, on a national basis, the use of formaldehyde in "Grana Padano" cheese provided that no residue is detectable when the cheese is marketed, may be extended to the whole Community.

## BACKGROUND

The Council Directive making a ninth amendment to the approximation of the laws of the Member States concerning the preservatives authorised for use in foodstuffs intended for human consumption (74/62/EEC of 17 December 1973)(1) permits Member States to maintain the provisions of their national laws relating to the use of formaldehyde in "Grana Padano" cheese provided that, when the product is marketed, no residue of formaldehyde is detectable in the finished product (2). This provision lapses after 31 December 1977 if no further action is taken and the Commission requested the Committee's advice on whether from the point of view of the health of the consumer the Directive could be modified to allow continuation of this practice on a Community basis.

## TECHNOLOGICAL DATA

"Grana Padano" cheese is produced in certain defined regions of Italy. It is a hard cheese, normally used for grating but sometimes eaten directly. Owing to local circumstances the method by which cheese of an acceptable quality is made involved the addition of small quantities of formaldehyde to the milk from which the cheese is produced. The formaldehyde suppresses the growth of undesirable microbes which, if left to proliferate, would cause holes ("blowing") in the finished cheese.

## TOXICOLOGICAL INFORMATION AND EVALUATION

Following consideration of toxicological data on hexamethylenetetramine (HMT) the Committee thought that it was applicable to formaldehyde since under acidic conditions or in the presence of proteins, HMT gradually decomposes to yield formaldehyde and ammonia (3) (4). There is a considerable amount of toxicological information on HMT and on formaldehyde (5)(6)(7)(8). This includes the results of biochemical, short-term, long-term and reproduction studies. The Committee agreed that as the residues of formaldehyde in "Grana Padano" cheese were minimal they were not likely to present a hazard to human health. No residue of formaldehyde should be detectable in cheese by an appropriate method with a limit of detection of 0.5 ppm.

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