The EFSA Journal (2006) 415, 1-10

Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the Commission related to

Use of formaldehyde as a preservative during the manufacture and preparation of food additives

Question Nº EFSA Q-2005-032

Adopted on 30 November 2006

SUMMARY

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food has been asked by the European Commission to issue an opinion on the safety in use of formaldehyde used as a preservative during the manufacture of food additives.

Formaldehyde has been reported to be used as an antimicrobial agent (preservative) in the production of carrageenan and alginates to avoid depolymerisation of raw material, and as an antioxidant and bleaching agent during the manufacturing of alginates.

Although the use of formaldehyde in the production of alginate and carrageenan is being phased out by some manufacturers, a maximum residue level of up to 50 mg/kg of formaldehyde in alginic acid and its salts has been requested by other manufacturers. No residue limit for formaldehyde in these additives has previously been evaluated for safety and therefore no residue limit is included in the current legislation for purity criteria of these additives.

The estimated dietary exposure to residual formaldehyde in alginates and carrageenan, based on data from the USA on the consumption of ice cream and ready to eat dairy dessert cream, examined by the Panel, is between 400 and 70 times lower than the Tolerable Daily Intake (TDI) value of 150 µg/kg bw set by the World Health Organisation (WHO) for drinking water.

No information was available to the Panel on residual formaldehyde in other gelling additives or on their actual use levels. However, the Panel considered an extreme worst case exposure scenario which assumes that an adult could eat 1 kg of food per day containing 2 % of any gelling agent containing 50 mg formaldehyde/kg. This exposure scenario would also include the uses of alginate and carrageenan outlined above. Under these conditions formaldehyde exposure levels would be 1 mg per person per day or for a 60 kg individual approximately 17 µg/kg bw/day, assuming an exposure to 1 kg of food per day containing gelling additives.

The estimated dietary exposure levels arising from this worst case exposure scenario would still be approximately 9 times lower than the TDI value of 150 µg/kg b.w. set by the WHO.

The Panel examined recent and previous evaluations of formaldehyde and concluded that there is no evidence indicating that formaldehyde is carcinogenic by the oral route. Considering that the potential dietary exposures estimates remain low compared to the toxicological reference values outlined above and that no systemic exposure to formaldehyde is to be expected at the estimated residual levels, the Panel estimates that exposure to gelling additives containing residual formaldehyde at the levels of 50 mg/kg of additive would be of no safety concern.

KEY WORDS

Formaldehyde, processing aid, food additives, preservative, CAS no. 50-00-0.

BACKGROUND

France informed the Commission in September 2004 that high levels of formaldehyde had been detected in some samples of gelling additives. After investigation with the manufacturers of these additives it is clear that formaldehyde is commonly used as a preservative during the manufacture of carrageenan and alginates and other similar additives. However, it appears that this use was not apparent when these additives were originally evaluated for safety and as a result no residue limit for formaldehyde is included in the relevant specific purity criteria.

The formaldehyde is added to the seaweed to prevent spoilage and as an alternative to total dehydration which the manufacturers avoid due to its high cost. The seaweed is then stored and processed in the appropriate manner. A manufacturer has requested a maximum residue level be set of 50 mg/kg in the additive and has provided data showing that analysis of batches of alginate (on the wet product) has consistently produced results below the detection limit (<20 mg/kg).

Formaldehyde in the form of hexamethylene tetramine (E 239) is currently permitted to be used in *Provolone* cheese as controlled by European Parliament and Council Directive 95/2/EC at a maximum level of 25 mg/kg residual amount, expressed as formaldehyde. Commission Directive 2002/72/EC for plastic materials in contact with food also sets a specific migration limit of 15 mg/kg for formaldehyde.

TERMS OF REFERENCE

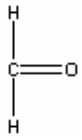
The Commission asks EFSA to issue an opinion on the safety in use of formaldehyde used as a preservative during the manufacture of food additives.

ASSESSMENT

CHEMISTRY AND TECHNICAL DATA

Formaldehyde

Synonyms: methanal, methylen oxide, oxymethylene, methylaldehyde, oxomethane
Chemical name: formaldehyde
CAS Registry Number: 50-00-0
Chemical formula: CH_2O
Chemical structure:



Description: Colourless gas at room temperature

Formaldehyde is typically used in aqueous solutions (formalin, formol or as its precursor hexamethylene tetramine) containing 30 to 50% formaldehyde. Methanol or other substances are typically added as stabilizers to reduce polymerisation, the former at concentrations up to 15%. In solid form formaldehyde is available as trioxane $(CH_2O)_3$ and paraformaldehyde, its polymer with 8-100 units of formaldehyde (IPCS 1989).

NATURAL OCCURENCE OF FORMALDEHYDE IN FOOD

Formaldehyde is found naturally at low levels in a wide range of foods such as fruits, vegetables, mushrooms and seafoods (IPCS, 1989; HEXPOC, 2005). It is also a normal product of human metabolism. In this assessment it is assumed that any residual formaldehyde in gelling additives remains unreacted when the gelling agent is added to food. This gives a conservative estimate of potential dietary exposure.

USE OF FORMALDEHYDE IN PRODUCTION OF ALGINIC ACID AND ITS SALTS

During purification of alginic acid, fresh brown seaweed is soaked in a 3% formaldehyde solution (based on seaweed dry matter), drained and stored for processing (Technical information document, 2004a). A further pre-treatment in a 2% formaldehyde solution is carried out to remove seaweed pigments. No further use of formaldehyde during the production process of alginates is reported. These treatments are followed by grinding and washing in acid water before production of alginates salts, which are then dried, milled and blended before packaging and dispatching (Technical information document, 2004a). The alginic acid extraction process involves washing, precipitation and drying steps of seaweeds extracts that should eliminate to some extent residual formaldehyde.

The main technological need for the use of formaldehyde in alginate production is to avoid microbial growth and spoilage of raw material stored during the seaweeds

harvesting season, extending from mid-May to mid-September (Technical information document, 2004a,b).

USE OF FORMALDEHYDE IN PRODUCTION OF CARRAGEENAN

A level of 5-10 kg formaldehyde/metric tonne of seaweed, without further details, has been reported in the production of high viscosity carrageenan (Technical information document, 2004b).

USE OF FORMALDEHYDE IN OTHER GELLING ADDITIVES

No specific information was available on the use of formaldehyde during production of other gelling additives. It has been reported by one manufacturer that formaldehyde is also used in Europe as preservative in the production of karaya-gum, karaya-flour, guar flour, and as a polyphenol-blocking agent in the production of karaya-gum.

FORMALDEHYDE RESIDUES LEVELS IN GELLING ADDITIVES

Measurements of residual formaldehyde in alginic acid produced in one factory during the period of June to October show that none of the analysed batches (70 batches) exceeded the limit of detection of the analytical method <20 mg/kg (AOAC method 931-08). Similar findings (<20 mg/kg) were reported for sodium alginate (46 batches tested), alginate blends (8 batches), potassium alginate (1 batch) and calcium alginate (1 batch) although the number of batches tested in the latter were less.

No data was available on residual levels of formaldehyde from other manufactured gelling additives (i.e. carrageenan, locust bean gum, guar gum, karaya-gum, etc).

ASSESSMENT OF EXPOSURE FROM THE USE OF GELLING ADDITIVES

Gelling additives covered by this opinion are authorised in foods following the *quantum satis* principle. This allows the use of an additive in most foodstuffs following good manufacturing practices, at a level not higher than is necessary to achieve the intended purpose. The Panel had precise data only on the levels of use of alginate and carrageenan additives used in ice cream and dairy dessert cream.

The consumption of alginate (without further specification) was estimated by a petitioner based on USA consumption data for ice cream (Technical information document, 2004b). A consumption volume of 240 ml/day of ice cream, corresponding to 120 g/day, with 0.35% alginate containing 50 mg/kg of residual formaldehyde was chosen as a conservative hypothesis. Under these conditions potential dietary exposure to formaldehyde was estimated to be 21 µg/day (0.35 µg/kg bw/day for a 60 kg individual).

The consumption of carrageenan was estimated by a petitioner based on USA consumption data on ready to eat dairy dessert cream (Technical information document, 2004b). A maximum consumption of 500 g/day of ready to eat dairy dessert cream, with 0.5% carrageenan containing 50 mg/kg of residual formaldehyde was chosen as a conservative hypothesis. Under these conditions potential dietary exposure to formaldehyde was estimated to be 125 µg/day (2.1 µg/kg bw/day for a 60 kg individual).

No information was available to the Panel on residual formaldehyde in other gelling additives or on their actual use levels. Therefore, to estimate potential formaldehyde exposure arising from these sources the Panel considered an extreme worst case exposure scenario which assumes that an adult could eat 1 kg of food per day containing 2 % of any gelling agent containing 50 mg formaldehyde/kg of additive. This exposure scenario would also include the uses of alginate and carrageenan outlined above. Under these conditions formaldehyde exposure levels would be 1 mg per person per day or for a 60 kg individual approximately 17 µg/kg bw/day, assuming an exposure to 1 kg of food containing gelling additives.

TOXICOLOGICAL EVALUATION

The present evaluation focuses on formaldehyde residues arising from its use as a processing aid during the production of some gelling additives. It is not the aim of this opinion to re-evaluate the toxicology of formaldehyde but rather to identify toxicological reference values pertaining to oral exposure. Extensive reviews on the toxicity of formaldehyde can be consulted elsewhere (EHC, 1989; CICAD, 2002; IARC, 2004; BfR, 2006).

Upon oral ingestion, formaldehyde in the blood is metabolised to formic acid within 90 seconds and half the ingested [^{14}C] radio-labelled dose is excreted within 12 hours through exhalation as carbon dioxide and via the urine and faeces in rats (BfR 2006). Remaining radioactivity can be found in several tissues, most probably due to metabolic incorporation into the single carbon pool and subsequent incorporation into biological macromolecules (BfR, 2006). In several mammalian species, including humans, formaldehyde concentrations in blood after exposure are comparable to physiological blood-levels (~ 0.1 mM) indicating that systemic availability of formaldehyde is low (BfR, 2006). Due to its high chemical reactivity and to its rapid cellular metabolism in lining cells, local effects of formaldehyde seem to play a more important role compared to systemic effects (BfR, 2006).

Carcinogenicity evaluations

Inhalation exposure:
There is strong evidence that formaldehyde causes tumours in the nasal epithelium of experimental animals (EHC, 1989; CICAD, 2002; IARC, 2004; BfR 2006). The mechanism by which formaldehyde induces nasal tumours is not fully understood. The weight of evidence from animal studies and human data supports the hypothesis that sustained cytotoxicity and regenerative cell proliferation are causal mechanisms in formaldehyde nasal carcinogenicity (CICAD, 2002; EHC, 1989). There is a correlation between the predominant non-neoplastic effects (cytotoxicity and increased cell proliferation), hyperplastic/dysplastic lesions and the most frequent tumour type (squamous cell carcinoma), concerning target epithelium and anatomical locations in the nasal cavity (BfR, 2006). Regenerative cell proliferation following formaldehyde-induced cytotoxicity might increase DNA replication and the likelihood of DNA replication errors resulting in mutations (CICAD, 2002). This mechanism may encompass a threshold-mediated response (Bolt, 2003).

Oral exposure:
The relevant long-term studies using oral administration of formaldehyde are discussed below.

Formaldehyde administered in drinking-water to groups of 20 male and female Wistar-rats for up to 104 weeks did not result in higher incidence of tumours compared to controls (Tobe *et al.*, 1989). Formaldehyde doses administered were 0, 0.02, 0.1 and 0.5 % (equivalent to 0, 10, 50 and 300 mg/kg bw/day). Non-neoplastic lesions (erosions and/or ulcers) in the forestomach and glandular stomach were observed in both sexes of the highest dose group (0.5 %). Squamous cell hyperplasia, hyperkeratosis and downward growth of basal cell were observed in the forestomach of the highest dose group whereas few of these changes were observed in the 0.1 % exposed group. A no-observed-adverse-effect level (NOAEL) of 0.02 % (10 mg/kg bw/day) based on these changes was identified.

Formaldehyde administered in the drinking-water to groups of 70 male and female Wistar-rats for up to 104 weeks did not result in significantly higher incidence of tumours compared to controls (Til *et al.*, 1989). The mean formaldehyde doses administered were 0, 1.2, 15 and 82 mg/kg bw/day for males, and 0, 1.8, 21 and 109 mg/kg bw/day for females. At the highest doses (82 and 109 mg/kg bw/day) formaldehyde caused gastric changes leading to severe damage to the gastric mucosa, including focal papillary epithelial hyperplasia and focal hyperkeratosis in the forestomach and chronic atrophic gastritis, focal ulceration and glandular hyperplasia in the glandular stomach. There was no evidence of a dose-related response. A NOAEL of 15 mg/kg bw/day based on the gastric changes was identified.

Formaldehyde administered in drinking-water to groups of 50 male and female Sprague-Dawley rats for up to 104 weeks, at the beginning of the experiment at concentrations of 10, 50, 500, 1000, 1500 mg/L (at high doses the intake of liquids decreased in male rats) was reported to induced haemolymphoreticular neoplasias (histiocytomas, lymphomas, leukaemia), malignant mammary tumours, testicular intersitial cell adenomas and to increase the total number of tumours (Soffritti *et al.*, 2002). In this study animals were observed for their entire life (up to 163 weeks). According to the authors no differences were observed in daily feed consumption, bw, and behaviour or survival rates between the groups and in non-oncological pathological treatment related changes.

However, several aspects of this study might limit the validity of the data and thus the conclusions that can be drawn. Doses were not corrected for decreased water uptakes or body weights and no data were presented on the incidence of tumour related mortalities and deaths associated with other non-neoplastic causes (i.e. unexpected infections or high mortality rates in controls) (BfR, 2006). Contrary to others studies (Til *et al.*, 1989, Tobe *et al.*, 1989) no local toxicity of formaldehyde (i.e. focal hyperkeratosis in the forestomach, chronic atrophic gastritis, focal ulceration in the glandular stomach) was reported in treated rats even at the highest dose tested (1500 mg/L). In this study only haemolymphoreticular neoplasias showed a dose-response relationship that while showing a positive trend in both sexes starting at 50 mg/kg bw/day, was nevertheless very flat over a wide dose range. However, the lack of data on background incidence of chronic inflammatory changes in the lungs of treated rats does not allow to evaluate the influence of chronic respiratory diseases in the development of lymphomas/leukemias, a

well known inducing factor (EFSA, 2006). Indeed, the lack of appreciation of this infectious parameter was identified as a major confounder in a recent review of a long-term study from the same laboratory using the same rat colony (EFSA, 2006). Moreover, data presented in Soffritti *et al.* (2002) would come from its own re-evaluation of a previous study on formaldehyde carried out by the authors in which the incidence of haemopoietic neoplasias were nearly half those reported in 2002 (BfR, 2006). The lack of explanation for this discrepancy raised concerns on the study's validity and credibility (BfR, 2006).

Evaluation of these studies indicates that currently, there is no definitive evidence to indicate that formaldehyde is carcinogenic when administered orally to laboratory animals (CICAD, 2002; BfR, 2006). Other evaluations have concluded more recently that the overall weight of evidence on systemic carcinogenicity of formaldehyde in animals is insufficient to indicate that formaldehyde has the potential to induce tumours of the haemopoietic system or the gastrointestinal tract after oral intake (BfR, 2006). Consistent with the cytotoxic effects seen at sites of contact for the inhalation route (see above), repeated oral administration of formaldehyde induces erosion/ulceration effects in the forestomach and glandular stomach and hyperplasia of the limiting ridge and glandular stomach (BfR, 2006). It can be concluded that such a mechanism may also encompass a thresholded response.
Reviews have concluded that Formaldehyde does not affect reproduction or gestational development parameters (IPCS, 1989; CICAD, 2002).

Genotoxicity evaluations

Safety evaluations have concluded that formaldehyde is genotoxic *in vitro* in both bacterial and mammalian cells (EHC, 1989; BfR, 2006; CICAD, 2002). Different *in vitro* genotoxic effects such as structural chromosomal aberrations, sister-chromatid exchanges, gene mutations, DNA strand breaks, DNA protein crosslinks, and DNA repair deficiencies have been demonstrated in mammalian cells exposed to formaldehyde (BfR, 2006). However, due to the high reactivity and efficient cellular metabolism of formaldehyde, any *in vivo* genotoxicity potential may be limited to directly exposed tissues and may not be readily observed in more distant tissues (BfR, 2006).

Most available *in vivo* genotoxicity studies dealing with formaldehyde focus on "local and/or systemic genotoxicity" animal models with exposure by inhalation. Examination of results and of the experimental conditions in more that 40 published *in vivo* genotoxicity studies indicates that any genotoxic potential of formaldehyde is localised to the immediate site of contact and is not expressed systemically in experimental animals. In human studies, local and systemic genotoxicity of formaldehyde is difficult to assess mainly because all human studies available considered inhalation exposure to formaldehyde and suffer from lack of description on levels of exposure and lack of information on co-exposures and other confounders (BfR, 2006).

As regards formaldehyde toxicity after inhalation, it was concluded (BfR, 2006) that human studies were not reliable to derive clear conclusions on the genotoxic potential of formaldehyde. Nevertheless it was considered, based on animal data from genotoxicity in the respiratory tract after inhalation, that formaldehyde can exhibit a genotoxic potential in directly exposed tissues in mammals and man; although no reliable data on

dose-effect relationships could be derived (BfR, 2006). Similar conclusions have been drawn from other evaluations in which, based on results from animal studies and on epidemiological studies in occupationally exposed populations, it was concluded that upon inhalation formaldehyde is weakly genotoxic at the site of contact with less evidence for distal effects (CICAD, 2002). Thus an extensive database in both humans and animals appears to confirm that critical effects attributed to formaldehyde occur at the initial site of exposure (CICAD, 2002; BfR, 2006).

Existing toxicological reference values by oral exposure

The WHO derived a drinking-water guideline value for formaldehyde of 900 µg/litre based on a tolerable daily intake (TDI) value of 0.15 mg/kg bw/day calculated from a NOAEL of 15 mg/kg bw/day in a 2-year study in rats causing stomach irritation and papillary hyperplasia associated with severe irritation (WHO, 1993). An allocation of 20% of the TDI to drinking water was used to derive the guideline value. This guideline value was confirmed in a more recent evaluation (WHO, 2004).

The US Environmental Protection Agency (EPA) derived a chronic oral exposure reference dose (RfD) of 0.2 mg/kg bw/day from the NOAEL of 15 mg/kg bw/day in the same 2-year study in rats (IRIS, 1990). The critical effects retained in the EPA evaluation where reduced weight gain, chronic atrophic gastritis, focal ulceration and glandular hyperplasia of the stomach found in the high-dosage groups (82 mg/kg bw/day).

Reference values from WHO and EPA evaluations were derived from the study of Til *et al*. (1989) mentioned before.

CONCLUSION

The Panel examined recent and previous evaluations of formaldehyde and concluded that there is no evidence indicating that formaldehyde is carcinogenic by the oral route.

The estimated dietary exposure to residual formaldehyde in alginates and carrageenan arising from data in USA on the consumption of ice cream and ready to eat dairy dessert cream are between 400 and 70 times lower than the TDI value of 150 µg/kg bw set by the WHO for drinking water.

No information was available to the Panel on residual formaldehyde in other gelling additives or on their actual use levels. However, the Panel considered an extreme worst case exposure scenario which assumes that an adult could eat 1 kg of food per day containing 2 % of any gelling agent containing 50 mg formaldehyde/kg. This exposure scenario would also include the uses of alginate and carrageenan outlined above. Under these conditions formaldehyde exposure levels would be 1 mg per person per day or for a 60 kg individual approximately 17 µg/kg bw/day, assuming an exposure to 1 kg of food per day containing gelling additives.

The estimated dietary exposure levels arising from this worst case exposure scenario would still be approximately 9 times lower than the TDI value of 150 µg/kg bw set by WHO.

Considering that the potential dietary exposures estimates remain low compared to the toxicological reference values outlined above and that no systemic exposure to formaldehyde is to be expected at the estimated residual levels, the Panel estimates that exposure to gelling additives containing residual formaldehyde at the levels of 50 mg/kg of additive would be of no safety concern.

DOCUMENTATION PROVIDED TO EFSA

Technical information on the use of formaldehyde in the production process of alginic acid and its salts. Danisco, December 2004a.

Technical information on formaldehyde. Degussa, December 2004b.

REFERENCES

BfR (2006). Schulte A, Bernauer U, Madle S, Mielke H, Herbst U, Richter-Reichhelm H-B, Appel K-E, Gundert-Remy U. Assessment of the carcinogenicity of formaldehyde [CAS No. 50-00-0]. Bundesinstitut für Risikobewertung. Berlin. http://www.bfr.bund.de/cm/238/assessment_of_the_carcinogenicity_of_formaldehyde.pdf

Bolt (2003). Bolt HM. Genotoxicity-threshold or not? Introduction of cases of industrial chemicals. *Toxicological letters* 140-141: 43-51, 2003.

CICAD (2002). Concise International Chemical Assessment Document 40. Formaldehyde. World Health Organisation. Geneva.

EC (1995). Directive 95/2/EC of 20 February 1995 amended.

EC (1996). Directive 96/77/EC of 2 December 1996 amended.

EC (2002). Directive 2002/72/EC of 2002.

EFSA(2006). Opinion of the Scientific Panel on Food Additives, Flavourings, Processing aids and Materials in contact with Food (AFC) on a new long-term carcinogenicity study on aspartame. *The EFSA Journal* 356: 1-44.
http://www.efsa.europa.eu/en/science/afc/afc_opinions/1471.html

EHC (1989) International Programme on Chemical Safety Environmental Health Criteria 89 Formaldehyde – WHO ISBN 92 4 154289 6
http://www.inchem.org/documents/ehc/ehc/ehc89.htm

HEXPOC (2005) Human Exposure Characterization of Chemical Substances; quantification of exposure routes – European Commission. Joint Research Centre. Institute for Health and Consumer Protection. Physical and Chemical Exposure Unit.

Ispra (VA) Italy - Yuri Bruinen de Bruin, Dimitrios Kotzias and Stylianos Kephalopoulos
http://www.jrc.ec.europa.eu/pce/documentation/eur_reports/report_EUR21501en2005.pdf

IARC (2004). Formaldehyde, 2-butoxyethanol and 1-*tert*-Butox-2-propanol. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 88: 2-9.

IPCS (1989). International Program on Chemical Safety. Environmental Health Criteria 89. Formaldehyde. World Health Organisation. Geneva.

IRIS (1990). Integrated Risk Information System. U.S. Environmental Protection Agency. http://punix1.sradev.com/iris/iris_dev/subst/0419.htm

Soffritti *et al*. (2002). Soffritti M, Belpoggi F, Lambertini L, Lauriola M, Padovani M, Maltoni C. Results of long-term experimental studies on the carcinogenicity of formaldehyde and acetaldehyde in rats. *Ann. N.Y. Acad. Sci*. 982: 87-105.

Til *et al.* (1989). Til HP, Woutersen RA, Feron VJ, Hollanders VHM, Falke HE. Two-year drinking water study of formaldehyde in rats. *Fd. Chem. Toxic.* 27: 77-87.

Tobe *et al.* (1989). Tobe M, Naito K, Kurokawa Y. Chronic toxicity study on formaldehyde administered orally to rats. *Toxicology* 56: 79-86.

WHO (1993) Guidelines for drinking-water quality. Second Edition. World Health Organisation. Geneva. p. 98.

WHO (2004) Guidelines for drinking-water quality. Third Edition. World Health Organisation. Geneva. p. 194.
http://www.who.int/water_sanitation_health/dwq/gdwq3/en/

SCIENTIFIC PANEL MEMBERS

Fernando Aguilar, Herman Autrup, Sue Barlow, Laurence Castle, Riccardo Crebelli, Wolfgang Dekant, Karl-Heinz Engel, Natalie Gontard, David Gott, Sandro Grilli, Rainer Gürtler, John Chr. Larsen, Catherine Leclercq, Jean-Charles Leblanc, F. Xavier Malcata, Wim Mennes, Maria Rosaria Milana, Iona Pratt, Ivonne Rietjens, Paul Tobback, Fidel Toldrá.