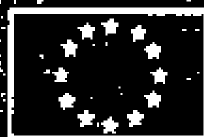
FOOD SCIENCE AND TECHNIQUES

Reports of the Scientific Committee for Food

(Thirty-sixth series)



EUROPEAN COMMISSION

European Commission

Food science and techniques

Reports of the Scientific Committee for Food

(Thirty-sixth series)

Opinions of the Scientific Committee for Food on:

Caustic sulphite caramel
Ammonia caramel
Carnauba wax
Coumarin
Di-2-ethylhexylphthalate
Di-2-ethylhexyladipate
3-monochloro-propane-1,2-diol (3-mcpd)
Polyethyleneglycol 6000
Neutron scanning (interrogation) devices
Iso-ascorbic acid
Acetylated oxidized starch
Cadmium
Esters used in plastics for food contact applications
Papain from papaya fruit used as a meat tenderizing agent

Directorate-General Industry
1997

Published by the
EUROPEAN COMMISSION
Directorate-General Industry

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Luxembourg: Office for Official Publications of the European Communities, 1997

ISBN 92-827-9580-2

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Printed in the United Kingdom

TABLE OF CONTENTS

Opinion on caustic sulphite caramel 1

Note 1

References 1

Opinion on ammonia caramel as a food colour 3

1. Terms of reference 3
2. Background 3
3. Current review 3
4. Discussion 3
5. Conclusion 4

Annex to opinion on ammonia caramel colour 5

1. Chronic toxicity and carcinogenicity 5
2. Genotoxicity 5
3. Short-term studies on ammonia caramel and THI in the rat 5
   1. 13-week study on ammonia caramel 5
   2. 4-week study on THI 6
4. Studies on lymphopenia, immunomodulation and interaction with vitamin B6 6
5. Study of ammonia caramel in human volunteers 8

References 8

Opinion on carnauba wax 11

1. Terms of reference 11
2. Background 11
3. Current review 11
4. Discussion 12
5. Conclusion 12

References 12

Opinion on coumarin 13

1. Terms of reference 13
2. Background 13
3. Discussion 14
4. Conclusions 16
5. Recommendations 17
6. Further research 17

References .... [illegible]

Opinion on di-2-ethylhexylphthalate 21

1. Terms of reference 21
2. Background 21
3. [illegible] .... [illegible]
4. [illegible] .... [illegible]
   [illegible] .... [illegible]

References .... [illegible]

Opinion on di-2-ethylhexyladipate 27

1. Terms of reference .... [illegible]
2. Background .... [illegible]
3. [illegible] .... [illegible]
4. Proposed [illegible] .... [illegible]
5. [illegible] .... [illegible]

References .... [illegible]

Opinion on 3-monochloro-propane-1,2-diol (3-mcpd) 31
1. Background 32
2. Conclusion 32

Opinion on polyethyleneglycol 6000 (PEG 6000) 35
1. Terms of reference 35
2. Background and discussion 35
3. Conclusion 35
References 36

Opinion on the food safety implications of surveillance systems 37
1. Terms of reference 37
2. Background 37
3. Discussion 37
4. Conclusions 40
References 41

Opinion on iso-ascorbic acid 43
1. Terms of reference 43
2. Background 43
3. Discussion and conclusions 43

Annex 45
Background 45
Biological data 46
Biochemical aspects 46
Vitamin C activity 48
Interaction of IAA and AA 48
Oxalate formation 49
Effects on metal absorption 50
Other biological effects 50
Toxicological studies 50
Acute toxicity 50
Comments 55
References 57

Opinion on acetylated oxidised starch 63
1. Terms of reference 63
2. Background 63
3. Specification 63
4. Subacute (dose range finding) study 64
5. Subchronic study 64
6. Comments 65
7. Conclusions 65

Opinion on cadmium 67
1. Terms of reference 67
2. Background 67
3. Conclusions 67
Dietary exposure 67
Carcinogenicity 69
Reference .... [illegible]

Report on certain esters used in plastics for food contact applications ... 71
1. Introduction ... 71
i) Hydrolysis studies ... 71
ii) 28-day oral studies ... 71
iii) Peroxisome proliferation studies ... 71
iv) Neurotoxicity studies ... 72
v) Reproduction and teratogenicity studies ... 72
vi) Mutagenicity studies ... 73
Classification of individual esters ... 73
Appendix ... 81
Definition of the SCF lists ... 81
Opinion on papain from papaya fruit (carica papaya) ... 85
1. Terms of reference ... 85
2. Background ... 85
3. Current review ... 85
4. Conclusion ... 86
References ... 87

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

Opinions on Caustic sulphite caramel, Ammonia caramel, Carnauba wax, Coumarin, Monochloro propanediol, Papain used as a meat tenderising agent

W GRUNOW

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H WALTON

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R. LOWERYS

M. VAHTER

L. VITTOZZI

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OPINION ON CAUSTIC SULPHITE CARAMEL

EXPRESSED ON 14 DECEMBER 1990

Note

At its 76th plenary meeting on 13 - 14 December 1990, the Scientific Committee for Food completed its evaluation of caustic sulphite caramel (caramel colour II). The opinion, which has not previously been published, was as follows:

> "The Committee considered caustic sulphite caramel in its 14th report and again in its 21st report. On the latter occasion, the Committee concluded that caustic sulphite caramel was temporarily acceptable pending provision of further information on the composition and homogeneity of caramels falling within this class.
>
> The Committee was provided with additional information indicating that the composition of caustic sulphite caramel is similar and intermediate to that of plain caramel (caramel colour I) and ammonium sulphite caramel (caramel colour IV). It was satisfied that the existing toxicity data on caustic sulphite caramel and ammonium sulphite caramel, with which it has many similarities, provide adequate documentation for the safety in use for both these classes of caramel.
>
> The Committee therefore decided to include caustic sulphite caramel within the ADI of 200 mg/kg bw it had already established for ammonium sulphite caramel. It stressed that the colours should fall within the specifications established when these classes of caramel were previously discussed."

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Reports of the Scientific Committee for Food 14th Series, EUR 8752, 1983

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Caramel Colour II: toxicological and chemical properties. Monograph prepared by the International Technical Caramel Association, Washington DC, August 1990 and submitted to the European Commission by the European Technical Caramel Association, 3 September [illegible]

OPINION ON AMMONIA CARAMEL AS A FOOD COLOUR

EXPRESSED ON 25 JUNE 1993, TEXT ADOPTED 17 JUNE 1994, REVISED 10 NOVEMBER 1994

1. Terms of reference

To advise on the safety in use in food of ammonia caramel (E 150c, caramel colour III).

2. Background

Caramel colours have been evaluated previously by the Committee and its opinion has been published in its report series numbers 1, 8, 14, and 21 (SCF 1974, 1979, 1984, and 1989).

In its most recent previous opinion, expressed on 10 December 1987, the Committee established an ADI of 0-200 mg/kg bw for ammonia caramel, but made it temporary pending a review of further studies and especially of the attempts to reduce the content of the lymphopenic compound THI (2-acetyl-4(5)-tetrahydroxybutylimidazole) from 25 mg of THI/kg to 10 mg of THI/kg ammonia caramel colour.

3. Current review

Since the last review by the SCF a considerable number of studies have been published on the colour itself and especially on the effect of THI on the lymphocytes and on the immune system as a whole.

The relevant industrial organisation, the European Technical Caramel Association, has submitted a review of the major safety studies to date on ammonia caramel (Chappel 1992a). Furthermore, the journal Food and Chemical Toxicology in 1992 dedicated an issue to studies on caramel colours (Chappel (ed.) 1992b).

The present review has concentrated on the most recent studies on the colour and on THI and has included two recent doctoral dissertations and a recently published study on human volunteers (Houben 1992, Gobin 1991, and Houben *et al.* 1992). A summary of the toxicological studies considered is given as an annex to this opinion.

4. Discussion

Neither the results of long-term studies in rats and mice, [illegible] of the available genotoxicity studies give rise to particular concern.

The major toxicological problem associated with the intake of ammonia caramel is the adverse effect of THI on the lymphocytes and the immune system as a whole. So far this effect has only been observed in rats and mice. It is believed that the effect would also occur in man, although humans may be less sensitive than rats (MacKenzie *et al.* [illegible]).

The effects of THI depend on the level of vitamin B_6 in the diet and for the estimation of a no-effect-level or NOAEL, the interaction with vitamin B_6 intake presents a difficulty. In the use of ammonia caramel as a food additive (with active "contaminant" THI) a safe level would be influenced by the level of vitamin B_6 in the diet of the actual consumer, which adds to the uncertainty.

In the most recent studies in rats on ammonia caramel and on THI the authors suggested NOAELs to be 20 g/kg bw for ammonia caramel and to be 120 µg/kg bw of THI in female rats. In male rats the NOAEL for THI was somewhat higher: 380 µg/kg bw (MacKenzie *et al.* 1992). It should, however, be emphasised that in these studies the immunotoxic effects found by others and mentioned below under (i) were not investigated. The NOAELs in the ammonia caramel study with high THI content (200 mg/kg of colour) and with THI itself might in fact have been lower if these other parameters had been included.

Houben's work (1992) suggest a potential Lowest Observable Adverse Effect Level of 20 µg/kg bw of THI. This would give a factor of 10 between this potential LOAEL and intake of THI if the ADI were to be set at 200 mg/kg bw for ammonia caramel with a THI content of max. 10 mg/kg of colour.

The question for the SCF was whether this could be judged satisfactory from a safety point of view. In reaching this judgement it should be borne in mind that:

(i) in the recent studies in rats effects other than lymphopenia have been detected including changes in immune function parameters (Houben 1992 and Houben *et al.* 1992). The detectable effects were on the thymus and peripheral lymphoid tissues and effects were observed in mitogenic stimulation assays *ex vivo*. These effects were clear and reproducible, and they are the basis for the difference between the apparent NOAEL in the MacKenzie *et al.* studies (1992) on THI and the results reported by Houben (1992).

(ii) in the studies in rats by MacKenzie *et al.* (1992) one of two samples of ammonia caramel used seemed to be a commercial sample with a THI content of 10 mg/kg colour. This THI content is exactly what the SCF requested in its 21st report (SCF 1989).

(iii) on the other hand a study in human volunteers, albeit of short duration (8 days), did not show any adverse effect of an oral dose of 200 mg/kg b.w. of caramel III with a THI content of 23 and 143 mg/kg caramel (Houben *et al.* 1992). The elderly male volunteers in the study were selected to be biochemically marginally deficient in vitamin B_6. The effects studied included, besides lymphocyte count and other haematological parameters, also some of the sensitive immunological parameters, which in rats were affected within a few days of low doses of THI (Houben 1992).

5. Conclusion

On the basis of the data received, the Committee decided to allocate a full ADI of 200 mg/kg bw for ammonia caramel with the proviso that the THI content should not exceed 10 mg/kg colour.

SUMMARY AND EVALUATION OF TOXICOLOGICAL STUDIES CONSIDERED

1. Chronic toxicity and carcinogenicity

It seems clear from the earlier evaluation of short- and long term studies that the major adverse effect of ammonia caramel is the lymphopenic and immunomodulatory effects chiefly caused by THI, but dependent on the dietary intake of vitamin B_6. Long-term studies in rats and mice have not indicated any carcinogenic potential.

2. Genotoxicity

Conflicting results have been published regarding genotoxic effects of ammonia caramel, which has been tested in various *in vitro* and *in vivo* systems covering different genetic endpoints. Negative or weak positive mutagenic effects in various strains of *Salmonella typhimurium* have been observed (Jensen *et al.* 1983, Yu *et al.* 1984, Aeschbacher 1986, Allen *et al.* 1992, Brusick *et al.* 1992). Conflicting results have been obtained with the yeast *Saccharomyces cerevisiae*, as ammonia caramel has been reported to increase the frequency of gene conversion in strain D7 up to 15 fold in non-toxic concentrations (Rosin *et al.* 1992), whereas two samples of ammonia caramel did not induce increased gene conversion using the yeast strain D4 (Brusick *et al.* 1992). Negative or weak positive clastogenic effects have moreover been reported in CHO cells (Allen *et al.* 1992, Brusick *et al.* 1992, and Stich *et al.* 1981) and in a test for gene mutation in L5178Y cells, both *in vitro*. *In vivo* studies were all negative (Brusick *et al.* 1992, Kawachi *et al.* 1980).

In the latest investigations, where well defined commercially important caramel colours have been tested, consistently negative results have been obtained with the Ames test. Moreover it appeared that the weak positive effects found in the test with mammalian cells *in vitro* were found at relatively high doses and in the absence of exogenous mammalian metabolic enzymes.

The overall pattern of genotoxicity found with ammonia caramel gives little cause for concern with respect to potential genotoxic effects in humans.

3. Short-term studies on ammonia caramel and THI in the rat (MacKenzie *et al.* 1992)

1. 13-week study on ammonia caramel

Groups of 20 rats (7 weeks old at the start) of each sex were given 0, [illegible] and [illegible] g low THI ([illegible] mg/kg) caramel and 2[illegible] g of high THI (250 mg/kg) in the drinking water for 13 weeks. The diet contained 35 mg of pyridoxine/kg diet. The treated rats had soft stools but no other treatment-related clinical effects or deaths were noted. There was not any treatment-related pathological changes in the large number of organs and tissues examined by gross and microscopical post mortem examination.

The clinical pathology tests performed were haematology, blood chemistry and urinanalysis. The only toxicologically significant finding was decreased white blood cell and lymphocyte counts, and increased neutrophil counts. This was seen at 2 weeks and at 6 weeks in animals given 20 g high THI caramel/kg bw with a return to normal values at 13 weeks.

This finding is consistent with an ADI of 200 mg/kg bw for the low THI containing caramel (10 mg THI/kg of colour).

2. 4-week study on THI

Groups of 20 rats of each sex were given 0, 0.9, 7.2 mg/kg bw THI and groups of 10 rats were given 0.1, 0.2, 0.5, 1.9 and 3.7 mg/kg bw THI in the drinking water. After 4 weeks 10 of the rats in 0, 0.9 and 7.2 mg/kg group were given water without THI for a 2 weeks recovery phase. The mean body weight of all test groups were decreased during 2. and 4. weeks of the study. These changes were ascribed to fasting for blood sampling and urinanalysis.

Gross and microscopical pathology of 10 animals of each group did not show treatment related effects.

Clinical pathology tests consisted of haematology, blood chemistry and urinanalysis. Haematology showed treatment related lowering of total white blood cell and lymphocyte counts and higher neutrophil counts. The haematological changes returned to normal by the 3rd day of the recovery phase and remained normal.

The NOEL for the only important haematological effect of THI noted, the reduction in lymphocyte count, was 380 µg/kg bw for males and 120 µg/kg bw for female rats. However, some more sensitive immunotoxicological parameters studied by G. Houben (1992) were not included.

4. Studies on lymphopenia, immunomodulation and interaction with vitamin B_6

(a). It was found that synthetic THI rapidly produces profound lymphopenia in the rat. The lymphopenia was shown to result in depletion of T and B lymphocytes from peripheral blood. No changes in T and B cell numbers in bone narrow and spleen was found, but in the thymus THI administration produced a decrease in immature cells (double positive CD_4+ and CB_8+ cells) and an increase in mature cells (single positive CD_4+ and CD_8+ cells). The lymphopenia by THI did not seem to be produced by reduced production of lymphocytes as the normal proliferative activity of all lymphoid organs was not affected - as measured by cytofluorometric analysis of 5-bromodeoxy-2'-uridine incorporated *in vivo* (Gobin 1991).

The immune competence of rats given THI was studied by assessing induced delayed hypersensitivity, graft versus host and mitogen proliferation assays to test T cell function. It can be concluded that THI suppressed cell mediated immunity. THI had no direct effect when added to lymphocyte cultures from untreated animals. However, lymphocyte cultures prepared from THI dosed rats did not proliferate *in vitro*, suggesting that THI might act through a mediator (Gobin 1991).

(b). In Houben's dissertation (1992) studies were performed in Wistar rats on ammonia caramel and THI under the influence of dietary pyridoxine. No difference in effects of caramel or THI on the rat immune system were observed, which confirmed results of earlier studies that the effects of ammonia caramel on the immune system are caused by THI. The effects were less pronounced or absent in rats fed a diet high in pyridoxine. THI did not induce obvious effects on vitamin B_6 status.

The effects induced by ammonia caramel and THI were reduced cell number in blood, spleen and lymph nodes, due to a comparable decrease in T and B lymphocyte numbers. In thymus an increase was seen in the number of mature medullary thymocytes and a decrease in the number of ER_3+ (recent thymus emigrants) in the spleen indicates a diminished migration of mature cells from thymus to the periphery. This increase in the number of mature cells was also indicated by an increase in mitogen-induced thymidine incorporation by thymocytes from THI exposed rats.

Further evidence for a change in the function of accessory cells was demonstrated in *ex vivo* studies as splenic cells from THI (and caramel) exposed rats did not show adequate response upon mitogenic stimulation.

Similar effects of THI and ammonia caramel administration to those observed in rats were observed in mice on a basic diet with 2-3 ppm vitamin B_6.

According to the review in this report spontaneous vitamin B_6 deficiency in humans is not seen frequently, but it may occur under certain conditions, secondary to disease or inborn errors in metabolism. Of more general interest is that several human populations may have an increased risk of marginal deficiency of vitamin B_6. Among these are chronic alcohol abusers (NB: heavy drinkers of dark beer may have a high intake of ammonia caramel), females on oral contraceptives, pregnant women, breast fed infants and elderly women and men. This last group was studied in a detailed study on human volunteers given ammonia caramel (see below).

In the concluding part of the report the author emphasises that a consistent NOEL for ammonia caramel and THI has not been reported yet, mainly due to the interference of vitamin B_6 in the diet. In addition, besides lymphopenia, other effects of these compounds have been observed on immune function parameters e.g. the immuno-histologically detectable effects are clear and reproducible on thymus and peripheral lymphoid tissues and the effects in *ex vivo* mitogenic stimulation assays. These seem only partly preventable by increasing vitamin B_6 to 10-12 ppm in the diet. The author concludes that the Lowest-Observed-Effect-Level in the rat so far suggests a No-Effect-Level for THI below 20 mg/kg bw in rats fed a diet with 2-3 ppm vitamin B_6. For an ammonia caramel with 25 mg of THI/kg this would be equivalent to an intake below 800 mg/kg bw (MacKenzie *et al.* 1992).

5. Study of ammonia caramel in human volunteers

In a double blind intervention study by Houben *et al.* (1992) groups of 8 male human volunteers (above 65 years of age) were given 0, 100 mg per kg bw (twice daily in a dessert) ammonia caramel (with 23 mg THI/kg) and 100 mg of ammonia caramel (with 143 mg THI/kg) in a dessert for 7 days. This dose is equivalent to the present temporary ADI. The participants were selected to be biochemically marginally vitamin B_6 deficient (decrease in plasma pyridoxal 5'-phosphate and increase in α-EAST). Ammonia caramel and high vitamin B_6 items were excluded from the diet 2 weeks prior to and during the experiment.

Base values of haematological and clinical-chemical parameters were obtained at two weeks before, one week before and at the start of the study. No effect of the intake of ammonia caramel with 23 and 143 mg of THI/kg were observed on blood lymphocyte numbers (total and within subsets) or on proliferative responses to mitogenic stimulation. In addition no effect was seen on other haematological parameters including serum immunoglobulin levels and on immunogen production *in vitro* by pokeweed mitogen-stimulated mononuclear blood cells. The authors emphasise that all effects on haematological and immunological parameters in rats are observed within a few days (see also Houben 1992) and that it, therefore, was found sufficient to conduct the study in humans for a period of seven days.

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OPINION ON CARNAUBA WAX

EXPRESSED ON 16 DECEMBER 1994

1. Terms of reference

To advise on the safety in use of carnauba wax.

2. Background

Carnauba wax has previously been evaluated by the Committee and on 19 October 1990 the substance was found temporarily acceptable as a glazing agent (SCF 26th Series Report, 1992). The acceptance was made temporary pending supplementary toxicological data and technical data on use.

3. Current review

Since then the WHO Joint Expert Committee on Food Additives (JECFA) has evaluated the same data as those evaluated by the SCF, that is, a 90-day rat study, a 28-week dog study, a combined reproduction and subchronic rat study, a rat teratogenicity study and *in vitro* mutagenicity studies in bacteria and yeast (WHO, 1993; Parent et al, 1983a, b; Rowland et al, 1982). The JECFA ADI of 7 mg/kg bw was based on the no-effect level from the combined reproduction and subchronic study, in which no adverse effects were observed up to the highest dose level tested of 1% in the diet (equivalent to 700 mg/kg bw).

No further toxicological data have been submitted to the SCF since its 26th Report, but the Committee has been informed that use of carnauba wax on confectionery is unlikely to exceed 200 mg/kg. Even with high intake of treated foods it would therefore be unlikely that intake would exceed 1-2 mg/kg bw/day.

4. Discussion

The Committee noted that the structure of the main components of carnauba wax (long-chain aliphatic esters) raises the possibility that this wax might behave like some mineral hydrocarbons. The key study (Parent et al, 1983b) in which full histopathological examinations were carried out on the control and high dose group of F1 rats was for the 13 week sub-chronic part of the study. [illegible] have been observed after ingestion of certain mineral hydrocarbons [illegible] reported. The Committee noted, however that these studies were completed before the effects of mineral hydrocarbons became known and that there were no absorption or metabolism data for carnauba wax. The Committee therefore requires information on the readiness of carnauba wax components to hydrolyse. If this cannot be shown then the Committee may require [illegible] studies.

Whilst the Committee has not received the toxicological data requested earlier, the information on use gives assurance that potential intake of carnauba wax will be well below the ADI allocated by JECFA and thus leaves a comfortable safety margin between intakes and the observed no-effect level in the combined reproduction and subchronic rat study. However, where there are no carcinogenicity studies, then the mutagenicity data should be adequate. There are studies on *S. typhimurium* TA 1535, 1537, 1538 and 98 which covers base-pair and frame-shift point mutation endpoints (although they did not use the TA 100 strain). But the Committee has not been made aware of any study covering the endpoint of chromosome aberrations.

5. Conclusion

It is recommended that subject to compliance with an acceptable specification the temporary acceptance of carnauba wax used as a glazing agent be extended pending submission of a mutagenicity test covering the endpoint of chromosome aberrations in mammalian cells *in vitro* and information on the readiness of carnauba wax ester components to hydrolyse. These studies should be carried out on material conforming to an appropriate and acceptable specification. This information should be submitted within one year.

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OPINION ON COUMARIN

(A CONSTITUENT OF NATURAL FLAVOURING SOURCE MATERIALS LIMITED BY ANNEX II OF FLAVOURINGS DIRECTIVE 88/388/EEC)

EXPRESSED ON 16 DECEMBER 1994

1. Terms of reference

To review the toxicity of coumarin in the light of recently completed studies and to consider whether the limits for coumarin in food set out in Annex II of the flavourings Directive 88/388/EEC (EEC, 1988) need to be amended.

2. Background

Coumarin is a naturally occurring benzopyrone. It occurs in various plants including tonka beans and sweet clover and in several natural flavouring source materials. Coumarin itself was originally used as a flavouring substance until the direct use of coumarin in food was prohibited in the USA in 1954 following reports of hepatotoxic effects in rats and dogs. It is still used in fragrances and tobacco. More recently, it has been used in the medical treatment of high protein lymphoedema and chronic infections such as brucellosis and tuberculosis. Coumarin has also been investigated in the treatment of renal cell carcinoma, malignant melanoma and prostate cancer.

Coumarin is listed in the 3rd Edition of the Council of Europe 'Blue Book' (Council of Europe, 1981) as an 'active principle'. The Council of Europe defines an active principle as a constituent of a natural flavouring source material for which, due to existing toxicological data, it appears necessary to set a maximum concentration limit in foodstuffs as consumed. No substance included in the list of active principles is approved by the Council of Europe for use as a chemically-defined flavouring substance in its own right. For coumarin, the general limits are < 2 mg/kg in food and beverages with specific exceptions of 10 mg/kg in 'special' caramels and 10 mg/kg in alcoholic beverages. It should be noted that the Council of Europe considered that the general limit in food and beverages should be less than 2 mg/kg, but that methods of analysis generally available at the time did not permit a lower level of detection.

The SCF examined the Council of Europe list of active principles in 1979 and considered that allowing the continued use of natural flavouring source materials with limitation of the active principles represented a [illegible] Council of Europe limits were endorsed with the [illegible] SCF limits for coumarin are included in Annex II of the EC Flavourings Framework Directive 88/388/EEC. That annex sets maximum limits for certain substances obtained from flavourings and other food ingredients with flavouring properties present in foodstuffs as consumed in which flavourings have been used. In other words, the limit only applies if flavourings have been added to a foodstuff but refers to the total amount of coumarin present in that foodstuff from either food ingredients or the flavouring. Annex II of the directive also contains a footnote indicating that the substances listed may not be added as such to foodstuffs or to flavourings.

Recently, a draft report of a US National Toxicology Program carcinogenesis bioassay on coumarin has been published (NTP, 1992a) and so the SCF has been asked to review coumarin. In the current review, the Committee has looked again at the earlier published toxicological studies on coumarin, as well as taking account of the recent NTP work and other studies published since our last review. The Committee was asked to consider whether the limits for coumarin in food, which apply when natural flavouring source materials are added to food, as set out in Annex II of the flavourings Directive, need to be amended. The SCF was not asked to consider coumarin as a chemically defined flavouring substance since it is currently prohibited for that purpose, nor was it asked to consider the context of its occurrence solely as a natural toxicant in foodstuffs.

3. Discussion

Coumarin is hepatotoxic to rats and dogs, causing liver enlargement, focal necrosis, fibrosis and bile duct proliferation. Other species however appear to be more resistant to the hepatotoxic effects of coumarin.

In rats, clear evidence of hepatotoxicity has been seen after dietary administration of doses in the range 87-125 mg/kg bw/day and above (HRC, 1984; Brune, 1984, Cohen, 1979) and after oral gavage doses of 25 mg/kg bw/day and above (NTP, 1992a). In addition to hepatotoxicity, studies have also shown coumarin caused cholangiocarcinomas and hepatocellular carcinomas. These were seen in both sexes at dietary doses of 230-340 mg/kg bw/day in two chronic studies on Sprague-Dawley rats in which doses ranging from 13-283 mg/kg bw/day (HRC, 1984) or 10-340 mg/kg bw/day (Brune, 1984) were given. Cholangiocarcinomas were not observed at doses of 87-110 mg/kg bw/day and below in these two studies. No cholangiocarcinomas or hepatocellular carcinomas were seen in the one chronic study in F344 rats in which coumarin was given by gavage at doses of 25, 50 or 100 mg/kg bw/day (NTP, 1992a). Several of the earlier 90 day and 2 year rat dietary studies have shown 1000 ppm (approximately 50 mg/kg bw/day) to be a no effect level for histological liver damage (Cohen, 1979). Changes in liver weight and serum liver enzyme levels however are seen from lower doses of 10-25 mg/kg bw/day when given by gavage (NTP, 1992a; Cohen, 1979). One of the more recent 2-year dietary studies has shown minor, atypical changes in the bile ducts of Sprague-Dawley rats at doses down to 10 mg/kg bw/day, the lowest dose tested (Brune, 1984).

In the dog oral doses of 25 mg/kg bw/day for over 100 days caused histological liver damage, 10 mg/kg bw/day being a no effect level (Hagan *et al*, 1967). Studies in limited numbers of baboons have found no evidence of histological liver damage and only slight evidence of reversible biochemical, histochemical or ultrastructural changes after oral dosing at 50 and 100 mg/kg bw/day for 3 weeks or dietary administration of doses up to 67.5 mg/kg bw/day for 2 years (Cohen, 1979). It is not known whether doses higher than these would produce histopathological changes in the baboon.

Only slight liver enlargement and no hepatotoxicity was found in CD1 mice given up to 280 mg/kg bw/day via the diet for 2 years (HRC, 1983). In a 2-year gavage study in B6C3F1 mice given 50, 100 or 200 mg/kg bw/day, syncytial alteration and centrilobular hypertrophy were seen in the liver at 200 mg/kg bw/day in females and in males at 100 mg/kg bw/day and above; syncytial alteration alone was seen at 50 mg/kg bw/day in male mice (NTP, 1992a). Significant increases in eosinophilic foci and hepatocellular adenomas were seen at 50 and 100 mg/kg bw/day but not at 200 mg/kg bw/day in female mice. In males, eosinophilic foci were increased at all doses but no hepatocellular adenomas were observed. In contrast to rats, no centrilobular necrosis was seen in mice of either sex. Similar increases in hepatocellular adenomas were seen in females in a 2-year gavage study in B6C3F1 mice using 3,4-dihydrocoumarin (which does not form an epoxide and causes no histopathological changes in rat liver) (NTP, 1992b). Also in contrast to the rat, coumarin did not cause liver necrosis in gerbils given a single i.p. dose of 125 mg/kg bw (Fentem *et al*, 1992), nor was it found to be hepatotoxic or hepatocarcinogenic in hamsters given up to 0.5% in the diet (approximately 600 mg/kg bw) for 2 years (Ueno and Hirono, 1981).

There are major interspecies differences in coumarin metabolism and these have been intensively studied to see if they might provide the explanation for the observed species differences in coumarin hepatotoxicity. In the rat, the major urinary metabolite is o-hydroxyphenylacetic acid from 3-hydroxycoumarin, whereas in baboons and man the major urinary metabolite is 7-hydroxycoumarin (Cohen, 1979). As in the rat, urinary excretion of 7-hydroxycoumarin is low in dogs, hamsters and some strains of mice (Cohen, 1979). One early theory of the mechanism of coumarin toxicity was that it would occur in species which did not form 7-hydroxycoumarin. However, the lack of hepatic effects in the hamster (Ueno and Hirono, 1981) suggests this is not the key difference. In the rat, recent studies indicate the involvement of a cytochrome P450 generated metabolite in the hepatotoxicity of coumarin, possibly the formation of a hypothetical 3,4-epoxide intermediate in the oxidation of coumarin to 3-hydroxycoumarin (Lake *et al*, 1989; Fentem *et al*, 1992a). Other recent evidence has suggested that the conjugation of a coumarin metabolite with glutathione provides a detoxification pathway in the rat (Huwer *et al*, 1991). Whilst the epoxide hypothesis is plausible and there are several strands of evidence to support it, once again the lack of hepatotoxicity in the hamster is puzzling since, like the rat, hamster microsomes produce o-hydroxyphenyl-acetaldehyde and 3-hydroxycoumarin, both of which can be derived from the 3,4-epoxide (Lake *et al*, 1992a). o-Hydroxyphenyl acetaldehyde is the major microsomal metabolite at high coumarin concentrations in many species including man (Lake *et al*, 1992a, Fentem *et al*, 1992b). 7-Hydroxycoumarin excretion in man is variable and can be as low as 10% (Cholerton *et al*, 1992). Alternative metabolic pathways used when 7-hydroxylation is low are unknown but *in vitro* studies on human liver microsomes suggest that at high coumarin levels, when the 7-hydroxylation pathway is saturated, then the o-hydroxyphenylacetaldehyde pathway may be favoured (Lake *et al*, 1992a, Fentem *et al*, 1992b). Clinical trials in man suggest that hepatotoxicity from coumarin is rare (Cox *et al*, 1989; Marshall [illegible] susceptible to coumarin [illegible]

Tumours have been observed at sites other than the liver. In a 2-year oral gavage study on F344 rats given 25, 50 or 100 mg/kg bw/day (NTP, 1992a), coumarin caused a dose-related increase in chronic nephropathy and a low incidence of renal adenomas (but not carcinomas) at 25 mg/kg bw/day (males) and 50 mg/kg bw/day (females). There was no clear dose response although, in males, this could have been due to the poor survival because of the nephropathy at the top two doses. Similar minor effects on the kidney were seen in a 2-year gavage study with 3,4-dihydrocoumarin in F344 rats (NTP, 1992b). No such effects were seen in the rat studies in which coumarin was given in the diet (HRC, 1984). In B6C3F1 mice given 50, 100 or 200 mg/kg bw/day coumarin by gavage for 2 years (NTP, 1992a), lung adenomas in both sexes and carcinomas in females were increased at the top dose. No such effects were seen in 2 year dietary studies in CD-1 mice given up to 280 mg/kg bw/day (HRC, 1983).

In mutagenicity studies *in vitro*, coumarin has tested negative in three Ames studies (Cohen, 1979), but positive or weakly positive in three others in one strain of Salmonella typhimurium, TA100, in the presence of metabolic activation (NTP, 1992a; Norman and Wood, 1981; Stoltz et al, 1982). A further study using the Ames spot test was inadequate for evaluation (Florin et al, 1980). Coumarin selectively inhibited excision repair in *E.coli* WP2 cells (Grigg, 1972). It increased the frequency of sister chromatid exchanges in Chinese hamster ovary (CHO) cells in the absence but not in the presence of metabolic activation and increased chromosome aberrations in CHO cells in the presence but not in the absence of metabolic activation (NTP, 1992a). Mutagenicity studies *in vivo*, comprising a sex linked recessive lethal assay in *Drosophila* (NTP, 1992a) and micronucleus tests in blood (NTP, 1993a) and bone marrow (Morris and Ward, 1992; Sterner and Korn, 1981) in mice, while negative, are of poor quality.

4. Conclusions

It may be concluded that coumarin is a carcinogen in rats via the oral route and possibly in mice. In rats, adenomas and carcinomas of the liver and bile duct and adenomas of the kidney have been observed. In mice, adenomas and carcinomas of the lung and liver adenomas have been observed. In reaching our recommendations (see below) the Committee gave particular weight to the occurrence of liver toxicity, including cholangiocarcinomas and hepatocellular carcinomas, confirmed in two chronic rat studies in which coumarin was administered by the dietary route. The results of the NTP gavage studies are more difficult to interpret: in rats only general liver toxicity, not tumours were seen, together with kidney adenomas, as well as nephropathy which is common in ageing rats; in the mouse significant dose-related increases were seen in lung tumours in both sexes, but significant increases in liver tumours were only seen in the low and mid-dose females. Both liver and lung tumours are common spontaneous occurrences in the strain of mouse used. Whilst not consistent with the dietary studies, the results from the gavage studies did not lessen our concern about the toxicity of coumarin.

A key issue in assessing the risk of coumarin to man is deciding whether or not coumarin is genotoxic. Particularly strong reassurance is needed that coumarin is not genotoxic *in vivo* when, in addition to positive *in vitro* studies, an epoxide has been postulated as a metabolic intermediate. The requirement for metabolic activation for a positive response in *Salmonella typhimurium* TA100 and in a study of chromosomal aberrations in CHO cells is consistent with the idea that activation to an epoxide may be required. However, metabolic activation did not appear to be required for the induction of SCEs in CHO cells *in vitro* (NTP, 1992a) but the positive response without S9 was weak and was not dose-related. The available *in vivo* mutagenicity studies, while negative, are not of a high enough standard to provide sufficient reassurance that coumarin is not active *in vivo*.

A further key consideration is whether the carcinogenicity seen in rats and mice, if due to an epoxide, is relevant to man. The Committee concluded that the epoxide route cannot be ruled out in man and need only be a minor pathway for genotoxic/carcinogenic effects to be of concern.

5. Recommendations

Taking into account the natural occurrence of coumarin in natural flavouring source materials, the carcinogenic activity of coumarin and the fact that a genotoxic mechanism cannot be excluded at this point in time, the Committee recommends that:

(i) the general limit in food and beverages for coumarin, which applies when it is present because natural flavouring source materials containing coumarin have been added, should be reduced to the currently achievable limit of detection for coumarin of 0.5 mg/kg;

(ii) action should be taken to reduce the higher levels which are currently permitted in certain traditional products.

6. Further research

The Committee considered that further research on coumarin would be desirable, particularly if any proposals to raise the general limit from that now recommended (the lowest achievable limit of detection) were to be considered. In this regard, further mutagenicity information could be particularly helpful. The Committee understands that new *in vitro* mutagenicity studies have been carried out recently, under the auspices of the Research Institute for Fragrance Materials, USA, on 7-hydroxycoumarin and ortho-hydroxyphenylacetic acid, which are the major metabolites in man and rat respectively. Final reports on these studies are pending. The Committee wishes to see these reports, but it should be stressed that these studies are on the end-stage metabolites only and thus they do not address the concern about the possible formation of an active epoxide intermediate. To address this concern, in the first instance, an *in vivo* bone marrow micronucleus test in mice and an *in vivo* liver UDS study in rats would be helpful.

Further research to address the more difficult issues could also be helpful but the Committee recognises that the resolution of these questions is less certain and could involve extensive work. These issues include whether the 3,4-epoxide is indeed responsible for the toxicity of coumarin, the extent to which it is produced in other species including man, whether a good animal model for man can be found from the metabolic viewpoint, what proportion of the human population has low 7-hydroxylase activity and how coumarin is metabolised in these people.

[illegible] likely risk to man will be possible without quantitative information on the [illegible] of coumarin in various natural flavouring source materials and foodstuffs to allow at least a rough estimate of coumarin intake in man.

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OPINION ON DI-2-ETHYLHEXYLPHTHALATE

EXPRESSED ON 16 DECEMBER 1994

1. Terms of reference

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

2. Background

This substance was first evaluated by the Committee (6th Report, 1978) as part of the review of the positive list of substances to be authorised in the manufacture of regenerated cellulose films intended to come into contact with foodstuffs. This evaluation was reviewed again by the Committee (14th Report, 1982) in the light of a considerable number of further studies and of the entire proceedings of the United States' National Toxicology Program (NTP) Conference on phthalates held in June 1981 (1). The Committee's conclusion at that time stated that DEHP had produced benign and malignant tumours of the liver in the mouse and the rat at the high doses used in the NCI/NTP studies. Although the *in vitro* mutagenicity tests gave conflicting results, the dominant lethal study (i.p.), an *in vivo* assay, indicated a possible genotoxic, though not dose dependent, effect. High oral doses in mice had also produced embryotoxic and teratogenic effects.

In view of these findings including the studies on mechanism and covalent binding for DEHP, the Committee excluded DEHP from the previously established group TDI for phthalates.

3. Review of additional data

A large number of additional investigations have now been provided for the Committee. They confirm that DEHP is hydrolysed to its monoester and 2-ethylhexanol predominantly in the intestinal tract and is subsequently partially absorbed. A considerable species difference exists in the degree of intestinal absorption. Further biotransformation occurs mainly in the liver showing again considerable species differences. In general, rodent species oxidise DEHP further than primates. Most species except the rat excrete the metabolites as glucuronides in the urine. The bulk of the compound is excreted in the urine and faeces within 24 hours, no tissue accumulation being observed (2,3).

The acute toxicity of DEHP is low, the major target organs being the liver and the testes (4). The most [illegible] finding is peroxisome proliferation in the liver, the occurrence of which again shows marked species differences. For example, it is found in the liver of rats but not of guinea pigs or marmosets and not in [illegible] human hepatocytes, suggesting that non-human primates and humans are less sensitive than rodents to chemical peroxisome proliferators (5,6).

Several oral studies in rats point to 50 mg/kg b.w. being the dose level at which liver weight increases and peroxisomal proliferations are induced. This is evidenced by ultramicroscopic changes in the liver and changes in the peroxisome-associated enzyme activities. Short term oral studies, including also dose levels below 50 mg/kg b.w. show inconsistent results. One 2-week study (7) by gavage in rats finds no increased liver weights or changes in hepatic palmitoyl CoA oxidation (PCoA), carnitine acetyltransferase or carnitine palmitoyltransferase activities at 25 mg/kg b.w.. Furthermore, no hepatic peroxisomal proliferation can be detected histochemically. However, 100 mg/kg b.w., the next dose level, affects biochemical indicators of peroxisomal proliferation.

A 7-day feeding study (8) reports a decrease in serum triglycerides at about 2.5 mg/kg b.w., an increase in carnitine palmitoyltransferase at about 5 mg/kg b.w. and an increased PCoA as well as increased carnitine acetyltransferase and carnitine palmitoyltransferase activities without any effect on liver weight at about 25 mg/kg b.w.. Ultramicroscopic hepatic changes are seen at about 250 mg/kg b.w.. Ultramicroscopic hepatic changes are seen at about 250 mg/kg b.w., the highest level tested. Because of lack of food consumption and age data of the rats, the dose levels were calculated from the dietary levels by using a factor of 20 instead of the usual 10 for short term studies in young rats.

The NOEL in a 3-week feeding study (9) in F344 rats, dosed with 0, 12, 109, 643, 1197 or 1892 mg/kg b.w. as calculated from the food intake, is reported as 12 mg/kg b.w. based on peroxisomal biochemical and ultramicroscopic parameters. Another 28-day feeding study (10) in F344 rats, dosed with 0, 24, 52, 115, 559, 1093 or 2496 mg/kg b.w., shows 52 mg/kg b.w. as NOEL for the induction of PCoA. However, the absolute liver weight is increased significantly at 52 mg/kg b.w. and higher and the relative liver weights are increased significantly at all dose levels in a dose-related manner. Histopathology of the liver shows a dose-related increase in hepatocyte basophilia at 52 mg/kg b.w. and higher doses.

A recent feeding study (11) in male Wistar rats used 0, 5, 18, 50, 180 or 500 mg/kg b.w. for either 2 or 4 weeks. The NOEL for induction of peroxisome associated enzymes lauric acid hydrolase and enoyl coenzyme-A hydratase is 5 mg/kg b.w.. Lightmicroscopic and electronmicroscopic morphometric analyses also show 5 mg/kg b.w. to be a NOEL. The NOEL for PCoA is 18 mg/kg b.w..

A 2-year feeding study (12) in rats with dose levels of 10, 100 and 1000 mg/kg b.w. shows slight increases at 10 mg/kg b.w. in the activities of CN-insensitive palmitoyl dehydrogenase in liver homogenates and in mitochondrial carnitine acetyltransferase. These changes become more pronounced at higher dose levels.

DEHP has no genotoxic potential. *In vitro* tests for gene mutations in bacterial, eukaryotic and mammalian cell systems, with or without S9, are negative. Similarly, *in vitro* tests for chromosomal aberrations and sister chromatid exchanges in mammalian cells and *in vivo* studies for chromosomal aberrations in somatic and germ cells are negative. There is no evidence for covalent binding to DNA, for induction of single strand breaks in DNA or for unscheduled DNA synthesis (13).

In long term studies, DEHP increased the incidence of hepatocellular adenomas and carcinomas in both mice and rats when given at high levels in the diet. The increases were dose related at doses of 3000 and 6000 mg/kg of diet in mice and 6000 and 12000 mg/kg of diet in rats. The lowest doses are equivalent to intakes of [illegible] mg/kg b.w. (1).

4. Proposed mechanism of action

The ability of some compounds, including DEHP, to induce hepatocellular carcinomas in rodents has been linked to their ability for inducing peroxisomal activity in the liver. Peroxisomal proliferation is in fact an early event during treatment with these compounds and appears to be the most sensitive change occurring in the liver at low doses. A causal association between peroxisomal proliferation and hepatic tumour development has been suggested (5,14). However, the underlying mechanism of carcinogenicity by peroxisomal proliferation is not known and may be complex. Suggestions include:

- oxidative stress and the induction of indirect DNA damage
- promotion of spontaneous preneoplastic lesions
- sustained growth stimulation.

All these mechanisms are considered to have a threshold. They are not mutually exclusive but could have an additive or synergistic effect (15).

Peroxisomal proliferation is associated in the peroxisomes with increased β-oxidation of fatty acids and similar compounds. This results in an increased production of hydrogen peroxide and/or other active oxygen species. The excess oxygen "leaks" from the peroxisomes and causes oxidative damage to other intracellular components. This may also lead to direct damage to the genetic material in the cell nucleus and subsequent neoplastic transformation. Alternatively, cumulative oxidative damage to intracellular membranes can lead to cell death and increased cell turnover, thus increasing the probability of spontaneous tumour formation (17).

Others claim that cell proliferation is unlikely to play a primary role in the hepatocarcinogenicity of peroxisome proliferators because a) the maximum proliferative response is very transient and b) chronic administration results in no or only a slight increase in replicative DNA synthesis. Furthermore, mitogen induced liver cell hyperplasia is less predisposed to become carcinogenic and several potent mitogens without a peroxisomal proliferative effect are not carcinogenic (16).

DEHP has adverse effects on reproduction. It causes foetotoxic and/or teratogenic effects in rodents. The NOEL for these effects in female mice, the most sensitive species, is 35 mg/kg b.w.. The NOEL for reproductive parameters in male and female mice is 15 mg/kg b.w. (18,19).

5. Conclusion

The overall NOEL for DEHP in the rat, based on the peroxisomal effects in the liver as measured by biochemical parameters and morphometric analysis in the sensitive species in the most recent study [illegible] kg b.w. An earlier study [illegible] b.w. as NOEL for hepatic peroxisomal effects. However, this study shows some deficiencies.

Bearing in mind the lack of genotoxicity in numerous assays using several mutagenic endpoints, the large margin between the doses of DEHP necessary to induce hepatic tumours (300-430 mg/kg b.w.) and those inducing hepatic peroxisomal activity, as well as the evidence for a threshold for increased hepatic peroxisomal activity enables the Committee to conclude that the very small intakes of DEHP from its uses in food packaging would not pose any carcinogenic hazard for man. Although the Committee acknowledges that some other studies might support a higher NOEL, it was unable to ignore the fact that lower doses still induced noticeable biochemical and electronmicroscopic changes and that some of these studies used different strains of rats.

The Committee also considered the suggestion that a safety factor of less than 100 might be applied in establishing a TDI for DEHP because of apparent differences in sensitivity with regard to peroxisomal enzyme induction and metabolism between subhuman primates, man and the laboratory rodents. It was not convinced, however, that the evidence was sufficiently weighty to accept this suggestion.

The Committee therefore retained as a matter of prudence a safety factor of 100 in establishing a TDI of 0.05 mg/kg b.w. for DEHP based on the NOEL for peroxisomal proliferation.

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OPINION ON DI-2-ETHYLHEXYLADIPATE

EXPRESSED ON 16 DECEMBER 1994

1. Terms of reference

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

2. Background

This substance was evaluated by the Committee (6th Report, 1978) as part of the review of the positive list of substances to be authorised in the manufacture of regenerated cellulose films intended to come into contact with foodstuffs. This evaluation was reviewed again by the Committee (14th Report, 1982) in the light of a considerable number of further studies and of the entire proceedings of the United States' National Toxicological Program (NTP) Conference on phthalates held in June 1981 (1). The Committee's conclusions at that time stated that the evidence regarding carcinogenicity was inconclusive because only in the mouse was there evidence of liver tumour induction. The only available mutagenicity test indicated mutagenic potential. Teratogenic and embryotoxic effects were only seen at high dose levels.

In view of these findings, including studies on mechanism and covalent binding to DNA for DEHA, the Committee excluded DEHA from the ADI previously established for three adipates.

3. Review of additional data

A number of additional investigations have now been provided for the Committee. They confirm that DEHA is rapidly absorbed by animal species, metabolised and excreted in the urine and to a lesser extent in the faeces and expired air within 48 hours. Absorption is incomplete after high doses. No tissue accumulation is observed. Rapid hydrolysis to the monoester and 2-ethyl-hexanol (EH) as well as of the monoester to adipic acid and EH occurs predominantly in the intestinal tract. EH is largely oxidised in the liver by ω-, ω-1- and β-oxidation to a series of metabolites. There are species differences in the biotransformation of DEHA. Rodents excrete largely ethylhexanoic, 5-hydroxy-2-ethylhexanoic and ethylhexanedioic acid, while monkeys excrete predominantly mono-2-ethylhexyl adipate and EH as the glucuronides (2).

DEHA has a low acute toxicity and causes little or no skin and eye irritation.

The liver is the major target organ in short-term studies in mice and rats, increased liver weight being observed at doses of 600 mg/kg b.w. and higher. In 2-3 week feeding studies in rats, using dose levels from 500-2000 mg/kg b.w., a hypolipidaemic effect is noted, indicated by the inhibition of cholesterol synthesis and an altered phospholipid synthesis. This effect is associated with hepatic peroxisome proliferation as observed electron-microscopically, increased activities of peroxisome-associated enzymes and an increased production of peroxisome proliferation-associated 80000 D polypeptide (PPA-80) (3).

The NOEL for the induction of peroxisomal proliferation in 3-4 week feeding studies in F344 rats is around 100 mg/kg b.w. based on the activities of hepatic peroxisomal-associated enzymes and ultramicroscopic changes. A further 30-day feeding study in male F344 rats shows a NOEL of 250 mg/kg b.w., based on ultramicroscopic changes and increased levels of peroxisome proliferation-associated 80000 D polypeptide. If PCoA oxidation is considered, then 250 mg/kg b.w. cause a three-fold rise in activity. A recent feeding study in male Wistar rats used 0, 20, 60, 200, 600 or 2000 mg/kg b.w. for two weeks. The NOEL for induction of peroxisonal associated enzymes was 200 mg/kg b.w. (14). Peroxisomal effects of DEHA are, however, moderate compared to those of DEHP. EH is considered to be the proximate peroxisomal proliferator. There is also a marked species difference for peroxisomal effects. *In vitro* studies with hepatocytes of rats, guinea pigs and marmosets show only in rat hepatocytes a clear effect (4,5). Non human primates and humans appear to be less sensitive than rodents to chemically induced peroxisome proliferation (6,7).

Although a dominant lethal assay (i.p.) in mice has been reported as showing a positive, dose-dependent effect (8), further assays in a range of systems are negative. Thus, *in vitro* bacterial assays with and without S9, *in vitro* mouse lymphocyte test for DNA damage with and without S9, an *in vivo* mouse micronucleus test and a sex-linked recessive lethal test in Drosophila show no genotoxic activity of DEHA. A covalent DNA-binding study in mouse liver and a cell transformation test in BALB/3T3 mouse cells are equally negative (3).

The previously assessed long-term studies in mice and rats, using dose levels of 1715 and 3570 mg/kg b.w. in mice and 600 and 1250 mg/kg b.w. in rats, showed only in mice a significantly increased incidence of liver tumours. A possible explanation might be the lower dose levels used in rats (9).

DEHA caused reduced fertility in a reproduction study in male mice at a high i.p. dose of 9220 mg/kg b.w. however, no testicular lesions have been reported in two 3-week feeding studies in rats and in 13-week and 2-year feeding studies in mice and rats (3). No effects on fertility of male and female rats are reported in a 1-generation reproduction study using dose levels up to 720 mg/kg b.w. although at that dose foetotoxic effects and, in parental animals, decreased growth and increased liver weights are noticeable (12). DEHA has no teratogenic effects in rats at dose levels up to 720 mg/kg b.w. but maternal effects are present. Foetotoxic effects appear at 110 mg/kg b.w. thus establishing as NOEL 55 mg/kg b.w. (13).

4. Proposed mechanism of action

The ability of some compounds, including DEHA, to induce hepatocellular carcinomas in rodents has been linked to their ability for inducing peroxisomal activity in the liver. Peroxisomal proliferation is in fact an early event during treatment with these compounds and appears to be the most sensitive change occurring in the liver at low doses. A causal association between peroxisomal proliferation and hepatic tumour development has been suggested (6,10). However, the underlying mechanism of carcinogenicity by peroxisomal proliferation is not known and may be complex. Suggestions include:

- oxidative stress and the induction of indirect DNA changes
- promotion of spontaneous preneoplastic lesions
- sustained growth stimulation.

All these mechanisms are considered to have a threshold. They are not mutually exclusive but could have an additive or synergistic effect (11)

Peroxisomal proliferation is associated in the peroxisomes with increased β-oxidation of fatty acids and similar compounds. This results in an increased production of hydrogen peroxide and/or other active oxygen species. The excess oxygen "leaks" from the peroxisomes and causes oxidative damage to other intracellular components. This may also lead to direct damage to the genetic material in the cell nucleus and subsequent neoplastic transformation. Alternatively, cumulative oxidative damage to intracellular membranes can lead to cell death and increased cell turnover, thus increasing the probability of spontaneous tumour formation (16).

Others claim that cell proliferation is unlikely to play a primary role in the hepatocarcinogenicity of peroxisome proliferators because a) the maximum proliferative response is very transient and b) chronic administration results in no or only a slight increase in replicative DNA synthesis. Furthermore, mitogen-induced liver cell hyperplasia is less predisposed to become carcinogenic and several potent mitogens without a peroxisomal proliferative effect are not carcinogenic (15).

5. Conclusion

The NOEL for DEHA in the rat based on the peroxisomal effects in the liver, as measured by biochemical parameters and electronmicroscopic analysis in this sensitive species, is around 100 mg/kg b.w. Bearing in mind the lack of genotoxic activity in all but one mutagenicity assay using several endpoints, the large margin between the doses of DEHA necessary to induce hepatic tumours only in mice (1715-3570 mg/kg b.w.) and those inducing hepatic peroxisomal activity ([illegible] mg/kg b.w.) as well as the evidence for a threshold for inducing hepatic peroxisomal activity enables the Committee to conclude that the very small intakes of DEHA from its use in food contact materials would not pose any carcinogenic hazard for man.

Foetotoxicity occurred in a teratogenicity study in rats at [illegible] mg/kg b.w. The NOEL was 30 mg/kg b.w. The Committee therefore used a safety factor of 100 to establish a TDI for DEHA of 0.3 mg/kg b.w.

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OPINION ON 3-MONOCHLORO-PROPANE-1,2-DIOL (3-MCPD)

EXPRESSED ON 16 DECEMBER 1994

1. Background

Chemical hydrolysates of vegetable proteins are commonly used as seasonings to improve the flavour of certain foods. Hydrolysis is usually achieved by treatment of crude proteins with hydrochloric acid. During this process, hydrochloric acid also interacts with glycerol released from vegetable fat yielding a number of chlorinated products, including 3-monochloro-propane-1,2-diol (3-MCPD).

3-MCPD is not the sole chloropropanol formed during the technological process currently used to produce hydrolysed vegetable protein. At its 62nd meeting (15 April 1988) the Committee examined 1,3-dichloro-2-propanol (DCP). Long-term toxicity/carcinogenicity studies in rats indicated a carcinogenic potential for this substance.

At its 74th meeting (28/29 June 1990) the Committee evaluated the contamination of hydrolysed plant proteins with 3-MCPD.

At that time, only limited information from a range of studies on 3-MCPD was available for assessment, including data on metabolism, acute toxicity, short-term and long-term toxicity, reproductive toxicity and genotoxicity. Although certain metabolites of 3-MCPD were identified, the metabolism of 3-MCPD was not yet fully understood. The data lacked well described toxicokinetic and mass balance studies. The available information, however, suggested that 3-MCPD was rapidly absorbed and circulated, crossed biological barriers and was distributed into many tissues and organs. No information on short-term toxicity in non rodent species was available. Chronic toxicity and carcinogenicity data on 3-MCPD were scarce. Reproduction studies focused mainly on the specific adverse effects of 3-MCPD on male fertility. The teratogenic potential of 3-MCPD had not been investigated. The results of special studies on genotoxicity remained inconclusive, although the substance was genotoxic in certain *in vitro* test systems.

It was not possible to establish an exposure limit for human consumers of 3-MCPD on the basis of this information. However, the Committee took into account that 3-MCPD was nephrotoxic, possessed an inhibitory effect on male fertility and showed genotoxic activity in some *in vitro* assays.

The Committee concluded that

> Based upon available analytical data the Committee recognised that the contents of 3-chloro-1,2-propanediol[1] (monochloro-propane-diol, MCPD) are highly variable among different hydrolysed vegetable protein preparations.

[1] 3-monochloro-propane-1,2-diol or 3-MCPD

Due to the present knowledge of toxicity of the compound and the information from the industry on present technology allowing contents below 10 mg MCPD per kg hydrolysed vegetable protein, the Committee is of the opinion that products with a higher content than this should be considered not acceptable."

In the meantime, a carcinogenicity study in rats has been completed. In this study, groups of 50 male and 50 female Fisher F 344 rats were exposed to drinking water containing 3-MCPD dissolved in tap water for 104 weeks. Average daily intakes of 1.1, 5.2, and 28.3 mg 3-MCPD/kg body weight for the males, and 1.4, 7.0 and 35.3 for the females were calculated on the basis of body weights, liquid consumption and drinking water analyses.

Treatment with 3-MCPD was accompanied by a dose-dependent increase in the incidence of tumours in the kidneys of animals of both sexes and in the testes, mammary and preputial glands of males. These results led to reconsideration of the above recommendation.

It had been argued that the kidney tumours were secondary to sustained organ toxicity. Furthermore, hormonal disturbances had been suggested to explain the appearance of tumours in the testes and mammary glands. However, no convincing experimental evidence was provided to support this assumption. It was also reported that attempts had been made to estimate the virtually safe dose of 3-MCPD by using log-probit extrapolation methods. However, the inaccurate dosing might render any attempt to quantitatively extrapolate the carcinogenicity data meaningless. The Committee is concerned by the large uncertainty of the dosing levels of the test substance during this study. As shown by the figures quoted in the footnote[2], within-dose group variations of up to a factor of eight were reported for the 3-MCPD concentration.

2. Conclusion

The Committee noted that several of the above described gaps of information had not been filled since it's first evaluation of 3-MCPD. However, based on the results of the recent carcinogenicity study and other available data, the Committee is now of the opinion that there is sufficient evidence for carcinogenicity of 3-MCPD in rats. In addition, 3-MCPD is mutagenic in the Ames test causing base pair substitutions in S. typh. TA 1535, positive in the mouse lymphoma TK locus assay, and possibly the Chinese hamster V79 HPRT test *in vitro*, and causes sister chromatid exchanges in V79 cells. In view of the clearly demonstrated *in vitro* genotoxicity and the tumorigenic effect in rats, 3-MCPD must be regarded as a genotoxic carcinogen. It also causes malignant transformations in mouse fibroblasts. While the role of nephrotoxicity and hormonally mediated effects of 3-MCPD cannot be

[2] Results of drinking water analyses indicated that the concentrations administered ranged between 0 and 22 ppm (control group), 0 and 89 ppm (low dose group), 58 and 495 ppm (intermediate dose group) and 241 and 626 ppm (high-dose group). The report states that the limit of detection of the analytical method applied was in the order of 10 ppm.

ruled out, conclusive evidence for the significance of such (secondary) hormonal mechanisms has not been provided. A safe threshold dose cannot be determined and 3-MCPD should be considered as an undesirable contaminant in food. Therefore residues of 3-MCPD in food products should be undetectable by the most sensitive analytical method[3].

Given the level of usage of hydrolysed plant proteins and the difficulty of determining 3-MCPD at the lower concentrations encountered in food products containing them, the Committee recommends that the presence of this substance be routinely controlled by analysis of the hydrolysed plant proteins themselves and that all efforts should be undertaken to develop technological methods leading to products not containing chlorinated propanols.

In view of the importance of controlling the presence of the substance in imported foods, the Committee further recommends that efforts be made to develop analytical methods allowing detection of 3-MCPD in finished foodstuffs at the levels likely to be encountered from use of contaminated hydrolysed plant protein.

[3] The Committee notes that, at present, methods using gas chromatography and electron capture detection can achieve a limit of detection of 0.05 mg/kg in liquid hydrolysed plant proteins containing 40% dry matter.

OPINION ON POLYETHYLENEGLYCOL 6000 (PEG 6000)

EXPRESSED ON 16 DECEMBER 1994

1. Terms of reference

The Committee has been entrusted with the task of carrying out an evaluation of the use of polyethyleneglycol 6000 (PEG 6000) in preparations for sweetener-based sodas.

2. Background and discussion

These preparations, which are supplied in the form of tablets notably containing around 3% of PEG 6000, are designed to produce when dissolved in water a soda-type beverage. In principle, their use is limited and they are not for the consumption of young children.

PEG 6000 is an excipient defined in the European pharmacopoeia, under the name of macrogol 6000, as a mixture of polymers with the general formula $H(OCH_2-CH_2)_nOH$ corresponding approximately to a molecular weight of 6000. The European pharmacopoeia stipulates the purity criteria with which this mixture of polymers must comply (1).

Most of the studies which have been carried out in this field are relatively old, and there is little extant information available on PEG 6000. However, it is generally acknowledged that the gastrointestinal resorption of polyethyleneglycols and their toxicity are inversely proportionate to their molecular weight (2).

PEG 4000, a polyethyleneglycol with a lower molecular weight than that of PEG 6000, has been administered for 12 months to a dog without any occurrence of side effects. PEG 4000 is used in the clinical field as a non absorbable marker. A 90 day study on the rat has revealed the innocuousness of PEG 6000 which, moreover, is used without any known toxic effects as an excipient in certain pharmaceutical preparations (3).

3. Conclusion

Taking into account its low resorption, the absence of known toxic manifestations and the limited exposure which could result from the recommended use, PEG 6000 (polyethyleneglycol 6000), as defined in the European pharmacopoeia, may be considered as acceptable for the limited requested use, i.e. as an excipient of sweetener-based tablets for the preparation of sodas.

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OPINION ON THE FOOD SAFETY IMPLICATIONS OF SURVEILLANCE SYSTEMS EMPLOYING NEUTRON SCANNING (INTERROGATION) DEVICES

EXPRESSED ON 3 MARCH 1995

1. Terms of reference

The SCF has been requested to consider whether the use of neutron scanning devices, and pulsed fast neutron analysis, (PFNA) in particular, would cause foodstuffs exposed to this type of interrogative irradiation to become a hazard to the health of the consumer.

2. Background

Cargo surveying systems presently used for baggage, luggage and freight inspection at airports, where security assurance is necessary, hitherto employed ionising radiation, commonly in the form of X-rays or of thermal neutron activation (TNA) systems, operating at low radiation doses or low mean neutron energies. UK regulations recognised the established safe use of such devices providing they impart a maximum radiation dose not greater than 0.5 Gy to the inspected material. The more recent need for non-invasive detection procedures for explosives or other contraband in large containers, heavy goods trucks and railway cars necessitates the employment of more powerful radiation interrogation systems. Because these large objects may also contain foods in transport, reassurance is now needed that such novel scanning procedures do not produce in the exposed foodstuffs any radiolytic products and induced radioactivity potentially deleterious to the consumer as well as the foodstuffs. The major concerns relate to the levels of induced radioactivity, the radiochemical changes, the likelihood of creating toxic effects, and the possible nutritional and organoleptic changes in the exposed food. The Committee was provided with a report on neutron-induced activation of food (1), a report on food safety effects of inspection by SAIC pulsed fast neutron analysis explosive detection system (2), and a paper on the irradiation of food and the induction of radioactivity (3) as background information.

3. Discussion

TNA systems use slow neutrons at mean energies much below 5 MeV and impart radiation doses well below 0.5 Gy, the present UK limit for exemption from irradiation control regulations, to the material inspected. X-ray scanning devices are limited to operate at a maximum energy of 10 MeV and at maximum dose levels of [illegible]. The PFNA systems have a far better penetrating power but require low

intensity neutron beams of high-energy neutrons, around 8 MeV, with an incident fast neutron flux of 5.0×10^5 neutrons $cm^{-2}s^{-1}$. In the worst case, a 5 minute directed search of a suspicious cargo, thus would impart to any foodstuff present a radiation dose of about 0.0063 Gy (i.e. less than 0.01 Gy)[*].

The physical basis for the PFNA is the stimulation by fast high-energy neutrons of the emission of gamma-rays characteristic of the different chemical elements in the irradiated target. These gamma-rays can be detected and thus identify the elements present in the target. Particular attention is given to the gamma-ray emissions from O, C and N atoms, because these elements, usually present in organic explosives at characteristic concentrations, yield distinct distribution patterns easily distinguishable from the patterns produced by foodstuffs. The scanning process involves the vertical sweeping by a collimated neutron beam, 20 cm in transverse diameter, of a horizontally moving container. The emitted gamma-rays are registered by an array of detectors linked to an appropriate computer system and provide a two-dimensional location of the elements emitting gamma-rays. Exact determination of the time of detection then gives information on the third spatial dimension along the direction of the neutron beam within the interrogated target. The resulting three-dimensional element distribution picture then indicates the presence and position of the suspected explosives mass within the scanned container. A normal fast scan of a container results in the neutron beam impinging on any spatial position for a maximum of 1 second. For directed search studies of suspicious locations from 10 s to a maximum of 5 min may be needed. Not more than 1% of a container is expected to undergo directed search.

The radiation effects of neutrons differ from those of ionising radiation, which latter removes electrons from irradiated atoms. Neutrons undergo elastic collisions with atomic nuclei, thereby being slowed and imparting recoil energy to the colliding atomic nuclei, which is then dissipated by interaction with electrons to yield radiochemical reactions products. Most neutrons thermalise (reach thermal equilibrium) in hydrogen-rich media by repeated collisions with protons. In other media neutrons are also captured by the atomic nuclei and then undergo nuclear reactions. Slow thermal neutrons are more effective in undergoing reactions with atomic nuclei than fast neutrons, particularly if neutron capture is involved. Neutron capture increases the atomic weight by 1. Fast neutrons tend to alter the atomic number of the colliding nucleus, thus creating a different chemical element. Neutrons therefore have a greater propensity to induce radioactivity in the scanned material and do not form any significant amounts of radiolytic products. The probabilities for the occurrence of the various nuclear reactions are tabulated extensively in the literature as nuclear cross sections for neutrons of different energies.

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[*] This dose is calculated from the effective equivalent dose of 21 mrem received by a man exposed for 1 s to a beam of fast high-energy neutrons at 8 MeV and an incident fast neutron flux of 5.0×10^5 neutrons $cm^{-2}s^{-1}$. As 1 mrem = 0.01 mSv, a 5 minute (300 s) exposure equals 21x300x0.01 = 0.63 mSv = 0.063 Sv. For irradiation by neutrons a dose of 10 Sv = 1Gy, hence 0.063 Sv = 0.063×10^{-1} = 0.0063 Gy. Effective equivalent doses are measured in Sv (Sieverts) and represent the biological effectiveness of the radiation. They are calculated from the formula GyxF, where F depends on the type of radiation, being 1 for X-rays, β rays, hard gamma rays, 20 for alpha-rays and 2-10 for neutron-rays.

The induced radioactivities for a large number of significant elements in food and the resultant effective equivalent doses to consumers of such neutron-interrogated food can be calculated, assuming a maximum absorbed dose of 0.5 Gy from irradiation with neutrons of energies 1, 2, 5, 8 and 14 MeV, and also assuming delay times in measuring the radioactivity of 5 min, 1 hr, 8 hrs, 1 day, 1 week and 1 month. The physical characteristics of the scanning neutron beam are not important for assessing the health risk to the consumer but the levels of radioactivity induced in the neutron-irradiated food and the effective equivalent radiation dose received when such food is ingested.

In order to calculate these parameters several paradigms were proposed. Apart from 17 specific foods, a theoretical food (mccf) containing the maximum credible concentrations of 81 key elements, and a theoretical reference food (rf), composed of 47 elements in concentrations based on their daily balance in the diet consumed by a "reference" man, were considered. Three configurations of containers were also considered: a semi-infinite slab, 1 kg food contained in a 20 kg luggage, and a 2 m high pallet of food. Using standard nuclear physics programmes and information in nuclear data banks the resulting neutron transport spectra and radioisotope inventories were calculated for these 19 foods considered exposed to neutron interrogation.

Although gross radioactivity is generally easier to measure than the amounts of individual radionuclides, this is a less important parameter in determining the radiological significance of the induced activities than the radioactive half-life and the radiation dose/unit activity intake of the various radionuclides present in the food.

Experimental methods to detect the additional induced radioactivity would require the application of much higher nuclear irradiation doses than is proposed in the PFNA screening procedure. Moreover, at the proposed maximum PFNA irradiation dose level of 0.01 Gy it is virtually impossible and extremely difficult to measure either the induced additional radioactivity or any radiolytic products by current analytical techniques or long-lived radicals by ESR. Therefore any changes in food exposed to neutron interrogation will be essentially undetectable and thus negligible.

The only way to obtain actual values of the above mentioned parameters for the assessment of the radiological risk from exposure by the ingestion of PFNA-screened food is the use of the well established, verified and routinely applied calculational methods discussed in the background information supplied to the SCF.

Under the conditions of assuming the worst scenario described above and an irradiation dose of 0.5 Gy, the calculations show that the radioactivity of the theoretical (mccf) food does not fall below the 0.4 Bq g^{-1} exemption limit for control of radioactive materials under the 1960 UK regulations even after a post-scanning delay of 1 month. This is largely due to its high K-content and the correspondingly high naturally occurring radioactivity. The activity of the theoretical rf food falls below the 0.4 Bq g^{-1} exemption limit in less than 1 week. The activity levels of the 17 specified foods vary after 1 week post-scanning from almost the mccf value to several orders of magnitude less depending on the elemental composition, and are generally below the 0.4 Bq g^{-1} exemption limit.

In estimating the effective equivalent dose to members of the public from exposure through ingestion of neutron-scanned food comparison should also be made with the international limit (ICPR) of 1 mSv per year averaged over a lifetime or with the more recent recommendation (NRPB) of a dose constraint, applied to a single source, of 0.3 mSv per year or with the advice (ACINF-UK) that irradiated foodstuffs with an activity content of less than 0.4 Bq g^{-1} giving rise to individual exposures of less than 5 µSv (0.005 mSv) per year should not be of concern to regulatory authorities. Furthermore, it has to be remembered that there will be always some delay between irradiation and consumption with a realistic minimum of 5 minutes. Also such scanned food represents only a small fraction of the daily diet.

Assuming prompt consumption of 1 kg of 5 minute-cooled neutron-scanned mccf food consumers would be subject to an exposure dose of 100 µSv/year, after 1 day-cooling it would be about 20 µSv/year and after 1 month-coolingabout 10 µSv/year. The no-regulatory-concern dose of 5 µSv/year would be reached by ingestion of 50 g of 5 minute-cooled or 500 g of 1 month-cooled mccf food.

Assuming prompt consumption of 1 kg of 5 minute-cooled neutron-scanned rf food consumers would be subject to an exposure dose of 2-5 µSv/year depending on the MeV of the scanning beam. After 1 day-cooling the exposure dose would be about 0.9 µSv/year and after 1 month-cooling about 0.2 µSv/year.

For the 17 specific foods a wide range of results arises but for most foods, after likely delays before consumption, exposure doses following ingestion of 1 kg neutron scanned food would be below 1 µSv/year. Doses above 5µSv/year could only occur with very few foods with a high initial Na, Cl, P, K, O, N and S content and which have been regularly neutron-scanned and marketed consistently to regular consumers.

The natural radioactivity levels in the diet are about 0.1 Bq g^{-1} and thus lower than those in foods immediately after neutron scanning. However the annual individual exposure doses arising from consumption of unirradiated food are of the order of 300 µSv, so that the contribution of less than 5 µSv/year from the likely small percentage in the total diet of neutron-scanned food is negligible. It should be noted that the total individual exposure doses due to natural background radioactivity are of the order of 2000 µSv (200 mrem) per year.

4. Conclusions

On the basis of the above information the SCF has concluded, that surveillance devices using neutron scanning, in particular PFNA systems operating at up to 14 MeV, which during interrogation do not impart to foodstuffs radiation doses greater than 0.01 Gy, raise no safety concerns with regard to the negligible induced additional radioactivity, radiolytic products, their toxicity, nutritional and organoleptic properties of the neutron interrogated foodstuffs. The estimated maximum effective equivalent radiation dose to the individual consumer would amount to less than 5 µSv from natural background radiation. However, in order to allay any consumer concerns it would be desirable to confirm the theoretically calculated induced radioactivity levels by testing food from a container which has been subjected to neutron scanning by a PFNA system for any induced radioactivity. A procedure should also be developed, which ensures that the same food items are not exposed repeatedly to neutron scanning, as this opinion of the committee applies only to foods which have been subjected to a single exposure of PFNA scanning.

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OPINION ON *ISO*-ASCORBIC ACID

EXPRESSED ON 3 MARCH 1995

1. Terms of reference

To provide clarification of the opinion expressed on iso-ascorbic acid (erythorbic acid) on 19 October 1990 (Scientific Committee for Food, Twenty-sixth Series of Reports).

2. Background

The Scientific Committee for Food has evaluated the safety in use of iso-ascorbic acid on two previous occasions. In its opinion of September 1984, the Committee concluded that the data then available were inadequate for full toxicological evaluation of the substance and, further, that the possibility of competitive interference with the absorption and distribution of ascorbic acid might be of significance for individuals with borderline intakes of this vitamin. On that occasion, the Committee was of the opinion that the use of iso-ascorbic acid in food and drink was not acceptable and did not establish an ADI (Scientific Committee for Food, Twenty-second Series of Reports). Subsequently, in its opinion of 19 October 1990, on the basis of the results of a long term study in rats and the satisfactory agreement of those findings with reported human nutritional experience, the Committee established an ADI of 6 mg/kg bw (Scientific Committee for Food, Twenty-sixth Series of Reports). The rationale for that conclusion was not presented *in extenso* in its published report.

In response to the request from the Commission to clarify its opinion on iso-ascorbic acid, the Committee sets out in the following text the reasoning supporting its conclusions of 19 October 1990 and also takes the opportunity to review its conclusions in the light of the recently adopted European Parliament and Council Directive 95/2/EC concerning food additives other than colours and sweeteners which establishes for the first time at European Union level provision for the use of iso-ascorbic acid in food.

3. Discussion and conclusions

Rats, mice, hamsters and rabbits lack the active carrier mediated absorption mechanism present in guineapigs and man. Intestinal absorption of iso-ascorbic acid has been shown to be less efficient in rodents compared to guineapigs and man. Retention of iso-ascorbic acid in the tissues and reabsorption in the kidneys is also poor, thus leading to its rapid elimination in the urine. Iso-ascorbic acid is therefore regarded as having low antiscorbutic activity. The evidence regarding iso-ascorbic acid interference with the ascorbic acid tissue stores or absorption is somewhat contradictory. Normal dietary intake from the presently suggested uses are not likely to lead to any significant deficiency in ascorbic acid. Only concentrations of iso-ascorbic acid at least an order of magnitude higher than those of ascorbic acid are likely to interfere significantly with ascorbic acid uptake. Human studies showed that daily doses of 600 mg had no adverse effects on ascorbic acid depletion in volunteers depleted of ascorbic acid.

The metabolism of iso-ascorbic acid has not been studied in detail but appears, at least qualitatively to be similar to that of ascorbic acid. Although formal multigeneration reproduction studies have not been carried out, other studies show that at least male reproductive function is not affected by orally administered iso-ascorbic acid. Iso-ascorbic acid is neither teratogenic nor genotoxic.

Despite the comparatively poor absorption of ingested iso-ascorbic acid by rodents, the doses employed in the available life-span studies in mice (Inai et al., 1989) are likely to have resulted in plasma levels of iso-ascorbic acid similar to those found in human volunteers given comparatively excessive doses for a prolonged period. In a 7 months study in mice, a dose of 15 g/kg b.w. gave plasma levels of 25 mg/l (Tsao & Salimi, 1983). In the life-span study in mice, no plasma levels were measured but the dose used was 3.3-7 g/kg b.w. In a study in human female volunteers, a dose of 10 mg/kg b.w. gave plasma levels of 3.5 mg/l. Normal dietary intakes, based on present technological uses, are around 0.13 mg/kg b.w. In the life-span rat study used to set the ADI doses of 0.625-1.25 g/kg b.w. were given but no plasma levels were measured. Therefore, at least one rodent study probably achieved adequate blood levels. Although the available life-span studies in mice and rats are inadequate in several respects in the light of modern requirements they did not produce any evidence for carcinogenicity or specific chronic toxic effects.

Overall, despite some deficiencies in the database, the Committee considered the use of iso-ascorbic acid to be acceptable because (i) although the metabolism of iso-ascorbic acid is not identical to ascorbic acid, it is similar, (ii) although rodents are less efficient at absorbing iso-ascorbic acid, there is some absorption in rats and mice (the species used in the toxicity studies) and (iii) no adverse effects have been reported from human exposure to date. As the NEL in the rat study was found to be lower than the minimal effect dose level in the mouse study, the ADI, previously established on the basis of the rat study, was confirmed.

ADI for man: 6 mg/kg b.w.

Current uses listed in the Directive (95/2/EC) are likely to result in dietary intakes well below the ADI. The Committee would like to be informed if new uses are being proposed which are likely to lead to intakes persistently exceeding the ADI.

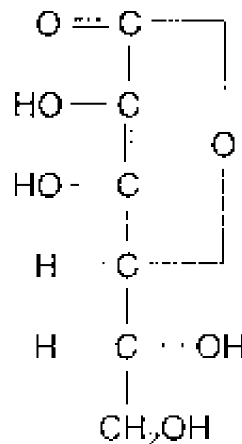
The data reviewed during the course of this evaluation are summarised in the annex to this text.

ANNEX

D-ISOASCORBIC (ERYTHORBIC) ACID AND ITS SODIUM, POTASSIUM AND CALCIUM SALTS

Background

IAA has the structural formula:



It is manufactured from Ca-2-keto-d-gluconate by a multistep process. The commercial product is crystalline IAA or Na IAA and is cheaper than ascorbic acid (AA).

It functions technologically as:

a) antioxidant to stabilise colour and flavour in fresh, frozen and canned fruits, to stabilise betacyanins, to prevent off-flavours in citrus flavoured carbonated drinks, beer, minced-fish

b) accelerator of nitrite cure for meats, for better shelf life (colour, flavour), reduction in residual nitrite, inhibition of nitrosamine formation.

Use levels:	fruit juices	80-200 mg/kg
	canned, frozen fruits	250-450 mg/kg
	beer	20-40 mg/l
	cured meats	200-500 mg/kg

IAA and Na IAA are permitted food additives in USA, Canada, Japan, Australia, New Zealand, Eastern Europe and some South American states either in fruit juices, fruits or cured meat products. In the EU their use is prohibited by the Antioxidants Directive and the Antioxidants in Food Regulations. IAA is also used in certain wines which cannot therefore be imported into the European Community.

A simple, quick and reliable analytical method distinguishes between AA and IAA (Hofman, B. 1980).

IAA and Na-IAA were evaluated by JECFA in 1961, 1973 and 1990. In the earlier evaluation an ADI of 5 mg/kg b.w. was established by that committee on the basis of a long-term study in rats (WHO, 1974) but this was changed to an ADI "not specified" (WHO, 1991) on the basis of new long-term toxicity and carcinogenicity studies in rats and mice as well as studies on the metabolic and nutritional interactions of IAA with AA including human studies.

Additional information has become available since that time which permits a re-appraisal of these substances.

The SCF evaluated IAA in 1990 and 1992 and established an ADI of 6 mg/kg b.w. (SCF 1990 and 1992).

Biological data

Biochemical aspects

Absorption, metabolism, distribution, excretion:

Many studies have been carried out in guineapigs which lack L-gulono-lactono-oxidase and cannot synthesise AA similar to man and the primates. Rats, hamsters and rabbits synthesise AA and do not appear to have an active transport mechanism for AA but absorb it and IAA by passive diffusion. Absorption of IAA from the gastrointestinal tract in man and guineapigs proceeds readily though less efficiently by the same active transport mechanism as for AA (Gould B.S.,1948; FASEB Report 1979; Hughes, R.E. & Jones, P.R. 1970). Comparison of plasma levels of IAA following prolonged administration orally to mice (Tsao, C.S. & Salimi, S.L. 1983) with the study in human volunteers (Sauberlich, H.E. et al, 1989) demonstrates similar plasma levels despite an order of magnitude lower intake in the human volunteers. Possibly the active carrier-mediated mechanism in man causes greater gut absorption and/or tubular reabsorption. The transport mechanism is located in the brush border membrane vesicles (Toggenburger, G. et al, 1979). Transport of L-AA and D-IAA is Na^{+}-dependent, electro-neutral and saturable. It is competitive and therefore potentially able to reduce AA uptake from the intestine, although IAA is a poorer substrate (Siliprandi, L. et al, 1979). Intact gut segments show a 16% decrease in AA influx if IAA is present at 10 times the AA concentration (Mellors, A.J. et al, 1977). However simultaneous oral administration of 1-C^{14}-IAA and 6-H^{3}-AA to guineapigs resulted in similar specific activities in the portal blood for 3.5 hours (Hornig, D. 1977). The metabolism of IAA has been little investigated. It probably follows the same pathways as AA. Rats fed IAA excreted dehydro-IAA (Fukushima, S. et al 1986) but no IAA-2-sulphate (Baker, E.M. et al 1973) in the urine. The final metabolites of IAA are probably CO^{2}, oxalic acid and C4- and C5-derivatives.

1-C^{14}-labelled IAA administered orally or i.p. to guineapigs was almost completely cleared from the body within 24 h. After i.p. administration over 75% appeared in the urine, 20% was exhaled, 3% was in the faeces. After oral administration some 54% was exhaled, 30% appeared in the urine and 4% in the faeces. Trace amounts (less than 1%) were found in the organs, chiefly liver, lung and kidneys (Hornig, D. 1975). Guineapigs given 40 mg/day for 2 months excreted at that time 1.9% of the ingested dose in their urine (Pelletier, O. 1969).

Dogs given 5 g Na-IAA excreted 19% within 24 h (Rivers, J.M. et al 1963). Rats excreted in their urine 10 times more C^{14}-labelled IAA compared to AA (Baker, E.M. et al 1973). However rats given 5% IAA in their diet only excreted twice as much as those given AA, only a 10% of the excreted material was unchanged IAA (Fukushima, S. et al 1984).

In human subjects an oral dose of 300 mg IAA did not affect the urinary AA level (Kadin, H. 1959). Loading tests on 6 volunteers with 165 or 300 mg IAA showed similar blood levels over 3 hours to those obtained with AA. Balance studies showed that 50-70% of the IAA test load compared to 35% of AA were excreted within 24 hours Excretion of IAA was more rapid and more complete suggesting little renal tubular resorption and no displacement of AA (Wang, M.M. 1962). Four males, partially depleted of AA were then given 50 mg IAA daily for 2 weeks, followed by 100 mg daily for 2 weeks. While all AA concentrations continued to fall throughout the 4 weeks, the urinary excretion of IAA increased considerably. A loading dose of 300 mg did not raise the white cell AA level, 50-60% of the load appearing in the urine. The fate of the retained portion is not known nor the function of the dehydro-IAA. White cell uptake appeared to be stereospecific (Rivers, J.M., 1963).

Tissues abstract IAA easily from the blood and reach 60-79% of the AA level but retain IAA less strongly (Hughes, R.E. et al, 1969; Hughes, R.E. et al, 1970). Oral graded doses of either AA or IAA, given to guineapigs over 16 days, produced detectable tissue levels of IAA only with doses of 20 or 100 mg/day. Less IAA was retained in the tissues compared to equivalent AA loading (Suzuki, E., et al 1988). IAA administered orally simultaneously with AA impaired AA uptake by guineapig tissues at doses of 200 mg/kg and above, thus reducing AA storage and bioavailability up to 50% in some organs (Hornig D. et al, 1974).

Transfer of IAA across membranes into the aqueous humour of the guineapig eye uses the same saturable transport mechanism as AA, although the affinity of IAA is smaller (Linner, E. et al, 1969). The transport mechanism for crossing the blood/brain barrier is similarly stereospecific, IAA having a lower affinity for the carrier than AA (Spector, R. et al, 1974). *In vitro* IAA also inhibits the accumulation of C^{14}-labelled AA in the isolated rabbit choroid plexus (Shibata, M.A. et al., 1989). Similar competitive inhibition of AA transport by IAA but with lower carrier affinity was noted for guineapig and rabbit ciliary bodies (Becker, B. 1967), cat retinal pigment cells (Khatami, M. et al, 1986), human placental microvillous membrane vesicles (Iioka, H. et al 1987) and rabbit ciliary epithelium (Chu, T.C., 1988). When tissue concentrations were examined after 9 days loading with IAA or AA, followed by 4 days depletion diet, the final tissue levels of IAA were much below those of AA despite higher doses of IAA. Thus the turnover rate of IAA was 4-5 times higher, the half-life correspondingly 4-5 times shorter (Pelletier, O. 1969).

IAA is rapidly eliminated in the urine and thus less toxic for man than AA (Gould B.S. 1948). Renal tubular reabsorption of IAA from the glomerular filtrate probably uses the same stereospecific, energy requiring transport system as for AA but is less efficient. IAA inhibits *in vitro* AA uptake into kidney cortex vesicles. IAA can therefore compete with and reduce AA reabsorption (Toggenburger, G. et al 1979). However dogs given a single i.v. dose of AA or IAA showed nearly identical plasma half-lives indicating that elimination and reabsorption had the same rate rather than being better for AA ([illegible] R. et al [illegible]). Daily oral doses of [illegible] mg IAA in adult women gave the same steady state plasma concentration as [illegible] mg AA (Sauberlich, H.E. et al [illegible]).

Vitamin C activity

In vivo IAA has from 1/20th to 1/40th of the antiscorbutic activity of AA. In one study, however, no such activity was seen (Reiff, J.S. et al 1956). Guineapigs on a scorbutogenic diet survived for 100 days on supplementary 10 mg IAA/day though weight gain was slightly reduced. Supplementary 1-2 mg AA/day produced full growth (Fabianek, J. et al 1967). Continuous dosage in drinking water or large daily doses adequately relieved or protected against scurvy. Single low oral doses showed little antiscorbutic effect because of the less efficient absorption of IAA in the gut and poor tissue storage (Hughes, R.E. et al 1970). AA-depleted guineapigs received 2 mg AA or 40 mg IAA daily for 2 months with full restoration of growth. Tissue levels of IAA were only about 1/2 of those of AA. This reduction in physiological activity *in vivo* may be apparent rather than real because of the less efficient uptake and faster urinary loss (Pelletier, O. et al 1969). Guineapigs on scorbutogenic diet kept well on 0.1% IAA in drinking water. IAA-pretreated animals were depleted twice as fast as AA-pretreated animals (Hughes, R.E. 1969; Hughes, R.E. 1971). The relative potencies of IAA and AA were compared in female guineapigs after a 6-day depletion diet. 100 mg IAA/day, 10 mg AA/day, 100 mg IAA + 0.5 mg AA/day or 100 mg IAA + 5 mg AA/day were then given for 9 weeks. Growth, SAP levels, collagen formation after wounding and tooth structure were normal. The effect of IAA was additive to subminimal AA levels and there was no competitive inhibition (Goldman, H.M. et al 1981). *In vivo* CCl_4-induced lipid peroxidation, as shown by pentane and ethane exhalation, was inhibited equally for 4 hours in deficient guineapigs by i.p. administered 75 mg IAA or AA as antioxidants (Kunert, K.J. et al 1983). IAA and AA were equally potent cofactors *in vitro* in promoting proline hydroxylation in cell free systems (Kurata, T. et al 1987; Kutnink, M.A. 1969). IAA and AA had the same effects on hepatic microsomal UDP-glucuronyltransferase (Neumann, C.M. et al 1988). To achieve equal potency in stimulating collagen synthesis in incubated intact cells required 8-10 times higher concentrations of IAA at low concentrations but equal concentrations of IAA and AA at 250 µM (Murad, S. et al 1981).

Interaction of IAA and AA

The possibility of an anti-vitamin C effect due to competition in the transport mechanism was examined in guineapigs, mice and human volunteers. Tissue concentrations in guineapigs receiving a semipurified diet plus either 0.1% AA or 0.1% AA + 2% IAA for 1 week showed deposition of IAA in organs with replacement of 1/2 of the AA. If followed by a treatment period with AA alone there was rapid loss of IAA (Pelletier, O. 1969). The effect of IAA on the turnover rate of 1-C^{14}-labelled AA, given as pretreatment to guineapigs on either scorbutic diet or with additional 0.1% AA or 2% IAA showed that the disappearance of labelled AA from brain, liver, heart, kidney, adrenals and spleen was accelerated more by IAA than AA for 6 days and the reverse for a further 4 days. IAA therefore increases AA turnover and does not spare AA (Pelletier, O. 1969). Guineapigs were given daily doses of 0, 20, 50, 100 or 400 mg IAA + 20 mg AA/day for 3 days and then loaded with oral single doses of 1-C^{14}-labelled AA. Tissue distribution after 6 hours showed a decreased C^{14} retention the higher the IAA dose (50 mg and higher) (Hornig, D. 1975). As the absorption rates of IAA and AA by guineapig gut are similar without competitive inhibition the reduction in tissue-AA could be due to faster metabolism and renal elimination. Using single oral doses of 1-C^{14}-labelled AA in guineapigs on deficient diet for 12 days and then for 16 days either 5 mg AA/kg b.w., 50 mg IAA/kg b.w. and 50 mg IAA + 5 mg AA/kg b.w. produced body weight depression in animals given IAA + AA or IAA alone. Respiratory C^{14}-CO_2 rose from 43% (AA) to 48% (IAA+AA) to 50% (IAA); urinary and faecal excretion were similar in the 3 groups. Hence IAA accelerated AA metabolism with a reduced half-life from 97 to 50 h. The body pool of AA was reduced by 30% by IAA + AA (Hornig, D. 1976)

IAA also stimulated hepatic decarboxylase (Hornig, D. 1977). Measuring tissue levels of AA and IAA by HPLC in guineapigs given either 0 or 5 mg AA/day, 100 mg IAA/day or 100 mg IAA + 5 mg AA/day showed lower tissue contents in animals given the mixture. Hepatic AA of IAA animals was the same as in the deficient animals and the disappearance rate of AA from tissues of IAA animals was the same as in deficient animals. Hence no increase in catabolism was noted but interference with uptake or alternatively there was an accelerated degradation of newly absorbed AA only (Arakawa, N. 1986).

Guineapigs were given a diet with 5 or 1 mg AA/day plus 0, 1, 5, 20 or 100 mg IAA/day. 100 mg IAA reduced tissue levels of the 5 mg AA/day animals but not of the 1 mg AA/day animals (Suzuki, E. 1988). 6 groups of guineapigs were given supplements of 1 mg AA + 1 mg IAA, 1 mg AA + 20 mg IAA, 5 mg AA + 1 or 5 or 20 or 100 mg IAA for 16 days. Body weights were similar in all groups. Liver AA of the 5 + 100 group was 50% of that of the 5 group. The activities of hepatic aniline hydroxylase, acid phosphatese, SAP, cytochrome P-450 were unaffected. At 1 mg AA the hepatic acid phosphatase and aniline hydroxylase activity were lower and restored in the 1+20 group (Suzuki, E. 1988). Groups of 4 male guineapigs were given 5 mg AA/day, 100 mg IAA/day or 100 mg IAA + 5 mg AA/day for up to 30 days, the control group was kept on deficient diet for 16 days. Hepatic aniline hydroxylase, cytochrome P-450, acid phosphatase, SAP activities were better in the supplemented groups but there was no difference between the 3 groups. Subsequently 3 depleted groups were given 5 mg AA/day, 100 mg IAA/day or 100 mg XAA + 5 mg AA/day for 20 days. All enzyme activities recovered similarly (Suzuki, E. 1989).

Groups of 4 female Swiss Webster mice were fed 5% IAA in the diet for 2 months followed by 10% IAA for 5 months. About 40% of endogenous hepatic AA and 31% of brain AA were replaced by IAA, thereby reducing the bioavailability of AA. Plasma levels remained unaffected. The body weight gain of IAA animals was reduced. Thus overall competitive replacement of AA occurred to a considerable degree (Tsao, C.S. et al 1983).

Male cynomolgus monkeys were kept for 8 weeks on a depletion diet and the repleated for 4 weeks with either 10 mg/kg b.w./day AA or 10 mg AA + 200 mg IAA/kg b.w./day. There was no difference in the blood ascorbate levels of the treated groups showing an absence of antagonistic action of IAA (Turnbull, J.D. et al 1979). 11 adult women were maintained for 21 days on a AA-free formula diet and then given gradually increasing AA supplements with or without 600 mg/day IAA. Some subjects became scorbutic and blood ascorbate was reduced markedly during depletion. Addition of 30 mg AA, 60 mg AA or 90 mg AA/day for 10 days was only effective in restoring blood AA levels when 90 mg were used. Addition of 600 mg IAA caused no adverse and perhaps a slight sparing effect (Sauberlich, H.E. 1989).

Oxalate formation

Conversion of AA to oxalate is [illegible] metabolic pathway. Reported values vary greatly but high intakes are mostly associated with increased urinary oxalate (S). Rats fed 25 g (~424 mg AA/kg b.w.) or [illegible] (~1960 mg AA/kg b.w.) ascorbyl palmitate/kg in the diet for 9 months were compared to a group given 1% IAA. 2 animals given ascorbyl palmitate had oxalate stones in the bladder (Harbaugh, [illegible]). Human volunteers given AA or IAA showed little degradation of IAA to oxalate. 90 mg/day AA increased urinary oxalate as much as 600 mg IAA/day (Sauberlich, H.E. 1989).

Effects on metal absorption

I.p. 1 g/kg b.w. of IAA or L-AA produced the same increase in mouse liver metallothioneines (56). Adult male volunteers given for 51 days a diet containing 200 g processed meat (uncured, nitrite cured, nitrite + 500 µg/g IAA cured sausage) showed no significant effects on the bioavailability and absorption of Fe, Zn, Cu, on serum Zn and serum Cu levels, plasma ferritin, transferrin or coeruloplasmin levels (Greger, J.L. 1984). Iron-deficient rats fed for 2 weeks on diets containing uncured meat or meat cured with 550 µg/g IAA, 156 µg/g nitrite or IAA + nitrite, showed no effect on iron absorption or incorporation into tissue (Lee, K. et al 1984; Lee, K. et al 1985; Mahoney, A.W. et al 1985). A similar test using constant Zn and Cu levels in the diets showed no effects on Zn and Cu utilisation, although high levels of AA in the diet of guineapigs, chicken and rats apparently depress Cu utilisation (Greger, J.L. 1985).

Other biological effects

2 groups of guineapigs were fed an AA-free diet with either skimmed milk or gluten as protein source for 10 days. One group received i.p. 800 mg/kg b.w. Na-IAA for 11 days, the other 5 mg AA/kg b.w. orally. No adverse effects or differences in body weight were seen. The adrenals had the highest IAA tissue level. Organ distribution of IAA was unaffected by the type of protein fed (Williams, H.S. 1972).

A group of 20 or more 2-days old suckling Sprague-Dawley rat pups were given by gavage for 10 or 25 days either saline, AA at various percentages of total milk solids consumed or IAA at 2% of total milk solids consumed. Lung collagen concentrations, lung weight and liver weight were unaffected. Rates of lung protein and elastin synthesis were lower by 25-30% in the AA and IAA treated pups. Pressure-volume curves were not affected. Lung tissue of AA-treated pups was less mature (Critchfield, J.W. 1988).

4 groups, each of 5 male guineapigs, were fed for 43 days diets containing 0.2% AA, 8.7% AA free acid, 8.7% mixed K, Na and Ca salts of AA or 8.7% mixed K, Na and Ca salts of IAA. 8.7% free AA acid but not the salts decreased bone density and urinary hydroxyproline. 8.7% free IAA did not cause bone demineralisation. No adverse effects and identical body weight gains to controls were noted (Bray, D. 1984).

Toxicological studies

Acute toxicity

Substance	LD_{50} (g/kg b.w.)		Reference
	mouse	rat	
isoascorbic acid	8.3	18.0	57
isoascorbyl palmitate	5.0	6.0	45

Short-term studies

Mouse:

5 groups, each of 6 weanling mice, received in their diet either 0, 2% 5% or 10% glucoascorbic acid, 10% AA or 5% IAA. Glucoascorbic acid caused severe scurvy with weight loss, diarrhoea and cutaneous haemorrhages at 5% and 10%, mild scurvy at 2% but normal growth and health were seen with 10% AA or 5% SAA (WHO 1974)

5 groups, each of 10 male and 10 female B6C3F1 mice aged 8 weeks, were given 0.625%, 1.25%, 2.5%, 5% and 10% Na-IAA in their drinking water for 10 weeks. 20 males and 20 females were the controls on distilled water. Males on 5% IAA gained less weight than controls, females on 5% IAA exceeded control weights. Histology showed marked atrophy of liver cells and lymphoid follicles of spleen (males at 5 and 10%, females at 10%) and hydropic degeneration of renal tubular epithelium at the same levels (Inai, K., 1989).

Rat:

2 groups, each of 10 male weanling Osborne-Mendel rats, were fed for 36 weeks 0 or 1% L-IAA in their diet. There were no significant differences from controls regarding growth, weight gain and mortality. Gross pathology and histopathology of lung, heart, liver, spleen, pancreas, stomach, small intestine, kidney, adrenal, testis and occasionally examination of colon, lymph node, bone, bone marrow, thyroid, parathyroid showed no specific lesions attributable to IAA (Fitzhugh, O.G. 1946). 5 groups, each of 10 male and 10 female F344 rats, were given 0.625%, 1.25%, 2.5%, 5% or 10% Na-IAA in their drinking water for 13 weeks. All rats on 10% refused to drink and died in 2-5 weeks. 3 males and 1 female on 5% died in 4 days. All other groups survived. 2.5% depressed body weight by 12% in males and 6% in females. No other parameters were reported (Abe, I et al 1984).

Long-term studies

Mouse

3 groups, each of 50 male B6C3F1 mice, were given 0, 1.25% or 2.5% Na-IAA in their drinking water. 3 groups, each of 50 female B6C3F1 mice, were fed 0, 2.5% or 5% in their diet for 96 weeks. Final sacrifice was at week 110 following 14 weeks recovery. Average body weights were similar to controls but final body weights were higher in survivors. Treated animals survived better. Tumour incidence at any site and time to tumour did not differ significantly from controls. The minimal effect level in male mice was 3.3 g/kg b.w. and in female mice 4 g/kg b.w.; reduced relative brain weight occurred at these doses. Hepatocellular adenomas and carcinomas were increased in male mice at 4.9 g/kg b.w. but the incidence in controls was unusually low and this effect disappeared after adjustment for survival. [illegible] toxic effects were not reported in detail (Inai, K., 1989).

Rat

2 groups, each of 10 rats (sex not stated), were fed diets containing 0 or 1% IAA for 2 years. No adverse effect on growth, mortality and histopathology (organs and lesions not listed) were observed (Lehman, A.J. 1951). 3 groups, each of 10 male weanling Osborne-Mendel rats, were given normal diet containing 5% lard with 0, 0.05% or 0.25% dietary IAA-palmitate for 2 years. Growth and mortality were similar to controls. Occasional haematology during the first year showed no toxic effects. Histopathology (organs and lesions not specified) showed no gross or microscopic differences from controls nor any distinct lesions attributable to treatment (Fitzhugh, O.G. 1946). 3 groups, each of 52 male and 52 female F344/Du Cr rats, were given for 96 weeks in their drinking water 0, 1.25% or 2.5% Na-IAA. 2.5% depressed body weight gains in both sexes but more in females during the 2nd year. Mean lifespan was similar in all groups. No specific tumours and no shortening of time-to-tumour attributable to treatment were noted. Chronic toxic effects were not properly investigated but a few lesions occurred in high dose rats (Abe, I. et al 1984).

Dog:

4 beagles received a total of 1 g IAA daily orally for 240 days. Another group of 4 beagles received 5 g IAA for 50 days followed by 2.5 g daily for 190 days. No toxicity was observed. Body weights, growth, haematology were normal. No gross or histopathological changes due to treatment were noted (Orahovats, P.D. 1957).

Reproduction studies

A formal multigeneration reproduction study has not been carried out with IAA.

Teratogenicity studies

Mouse:

Pregnant CD-1 mice were given IAA at 0, 10.3, 47.6, 221.9 or 1030 mg/kg b.w. by gavage from day 6 to 16 of pregnancy. No adverse effects were noted on maternal or foetal survival and no soft tissue or skeletal teratogenic effects were noted (FDRL 1974).

Rat:

Pregnant Wistar rats were given IAA at 9.0, 41.8, 194.0 or 900 mg/kg b.w. in their diet from day 6 to 16 of pregnancy. No adverse effects on maternal or foetal survival and no soft tissue or skeletal teratogenic effects were noted (FDRL 1974).

Pregnant Wistar rats were given diets containing 0, 0.05%, 0.5% or 5% IAA from day 7 to 14 of pregnancy. No adverse effects were noted on body weight gain, foetal deaths, litter numbers, foetal sex ratio, foetal body weight and placental weight. No soft tissue or skeletal teratogenic effects were seen. Postnatal development was unaffected (Ema, M. 1985).

Chicken:

IAA had embryotoxic effects closely related to the dose injected into the air sac or yolk of fertilised eggs. Embryonic mortality was increased and more abnormal chicks hatched but histopathology was inconclusive. The LC_{50} at 96 hours via air cell was 84 mg/kg (Naber, E.C. 1975; Hwang, U.K. et al 1974, Newell, G.W. et al 1974).

Mutagenicity studies

1. *Bacterial tests:*

A salmonella reverse mutation test with TA 100 +/- S9 using IAA free acid was weakly positive and dose dependent up to 20 mg/plate. Na-IAA and AA were negative up to 5 mg/plate (Ishidate, M., 1984).
A salmonella reverse mutation test (strains not mentioned) was negative up to 0.5% (w/v) IAA (Litton Bionetics 1976)
A salmonella reverse mutation test in TA 1530, 1535, 1536, 1537, 1538 +/S9 was negative (Newell, G.W. 1974)
A salmonella reverse mutation test in TA 98 was negative for Na-IAA (Kawachi, T. et al 1980).
A salmonella reverse mutation test in TA 98 and TA 100 +/- S9 was negative for Na-IAA (Peters, J.H. et al. 1983).

2. *Yeast tests*

IAA was mutationally inactive against Sacch. cerev. at concentrations of 2% and 4% (Litton Bionetics 1976).
Concentrations of 5% Na-IAA did not increase mitotic recombination frequency in Sacch. cerev. D3 (Newell, G.W. et al 1974)

3. *Host mediated assays*

Na-IAA was not mutagenic in a host-mediated assay using Salm. typhim TA 1530 or Sacch. cerev. D3 (Newell, G.W. et al 1974)

4. *In vitro mammalian cell assays*

Na-IAA and IAA were inactive in Chinese hamster fibroblast (CHL) cells for chromosomal aberrations up to 0.25 mg/ml and 48 hour incubation (Ishidate, M. et al 1984). Na-IAA caused [illegible] chromosomal aberrations in CHL fibroblasts with S9 (Matsuoka, A. et al 1979). No chromosomal aberrations [illegible] were [illegible] human fibroblasts (HE 2144) at doses of [illegible] mg Na-IAA [illegible] 48 hours (Kawachi, T. et al 1980; Sasaki, M. et al 1980).

5. Micronucleus test

A test in rat bone marrow was reported positive for Na-IAA but insufficient details are reported to assess validity (Kawachi, T. et al 1980).
A test in mice using i.p. IAA up to a single dose of 1500 mg/kg b.w. or 4 daily doses of 750 mg/kg b.w. was negative (Hayashi, M. et al 1988).

6. Dominant lethal test

Na-IAA was tested in rats at dose levels of 0, 0.2, 1.0 and 5.0 g/kg b.w. given either once or on 5 successive days. The positive control was 0.2 mg TEM/kg b.w. given i.p., breeding being carried out over 8 weeks. No treatment-related changes in early and late foetal deaths, corpora lutea and preimplantation losses were noted. Occasional significant differences were neither time- nor dose-dependent (Newell, G.W. et al 1988).

7. Heritable translocation test.

Adult male mice were treated for 7 weeks with dietary levels of 1% or 5% Na-IAA, positive control being 4 weeks' treatment with TEM in the drinking water. 100 F_1 males from each group were mated with 3 females each and the nonbreeders, presumptive steriles and partially steriles identified. Rebreeding left 3 controls, 20 TEM and 3 IAA treated males. Cytogenetic evaluation of meiotic chromosomes showed heritable reciprocal translocations only in the TEM-treated males. There was no effect on male fertility (Jorgenson, T.A. et al. 1978; Newell, G.W. et al 1988).

Studies on mutagenicity modulation

The modulating effect of human urine concentrates on the mutagenic or promutagenic activities of 2-acetylaminofluorene, 2-anthramine and 2,3,6-trinitrotoluene on Salm. typhim. TA 98 and TA 100 were not changed consistently, whether 30, 60 or 90 mg AA or 600 mg IAA/day were ingested additionally (Peters, J.H. et al 1983).

Studies on tumour promotion

Using various models of initiator followed by IAA or Na-IAA no promoting effect of AA or IAA could be demonstrated. An enhanced carcinogenic response could be observed only when Na-AA and Na-IAA were given at 5% in the diet. Promotion effects were limited to forestomach, colon and urinary bladder. This suggests that the Sodium ion rather than the AA or IAA are responsible (Fukushima, S., et al. 1986, Fukushima, S., et al. 1986, Fukushima, S., et al. 1986, Fukushima, S., et al. 1987, Shibata, M.A. et al. 1989). Electronmicroscopy of rat bladder epithelium showed hyperplasia only in animals given 5% Na-AA or Na-IAA but not with 5% AA (Fukushima, S. et al. 1986). Similarly only Na-AA but not AA increased DNA synthesis in the bladder epithelium (Shibata, M.A. et al. 1989).

Comments

Antiscorbutic activity

Although the apparent antiscorbutic activity of single doses of IAA is about 5% of that of AA, large doses over prolonged periods will relieve the symptoms of scurvy. IAA competitively inhibits the stereospecific carriers of the transport mechanism of AA in the gut mucosa and membranes of different tissues but is a poorer substrate with about 1/5th affinity for the carrier. Hence higher IAA concentrations are needed to achieve antiscorbutic effects. AA in the tissue compartment with low affinity and high turnover (adrenals, lung, kidneys, testis, eye) is easily replaced by IAA but not in the tissue compartment with high affinity and low turnover (brain). IAA reduces the AA body pool, increases AA catabolism and reduces AA half-life by more rapid elimination of AA. IAA in adequate amounts has AA potency as shown in enzyme studies but no conclusions are possible regarding any effect on the bioavailability of AA, the evidence being contradictory.

Absorption

The intestinal absorption of IAA proceeds by an active carrier-mediated mechanism in guineapigs and man but by passive diffusion in rats, mice, hamsters and rabbits. Comparison of the plasma levels of IAA following prolonged oral administration to mice in one study (Tsao, C.S. et al 1983) with another study in human volunteers (Sauberlich, H.E. et al 1989) demonstrates similar plasma levels despite an order of magnitude lower intake in the human volunteers. This difference could be due to the active carrier-mediated mechanism in man leading to greater gut absorption and/or tubular reabsorption. No study is available which enables a direct comparison of the rates of absorption by the two mechanisms in rodents and man. If the present uses of IAA were to be considerably extended adequate studies on comparative absorption rates in rats and man as well as the establishment of oral dose levels to achieve appropriate plasma levels in rats and mice would be essential.

There is no evidence for IAA interference with Fe, Zn or Cu absorption from cured meat.

Metabolism

Although no specific detailed studies have been carried out on the metabolism of IAA, urinary dehydro-IAA was detected in one rat study, indicating that IAA can be a substrate for ascorbate oxidase. In another rat study, using both 14-C-labelled IAA and AA, the metabolite ascorbate-2-sulphate but no isoascorbate-2-sulphate was detected in the urine. In man, oxalate excretion is greater with AA than with IAA (Sauberlich, H.E. et al 1989). Although this demonstrates certain differences in the metabolism of IAA and AA in rat and man it is likely that there are at least qualitative though not quantitative similarities. In interpreting the relevance of toxicity studies in rats to man it should be noted that the ascorbyl-glucuronate pathway is absent in man. Further studies to more fully elucidate the metabolism of IAA would be desirable, bearing in mind however that even the metabolism of AA has not been completely established.

Excretion

IAA is largely excreted in the urine, renal tubular reabsorption being less efficient but using the same transport mechanism as AA. IAA could therefore compete with and reduce AA reabsorption. Experiments on human volunteers show no depletion of AA due to IAA intake but the animal evidence is contradictory.

Toxicity/Carcinogenicity

The more recent short-term study in mice did not show any specific toxic effects up to 2.5% IAA in their drinking water, higher doses causing reduced weight gain as well as liver, kidney and spleen toxicity. The available recent carcinogenicity study in mice (Inai, K. et al 1989) resulted in a minimal effect level of IAA for male mice of 3.3 g/kg b.w. and female mice of 4 g/kg b.w., when administered in their drinking water (reduced relative brain weights occurred at these doses). Hepatocellular adenomas and carcinomas were increased in male mice at 4.9g/kg b.w./day but the incidence in controls was unusually low and the effect was no longer apparent after adjustment for survival. Chronic toxic effects were not reported in detail, although it was stated that no significant histopathological differences were noted between treated and control groups.

An early 36-weeks feeding study (Fitzhugh, O.G. et al 1946) in rats used only male animals and a single dose level. The reported findings in gross pathology and histopathology showed no evidence of any specific toxic effects attributable to IAA. This study was however inadequate by modern requirements. Another more recent short-term study (Abe, I. et al 1984) pointed to a NEL of about 1.25% in the drinking water, higher doses causing loss of weight. As no other parameters were reported, this study is also regarded as inadequate.

The recent life-span carcinogenicity study in rats (Abe, I. et al 1984), using administration in the drinking water, did not show any carcinogenic effects, the NEL being 1.25%, higher doses causing reduced bodyweight gain. Chronic toxic effects were not properly investigated - no incidences were reported although a few lesions occurring in high dose rats were described. The inadequacies of this recent study regarding chronic toxic effects is partially offset by the life-span study in mice mentioned above. The latter study stated there was no evidence for the occurrence of specific toxic lesions attributable to dietary administration of IAA, although again no incidences of lesions were given.

Reproductive toxicity

A formal multigeneration-reproduction study has not been carried out with IAA. However, the dominant lethal study in rats and the heritable translocation study over several generations in mice show no evidence of any significant interference by IAA at least with male reproductive function, as only the males were treated. No conclusions on female reproductive function can be derived from these studies. 2500 mg/kg b.w. in rats and 1030 mg/kg b.w. in mice caused no teratogenic effects.

Genotoxicity

IAA was adequately and extensively tested for somatic and germ cell mutagenicity in several *in vitro* and *in vivo* systems and was shown to have no genotoxic potential. IAA has also no tumour-promoting activity in several model systems testing for promoter activity.

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Opinion on acetylated oxidised starch

EXPRESSED ON 2 JUNE 1995

1. Terms of reference

To advise on the acceptability with respect to safety in use of acetylated oxidised starch.

2. Background

A submission was received on 14.06.1991 requesting permission to use acetylated oxidised starch (AOS) as a food additive. No toxicity data were submitted at that time in support of this request. A W/G of the SCF considered the application at their meeting of 7-8.11.1991. It noted that acetylated starch and oxidised starch (starches in group B, see second Series of Reports, 1976) were already considered acceptable in their own right. However, to have more complete reassurance of the safety of a modified starch subjected to a combined treatment of acetylation and oxidation, it required a 90-day feeding study and a more precise specification setting out the degree of modification as well as proposing maximum limits for residues of reactants and byproducts such as sodium chloride, sodium chlorate, sodium sulphate and sulphur dioxide.

At the 81st meeting of the SCF of 9-10.12.1991 the Committee considered AOS temporarily acceptable pending receipt of the results of a 90-day feeding study and an adequate specification within 2 years.

A full report on a 90-day feeding study in rats has now been submitted early in 1994 together with a specification for those products which had received the highest treatment and which were the subject of the 90-day study.

3. Specification

Degree of substitution for acetyl	[illegible] to 0.10 mol/mol or [illegible] w/w
Degree of substitution for carboxyl	[illegible] mol/mol or [illegible] w/w
Residual reactants	[illegible] maximum [illegible] ppm [illegible] [illegible] equivalent to less than [illegible] NaCl [illegible] other residues and byproducts [illegible] ppm

4. Subacute (dose range finding) study

4 groups, each of 5 male Wistar rats, 4-5 weeks old and housed 5 per cage, were fed for 2 weeks in their diet either 0%, 10%, 30% or 50% extruded AOS. 1 additional group was fed 30% non-extruded AOS. Only body weight gain, food intake, food efficiency and the weight of filled and empty caeca were determined. Weight gain, food intake and food efficiency were comparable among all groups. The absolute and relative filled and empty caecal weight was increased in the groups fed 30% and 50% AOS and in the group fed 30% nonextruded AOS. Animals given 50% AOS showed diarrhoea and considerable caecal enlargement. On the basis of these findings the dose range for the 90-day study was set at 0%, 5%, 10% and 30% in the diet.

5. Subchronic study

4 groups, each of 10 male and 10 female Wistar rats, aged 4-5 weeks, were fed for 13 weeks in their diet 0%, 5%, 10% or 30% extruded AOS. Body weight gain and food consumption were measured weekly, urinalysis was carried out in week 12, ophthalmoscopy was performed on all groups at the start and in controls and the top dose group in week 12. Full haematology and clinical chemistry including ALP, ASAT, ALAT, GGT estimations were performed once in week 14. Relative organ weight were determined in all dose groups for testes, pituitary, thyroid, adrenals, kidneys, thymus, brain, spleen, heart, liver and empty and full caecum. Histopathology of 33 organs was performed on all animals of the control and top dose group. In all other test groups only kidney, liver, lung and urinary bladder were examined.

No abnormal clinical signs or behavioural abnormalities were observed. No mortalities occurred. Body weight gain, food intake and food efficiency were comparable among test and control groups. There were no treatment related changes in the haematological, blood clotting and clinical chemistry parameters and all values were comparable between control and test groups. Urinalysis showed no treatment-related changes. All organ weights were comparable between test and control groups, but the absolute and relative weights of filled and empty caecum were increased in the 30% test group. One male rat in the 30% group had a dilated caecum.

Some males and females in all groups except the females given 5% AOS showed a slight increase in the incidence of hyperplasia of the transitional renal pelvic epithelium; particularly in top dose males and females compared to controls. However none of the increases was statistically significant. 4 out of 10 males in the 30% test group showed focal hyperplasia of the bladder epithelium. This was not seen in any other test group nor in the control group. Mineralisation in the pelvic renal epithelium as well as some renal cortical and cortico-medullary mineralisation was seen in all groups and especially in the top dose female group. There were a few aggregates of RES cells in the liver of males of the 5% dose group and less so in the 10% and 30% dose groups as well as in all female test and control groups. A dose-response relationship was not discernible. This finding is common for the strain and age of the rats used.

6. Comments

The increased weight of filled and empty caecum in rats of the top dose was not associated with any histopathological changes in the caecal wall or elsewhere in the GI-tract. It is a well-known response to high dietary levels of poorly digested carbohydrates in rats, the probable mechanism being the increased osmotic load from the SCFA produced by microbial degradation and the associated water retention.

The changes in the urothelial epithelium of the renal pelvis are similar to those found in feeding tests with other chemically modified starches in rats and in mice and are associated with mineralisation. The hyperplasia of the urinary bladder epithelium, seen only in males of the top dose group is probably treatment-related and a consequence of bladder irritation by calculi. There were no other significant pathological findings in any of the test groups.

7. Conclusions

The NOEL in this study was 10% of AOS in the diet. In the absence of any specific adverse findings associated with the feeding of AOS the Committee is of the opinion that AOS can be included among the other modified starches in group B which is considered acceptable (13th Series of reports of the Scientific Committee for Food, 1982) and for which the establishment of individual ADIs was judged to be unnecessary provided the technological usage remained at present-day levels, an aspect to be kept under review by the Commission. The specification supplied is adequate.

OPINION ON CADMIUM

EXPRESSED ON 2 JUNE 1995

1. Terms of reference

The Committee is asked to evaluate the risk to human health arising from dietary exposure to cadmium with particular reference to exposure from animal meat and organs.

2. Background

Concern over reports of high levels of cadmium in the organs and meat of horses imported into the European Union prompted the Commission to ask the Committee to carry out a general review of risk to public health arising from dietary exposure to this element.
The Committee endorsed the JECFA PTWI of 7 µg/kg b.w in its opinion expressed on the 7th December 1989 (SCF Report, 26th Series).

3. Conclusions

Dietary exposure

The Committee noted that its ability to assess the dietary exposure to cadmium in EU was greatly limited by the availability of reliable data on the consumption of relevant foodstuffs by individuals. This is particularly important in the case of cadmium since, with the exception of cigarette smoke and work place air, the contribution of non-dietary media contaminated sources to the exposure of the general population is negligible in comparison to the diet.

The average dietary exposure of European populations to cadmium, as indicated by monitoring programmes or from studies in smaller population groups, ranges from 100 to 400 µg/person/week (23 to 95% of the PTWI for a 60 kg person). Estimates vary appreciably even in the same country. The overall conclusions may therefore need to be reviewed in the light of better information.

In relation to the specific question regarding exposure from animal muscle and organs, the Committee noted that the cadmium contents of muscle, liver and kidney were largely independent of the species with the notable exception of the horse for which cadmium levels are considerably higher. The cadmium content of liver and kidney is known to increase with age and the Committee concluded that these higher levels arise from the tendency to slaughter horses at a considerably greater age than most other animals intended for meat production. Similar considerations may therefore be expected to apply to other species slaughtered at relatively greater age but no data were available to the Committee to support this assertion.

The following table summarises the reported consumption data and cadmium levels in animal muscle and organs. The ranges of maximum limits (MLs) in force in the Member States are included for comparison.

Meat Product	*Range of MLs in the EU (µg/g)*	*Weekly Consumption (g)*		*Cadmium content (µg/g)*	
		Average	*High*	*Average*	*High*
Muscle[1]	0.05 - 1	1050	1750	0.03	0.2
Horse muscle	0.2 - 1	70	175	0.13	0.5
Liver[1]	0.2 - 1	84	210	0.1	0.5
Horse liver[4]	0.2 - 1	14	35[3]	10	20
Kidney[1]	0.2 - 2.5[2]	42	105	0.3	3
Horse kidney[4]	0.5 - 1	7	18[3]	50	150

[1] values for consumption and cadmium content excluding horse meat and organs

[2] no separate ML for horse kidney in the countries with this limit

[3] assumed to be 2.5 times average consumption

[4] derived from data on horse offal and assumes consumption of liver and kidney in the ratio 2 to 1

On the basis of a PTWI of 7 µg/kg b.w. and mean body weight of 60 kg:

- for average and high consumers of meat in general and non-horse organs, the cadmium intake ranges from 2 to 13% of the PTWI for products having average cadmium contents and from 8 to 83% for those having high cadmium contents.
- for average and high consumers of horse liver and horse kidney, the cadmium intake ranges from 34 to 208% of the PTWI for products having average cadmium contents and from 67 to 645% for those having high cadmium contents.

It is stressed that the above estimates are based on the reported current dietary consumption patterns and increase in the proportion of horse meat and offal consumed would lead to greater cadmium exposure.

The Committee concludes that for a significant part of the population, exposure to cadmium from dietary sources alone is at a level close to the PTWI. It therefore recommends greater effort to reduce dietary exposure and the continuation of regular and comprehensive monitoring of this element in the diet of the population of the European Union.

In the particular case of horse meat and organs, it appears that at the current levels of consumption by individuals, products sold in conformity with typical maximum residue limits in force in the Member States would not pose a risk. However, the great majority of horse liver and kidneys have cadmium contents which greatly exceed these limits and their consumption is likely to lead to unacceptable levels of cadmium exposure.

Carcinogenicity

During the course of this evaluation, the Committee noted that, since its previous opinion of December 1989, the results of a number of carcinogenicity studies had become available and that IARC had recently classified cadmium and its compounds as human carcinogens in Group 1 (IARC 1993). The Committee therefore reviewed the aspect of carcinogenicity in order to complete its re-evaluation of the toxicology of cadmium from all dietary sources as a matter of urgency.

Cadmium is a cumulative element with a biological half-life of 10-20 years in humans. Kidney disfunction has so far been considered to be the critical effect of long term exposure to cadmium and quantitative risk assessments have been performed accordingly.

Recently, IARC classified cadmium and cadmium compounds in Group 1 (Human carcinogens), based on evidence from human studies, mainly those on lung cancer associated with cadmium inhalation in the work place, and from animal studies (mainly two inhalation studies in rats producing malignant tumours and one oral study in male rats producing leukemias, interstitial cell tumours of the testis and proliferative lesions of the prostate). The IARC classification is qualitative only. Supportive evidence was also provided by the genotoxic effects induced in several eukaryotic cells, *in vitro* and *in vivo*, including human cells.

Concerning the interpretation of animal and human data and in particular the question whether cadmium should be considered only or primarily carcinogenic by inhalation, a number of important issues should be taken into account:

- the uncertainties on the mechanisms of cadmium carcinogenicity;
- the limitations of the currently available animal studies involving oral exposure;
- the need for clarification of the role of metallothionein, particularly in relation to carcinogenicity and genotoxicity;
- the role of various cadmium compounds and their bioavailability;
- interaction with other metals (Zn, Fe, Cu).

The available data do not allow adequate quantitative assessment of carcinogenic risk. However, the carcinogenic risk associated with oral exposure appears to be much lower than that from direct inhalation.

Concerning the genotoxicity data, despite some conflicting results, overall the data suggest that the genotoxic activity is a common trait of cadmium compounds. However, different types of evidence suggest indirect modes of action (e.g. oxidative damage by reactive oxygen species, interaction with critical thiol groups of proteins, inhibition of DNA repair enzymes) especially at the chromosome and genome level.

The Committee concluded that, at present it cannot exclude a carcinogenic risk from dietary exposure to cadmium, and is therefore unable to establish a safe level for cadmium in food. Epidemiological data collected on environmentally exposed populations during the last two decades have provided a sound basis to conclude that alterations of kidney function should be regarded as the critical effect of cadmium. For this reason, and in view of the narrow safety margin between the cadmium body burden of the general population in the Europe Union and the critical level for renal disfunction, there is a need to keep dietary exposure to cadmium as low as possible.

The present PTWI of 7 µg/kg b.w. is still considered to be valid for renal dysfunction but should be re-evaluated as soon as further information concerning carcinogenicity or mutagenicity becomes available.

Reference

International Agency for Research on Cancer (IARC) Monograph on the evaluation of carcinogenic risks to humans. Vol 58. Beryllium, cadmium, mercury and exposures in the glass manufacturing industry. Lyon 1993.

REPORT ON CERTAIN ESTERS USED IN PLASTICS FOR FOOD CONTACT APPLICATIONS

OPINION EXPRESSED ON 2 JUNE 1995

1. Introduction

In the past, the SCF has evaluated a number of esters including esters of adipic, azelaic, citric, phosphoric, phosphorous, phthalic, sebacic, sulphosuccinic, thiodipropionic and trimellitic acids. Several of these have already been classified in lists 1, 4A or 6A and they do not appear in this report. For esters not classified in the above lists, the SCF has made recommendations about the studies which need to be carried out in order that a full evaluation can be made. All these recommendations, previously published in separate documents are brought together in this report. The corresponding Community Directive reference number precedes the entry for each substance. The definitions of the lists are given in the Appendix.

> N.B. For a number of the substances, the additional requested data have been made available but have not been evaluated at the time of adoption of the present report.

i) Hydrolysis studies

These have been requested for those monoesters which may hydrolyse to substances which are already in lists 0, 1, 2 and 3 (see Practical Guide N 1, 2 April 1993).

ii) 28-day oral studies

These have been requested in some instances because some subchronic data were already available but were not adequate to establish a TDI. Where 28-day studies have been requested, the wg expects that group sizes, histopathology, haematology, clinical chemistry and urinalysis will conform to the present day standards of 90-day studies. However, note that 28-day or 90-day studies need only be provided if migration exceeds 0.05 mg/kg of food (see Practical Guide N 1, 2 April 1993).

iii) Peroxisome proliferation studies

These have been requested, in principle, for all alkyl esters because there is evidence to indicate that both branched and unbranched esters of differing chain lengths may cause peroxisomal proliferation. These data have not been requested for cycloaliphatic or aromatic esters because there is no evidence to suggest that they cause peroxisomal proliferation.

However, for linear chain esters a study of peroxisome proliferation is not required provided the restriction resulting from other toxic effects is ≤ 0.05 mg/kg b.w. If there are no other toxic effects which invoke a restriction of ≤ 0.05 mg/kg b.w., then in the absence of a peroxisome proliferation study, the SCF will set a restriction at 0.05 mg/kg b.w. This approach is based on the assumption that di-2-ethylhexyl phthalate (DEHP, which has a restriction of 0.05 mg/kg b.w. based on peroxisome proliferation) is considered to be the most potent peroxisome proliferator among esters used as monomers and additives in food contact materials.

For esters in general, a study of peroxisome proliferation will not be required if structure-activity arguments, based on adequate scientific evidence, can be provided which show that peroxisome proliferation would not be expected to occur. This approach is based on the growing knowledge about structure-activity relationships for peroxisome proliferation.

Where peroxisome proliferation studies are conducted, they should be carried out in the rat and dosing should be a minimum of 14 days in duration. At least the following two markers should be measured: palmitoyl CoA oxidation and lauric acid 12-hydroxylase. The following methods to assess these activities are recommended:

Palmitoyl-CoA oxidation: a spectrophotometric assay based on the methods of either Lazarow or Bronfman.

Lazarow PB and DeDuve C. Proceedings of the National Academy of Sciences 73 (1976), 2043-2046.

Lazarow PB. Methods in Enzymology 72 (1981), 315-319.

Bronfman M, Inestrosa NC and Leighton F. Biochemical and Biophysical Research Communications 88 (1979), 1030-1036.

Lauric acid 12-hydroxylase: a radiometric assay using radiolabelled substrate and HPLC to separate individual hydroxy metabolites from unmetabolised substrate.

Parker GL and Orton TC. In: Biochemistry Biophysics and Regulation of Cytochrome P-450. Eds: Gustafsson J A, Duke JC, Mode A and Rafter J. pp 373-377. Elsevier/North Holland, Amsterdam (1980).

Sharma R, Lake BG, Foster J and Gibson GG. Biochemical Pharmacology 37 (1988), 1193-1201.

However, note that these data need only be provided if migration exceeds 0.05 mg/kg of food (see Practical Guide N.1, 2 April 1993).

iv) Neurotoxicity studies

These have been requested for phosphoric acid and phosphorous acid esters because there is evidence that a number of substances in the group have neurotoxic properties. Measures of anticholinesterase activity plus either a standard test in chickens for delayed neuropathy or measurement of inhibition of neuropathy target esterase (NTE) would be appropriate. However, note that these data need only be provided if migration exceeds 0.05 mg/kg of food.

v) Reproduction and teratogenicity studies

These have been requested because there is evidence that a number of substances in the group have adverse effects on reproduction and/or are teratogenic. However, note that, with the exception of substances having a t-TDI, these data need only be provided if migration exceeds 5 mg/kg of food (see Practical Guide N.1, 2 April 1993).

vi) Mutagenicity studies

These have been requested according to the Practical Guide N.1 (2 April 1993), which requires 3 particular types of test, irrespective of the level of migration.

N.B. DEHA and DEHP have not been included in this re-evaluation because the SCF will publish separate opinions on them. However, the group restriction of 0.05 mg/kg b.w. for certain of the esters listed below (which are suspect for peroxisome proliferation) is based on the most recent evaluation of the peroxisome proliferation activity of DEHP, for which a TDI of 0.05 mg/kg b.w. has been agreed.

Classification of individual esters

N.B. With the exception of substances having a t-TDI, for all remaining esters mentioned below it is necessary to provide migration data according to the Practical Guide N.1, 2 April 1993. In the case of substances having a t-TDI, the studies specified are required in order to establish a full TDI.

1. The following substances are classified in List 6B with a group restriction of 0.05 mg/kg b.w and require:

   a) toxicological data depending on level of migration (see SCF guidelines)
   and

   b) if the migration exceeds 0.05 mg/kg also the additional study specified below:
   - peroxisome proliferation study

   c) any other data specified beside the substance.

Monomers

12190 Adipic Acid, Di-n-Decyl Ester
12220 Adipic Acid, Diisodecyl Ester
Needed: Specifications on identity
12235 Adipic Acid, Dimethyl Ester
12250 Adipic Acid, Di-n-Octyl Ester
12910 Azelaic Acid, Dimethyl Ester
24340 Sebacic Acid, Di-n-Decyl Ester
24370 Sebacic Acid, Dimethyl Ester

Additives

32000	Adipic Acid, Bis(6-Methylheptyl) Ester
32080	Adipic Acid, n-Decyl n-Octyl Ester
32240	Adipic Acid, Dibutyl Ester
2320	Adipic Acid, Di-n-Decyl Ester
32480	Adipic Acid, Diisobutyl Ester
	Available: Inadequate 2 year oral rat study
32560	Adipic Acid, Diisodecyl Ester
	Needed: Specifications on identity
32720	Adipic Acid, Diisooctyl Ester
	Needed: Specifications on identity
32760	Adipic Acid, Dimethyl Ester
32800	Adipic Acid, Di-n-Nonyl Ester
32840	Adipic Acid, Di-n-Octadecyl Ester
32880	Adipic Acid, Di-n-Octyl Ester
36320	Azelaic Acid, Bis(2-Ethylhexyl) Ester
	Available: Inadequate 90 day study
36400	Azelaic Acid, Bis(6-Methylheptyl) Ester
36520	Azelaic Acid, Diisooctyl Ester
	Needed: Specifications on identity
36560	Azelaic Acid, Di-n-Octyl Ester
44280	Citric Acid, Dioctadecyl Ester
	Needed: Specifications on identity
44560	Citric Acid, Tributyl Ester
44720	Citric Acid, Tri-n-Octadecyl Ester
44800	Citric Acid, Tris(2-Ethylhexyl) Ester
74800	Phthalic Acid, Dialkyl (C7-C11) Esters
	Needed: Specifications on identity
75280	Phthalic Acid, Diisobutyl Ester
75640	Phthalic Acid, Di-n-Decyl Ester
75680	Phthalic Acid, Di-n-Nonyl Ester
75760	Phthalic Acid, Di-n-Octadecyl Ester
76005	Phthalic Acid, Mixed Esters with Butyl Glycolate and Butanol
	Available: 30 day and 1 year oral rat studies and mutagenicity studies which are all inadequate.
76085	Phthalic Acid, Mixed Esters with Ethyl Glycolate and Ethanol
	Available: 4 month and 2 year rat and 1 year dog studies which are all inadequate.
76160	o-Phthalic Acid, n-Pentyl Benzyl Ester
85200	Sebacic Acid, Bis(6-Methylheptyl) Ester
85440	Sebacic Acid, Dimethyl Ester

85520 Sebacic Acid, Di-n-Octyl Ester
Available: data inadequate (extract from Lefaux book).
91560 Sulphosuccinic Acid, Bis(1,3-Dimethylbutyl) Ester, Sodium Salt
91570 Sulphosuccinic Acid, Bis(2-Ethylhexyl) Ester
91630 Sulphosuccinic Acid, Dihexyl Ester, Sodium Salt
91650 Sulphosuccinic Acid, Diisobutyl Ester, Sodium Salt
91665 Sulphosuccinic Acid, Diisodecyl Ester, Sodium Salt
91672 Sulphosuccinic Acid, Diisotridecyl Ester, Sodium Salt
91680 Sulphosuccinic Acid, Dioctyl Ester, Sodium Salt
91720 Sulphosuccinic Acid, Dipentyl Ester, Sodium Salt
91760 Sulphosuccinic Acid, Ditridecyl Ester, Sodium Salt
91800 Sulphosuccinic Acid, Isodecyl Ester, Disodium Salt
93000 Thiodipropionic Acid, Bis(2-Ethylhexyl) Ester
93040 Thiodipropionic Acid, Dibehenyl Ester
93120 Thiodipropionic Acid, Didodecyl Ester
93200 Thiodipropionic Acid, Dihexadecyl Ester
93280 Thiodipropionic Acid, Dioctadecyl Ester
93360 Thiodipropionic Acid, Ditetradecyl Ester
95440 Tris(2-Ethylhexyl) Acetylcitrate

2. The following substances are classified in List 6B with a group restriction of 0.05 mg/kg b.w. and require:

a) toxicological data depending on level of migration (see SCF guidelines)
and
b) if the migration exceeds 0.05 mg/kg the additional studies specified below:
- peroxisome proliferation study
- neurotoxicity studies

72720 Phosphoric Acid, Di-n-Hexadecyl Esters
72760 Phosphoric Acid, Di-n-Nonyl Ester
73840 Phosphoric Acid, Triisobutyl Ester
74080 Phosphorous Acid, Triisodecyl Ester

3. The following substances (which are not peroxisome proliferators) are classified in List 6B with a group restriction of 0.05 mg/kg b.w. because they are suspect for neurotoxicity and require:

a) toxicological data depending on level of migration (see SCF guidelines)
and
b) if the migration exceeds 0.05 mg/kg the additional studies specified below:
- neurotoxicity studies

72700	Phosphoric Acid, Cresyl Diphenyl Ester
72840	Phosphoric Acid, Diphenyl-p-Tolyl Ester
73920	Phosphoric Acid, Triphenyl Ester
74020	Phosphorous Acid, 2-tert-Butyl-alpha-(3-tert-Butyl-4-Hydroxyphenyl)-p-Cumenyl Bis(4-Nonylphenyl) Ester
74040	Phosphorous Acid, Diphenyl Ester
74160	Phosphorous Acid, Tris-2 (Cyclohexylphenyl) Ester
74320	Phosphorous Acid, Tris[(3-Ethyl-3-Oxetanyl)-Methyl] Ester

4. The following substances are classified in List 6B with a group restriction of 0.05 mg/kg b.w. and require:

   a) toxicological data depending on level of migration (see SCF guidelines)

   and

   b) the additional study specified below, if any:

32160 Adipic Acid, Dialkyl Esters (C7-C9)

Available: 90-day oral rat study.

Needed: Specifications in the first instance and if migration exceeds 0.05 mg/kg peroxisome proliferation study of the specified substance too.

32640 Adipic Acid, Diisononyl Ester

Available: 90 day oral rat and dog studies and two mutagenicity studies.

Needed: Specifications and test for chromosome aberrations in mammalian cells *in vitro* in the first instance and, if migration exceeds 0.05 mg/kg, peroxisome proliferation study of the specified substance too.

36480 Azelaic Acid, Di-n-Hexyl Ester

Available: 90-day and 2 year oral rat and 1 year oral dog studies.

Needed: If migration exceeds 0.05 mg/kg, peroxisome proliferation study too.

73600 Phosphoric Acid, Tributoxyethyl Ester

Available: Ames test, 14 day and 18 week oral rat studies.

Needed: Full report of 18 week oral rat study by Monsanto (1987).

73680 Phosphoric Acid, Tributyl Ester

Available: Ames tests and several subchronic oral rat studies.

Needed: If migration exceeds 0.05 mg/kg, peroxisome proliferation and neurotoxicity studies too.

74000 Phosphoric Acid, Tris(2-Ethylhexyl) Ester

Available: Ames test, 90-day and 2 year oral mouse and rat studies.

Needed: If migration exceeds 0.05 mg/kg, peroxisome proliferation and neurotoxicity studies too.

75200 Phthalic Acid, Di-n-Heptyl Ester

Available: Ames test.

Needed: If migration exceeds 0.05 mg/kg, peroxisome proliferation study too.

75600 Phthalic Acid, Dimethyl Ester

Available: Limited oral rat chronic toxicity/carcinogenicity study, oral teratogenicity studies in rats and mice, Ames test.

75840 Phthalic Acid, Di-n-Octyl Ester

Available: Ames test, peroxisome proliferation study, oral mouse reproduction study, inadequate oral rat 90-day study, inadequate oral rat chronic toxicity/carcinogenicity study.

75920 Phthalic Acid, Di-n-Tridecyl Ester

Available: Ames test.

Needed: If migration exceeds 0.05 mg/kg, peroxisome proliferation study too.

85120 Sebacic Acid, Bis(2-Ethylhexyl) Ester

Available. Ames test and 3 week oral rat study.

Needed: If migration exceeds 0.05 mg/kg, peroxisome proliferation study too.

85360 Sebacic Acid, Dibutyl Ester

Available: 2-year oral rat study and reproduction study in rats both inadequate, 3 mutagenicity tests (reports not available).

Needed: Mutagenicity reports in first instance and, if migration exceeds 0.05 mg/kg, peroxisome proliferation study too

94800 Trimellitic Acid, Tris(2-Ethylhexyl) Ester

Available. Ames test and indication of peroxisome proliferation.

Needed If migration exceeds 0.05 mg/kg, peroxisome proliferation study too.

[Waiting list as it is a new substance.]

5. The following substances are classified in List 2 but their requirements have been modified as follows:

74560 Phthalic Acid, Benzyl Butyl Ester

12 t-TDI 0.1 mg/kg b.w.

Available: 6-month oral rat, carcinogenicity and peroxisome proliferation studies *in vitro* (RIVM 1987, September)

Needed. *In vivo* peroxisome proliferation study, reproduction and teratogenicity studies

74880 Phthalic Acid, Dibutyl Ester

12 t-TDI 0.05 mg/kg b.w.

Available Limited 90-day and 1-year oral rat studies, oral reproduction and teratogenicity studies, limited mutagenicity studies (RIVM Report, May 1988)

Needed: Tests for gene mutation and chromosome aberrations in mammalian cells *in vitro*, and, if migration exceeds 0.05 mg/kg 28 day oral study and peroxisome proliferation study too

74960 Phthalic Acid, Dicyclohexyl Ester

L2 t-TDI 0.1 mg/kg b.w.

Available: Three 90-day oral rat studies, limited *in vitro* mutagenicity studies (RIVM, 1988)

Needed: Tests for gene mutation and chromosome aberration in mammalian cells *in vitro* and reproduction and teratogenicity studies.

75040 Phthalic Acid, Diesters with Hexadecanol and/or Octadecanol

L2 Group t-TDI 0.15 mg/kg b.w.(with 76120)

Available: 3-month oral rat and teratogenicity studies, Ames test (RIVM doc. 1990-09-11; CS/PM/529).

Needed: Tests for gene mutation and chromosome aberration in mammalian cells *in vitro*, reproduction study and peroxisome proliferation study.

75120 Phthalic Acid, Diethyl Ester

L2 t-TDI: 0.2 mg/kg b.w.

Available: 3-month oral rat, mutagenicity, ip teratogenicity and peroxisome proliferation studies (Fd Cosmet Toxicol 1978, 16, 415-422; RIVM 1986, June).

Needed: Reproduction and teratogenicity studies.

76120 Phthalic Acid, n-Hexadecyl n-Octadecyl Ester

L2 Group t-TDI 0.15 mg/kg b.w. (with 75040).

6. The following substances are classified in list 7 with no restriction and require the data specified below.

33040 Adipic Acid, Mono-n-Octadecyl Ester, Calcium Salt

Needed: Hydrolysis studies.

44320 Citric Acid, Monoisopropyl Ester

Needed: Hydrolysis studies and reports from Duel et al, 1951.

44400 Citric Acid, Mono-n Octadecyl Ester

Needed: Hydrolysis studies and reports from Duel et al, 1951.

73360 Phosphoric Acid, Mono-n-Hexadecyl Ester

Needed: Hydrolysis studies

[Waiting list as it is a new substance.]

73440 Phosphoric Acid, Mono-n-Hexyl Ester

Needed: Hydrolysis studies

7. The following substances (which are not peroxisome proliferators) are classified in List 8 with no restriction and require data according to SCF guidelines:

12850	Azelaic Acid, Bis(2-Hydroxyethyl) Ester
12940	Azelaic Acid, Diphenyl Ester
23178	Phosphorous Acid, Triphenyl Ester
24400	Sebacic Acid, Diphenyl Ester
91580	Sulphosuccinic Acid, Dicyclohexyl Ester, Sodium Salt
91780	Sulphosuccinic Acid, ([2-(12-Hydroxy-1-Oxooleyl)-Amino]Ethyl] Ester, Disodium Salt

8. The following substances are classified in list 9 with a group restriction of 0.05 mg/kg b.w.:

Monomers

25565	2,2,4-Trimethyladipic Acid, Methyl Esters
25566	2,4,4-Trimethyladipic Acid, Methyl Esters

Additives

31760	Adipic Acid, Alkyl, Primary (C4-C15) Esters
32920	Adipic Acid, Esters with Diols from C2-C6
32963	Adipic Acid, Mixed Esters with 1,2-Propyleneglycol and Alcohols, Monoh., Sat., Linear (C8-C10) [Waiting list as it is a new substance]
36240	Azelaic Acid, Alkyl, Primary (C1-C12) Esters
44240	Citric Acid, Alkyl, Primary (C2-C12) Esters
72880	Phosphoric Acid, Ethanolamine Hexyl Branched and Linear Ester
73200	Phosphoric Acid, Mono- and Diesters with Alcohols, Aliph. (C9-C18), Diethanolamine Salt
73280	Phosphoric Acid, Mono- and Diesters with Alcohols, Aliph. (C9-C18), Salts
[illegible]	Phosphoric Acid, Mono- and Diesters with Alcohols, Aliphatic, Monohydric, Saturated (C2-C4)
[illegible]	Phosphoric Acid, Mono- and Diesters with Alcohols, Monohydric, Saturated, Primary, Linear (C12-C18), Diethanolamine Salt
[illegible]	Phosphoric Acid, Mono- and Diesters with Alcohols, Monohydric, Saturated, Primary, Linear (C12-C18), Salts
[illegible]	Phosphoric Acid, Nonyl Ester, Sodium Salt
[illegible]	Phosphoric Acid, Octadecyl Esters [Waiting list as it is a new substance]
[illegible]	Phosphoric Acid, Trialkyl(C4-C16) Ester
73760	Phosphoric Acid, Triethanol Ester
[illegible]	Phosphoric Acid, Tris(Alkoxyalkyl)(C3-C8) Ester
74060	Phosphorous Acid, Trialkyl(C8-C12) Ester

74600	Phthalic Acid, Bis(Alkoxyalkyl C3-C18) Ester
74760	Phthalic Acid, Bis(Methylcyclohexyl) Ester
75520	Phthalic Acid, Diisooctyl Ester
76000	Phthalic Acid, Mixed Esters with Butyl Glycolate and Alcohols, Aliph., Monoh., (C1-C4)
76080	Phthalic Acid, Mixed Esters with Ethyl Glycolate and Alcohols, Aliph., Monoh., (C1-C4)
85040	Sebacic Acid, Alkyl(C6-C12) Esters
91540	Sulphosuccinic Acid, Alkyl(C4-C20) Esters, Salts
94680	Trimellitic Acid, Trialkyl(C1-C8) Ester
94720	Trimellitic Acid, Trialkyl(C7-C9) Ester
94760	Trimellitic Acid, Triisooctyl Ester

Appendix

Definition of the SCF lists

Substances for which the committee was able to express an opinion

List 0

Substances, e.g. foods, which may be used in the production of plastic materials and articles, e.g. food ingredients and certain substances known from the intermediate metabolism in man and for which an ADI need not be established for this purpose.

List 1

Substances, e.g. food additives, for which an ADI, a temporary ADI (t-ADI), a MTDI, a PMTDI, a PTWI or the classification "acceptable" has been established by this Committee or by JECFA.

List 2

Substances for which a TDI or a t-TDI has been established by this Committee.

List 3

Substances for which an ADI or a TDI could not be established, but where the present use could be accepted.

Some of these substances are self-limiting because of their organoleptic properties or are volatile and therefore unlikely to be present in the finished product. For other substances with very low migration, a TDI has not been set but the maximum level to be used in any packaging material or a specific limit of migration is stated. This is because the available toxicological data would give a TDI which allows that a specific limit of migration or a composition limit could be fixed at levels very much higher than the maximum likely intakes arising from present uses of the additive.

List 4

Substances for which an ADI or a TDI could not be established, but which could be used if the substance migrating into foods or in food simulants is not detectable by an agreed sensitive method.

List 5

Substances which should not be used.

Substances for which there were insufficient toxicological or technological data to enable the Committee to express an opinion

List 6

Substances for which there exist suspicions about their toxicity and for which data are lacking or are insufficient.

The allocation of substances to this list is mainly based upon similarity of structure with that of chemical substances already evaluated or known to have functional groups that indicate carcinogenic or other severe toxic properties.

- Section 6A: Substances suspected to have carcinogenic properties. These substances should not be detectable in foods or in food simulants by an appropriate sensitive method for each substance.
- Section 6B: Substances suspected to have toxic properties (other than carcinogenic). Restrictions may be indicated.

List 7

Substances for which some toxicological data exist, but for which an ADI or a TDI could not be established. The required additional information should be furnished.

List 8

Substances for which no or only scanty and inadequate data were available.

List 9

Substances and groups of substances which could not be evaluated due to lack of specifications (substances) or to lack of adequate description (groups of substances).

Groups of substances should be replaced, where possible, by individual substances actually in use. Polymers for which the data on identity specified in "SCF Guidelines" are not available.

List W

"Waiting list". Substances not yet included in the Community lists, as they should be considered "new" substances, i.e. substances never approved at national level. These substances are not susceptible to be included in the Community list, lacking the data requested by the Committee.

On the basis of the data lacking the waiting list is subdivided into:

List W7

Substances for which some toxicological data exist, but for which an ADI or a TDI could not be established. The required additional information should be furnished.

List W8

Substances for which no or only scanty and inadequate data were available.

List W9

Substances and groups of substances which could not be evaluated due to lack of specifications (substances) or to lack of adequate description (groups of substances).

OPINION ON PAPAIN FROM PAPAYA FRUIT (*CARICA PAPAYA*)

USED AS A MEAT TENDERISING AGENT

EXPRESSED ON 2 JUNE 1995

1. Terms of reference

To advise on the safety of the use of papain as a meat tenderiser by injection into beef cattle after stunning and before bleeding.

2. Background

Papain was previously evaluated and found acceptable for chill-proofing of beer on the basis that it is a constituent of a part of a plant (papaya fruit) commonly consumed as a food source (80th meeting of the Scientific Committee for Food, October 1991). This was in accordance with the principles established by the Committee in its guidelines for the presentation of data on food enzymes (CEC, 1992).

The use of papain as a meat tenderiser by injection in solution into live animals before slaughtering has previously been practised in some Member States but has been discontinued on animal welfare grounds. The Committee has now been presented with a dossier describing an alternative method where the animals are stunned and pithed before they are injected with a sterile solution of papain in an oxidised, inactivated form and, shortly after, hoisted to the bleeding rail and then stuck.

According to the dossier, this treatment would result in residue levels of papain of less than 1 mg/kg in muscle and up to 5 mg/kg in offal.

3. Current review

Papain has been in use for many years for medical purposes, in cosmetics and for various food purposes. Since it is an enzyme naturally present in food, papain has not been subject to the toxicity testing which is normally performed on food additives. With the exception of allergic reactions, cases of adverse effects reported in the literature (damage to the oesophagus following impaction of treated meat) have all been associated with high exposure from uses not comparable to the proposed one and it is unlikely that the small residues resulting from the proposed use would cause any such effects.

The Committee is aware of reports in the literature indicating that papain has been associated with allergic reactions but the majority of cases were not relevant to oral exposure, mostly being associated with occupational exposure by the airborne route. Only a few cases of allergic responses to oral administration of papain have been reported (Mansfield and Bowers, 1983, Mansfield et al, 1985).

The Committee considered the evidence for the claim that the enzyme is not significantly activated until papain-treated meat is cooked. We were informed that:

- Papain is injected into the stunned animal in a form inactivated by oxidation and will not be activated until the oxygen tension in the carcass is sufficiently low.
- This conversion to the activated form only occurs when the normal processes taking place in the carcass after death have also reduced the pH and rapid chilling has considerably reduced the temperature. Both these changes reduce the level of activity of activated papain.
- Papain protease, unlike many other enzymes, has its optimum activity at 50-70°C, this optimum activity being considerably higher than that at ambient temperature.
- Traditional ageing of meat by storing over days/weeks involves the release of intrinsic proteases which, like papain, also degrade proteins.

The Committee considered the question of whether meat treated with papain may be more susceptible to microbial deterioration. Enhanced protein degradation by papain increases soluble nitrogen content of meat which may stimulate microbial growth, mainly of those bacterial genera with high nutritional requirements. In one study, no significant differences were found between bacterial counts of surface samples of papain tenderised sirloin strip steaks and counts of control steaks. However, the internal meat tissue of papain tenderised beef cuts was frequently contaminated with bacteria whereas all samples of control beef cuts were free from bacteria (Anonymous, 1961). It has been shown experimentally that poor keeping quality of papain tenderised meat was associated with deep penetration of bacteria (Thomas, 1966). Spoilage problems with this type of meat were experienced after relatively short periods of storage (Hudson & Roberts, 1974).

Formation of biogenic amines in fermented sausages is well documented (Eitenmiller et al. 1978; Eerola et al. 1993). Addition of an exogenous proteinase to the ingredients of dry fermented sausages enhanced the formation of free amino acids, and the higher amount of substrate in sausages with added proteinase available for the decarboxylase activity of the microbial flora resulted in an increase of up to nine-fold in tyramine content compared to that found in control sausage (Diaz et al. 1993). If papain-tenderized meat is used for the preparation of fermented meat products, such as sausages, salami, etc., an increase in the amount of biogenic amines formed seems likely to occur.

The Committee also considered whether meat treated with papain but otherwise unprocessed could give cause for health concern with respect to degradation of proteins. However, since papain degrades proteins to components which are also products of normal digestion, the Committee does not consider that the consumption of meat treated with papain would give any reasons for concern in this respect.

4. Conclusion

While the Committee recognises that considerations of animal welfare, organoleptic meat quality and appropriate consumer information are relevant factors to be considered, they do not fall within the remit of the SCF and should be considered by other relevant bodies.

The Committee noted that present rules would require that tenderised meat could not be marketed as fresh meat and that such meat should be properly labelled. If the use of injected papain as a meat tenderiser is authorised, then the Committee wishes to draw to the attention of the Commission the desirability of explicit labelling so that consumers who have acquired an allergy towards papain have an opportunity to avoid papain-treated meat.

Given the general principle that enzymes derived from normal food sources may be considered as posing no health problems, and the lack of reports of adverse effects of the enzyme other than very occasional cases of allergic reactions, the Committee considers the use of papain as a meat tenderiser, administered to stunned and pithed animals before bleeding, is acceptable from the point of view of the safety of papain *per se*, provided the general provisions in the SCF guidelines on enzymes (CEC, 1992) are followed. The bacteriological safety of the use of papain injection into beef as a meat tenderising procedure should be confirmed by pilot testing under different conditions, representative of actual hygienic conditions of slaughterhouses in the different member states of the EU. The safety of the use of papain-treated meat in the manufacture of fermented meat products with regard to the formation of biogenic amines should also be confirmed by comparison with the same products made from control untreated meat

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European Commission

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

Luxembourg: Office for Official Publications of the European Communities

1997 — VIII, 88 pp. - 16.2 x 22.9 cm

Food science and techniques series

ISBN 92-827-9580-2

Price (excluding VAT) in Luxembourg: ECU 17

The Scientific Committee for Food was established by Commission Decision 74/234/EEC of 16 April 1974 (OJ L 136, 20.5.1974, page 1), replaced by Commission Decision 95/273/EC of 6 July 1995 (OJ L 167, 18.7.1995, page 22), to advise the Commission on any problem relating to the protection of the health and safety of persons arising or likely to arise from the consumption of food, in particular on nutritional, hygienic and toxicological issues.

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

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

The present report deals with opinions on:

— Caustic sulphite caramel

— Ammonia caramel

— Carnauba wax

— Coumarin

— Di-2-ethylhexylphthalate

— Di-2-ethylhexyladipate

— 3-monochloro-propane-1,2-diol (3-mcpd)

— Polyethyleneglycol 6000

— Neutron scanning (interrogation) devices

— Isoascorbic acid

— Acetylated oxidized starch

— Cadmium

— Esters used in plastics for food contact applications

— Papain from papaya fruit used as a meat tenderizing agent