European Food Safety Authority

EFSA Journal 2014;12(6):3696

SCIENTIFIC OPINION

Scientific Opinion on the re-evaluation of hexamethylene tetramine (E 239) as a food additive [1]

EFSA Panel on Food additives and Nutrient Sources added to Food (ANS)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Hexamethylene tetramine (HMT) is a food additive, currently only permitted in EU for use in Provolone cheese. The maximum permitted level is 25 mg/kg residual amount, expressed as formaldehyde, the break down product of HMT under acidic conditions. HMT has been previously evaluated by the Joint Expert Committee on Food Additives (JECFA, 1974) who established an ADI of 0.15 mg/kg bw/day based on a reproductive study with a NOEL of 15 mg/kg bw/day. Due to the limitations in the database the Panel could not identify a critical study and therefore to derive an ADI. However, the Panel noted that the exposure to formaldehyde from HMT of high level consumers of Provolone cheese equalled 18 µg formaldehyde/kg bw/day in adults and could be as high as 87 µg formaldehyde/kg bw/day in children according to a theoretical conservative assumption that all ripened cheese consumed was Provolone cheese. Considering the estimated exposure from the very limited permitted use, the toxicological database on HMT, the data from use of HMT therapeutically, the available oral toxicity and toxicokinetic data of formaldehyde and the magnitude of the potential effect on intracellular formaldehyde levels arising from this use of HMT, the Panel concluded that the use of HMT in Provolone cheese at the MPL of 25 mg/kg residual amount, expressed as formaldehyde, would not be of safety concern. However the Panel considered that any increase in the permitted uses of HMT or increases in the MPL of 25 mg /kg residual amount, expressed as formaldehyde would need detailed assessment which might require new toxicity data as well as use levels and/or an evaluation of its impact on formaldehyde levels in vivo.

KEY WORDS

hexamethylene tetramine (HMT), E 239, CAS Registry Number is 100-97-0, formaldehyde, Provolone cheese

---

[1] On request from the European Commission, Question No EFSA-Q-2011-00458, adopted on 13 May 2014.

[2] Panel members: Fernando Aguilar, Riccardo Crebelli, Birgit Dusemund, Pierre Galtier, David Gott, Ursula Gundert-Remy, Jürgen König, Claude Lambré, Jean-Charles Leblanc, Alicja Mortensen, Pasquale Mosesso, Rose Martin, Dominique Parent-Massin, Agneta Oskarsson, Ivan Stankovic, Paul Tobback, Ine Waalkens-Berendsen, Rudolf Antonius Woutersen, Matthew Wright. Correspondence: fip@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the Working Group B on Food Additives and Nutrient Sources added to Food: Fernando Aguilar, Martin Bakker, Polly Boon, Riccardo Crebelli, Birgit Dusemund, David Gott, Torben Hallas-Møller, Jürgen König, Oliver Lindtner, Daniel Marzin, Alicja Mortensen, Agneta Oskarsson, Iona Pratt †, Paul Tobback, Ine Waalkens-Berendsen, Rudolf Antonius Woutersen for the preparatory work on this scientific opinion and the EFSA staff: Anna Christodoulidou and Federica Lodi for the support provided to this scientific opinion.

† Deceased.

Suggested citation: EFSA ANS Panel (EFSA Panel Food additives and Nutrient Sources added to Food), 2014. Scientific Opinion on the re-evaluation of hexamethylene tetramine (E 239) as a food additive. EFSA Journal 2014;12(6):3696, 39 pp. doi:10.2903/j.efsa.2014.3696

Available online: www.efsa.europa.eu/efsajournal

SUMMARY

The ANS Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluation, reviews and additional literature that became available since then. An additional source of information was the registration dossier provided by industry for HMT under the REACH Regulation 1907/2006, published by the European Chemicals Agency (ECHA, 2011). The Panel noted that not all original studies on which previous evaluations or reviews were based were available for re-evaluation by the Panel.

Specifications for HMT have been defined in Commission Regulation (EU) No 231/2012 and by JECFA (2006). HMT is described as colourless or white crystalline powder. The purity is specified as not less than 99% anhydrous. Under acidic aqueous conditions HMT can yield formaldehyde and ammonia.

HMT (E 239) is currently only permitted for use in Provolone cheese at a maximum level of 25 mg/kg residual amount, expressed as formaldehyde. The formaldehyde released from HMT under acidic conditions or in cheese can react with proteins.

HMT has been previously evaluated by the Joint Expert Committee on Food Additives (JECFA) in 1962, 1965, 1967, 1972 and 1974 (JECFA, 1962, 1965, 1967, 1972 and 1974). JECFA established an ADI of 0.15 mg/kg bw/day based on a reproductive study with a NOEL of 15 mg/kg bw/day (JECFA, 1974). HMT has not been directly evaluated as a food additive by the Scientific Committee on Food (SCF). In 1977, the SCF referred to HMT during its evaluation of the use of formaldehyde in grana padano cheese, since HMT decomposes to form formaldehyde under acidic conditions or in the presence of proteins (SCF, 1977). More recently, TemaNord in 2002 (TemaNord, 2002), the United States Environmental Protection Agency (EPA) in 2006 (EPA, 2006) and the Federal Institute for Occupational Safety and Health (BAuA, Germany) in 2008 (BAuA, 2008) reviewed the safety of HMT.

Under acidic conditions, HMT is converted to formaldehyde, which in turn would be converted into formic acid. Overall, both in animal and human studies, formaldehyde is rapidly absorbed and converted to formic acid. The rate of oxidation of formaldehyde to formic acid was comparable in all animal species, with a half-life of only 1 minute. The elimination half-life of formic acid is reported to vary from 55 minutes in animals to 90 minutes in humans and can be excreted via the kidneys or further oxidised to CO_2 and water (JECFA, 1962, 1965, 1972, 1974; BAuA, 2008). In humans, about 88% of the administered oral dose of 1 g HMT was absorbed within 12 hours and excreted mostly unchanged (about 82% of recovery) in the urine in 24 hours. The maximum serum concentration (35.2 mg/L) after a single dose was achieved within 1 hour and the mean elimination half-life in blood was reported to be 4.3 hours. Approximately 10-20% of an oral dose of HMT is converted to formaldehyde. HMT can pass the placenta and is detectable in breast milk of breastfeeding women; however, no accumulation was reported. Formaldehyde formation from HMT was dependent on pH , formaldehyde generation prior to absorption would be relevant following oral ingestion as the pH of the stomach is acidic and has been estimated as 10-20% of the dose. Further down the gastrointestinal tract, the pH is neutral with nearly no generation of formaldehyde. HMT can also be converted into formaldehyde in urine and the rate of conversion was pH dependent (BAuA, 2008).

Results from animal experiments and limited data in humans indicate that HMT is of very low to moderate acute toxicity.

There is limited information available on the subchronic toxicity of HMT. None of the studies provided data on haematology and clinical chemistry; data on histopathology were limited. However, body weight gain, food consumption, survival, organ weights, gross pathology and histopathology were generally unaffected following exposure to HMT. The only treatment related clinical observation in studies with rats was a yellow staining of the perineal hair in some cases and decreased body weight gain or weight loss in a 15 weeks study in rabbits.

With regards to the genotoxicity, HMT was weakly positive in bacterial gene mutation assays and in an indicator tests in yeast and at high doses in tests for chromosomal aberrations and sister chromatid exchanges in mammalian cells in vitro (BAuA, 2008). In vivo, negative results were obtained in chromosomal aberration tests in mouse bone marrow by the oral route and in the dominant lethal test in mice by i.p. administration, indicating that the genotoxic activity elicited by HMT in vitro is not systemically expressed in vivo. The Panel noted that HMT may be partially converted in the stomach into formaldehyde which, at high doses, is genotoxic in vivo at the site of first contact. In this respect the Panel noted that HMT used as food additive breaks down into formaldehyde during cheese-making and storage, and that *in situ* formed formaldehyde largely reacts with amino groups of milk proteins. Thus, the exposure to formaldehyde resulting from the use of HMT as food additive is expected to be negligible, much lower than resulting from other authorized uses or from normal mammalian metabolism (878-1310 mg/kg bw/day assuming a half -life of 1-1.5 min; EFSA, 2014). Overall, the Panel concluded that the proposed use of HMT as food additive does not raise concern for genotoxicity.

Available information on the chronic toxicity of HMT was limited. None of the studies provided data on haematology and clinical chemistry. Body weight gain, food consumption, organ weights, gross pathology and histopathology were unaffected following exposure to HMT. However, survival rates and growth were significantly decreased in a study in CTM mice treated with 12.5 g HMT/kg bw/day for 30 weeks. The only treatment related clinical observation in studies with rats was occasional yellow staining of the perineal hair. Overall, HMT was not carcinogenic in experimental animals treated at doses up to 2.5 g HMT/kg bw/day (Brendel, 1964; Natvig et al., 1971; Della Porta, 1968; Lijinsky and Taylor, 1977). For all the studies, the NOAELs for HMT corresponded to the highest dose tested. The BAuA (2008) evaluation reported that the existing long-term/carcinogenicity studies on HMT were not in line with the current guidelines on carcinogenicity and/or combined chronic toxicity/carcinogenicity. However, the data submitted were considered useful in assessing the carcinogenic potential of HMT. In addition, considering the negative results from in vivo genotoxicity testing, BAuA concluded that HMT was not considered as carcinogenic for experimental animals (BAuA, 2008). The BAuA evaluation (2008) also reported that one valid cancer study with formaldehyde administered via drinking water to rats did not show an increased tumour incidence in any organ (Til et al., 1989). The Panel concluded that the formation of formaldehyde from HMT should not be of concern with regards to carcinogenicity. The relevant long-term studies using oral administration of formaldehyde have been discussed in the EFSA evaluation in 2006 (EFSA, 2006).

A large set of data have been described on the reproductive and developmental toxicity of HMT in rats, dogs, and human. Overall, the information available is limited, due to the use of low numbers of animals, limited number of reproductive and developmental parameters recorded, and teratogenicity not properly assessed. However, data available indicated that HMT did not present the potential to induce adverse effects on the fertility in rats. Both the EPA (EPA, 2006) and the BAuA evaluations (BAuA, 2008) considered a NOAEL of 1.5-2.5 g HMT/kg bw/day for reproductive toxicity in rats, based on the study by Della Porta et al. (1970). With regards to developmental toxicity, in both rats and beagle dogs adverse developmental effects observed during the postnatal period were preweaning mortality and postnatal growth retardation. The BAuA evaluation (BAuA, 2008) reported NOAEL values for developmental toxicity for rats (Natvig et al., 1971) and dogs (Hurni and Ohder, 1973) of 100 mg HMT/kg bw/day (the highest dose) and 15 mg HMT/kg bw/day, respectively. However, the EPA (2006) evaluation concluded that there are many inconsistencies on the results of the dog study, since the effects were not consistent with the dose levels, and no details have been provided to clarify these inconstancies. Therefore, the EPA (2006) did not take into account the dog study for their risk assessment (only the rat studies by Della Porta, 1970 and Berglund, 1966). In humans, the study by Furness et al. (1974) showed that no treatment-related abnormalities during the pregnancy or the development of the children had been reported. Negative findings were also found in the study by Siffel and Czeisel (1995). However, in the surveillance study by Briggs et al. (1994), 3.8% (of 209 newborn whose mothers had been treated with HMT during the first trimester) showed birth defects (BAuA, 2008). The BAuA evaluation (2008) concluded that, overall, all the studies reported did not sufficiently meet the requirements for a sound risk assessment evaluation with respect to reproductive

and developmental toxicity. The Panel concluded that despite limitations in the database on reproductive and developmental toxicity, the available data were sufficient for evaluating the single permitted use and use levels. The EFSA Opinion (EFSA, 2006) on formaldehyde reported that formaldehyde does not affect reproduction or gestational developmental parameters (IPCS, 1989; CICAD, 2002).

No adverse effects have been reported in patients receiving HMT for long-term prophylaxis or therapy as urinary antibacterial-antiseptic substance at dose levels of 2 to 4 g/day (corresponding to 28 to 57 mg/kg bw/day) for up to 4 weeks (corresponding to a NOAEL of 57 mg/kg bw/day). However, with a higher dose of 8 g/day (corresponding to 114 mg/kg bw/day) over 3 to 4 weeks clinical symptoms such as bladder irritation, painful and frequent micturition, albuminuria and haematuria were reported in some individuals. With regards to the use of HMT as a drug in humans there is no information available on the formation of tumours in the urinary tract or in other organs or tissues (BAuA, 2008).

In humans, skin sensitizing properties of HMT have been reported. Following skin contact acute dermatitis was the main symptom. Other reports described a number of cases in which allergic symptoms of the respiratory system were also reported following HMT exposure. However, in all cases exposure to other chemicals occurred simultaneously, therefore the induction of specific respiratory hypersensitivity by HMT cannot be clearly demonstrated. Regarding data available on effects of HMT on human following occupational exposure (by inhalation or skin contact), human data available do not provide any conclusive information on the association between HMT occupational exposure and cancer in humans, since toxic effects in humans at the workplace have only been reported after repeated exposure to mixtures of several compounds rather than HMT alone (BAuA, 2008).

The estimated mean exposure to HMT (expressed as formaldehyde) via consumption of Provolone cheese was low for the total population: on average 0.3 µg formaldehyde/kg bw/day. For consumers only of Provolone cheese the mean exposure ranged from 5 µg formaldehyde/kg bw/day in toddlers up to 20 µg formaldehyde/kg bw/day in children (95^{th} percentiles: 18 µg formaldehyde/kg bw/day for adults). According to a theoretical conservative assumption that all ripened cheese consumed was Provolone cheese, the highest estimated exposure, using the 95^{th} percentile of consumers only combined with the MPL, equalled 87 µg formaldehyde/kg bw/day in children.

The Panel concluded that due to the limitations in the toxicological database a critical study could not be identified and therefore it was not possible to derive an ADI. The exposure to formaldehyde from HMT of high level consumers (95^{th}) of Provolone cheese equalled 18 µg formaldehyde/kg bw/day in adults and could be as high as 87 µg formaldehyde/kg bw/day in 3-9 year old children according to a theoretical conservative assumption that all ripened cheese consumed was Provolone cheese. These exposures were around 1000 fold lower than formaldehyde exposure corresponding to the human therapeutic doses of 57 mg HMT/kg bw/day not associated with adverse effects in humans. Based on the:

- estimated exposures,
- consideration of the overall toxicological database on HMT,
- oral toxicity and toxicokinetic data of formaldehyde,
- the magnitude of the potential effect on intracellular formaldehyde levels arising from this use of HMT

The Panel concluded that the use of HMT in Provolone cheese at the MPL of 25 mg/kg residual amount, expressed as formaldehyde, would not be of safety concern.

However the Panel considered that any increase in the permitted uses of HMT or increases in the MPL of 25 mg /kg residual amount, expressed as formaldehyde would require detailed assessment which might require new toxicity data as well as use levels and/or an evaluation of its impact on formaldehyde levels in vivo.

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY EUROPEAN COMMISSION

Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union. In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the European Union before 20 January 2009 has been set up under Regulation (EU) No 257/2010[4]. This Regulation also foresees that food additives are re-evaluated whenever necessary in light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU[5] of 2001. The report "Food additives in Europe 2000[6]" submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for the re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with the highest priority.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of the adoption of Regulation (EU) 257/2010 the 2003 Terms of Reference are replaced by those below.

TERMS OF REFERENCE AS PROVIDED BY EUROPEAN COMMISSION

The Commission asks the European Food Safety Authority to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedures and deadlines that are enshrined in Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

[4] OJL 80, 26.03.2010, p19
[5] COM(2001) 542 final.
[6] Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers, TemaNord 2002:560.

ASSESSMENT

1. Introduction

The present opinion deals with the re-evaluation of the safety of hexamethylene tetramine (HMT) (E 239) when used as food additive.

HMT is a food preservative authorised to be used only in Provolone cheese, in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives, at a maximum level of 25 mg/kg residual amount, expressed as formaldehyde (EFSA, 2006).

HMT has been previously evaluated by the Joint Expert Committee on Food Additives (JECFA) in 1962, 1965, 1967, 1972 and 1974 (JECFA, 1962, 1965, 1967, 1972 and 1974). JECFA established an ADI of 0.15 mg HMT/kg bw/day based on a reproductive study with a NOEL of 15 mg HMT/kg bw/day (JECFA, 1974). HMT has not been directly evaluated as a food additive by the Scientific Committee on Food (SCF). In 1977, the SCF referred to HMT during its evaluation of the use of formaldehyde in grana padano cheese, since HMT can decompose to form formaldehyde under acidic conditions or in the presence of proteins (SCF, 1977).

The Panel on Food Additives and Nutrient Sources added to Food (ANS) was not provided with a newly submitted dossier and based its evaluation on previous evaluations, reviews and additional literature that became available. No data was submitted following a public call for data.[7]

2. Technical data

2.1. Identity of the substances

HMT (E 239) is a preservative, its molecular formula is $C_6H_{12}N_4$ and its molecular weight is 140.19 g/mol. The CAS Registry Number is 100-97-0 and EINECS number is 202-905-8. The chemical name is 1,3,5,7-tetraazatricyclo [3.3.1.1^{3,7}]-decane.

The structural formula of HMT is given in Figure 1:

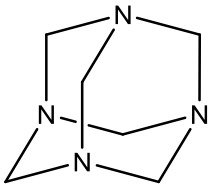

Figure 1: Structural formula of HMT

HMT is a colourless or white crystalline powder. HMT sublimes at temperatures > 250°C (Haynes and Lide, 2010). It is very soluble in water (667 g/L (BAuA, 2008)), soluble in ethanol, acetone and chloroform and slightly soluble in diethyl ether and benzene (Haynes and Lide, 2010). The partition coefficient is -4.15 (BAuA, 2008). It has over 30 synonyms; some of the most common ones are formin, hexamine, urotropin and methenamine.

Up to six molecules of formaldehyde can be released from each molecule of HMT.

[7] Call for scientific data on food additives permitted in the EU and belonging to the functional classes of preservatives and antioxidants. Published: 23 November 2009 and modified on 5 February 2010. Available from: http://www.efsa.europa.eu/en/dataclosed/call/ans091123a.htm

2.2. Specifications

Specifications have been defined by Commission Regulation (EU) No 231/2012[8], JECFA, 2006c; TemaNord, 2002)[9] and by the JECFA (2006, this is the compendium of JECFA specifications incorporating the original HMT specification defined in 1973). Metals and arsenic specifications were revised at the 63rd JECFA (2005). These are detailed in Table 1.

Table 1: Specifications for HMT (E 239) according to Commission Regulation (EU) No 231/2012 and by JECFA (JECFA, 2006)

	Commission Regulation (EU) No 231/2012	**JECFA (2006)**
Description	Colourless or white crystalline powder	Nearly odourless, colourless lustrous crystals, or white crystalline powder
Assay	Not less than 99% on the anhydrous basis	Not less than 99.0% on the dried basis
Identification		
Solubility	-	Freely soluble in water and soluble in ethanol
Formaldehyde test	Positive	Heat a 1 in 10 solution of the sample with dilute sulphuric acid TS. Formaldehyde is liberated, recognizable by its odour and by its darkening of paper moistened with silver ammonium nitrate TS.
Ammonia test		Passes test
Sublimation point	Approximately 260°C	-
Purity		
Loss on drying	No more than 0.5% after drying at 105°C in vacuum over P_2O_5 for two hours	No more than 2.0% over P_2O_5 for four hours
Sulphated ash	Not more than 0.05%	No more than 0.05% Test 2 g of the sample (method I)

[8] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p 1-295

[9] Commission Directive 2008/84/EC of 27 August 2008 laying down specific purity criteria on food additives other than colours and sweeteners. OJ L 253, 20.9.2008, p.1

	Commission Regulation (EU) No 231/2012	JECFA (2006)
Sulphate	Not more than 0.005% expressed as SO_4	-
Chlorides	Not more than 0.005% expressed as Cl	-
Ammonium salts	Not detectable	Colour comparison with a reference standard should not be darker.[a]
Arsenic	Not more than 3 mg/kg	-
Lead	Not more than 2 mg/kg	No more than 2 mg/kg
Mercury	Not more than 1 mg/kg	-

(a): Add 1 mL of Nessler's reagent TS to 10 mL of a 5% solution of the sample. The mixture should not be darker than a mixture of 1 mL of the reagent in 10 mL of water.

The Panel noted that the European Pharmacopeia for use of HMT as a pharmaceutical contains a limit for free formaldehyde in HMT (50 mg/kg).

There are a number of differences between the EU and JECFA specifications;

- The 'loss on drying' is no more than 0.5% (2 hours) and not more than 2.0% (4 hours) in the EU and JECFA specifications respectively.
- No limits for sulphate and chloride content in the JECFA specification are provided.

2.3. Manufacturing process

A process for hexamethylenetetramine production was described by Meissner et al. (1954). In this process, HMT is produced by a direct addition reaction between formaldehyde and ammonia in the gaseous phase. The reaction slurry obtained after the addition reaction is subjected to a continuous centrifugation in which the crystals are separated, washed and dried. In the process crystallised HMT is produced continuously (purity 98 %).

To separate the very small amount of side products formed in the reaction, the mother liquor from the reactor is passed over adsorption filters and continuously purified. The process is further detailed in the paper cited above.

Other production processes have been described in literature (Smolin & Rapoport, 1959; Dan et al. 2011; Kovac Kralj; 2013; Taghdiri & Zamani, 2013).

2.4. Methods of analysis in food

Several methods have been reported in the literature for the detection of HMT in food. A bioassay uses *Staphylococcus aureus*, a yoghurt producing lactic acid organism and 2,3,5-triphenyltetrazolium chloride as indicator of bacterial growth (Kotter, 1959), steam distillation has been used followed by colour reaction (Dumitrescu, 1975) and chromatographic methods (Kovacs and Denker, 1962; Sandoval, 1960). Infrared (IR) spectroscopy has been used for the detection (Paseiro Losada et al., 1989). Colorimetric methods for the determination of hexamethylene tetramine amongst other preservatives have been also reported (Engst, 1969). One of the most recent methods reported in the

literature is a simultaneous determination of methylene blue, HMT and resorcinol in pharmaceutical formulations by first derivative UV spectroscopy (Onur and Acar, 1992). Potentiometric titration has been used for the determination of HMT in the presence of large quantities of urea, formaldehyde and ammonium hydroxide (Ganatra et al., 1998).

2.5. Reaction and fate in food

HMT is unstable under acidic conditions.

Hutschenreuter (1956), when studying the fate of HMT in fish marinades, found that under acidic conditions HMT decomposes with the formation of formaldehyde and ammonia and that the decomposition of HMT is enhanced when protein material (in casu fish proteins) is present. This is stated to be due to the rapid reaction of formaldehyde with amino acids (such as histidine or tryptophan) present in proteins.

In a review by Restani and Galli (1991) on the oral toxicity of formaldehyde it is stated that HMT liberates formaldehyde in the stomach under acidic conditions. HMT decomposes gradually yielding ammonia and formaldehyde which are stated to be normal body constituents. It is further indicated that formaldehyde is a very reactive compound and that it reacts with different macromolecules such as proteins and nucleic acids.

Formaldehyde in food systems such as cheese subsequently reacts and can form a variety of products such as spinacine (Restani and Galli, 1992). The Panel noted that the latter paper contains determinations of tolerance level of spinacine in cheese which is not supported by experimental evidence.

2.6. Case of need and proposed uses

Maximum Permitted Levels (MPLs) of HMT (E 239) have been defined in the Annex II of Regulation (EC) No 1333/2008[10] on food additives for use in foodstuffs.

Currently, HMT (E 239) is an authorised food additive in the EU in Provolone cheese at a maximum residual allowed use level of 25 mg/kg residual amount, expressed as formaldehyde.

Table 2 summarises foods that are permitted to contain 4-HR (E 586) and the corresponding MPLs as set by Annex II of Regulation (EC) No 1333/2008.

Table 2: MPL of HMT (E 239) in foods according to the Annex II of Regulation (EC) No 1333/2008

Category number	Food	restrictions/exception	Maximum level (mg/L or mg/kg as appropriate)
1.7.2	**Ripened cheese**	only Provolone cheese	25 mg/kg residual amount, expressed as formaldehyde

Commission Regulation No 10/2011 on plastic materials and articles intended to come into contact with food[11] also set a specific migration limit of 15 mg/kg for formaldehyde. HMT (E 239) can be used outside the EU as a preservative in other cheeses (ripened cheeses, including rind) if technologically justified and only within the functions and limit specified (JECFA, 2006).

[10] Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 354, 31.12.2008, p. 16

[11] Commission Regulation (EU) No 10/2011 of 14 January on plastic materials and articles intended to come into contact with food. OJ L 12/1, p 1-89

2.6.1. Actual and reported level of use of HMT (E 239)

Most food additives in the EU are authorised at a specific MPL. However, a food additive may be used at a lower level than the MPL. In the framework of Regulation (EC) No 1333/2008 on food additives and of Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives, EFSA issued a public call for usage level and concentration data in food and beverages on HMT (E 239) in March 2013 with deadline November 2013. Data requested included present use and use patterns (i.e. which food categories and subcategories, proportion of food within categories/subcategories in which it is used, actual use levels (typical and maximum use levels) and concentration data (analytical/monitoring data).

However, no data on HMT (E 239) were received during this call. The below exposure assessment is therefore only based on the MPL of HMT (25 mg/kg) expressed as residual formaldehyde levels.

2.7. Information on existing authorisations and evaluations

HMT (E 239) has been evaluated by JECFA in 1962, 1965, 1967, 1972 and 1974 (JECFA, 1962, 1965, 1967, 1972 and 1974). JECFA established an ADI of 0.15 mg/kg bw/day based on a reproductive study with a NOEL of 15 mg/kg bw/day (JECFA, 1974).

HMT (E 239) has not been directly evaluated as a food additive by the SCF. In 1977, the SCF referred to HMT during the evaluation of the use of formaldehyde in grana padano cheese, since HMT can decompose to form formaldehyde under acidic conditions or in the presence of proteins (SCF, 1977).

HMT (E 239) has also been reviewed by TemaNord (TemaNord, 2002). TemaNord reported that HMT (E 239) was positive in several mutagenicity tests and noted one incidence of increased tumours in one of the long-term studies and recommended a re-evaluation of HMT (E 239).

In 2006, the United States Environmental Protection Agency (EPA) reassessed HMT when used as an inert ingredient in pesticide formulations (EPA, 2006). The EPA concluded that no harmful effects would result from the exposure to HMT when used as an inert ingredient in pesticide formulations also taking into account dietary exposure and all other non-occupational sources of pesticide exposure (EPA, 2006).

Formaldehyde in drinking-water has been considered in the Guidelines for Drinking-water Quality (WHO, 2008). Concentrations up to 30 µg/L have been found in ozonated drinking-water. It was not considered necessary though to set a formal guideline value for formaldehyde in view of the significant difference between the expected concentrations of formaldehyde in drinking-water and the tolerable concentration (WHO, 2008).

The European Food Safety Authority (EFSA) issued two opinions on the safety in use of formaldehyde for poultry (EFSA, 2004) and used as a preservative during the manufacture of food additives (EFSA, 2006). The toxicity of formaldehyde has also been assessed by other international bodies (EHC, 1989; Health Canada, 1999; CICAD, 2002; Afssa, 2004; IARC, 2006; BfR, 2006).

Commission Regulation No 10/2011 on plastic materials and articles intended to come into contact with food[12] also set a specific migration limit of 15 mg/kg for both formaldehyde and HMT. HMT (E 239) can be used outside the EU as a preservative in other cheeses (ripened cheeses, including rind) if technologically justified and only within the functions and limit specified (JECFA, 2006).

2.8. Exposure assessment

Food consumption data used for exposure assessment

[12] Commission Regulation (EU) No 10/2011 of 14 January on plastic materials and articles intended to come into contact with food. OJ L 12/1, p 1-89

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with data from national information on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a).

The food consumption data gathered by EFSA were collected using different methodologies and thus direct country-to-country comparison should be made with caution.

For calculation of chronic exposure, intake statistics have been calculated based on individual average consumption over the total survey period excluding surveys with only one day per subject. High level consumption was only calculated for those foods and population groups were the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011b). The Panel estimated chronic exposure for the following population groups: toddlers, children, adolescents and adults. Calculations were performed using individual body weights.

Thus, for the present assessment, food consumption data were available from 26 different dietary surveys carried out in 17 different European countries as mentioned in Table 3:

Table 3: Population groups considered for the exposure estimates of HMT, expressed as formaldehyde.

Population	**Age range**	**Countries with food consumption surveys covering more than one day**
Toddlers	from 12 up to and including 35 months of age	Bulgaria, Finland, Germany, Netherlands
Children[13]\	from 36 months up to and including 9 years of age	Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden
Adolescents	from 10 up to and including 17 years of age	Belgium, Cyprus, Czech Republic, Denmark, France, Germany, Italy, Latvia, Spain, Sweden
Adults	from 18 up to and including 64 years of age	Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Spain, Sweden, UK
The elderly[13]	from 65 years of age and older	Belgium, Denmark, Finland, France, Germany, Hungary, Italy

Consumption records were codified according to the FoodEx classification system (EFSA, 2011a). Nomenclature from FoodEx classification system has been linked to the Food Classification System as presented in the Annex II of Regulation (EC) No 1333/2008, part D, to perform exposure estimates.

[13] The terms "children" and "the elderly" correspond respectively to "other children" and the merge of "elderly" and "very elderly" in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011b).

2.8.1. Exposure to HMT (E 239) from its use as food additive

Exposure to HMT (E 239) from its use as a food additive in Provolone cheese was calculated using the Maximum Permitted level (MPL) of 25 mg/kg residual amount, expressed as formaldehyde, combined with national consumption data for the five population groups (Table 3).

Provolone cheese is not separately codified in the Food Classification System (FCS) of Annex II of Regulation (EC) No 1333/2008, part D. To avoid an overestimation of the exposure by assuming that the food group 1.7.2 Ripened cheese of the FCS consists of only Provolone cheese, the exposure assessment was performed using the food consumption data of the Comprehensive database codified according to FoodEx. In this classification system, Provolone cheese is codified as such. Examination of this database showed that this cheese is solely codified in the Italian survey. Based on these consumption data an exposure assessment was performed per population group.

Mean exposure to HMT was calculated using the mean consumption of Provolone cheese per population group for both the total population and the consumers only. The high level exposure was calculated per population group using the 95th percentile (P95) of the total population and consumers only. These consumption levels were combined with the MPL of 25 mg/kg residual amount, expressed as formaldehyde. The high level of exposure was only calculated when the number of individuals per population group was at least 60 (EFSA, 2011b).

Table 4 summarises the estimated exposure to HMT, expressed as formaldehyde, from its use as food additive in Provolone cheese in the five population groups living in Italy. The mean exposure to HMT, expressed as formaldehyde, for the total population was similar in all population groups (average 0.3 µg/kg bw/day). For the consumers only, the mean intake of HMT, expressed as formaldehyde, from Provolone cheese was highest in the population group children: 20 µg/kg bw/day. The high level of exposure for consumers only could only be calculated for the adults and equalled 18 µg/kg bw/day (Table 4).

In the absence of data at that level of detail from other EU countries, it was assumed that the whole food group 1.7.2 Ripened cheese of the FCS consists of only Provolone cheese, the exposure can be calculated using the food additives intake model (FAIM), available on the EFSA website (http://www.efsa.europa.eu/en/topics/topic/additives.htm). Using this conservative assumption the highest exposure that could be calculated, using the P95 of consumers only combined with the MPL, equalled 87 µg/kg bw/day in children. Since Provolone cheese is probably only a niche product in many countries this figure is likely to be an overestimate.

Table 4: Summary of anticipated exposure to residual amounts of HMT (expressed as µg/kg bw/day of residual level of formaldehyde), from its use as food additive in Provolone cheese using the MPL in five population groups from survey in Italy:

	Toddlers (12-35 months)	**Children (3-9 years)**	**Adolescents (10-17 years)**	**Adults (18-64 years)**	**The elderly (>65 years)**
No subjects					
Total population	36	193	247	2313	518
Consumers only	2	5	9	73	21
Estimated exposure using MPL					
Total population					
• Mean	0.3	0.5	0.3	0.3	0.4
• High level[14]	-	-	-	-	-
Consumers only					
• Mean	5.0	20	8.3	8.1	9.5
• High level[14]	-	-	-	18	-

2.8.2. Exposure from other sources

Formaldehyde is permitted in Food Contact Materials (FCM) with a specific migration limit of 15 mg/kg (Regulation (EU) No 10/2011). Assuming the daily amount of consumed food which has been in contact with FCM being 1 kg, the additional exposure from FCM is 15 mg/day, which corresponds to 214 µg/kg bw/day for adults (70 kg), 283 µg/kg bw/day for adolescents (53 kg), 652 µg/kg bw/day for children (23 kg) and 1250 µg/kg bw/day for toddlers (12 kg).

Furthermore, HMT is used as a urinary tract antibacterial-antiseptic drug as well as for long-term prophylaxis of urinary tract infections.

2.9. Uncertainty analysis

Uncertainties in the exposure assessment of HMT have been discussed above. According to the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties have been considered and summarised below:

[14] 95th percentile could not be calculated because less than 5% of the Italian population in the database were consumers or because the number of subjects in the sample was less than 60

Table 5: Qualitative evaluation of influence of uncertainties

Sources of uncertainties	Direction(a)
Consumption data: different methodologies / representativeness / under reporting / misreporting / no portion size standard	+/-
Use of data from food consumption survey of few days to estimate long-term (chronic) exposure	+
Use of the MPL as the residual concentration of HMT (expressed as mg/kg of residual level of formaldehyde) present in provolone cheese.	+

(a): + = uncertainty with potential to cause over-estimation of exposure; - = uncertainty with potential to cause underestimation of exposure.

3. Biological and toxicological data

The present opinion summarises the major studies on HMT evaluated by the SCF (1977), JECFA (JECFA, 1962, 1965, 1972, 1974) and reviewed by TemaNord (2002). More recently, HMT has also been reviewed and evaluated by EPA (2006) and BAuA (2008), and these evaluations contain studies not included in the previous evaluations by SCF (1977), JECFA (1962, 1965, 1972 and 1974) and TemaNord (2002). An additional source of information was the registration dossier provided by industry for HMT under the REACH Regulation 1907/2006, published by the European Chemicals Agency (ECHA, 2011). The present document also reports the new literature data.

Since under acid conditions or in the presence of proteins, HMT is converted to ammonia and formaldehyde (Hutschenreuter, 1956), and HMT generated free formaldehyde in the stomach (Malorny & Rietbrock, 1963), the SCF considered toxicological information on formaldehyde and its main metabolite formic acid was relevant to the assessment of HMT (SCF, 1977). The BAuA evaluation (2008) stressed that formaldehyde formation from HMT is strongly dependent on acidic pH values. Therefore, formaldehyde generation would be relevant following oral ingestion as the pH of the stomach is acidic (BAuA, 2008). At the authorised levels of use, the levels of ammonium ion produced would not be relevant for risk assessment.

In 2006, EFSA evaluated the safety of formaldehyde used as a preservative during the manufacture of food additives. The opinion did not re-evaluate the toxicology of formaldehyde *per se* but identified toxicological reference values for oral exposure (EFSA, 2006). The toxicity of formaldehyde has been extensively addressed by other international organizations (EHC, 1989; Health Canada, 1999; CICAD, 2002; Afssa, 2004; IARC, 2006; BfR, 2006).

3.1. Absorption, distribution, metabolism and excretion

3.1.1. In vitro and animal studies

Both JECFA (1962, 1965, 1972, and 1974) and BAuA (2008) described a series of toxicokinetics studies on HMT, formaldehyde and formic acid both in vitro and in animals and the results are summarised below.

3.1.1.1. HMT

It has been reported that under acid conditions, or in the presence of proteins, HMT is converted to ammonia and formaldehyde (Hutschenreuter, 1956; Restani and Galli, 1991), and that HMT also generated free formaldehyde in the stomach (Malorny & Rietbrock, 1963).

Musher et al. (1974) as well as Strom et al. (1993) studied HMT (as methenamine) metabolism in vitro and examined the effect of the pH in the urine on the conversion rate of HMT to formaldehyde (BAuA, 2008). Both groups showed that, under acidic condition, HMT was hydrolyzed to formaldehyde at a rate mainly dependent on pH. Musher et al. (1974) reported that concentrations of formaldehyde higher than 25 µg/mL could be achieved in urine containing more than 0.6 mg HMT/mL at pH ≤ 5.7 or more than 1 mg/mL at pH ≤ 5.85. In another study, the half-life of HMT conversion to formaldehyde was shown to increase approximately 20 fold from 20 hours at pH 5.0 to about 400 hours at pH 6.5 (Strom et al., 1993).

In mice orally administered radioactive HMT (as hexamine), 80% of the radioactivity was excreted in the urine within 3-4 hours, mainly unchanged and partially as free formaldehyde. Only 2% was excreted via the lungs as CO_2. After 22 hours, 4% of the radioactivity was still present in the body. HMT was also found in the liver and bones of the fetuses of pregnant mice (Schlede, 1966).

3.1.1.2. Formaldehyde and formic acid

The EFSA evaluation (EFSA, 2006) summarised information on the toxicokinetics of formaldehyde in animal species taken from the BfR evaluation (BfR, 2006) reporting that, following oral ingestion in rats, formaldehyde in the blood was converted to formic acid within 90 seconds and half the ingested ^{14}C radio-labelled dose was eliminated as CO_2 within 12 hours, and via the urine and faeces in rats (EFSA, 2006). The remaining radioactivity was found in several tissues, possibly due to metabolic incorporation into the single carbon pool and consequent inclusion into biological macromolecules (EFSA, 2006). It was also reported that, in different mammalian species, including humans, levels of formaldehyde in blood were similar to physiological blood-levels (~ 0.1 mM) indicating that systemic availability of formaldehyde was low (EFSA, 2006). Due to its high chemical reactivity and to its rapid metabolism in lining cells, local effects of formaldehyde appeared to play a more significant role compared to systemic effects (EFSA, 2006).

EFSA, 2014, evaluated the oral internal dose of formaldehyde in humans from endogenous production, food-derived from target animals exposed to formaldehyde-treated feed and formaldehyde generated from dietary sources of methanol, including from food additives such as aspartame. Endogenous turnover of formaldehyde was estimated to be approximately 0.61-0.91 mg/kg bw per minute and 878-1310 mg/kg bw per day assuming a half life of 1-1.5 min (EFSA, 2014).

3.1.2. Human studies

3.1.2.1. HMT

The 2008 BAuA evaluation (BAuA, 2008) described a series of toxicokinetics studies on HMT (as methenamine) in human volunteers (BAuA, 2008) and the results are summarised below.

After oral administration of a single 1 g dose of methenamine hippurate to four volunteers, the maximum plasma concentration (70 – 100 µmol/L) was reached within 1 to 2 hours, and the mean elimination half-life was about 4 hours. The average distribution volume was close to the total body water in adults (about 0.6 L/kg). The renal clearance values (mean 71 mL/min) were lower compared to the plasma clearance value (mean 93 mL/min). In cross-over experiments over 6 days, after multiple dosing of tablets and granules of methenamine hippurate (1 g/12 hours), about 80% of the dose administered in each period was recovered in the urine within 12 hours (Allgen et al., 1979). The recovery of HMT in urine was slightly higher from tablets (total mean value = 83 ± 1.9%) than from granules (total mean value = 78 ± 1.8%). Methenamine hippurate (1 g tablets as a single dose) was also administered to 8 healthy pregnant women during labour and for lactational transfer in 4 nursing

mothers. Data showed that in umbilical cord plasma HMT concentrations were initially low, but after 4 hours were about at the same level as that in maternal plasma, indicating that HMT slowly passed the placenta without accumulation in the fetal circulation. However, HMT concentrations in the amniotic fluid were low and varied (between 4 and 63 µmol/L), and there was no correlation to the concentration in umbilical cord or maternal plasma. The authors noted that the study design meant umbilical cord samples were taken at different times post-dosing. The HMT concentrations in breast milk (range 48-52 µmol/L) were also found to be in the same range as in maternal plasma five hours after dosing. Therefore, the authors concluded that no accumulation of HMT occurred in milk, and could be safely given to pregnant and breastfeeding women. The amount of HMT uptake by the child during a respective meal was calculated to be far below the usual therapeutic doses (of 5-10 mg/kg bw) given to adults (Allgen et al., 1979).

In a crossover study (Klinge et al., 1982), two formulations of methenamine hippurate were administered to ten healthy volunteers (6 women and 4 men) as a single dose (1 g) on the first day and then 1 g twice a day for 8 days. After a week of wash out period, the second formulation was administered for another 8 days. The serum maximum concentration of 35.2 mg/L after a single dose was achieved within 1 hour and the mean serum elimination half-life was reported to be 4.3 hours. The distribution volume was 0.56 L/kg, and no accumulation was reported. After a single dose, about 82% was recovered in the urine within 24 hours, and during each 12 hours dosing intervals, about 88% was recovered. In the urine, the average minimum concentration did not go below 150 mg/L (Klinge et al., 1982).

In another cross-over study, Gollamudi et al. (1981) measured the urinary excretion of both HMT and formaldehyde for 48 hours after the oral administration of 10 different HMT formulations (as methenamine or its mandelate or hippurate) containing between 0.439-0.500 g of HMT to ten male human volunteers. It was reported that there were no significant differences among HMT and its various salts on the total excretion of free formaldehyde (ranging from 5.5 to 8.7% of the oral dose) in the urine at 48 hours. However, differences were noted among the different formulations on cumulative excretion of total HMT (varying from 16 to 83% of the oral dose) (Gollamudi et al., 1981).

The BAuA evaluation (BAuA, 2008) reported that there was evidence that approximately 10-20% of an oral dose of HMT was converted to formaldehyde and ammonia in the stomach (Gleckman et al. (1979). In another study, methenamine mandelate was administered to 13 healthy men as oral doses of 1 g, 4 times/day. The average content of free formaldehyde collected in the urine was about 6% (varying from 3.2 to 16.6%) (Gandelman et al., 1967).

Greenfield et al. (1969) examined the urine excretion of formaldehyde in 8 healthy male volunteers receiving orally, on separate days, 390 g of HMT, 1 g-dose of methenamine mandelate (480 mg HMT, 520 mg mandelic acid), methenamine sulfosalicylate (390 mg HMT, 610 mg sulfosalicylate), methenamine hippurate (470 mg HMT, 530 mg hippuric acid) in a 8-hours crossover study. In a second 24-hours crossover study, 4 g methenamine mandelate or methenamine sulfosalicylate, and 2 g of methenamine hippurate were given orally, on separated days, to 4 volunteers. Data showed that peaks levels of free formaldehyde occurred at 2 hours in the 8-hours study, and at 4-6 hours in the 24-hours study. According to the authors, this delay in formaldehyde release (especially marked in the 24-hours study with methenamine mandelate) was mainly due to the different types of formulations which could delay the absorption of the drug until the lower small intestine. In the same study, it was also reported a direct influence of pH on formaldehyde release from HMT (0.5 mg/mL). At pH of 5.4-5.7, 108 µg/mL of formaldehyde was recovered. However, substantial production of formaldehyde (37 µg/mL) occurred also at slightly alkaline pH (8.1-8.2) (Greenfield et al., 1969).

3.1.2.2. Formaldehyde and formic acid

More recently, the BAuA evaluation (2008) reported that, following oral ingestion, formaldehyde can be absorbed and converted to formic acid within 90 seconds. The elimination half-life of formic acid was reported to be 90 minutes. Formic acid can be excreted through the kidney as its sodium salt or is

further oxidised to CO_2 and water (Pandey et al., 2000; BAuA, 2008). The rate of conversion of formic acid to CO_2 and water was reported to be approximately 50% more rapid in rodents than in primates (Pandey et al., 2000; BAuA, 2008). The relative slow metabolism of formic acid in humans can lead to metabolic acidosis, due to accumulation of formic acid (Pandey et al., 2000; BAuA, 2008). The same authors reported two other studies in human. In the first study in which 11 volunteers were given formate intravenously (dose not specified), the average elimination half-life of formate was 55 minutes. In a second study in which 11 volunteers were given formate orally, the half -life was reported to be 45 minutes (dose of 3 g of formate), and 46 minutes (dose of 4.4 g for formate) (Pandey et al., 2000; BAuA, 2008).

3.1.3. Summary on the ADME of HMT and formaldehyde

In summary, under acidic conditions, HMT was converted to formaldehyde, which in turn would be converted into formic acid. Overall, both in animal and human studies, formaldehyde is rapidly absorbed and converted to formic acid. The rate of oxidation of formaldehyde to formic acid was comparable in all animal species, with a half-life of only 1 minute. The elimination half-life of formic acid is reported to vary from 55 minutes in animals to 90 minutes in humans and can be excreted via the kidneys or further oxidised to CO_2 and water (JECFA, 1962, 1965, 1972, 1974; BAuA, 2008). In humans, about 88% of the administered oral dose of 1 g HMT was absorbed within 12 hours and excreted mostly unchanged (about 82% of recovery) in the urine in 24 hours. The maximum serum concentration (35.2 mg/L) after a single dose was achieved within 1 hour and the mean elimination half-life in blood was reported to be 4.3 hours. Approximately 10-20% of an oral dose of HMT is converted to formaldehyde. HMT can pass the placenta and is detectable in breast milk of breastfeeding women; however, no accumulation was reported. Formaldehyde formation from HMT was dependent on pH , formaldehyde generation prior to absorption would be relevant following oral ingestion as the pH of the stomach is acidic and has been estimated as 10-20% of the dose. Further down the gastrointestinal tract, the pH is neutral with nearly no generation of formaldehyde. HMT can also be converted into formaldehyde in urine and the rate of conversion was pH dependent (BAuA, 2008).

3.2. Toxicological data

There are studies in the literature on HMT toxicity via other routes of administration (e.g. inhalation, dermal, subcutaneous). As these routes of exposure were not directly relevant to the toxicity of HMT from food additives, further details on these studies were not considered in this opinion.

3.2.1. Acute oral toxicity

The JECFA (1962, 1965, 1972, 1974), the EPA (2006) and the BAuA (2008) evaluations provided summary information on the acute oral toxicity of HMT and formic acid and its salts, and the summary is presented below (JECFA, 1962, 1965, 1972, 1974; EPA, 2006; BAuA, 2008). A few more studies were also reported in a dossier submitted to ECHA (ECHA, July, 2011. Online access: http://apps.echa.europa.eu/registered/registered-sub.aspx#search).

In rats LD_{50} values of 10 g/kg bw or greater have been reported whilst values in mice were lower at around 2 g/kg bw.

Two case report studies have been found in the literature. Tanaka and Kitajima (1976) reported a case study in which a man suffering from renal disturbance, died of post-operative bleeding accompanied by uremia, apparently related to treatment with HMT. Severe interstitial nephritis (e.g. intense cell infiltration, absence of glomerular involvement and marked softening and enlargement) of the kidney) was reported (Tanaka and Kitajima, 1976). Another more recent case report described a photosensitivity reaction to methenamine hippurate in a 70-year-old woman after taking methenamine hippurate for several years to prevent urinary tract infections. An erythematous and blistering rash on the sun-exposed areas of her face, trunk and upper limbs was reported (Selvaag and Thune, 1994).

3.2.2. Short-term and subchronic toxicity

The BAuA evaluation (2008) describes a few short-term/subchronic studies on HMT, which are summarised below.

Male and female rats (cPah strain, 5/sex) were treated by gavage with 1280 and 1780 mg HMT /kg bw/day respectively (purity unspecified) for 90 days. Haematology and clinical biochemistry parameters were not measured. There was no HMT-induced mortality and no differences from controls in behaviour, body weight gain and food consumption in rats of both sexes treated with HMT. The only clinical sign observed in animals given HMT was a citrus-yellow discolouration of the fur. No differences in macroscopic lesions in the main organs of treated and controls animals were observed. However, no data on HMT-induced histopathological findings were available (Brendel, 1964). The yellow discolouration of fur observed in animals was attributed to a reaction between formaldehyde in the urine and kynurenine in the rat hair (Restani and Galli, 1991).

10-week old Wistar rats (10/sex) received a high concentration of 5.0% HMT (equivalent to 5000 mg/kg bw/day, based on body weights of 250 g in males and 200 g in females and an average water consumption of 10% of the body weight) daily in the drinking water for two weeks with a subsequent 102-week treatment-free period. About half of the rats of both sexes died within one week after treatment. Specific causes of death were not reported. Surviving rats recovered rapidly and showed no toxic effects. The only treatment related clinical observation was a citrus-yellow discolouration of the hair coat which was considered of no toxicological relevance. Data on haematology and clinical biochemistry were not available. Growth, necropsy and histopathology of the treated animals showed no specific changes due to HMT (Della Porta et al., 1968).

The EPA evaluation (EPA, 2006) reported two other studies in mice and rabbits. Mice fed HMT at doses up to 5 g/kg bw for 10 days showed no toxic effects (CIR, 1992). A study in rabbits fed HMT intermittently at a dose of 525 mg/kg bw for 15 weeks showed that HMT induced decreased body weight gain or weight loss (RTECS, 2005 as referenced in EPA 2006).

Short-term toxicity of spinacine.

Short term toxicological studies with spinacine, the most abundant end-product of formaldehyde in cheese (derived from the N-terminal histidine residue in gamma 2-casein) showed a NOAEL of 300 mg/kg bw/day. From these results, the authors suggested a Tolerance Level (TL) of 1800 mg spinacine/kg cheese. The authors concluded that there was no appreciable health risk from consumption of cheese made using formaldehyde (Grana Padano) or HMT (Provolone) (Restani and Restelli, 1992).

In conclusion, there is limited information available on the subchronic toxicity of HMT. None of the studies provided data on haematology and clinical chemistry; data on histopathology were limited. However, body weight gain, food consumption, survival, organ weights, gross pathology and histopathology were generally unaffected following exposure to HMT. The only treatment related clinical observation in studies with rats was a yellow staining of the perineal hair in some cases and decreased body weight gain or weight loss in a 15 weeks study in rabbits.

3.2.3. Genotoxicity

Previous evaluations

The JECFA evaluation (JECFA, 1974) reported that both HMT (Auerbach, 1951) and formaldehyde (Rapoport, 1946 as reported in JECFA 1974) were shown to be mutagenic in *Drosophila melanogaster*.

In 1991 it was reported that HMT did not act as a clastogen on *Vicia faba* roots and V79 cells but increased the frequency of sister-chromatid exchanges in V79 cells in the absence of metabolic

activation (Girmanova et al., 1991). HMT was found positive in Ames test in *S. typhimurium* strains TA 98 and TA 100 without metabolic activation systems and to be DNA-damaging in the rec assay on *Bacillus subtilis* spores strain H17 and M45 in absence of metabolic activation. High doses of HMT were reported to induce chromosomal aberrations both in mouse lymphocyte cultures and in human HeLa cell line. Moreover, morphological transformation was induced in baby hamster kidney cells. Mutagenic effects were not observed in a dominant lethal test in C3H mice (Loeper and Berzins, 1995).

The EPA evaluation (EPA, 2006) reported a study by Orstavik and Honglso (1985) showing that extracts of a sealer compound (AH26), which contains also 25% of HMT, were mutagenic in strains TA 100 in a dose-dependent manner, with or without S9 metabolic activation. However, HMT was negative for mutagenicity when tested individually (Orstavik and Honglso, 1985).

The Panel noted that some of the test systems considered in previous evaluations, i.e. cytogenetic tests in plant cell, the *rec* assay in bacteria, and cell transformation in BHK cells, have received insufficient validation or are considerd unreliable for genotoxicity assessment.

More recently, the BAuA reported a series of in vitro and in vivo studies with HMT (BauA, 2008), whose results are summarised below:

In vitro assays with bacteria

In bacterial gene mutation assays with HMT, weak positive effects with and without S-9 mix were reported for *S. typhimurium* strains TA 97, TA 98 and TA 100 (approx. 2-fold increases compared to control values) only at high concentrations, *viz*. from 10,000 µg/plate (Zeiger et al., 1992). In another study, increased numbers of mutant colonies (compared to the control value) were observed only at concentrations higher than 5000 µg/plate in *S. typhimurium* strains TA 98 and TA 100 (Shimizu et al., 1985), while for strains TA 1535, TA 1537, TA 1538 and WP2uvrA up to 10 000 µg/plate negative results were observed with and without S-9 mix (BAuA, 2008). In a screening of rubber chemicals HTM, tested up to 5000 µg/plate, was negative in *S. typhimurium* strains TA 98 and TA 100 with and without metabolic activation (Crebelli et al., 1984).

In vitro assays with mammalian cells

In addition to the study by Girmanova et al (1991) mentioned by TemaNord, BauA quoted a poorly documented chromosomal aberration assay with HeLa cells in which negative results were found up to concentrations of 1 mmol/L; higher concentrations were found to induce strong cytotoxic effects (Baldermann et al., 1967). Dooley et al. (1985) reported a negative L5178Y $TK^{+/-}$ mouse lymphoma assay with HMT under addition of formaldehyde dehydrogenase and NAD+ to the test system (limited information, abstract only). The Panel noted the limitations in study protocol and/or reporting of the in vitro studies in mammalian cells.

Takahashi and Ono (1993), as reported to ECHA[15] in which negative effects of HMT on strains TA 98 and TA 100 were observed, with or without metabolic activation. Another study in which HMT was tested in a GreenScreen® assay in *S. cerevisiae* at different concentrations up to cytotoxic concentrations (highest dose: 300 µg/mL) in the absence of mammalian metabolic activation was also reported. HMT was shown to be genotoxic in the GreenScreen Assay at a lowest effective concentration of 150 µg/mL (Cahill et al., 2004).

In vivo assays with mammals

[15] (ECHA, methenamine, 2014 http://apps.echa.europa.eu/registered/data/dossiers/DISS-9d86c041-f4b7-44cd-e044-00144f67d249/DISS-9d86c041-f4b7-44cd-e044-00144f67d249_DISS-9d86c041-f4b7-44cd-e044-00144f67d249.html)

HMT was found to be negative in a chromosomal aberration test in mouse bone marrow with acute or repeated oral administration (Vujosevic et al., 1986). In the acute experiment HMT was given as a single oral dose at 618 mg/kg (corresponding to 1/3 of LD_{50}-value) and two lower doses at 6, 12 and 24 hours before sacrifice; in the repeat dose experiment, HTM was given in 5 daily administrations with sacrifice 6 hours after last dosing. No information about clinical symptoms or cytotoxic effects was reported by the authors. However, from the toxicokinetic data available BAuA concluded that the target organ was exposed to HMT (BAuA, 2008).

Negative results were shown in a dominant lethal assay in mice after single intraperitoneal doses up to 10 g HMT /kg bw. A second assay, in which oral doses of 25 g/kg (maximum tolerated dose) were administered, was considered not valid, due to higher frequencies of live implants in treated animals than in control animals (Baldermann et al., 1967). No positive controls were included (BAuA, 2008).

With regards to the genotoxicity, HMT was weakly positive in bacterial gene mutation assays and in an indicator tests in yeast and at high doses in tests for chromosomal aberrations and sister chromatid exchanges in mammalian cells in vitro (BAuA, 2008). In vivo, negative results were obtained in chromosomal aberration tests in mouse bone marrow by the oral route and in the dominant lethal test in mice by i.p. administration. Based on these findings, the Panel concluded that the weak genotoxic potential elicited by HMT in vitro is not expressed in two limited in vivo assays.

3.2.3.1. Genotoxicity of formaldehyde.

Different safety evaluations reported on the genotoxic effects of formaldehyde in different in vitro assays such as structural chromosomal aberrations, sister-chromatid exchanges, gene mutations, DNA strand breaks, DNA protein crosslinks, and DNA repair deficiencies in both bacterial and mammalian cells (EHC, 1989; CICAD, 2002; IARC, 2006; BfR, 2006), as cited in the EFSA evaluation (EFSA, 2006). The EFSA evaluation (EFSA, 2006) also reported that most available in vivo genotoxicity assays on formaldehyde are based on "local and/or systemic genotoxicity" animal models following exposure by inhalation. The majority of these in vivo genotoxicity studies indicated that any genotoxic potential of formaldehyde was limited to the site of contact and did not occur systemically in experimental animals (BfR, 2006). The site of contact seems to be the preferred target of genotoxicity also after oral exposure: in a study in rats treated orally with formaldehyde (200 mg/kg bw), induction of micronuclei and nuclear anomalies in stomach, duodenum, ileum and colon compared to controls were observed (Migliore et al., 1989; BAuA, 2008). These effects were more pronounced in the stomach, decreasing progressively at other sites of the gastrointestinal tract as the distance from the stomach increased (BAuA, 2008).

3.2.4. Chronic toxicity and carcinogenicity

The JECFA evaluation (JECFA, 1972) reported a long-term/carcinogenic study in which NMRI/Han albino mice (30/sex/group) were fed 0% or 1% HMT or 0.15% formaldehyde for 2 years. Benign and malignant tumours were reported in 43 animals: 20 in the HMT group, 12 in the formaldehyde group and 11 in the control group. Except for 1 control male and 2 males in the HMT group, all tumours occurred in females. 29 out of 36 malignant tumours were subcutaneous carcinomas and adenocarcinomas (Kewitz and Welsch, 1966 as reported in JECFA, 1972). The same author conducted a further study in groups of 50 female mice fed HMT at levels of 0%, 0.1%, 0.5% and 1% for 31 weeks. No differences in tumour incidence between the groups were observed (Kewitz, 1966). No further information is provided. In vitro formation of carcinogenic nitrosamine has been reported as a result of the interaction of nitrite with HMT at pH between 1-3 (JECFA, 1974).

In other studies in mice, HMT was tested in three different strains: CTM, outbred; C3Hf/Dp, inbred; and SWR/Dp, inbred. 96 males and 102 females CTM mice (10 weeks old) received 0, 0.5, 1, or 5% HMT in drinking water for 30 or 60 weeks. In the C3Hf/Dp mice (5 weeks old) 49 males and 44 females received 1% HMT in the drinking water over a period of 60 weeks (calculated daily intake of 2.5 g HMT/kg bw/day in either sex). In the SWR/Dp mice (7 weeks old), 29 males and 27 females mice received 1.0% HMT in drinking water for 60 weeks (calculated daily intake of 2.5 g HMT/kg

bw/day in either sex). Mice were observed up to 100 weeks of age. No significant differences were observed in body weight gain between control and HMT-treated SWR and C3Hf strain groups. Water intake was similar in both control and HMT-treated groups. No data on haematology and clinical biochemistry were reported. Treatment of CTM mice with 5% HMT (12.5 g HMT/kg bw/day) for 30 weeks showed a significant reduction in survival rates and slight reduction of growth in the surviving animals. Slight retardation of growth was also seen in SWR mice treated with 1% HMT (2.5 g HMT/kg bw/day). The effect on growth in SWR mice was very small, and not statistically significant, and no findings were noted at necropsy and microscopy. In addition, there were no HMT-related gross and microscopic findings in mice of all tested strains (Della Porta et al., 1968). Therefore, based on these results the BAuA evaluation (2008) established a NOAEL for mice of 2.5 g HMT/kg bw/day HMT in either sex (BAuA, 2008). The EPA evaluation (2006), however, reported for the same study a NOAEL of 12.5 g HMT/kg bw/day, the highest dose tested, and no LOAEL was established. No carcinogenic activity was reported (EPA, 2006).

TemaNord (2002) described chronic toxicity studies in dogs and rats receiving oral HMT in a dosage of 50-200 mg HMT/kg daily and 0.8-6.4 g HMT /kg daily, respectively. Gastric and bladder irritation occurred with some hemorrhagic sites and ulceration (TemaNord, 2002).

The BAuA evaluation (2008) reported different long-term studies in animals following oral exposure. These studies are only shortly reported and have not been evaluated in details in the original document by the Panel because they are not considered an adequate basis for establishing health based guidance values.

BD (cPah) rats (15/sex) were treated with an average calculated dose of 1130 mg HMT/kg bw/day for males and 1570 mg HMT/kg bw/day for females. Haematology and clinical biochemistry parameters were not measured. Except for citrus-yellow fur discolourations, no difference in macroscopic and microscopic findings in organs or in body weight gain between treated and control groups of both sexes were observed. No tumours were reported (Brendel, 1964). Based on the results of this study, the BAuA evaluation (2008) reported a NOAEL of approximately 1130 mg HMT /kg bw/day in males and 1570 mg/kg bw/day in female BD (cPah) rats, the only doses tested(BAuA, 2008).

Outbred Wistar rats (10 weeks old, 48/sex) received either 0 or 1.0% HMT in the drinking water for 104 weeks (calculated intake 1.5-2 g HMT/kg bw/day in males and 2-2.5 g HMT/kg bw/day in females). After the end of treatment period rats were observed for a subsequent treatment-free period of up to 3 years of age. Body weights showed no significant differences between controls and HMT treated groups. Water intake was also comparable in both control and HMT treated test groups. There were no data on haematology and clinical biochemistry. Survival was 84% in HMT-treated and untreated animals at the end of the experiment. In all HMT-treated rats a yellow colouration of the coat was observed. No pathological lesions related to HMT treatment were observed in rats that died during the study or sacrificed at the end of the test (Della Porta et al., 1968). Based on these results, the BAuA evaluation (2008) reported a NOAEL of 1.0% (calculated intake 1.5-2 g HMT/kg bw/day in males and 2-2.5 g/kg bw/day in females), the only doses tested.

In another experiment, outbred Wistar rats (10 weeks old, 12/sex) received a high concentration of 5.0% HMT (equivalent to 5 g HMT/kg bw/day, based on body weights of 250 g in males and 200 g in females and an average water consumption of 10% of the body weight) daily in the drinking water for two weeks with a subsequent 102-week treatment-free period. 50% mortality was observed after 2 weeks, but no other treatment-related histopathological changes were recorded (BAuA, 2008). Therefore, the BAuA evaluation (2008) reported a LOAEL of 5 g/kg bw/day). The EPA evaluation (2006) reported for the same study a LOAEL of 7.25 g/kg bw/day (EPA, 2006). The Panel noted that these reported intakes appeared high based on the available information on normal water consumption in rats.

Wistar rats (two months old, 16/sex/group) were given in the diet either 0 or 0.16% HMT (equivalent to 100 mg/kg bw/day) from weaning to natural death. Data on haematology and clinical chemistry

were not available. No significant differences between controls and HMT-treated animals were shown with regards to general health and behaviour. Only a yellow staining of the perianal hair was observed in one male and three female rats treated with HMT. No significant differences were observed between HMT treated animals and control with respect to body weight, muscular activity, organ weights, histopathological findings, life-span and causes of death (Natvig et al., 1971). In an additional palatability experiment, albino rats (10-12 week old) were allowed to choose between food containing HMT and the same food without HMT for a 28-day period (the two types of food were consumed in a similar amount). After a 120-day period during which they were fed only the HMT enriched diet, the animals were again allowed to choose between the two diets in a second 28-day trial. The addition of HMT had no effect on palatability of the diet (Natvig et al., 1971). Therefore, in this chronic oral toxicity study in Wistar rats the BAuA evaluation (2008) reported a NOAEL of 100 mg HMT/kg bw/day for both sexes (BAuA, 2008).

Sprague-Dawley rats (8-10 weeks old, 15/sex/group) were given 0.1% HMT in drinking water. Each group received a total dose of 5 g HMT equivalent to 80 mg HMT/kg bw/day in males and 100 mg HMT/kg bw/day in females, based on a body weight of 250 g in males and 200 g in females), either with or without 0.2% sodium nitrite (total dose of 10 g nitrite per rat) on 5 days/week for a period of 50 weeks. There were no data on haematology and clinical biochemistry. No significant differences in the survival rate were reported. No tumours findings were reported either in animals fed HMT alone or in combination with nitrite (Lijinsky and Taylor, 1977). Based on these data, the BAuA evaluation (2008) reported a NOAEL for HMT of 80 mg HMT/kg bw/day for males and 100 mg HMT/kg bw/day for females (BAuA, 2008).

In a comparative study in cats, one group of two male and three female cats received a diet containing 1250 mg HMT/kg diet. Each cat received a total dose of 180 g HMT for 742 days. The mean dose per cat for a period of two years was estimated to be approximately 61 mg HMT/kg bw/day (assuming a mean body weight of 4 kg for both sexes). Another group of cats, one male and three females, were fed a diet containing 374 mg formaldehyde/kg diet for 106 weeks (equivalent to approximately 20.88 mg formaldehyde/kg bw/day for both sexes, based on feed consumption resulting in a total dose of 62 g/cat). A third group of three male and female cats were the control. One female in the formaldehyde group died after seven months of pleurisy and a female in the HMT group died after twenty-three months of a pyrogen infection in the nasal cavity and paranasal sinuses. No treatment-related effects were found concerning food consumption, body weight gain, or behaviour in animals treated with HMT. There were no data on haematology, biochemistry and histopathology (Kewitz, 1966, unpublished report). Based on the results reported, the BAuA evaluation (2008) reported a NOAEL of 61 mg HMT/kg bw/day for male and female cats (BAuA, 2008), the only dose tested.

In summary, available information on the chronic toxicity of HMT was limited. None of the studies provided data on haematology and clinical chemistry. Body weight gain, food consumption, organ weights, gross pathology and histopathology were unaffected following exposure to HMT. However, survival rates and growth were significantly decreased in a study in CTM mice treated with 12.5 g HMT/kg bw/day for 30 weeks. The only treatment related clinical observation in studies with rats was occasional yellow staining of the perineal hair. Overall, HMT was not carcinogenic in experimental animals treated at doses up to 2.5 g HMT/kg bw/day (Brendel, 1964; Natvig et al., 1971; Della Porta, 1968; Lijinsky and Taylor, 1977). For all the studies, the NOAELs for HMT corresponded to the highest dose tested. The BAuA (2008) evaluation reported that the existing long-term/carcinogenicity studies on HMT were not in line with the current guidelines on carcinogenicity and/or combined chronic toxicity/carcinogenicity. However, the data submitted were considered useful in assessing the carcinogenic potential of HMT. In addition, considering the negative results from in vivo genotoxicity testing, BAuA concluded that HMT was not considered as carcinogenic for experimental animals (BAuA, 2008). The BAuA evaluation (2008) also reported that one valid cancer study with formaldehyde administered via drinking water to rats did not show an increased tumour incidence in any organ (Til et al., 1989). The Panel concluded that the formation of formaldehyde from HMT should not be of concern with regards to carcinogenicity.

The relevant long-term studies using oral administration of formaldehyde have been discussed in the EFSA evaluation in 2006 (EFSA, 2006).

The EFSA 2006 evaluation reported that the evaluation of these studies indicated that currently, there is no definitive evidence to indicate that formaldehyde is carcinogenic when administered orally to laboratory animals. Other evaluations 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. Consistent with the cytotoxic effects seen at sites of contact for the inhalation route, repeated oral administration of formaldehyde induces erosion/ulceration effects in the forestomach and glandular stomach and hyperplasia of the limiting ridge and glandular stomach. Such a mechanism may also include a threshold response. Therefore, the EFSA evaluation (2006) concluded that: 'The Panel examined recent and previous evaluations of formaldehyde and concluded that there was no evidence indicating that formaldehyde is carcinogenic by the oral route' (EFSA, 2006).

3.2.5. Reproductive and developmental toxicity

The 1972 JECFA evaluation (JECFA, 1972) reported several reproductive and developmental studies in different animal species and these data are described below.

In a 5-generation study lasting three and half years, a total of 80, 80 and 245 rats were given 0, 5 and 50 mg HMT/kg day in the drinking-water. No treatment-related changes were found (Malorny, 1966).

In an unpublished study submitted to JECFA (1974) by Berglund (1966) rats (10/sex) were fed doses equivalent[16] to 0, 20, 40 and 80 mg HMT/kg bw/day for 2 years. There were no effects at any dose on growth, 2-year survival, reproduction and viability of offspring. No specific pathological changes were observed at any dose level (Berglund, 1966).

The JECFA described studies in dogs with small numbers of animals dosed with HMT and formaldehyde. The limited reports of these studies reported no consistent effect of HMT or formaldehyde on the parameters measured (Kewitz, 1966, Tierfarm, 1969).

The BAuA evaluation (BAuA, 2008) described other studies on reproductive and developmental toxicity of HMT in animals and in human. The results of these studies are summarised below.

Reproduction was investigated in a lifetime feeding study on Wistar rats fed with a standard diet of 0.16% HMT (corresponding to about 100 mg HMT/kg bw/day) (Natvig et al., 1971). After three months of treatment, 16 males and 16 females of the treated group were mated. 16 males and 16 females of the F1 generation were fed with the same diet as their parents from weaning. For the F1 generation, no effects on average litter size were noted, and no significant differences from the controls were found on muscular activity, general health, mean body weights at 7, 15, and 18 weeks of age and for relative organ weights (liver, kidney, adrenals, gonads) at 18 weeks of age (Natvig et al., 1971). However, BAuA reported that no other parameters were evaluated and data available were limited (BAuA, 2008).

Della Porta et al., (1970) conducted a further study on transplacental toxicity and carcinogenesis in two independent experiments for one and for three successive generations in Wistar rats exposed to HMT via drinking water. In the first experiment 12 females and 6 males were given 1% HMT in drinking water (daily intake of approximately 1.5-2 g HMT/kg bw/day for males and of 2-2.5 g HMT/kg bw/day for females) during two weeks prior to mating through gestation and lactation. A similar untreated group of 12 females and 6 males served as controls. Within 25-30 days after mating 11 treated females and 11 controls became pregnant and delivered 110 and 118 pups, respectively. After delivery pups of both groups were reduced to 8 offspring per litter (treated group 47 males/38 females, control group 37 males/46 females). After weaning, offspring (24/sex) were continued on 1%

[16] Calculated based on a default value of 0.05 (EFSA Scientific Committe, 2012)

HMT in drinking water up to the 20th week of age. In treated males up to postnatal week 9 and in treated females up to postnatal week 13, the body weights were significantly lower than those of controls. However, at the beginning of the postweaning, the initial body weights of the offspring of treated females were already lower than those of the offspring of the controls, indicating that growth deficits were already present. No differences in body weights were recorded at the end of the 20 weeks. No differences were noted between treated and control groups on organ weight and gross or microscopic findings at the end of the treatment (Della Porta, 1970). Based on the temporary weight loss, the EPA evaluation (2006) established a NOAEL of 1% HMT (corresponding to 1 500-2 000 mg HMT/kg bw/day for males and of 2 000-2 500 mg HMT/kg bw/day for females). The weight loss could be attributed to the decreased palatability of the drinking water (EPA, 2006).

In a second experiment (Della Porta et al., 1970) rats were given 1% HMT in drinking water for three successive generations, up to the age of 40 weeks in the F1 and F2 groups and of 20 weeks for the F3 group. Afterwards, all groups were kept under observation up to week 130 of their lifetime. The F0 generation group consisted of one male and two females that were given 1% HMT in drinking water during four weeks before mating. The treatment of the females continued until two litters of ten pups each had been weaned. The descendant F1 groups consisted of 13 males and 7 females. The females were mated to 3 males of their group. One dam died during delivery while the remaining 6 dams gave birth to a total of 36 pups from which 10 died during lactation. The resulting F2 group consisted of 15 males and of 11 females. These females were mated to 4 males of their group and delivered a total of 99 pups from which only 12 males and 12 females were further raised to form the F3 group. An additional group of 5 females was given 2% HMT from mating through lactation. They delivered a total of 49 pups from which 16 animals per sex were continued on 2% HMT for 50 weeks. A group of 48 males and 48 females were the untreated control. All groups were observed for over two years of age. No differences on survival rates and on body weights of all offspring generations were reported. No evidence of carcinogenicity was found (Della Porta, 1970). No other parameters on reproductive endpoints have been recorded since this study had been primarily directed to elucidate potential carcinogenicity (BAuA, 2008).

In another study, a group of 9 females Alpk:AP (Wistar-derived rats) were given HMT daily by gavage at dose of 1000 mg HMT/kg bw during gestation day 7 to 17 (Wickramaratne, 1987). A decrease in body weight gain was noted in treated animals. 5 pregnant females of the treated group showed no difference in mean litter size, survival of pups and pup postnatal weight gain. However, the BAuA evaluation (2008) reported that the number of dams for which offspring could be evaluated was limited and no rationale was provided to explain the reason why only 5 out of 9 sperm-positive females produced litters (BAuA, 2008).

HMT was given to 51 female beagle dogs at dietary concentration of 600 or 1250 mg HMT/kg diet (equivalent to doses of 15 or 31 mg HMT/kg bw/day, assuming an average body weight of 12 kg) from days 4 to 56 after mating (Hurni and Ohder, 1973). 9, 8, and 8 females provided litters from the control, 15 or 31 mg HMT/kg bw/day groups, respectively. Further groups were treated with formaldehyde (125 and 375 mg formaldehyde/kg diet). Pregnancy rates, mean length of gestation, mean litter size or body weight gains of the mothers were not affected by treatment. The mean litter size was within the normal range for all groups (controls: 6.7; formaldehyde, 125 mg formaldehyde/kg diet: 5.4; formaldehyde, 375 mg formaldehyde/kg diet: 7.1; HMT, 600 mg HMT/kg diet: 6.3; HMT, 1250 mg HMT/kg diet: 7.0). In the highest dose group of HMT, the percentage of stillborn pups was higher than in the other groups (10 stillbirths out of 56 pups compared to four stillbirths out of 60 control pups), due to the fact that only two pups in one litter of nine pups were born alive. No skeletal or any other malformation was recorded in any of the stillborn pups. During the first month a retardation of growth and an increase in mortality were noted in the highest dose of HMT no data provided). In the same group the percentage of pups surviving to weaning was lower than in the other groups (33 out of 46 pups survived compared to 49 out of 56 control pups). At both dose levels of HMT, birth weight (equal to 90-92% of control pup birth weights) and post-natal growth (equal to 91-94% of control pup weights in the 8th week) were slightly decreased. All dogs observed for a longer period were reported to be normal in behaviour and general appearance. No malformations were found

in dogs observed up to 9 months. 18 other dogs transferred to the breeding colony and observed for nearly 2 years did not show any signs of physiological or skeletal abnormalities or disorders of reproduction (Hurni and Ohder, 1973). Based on these data, the BAuA identified a NOAEL of 15 mg HMT/kg bw/day. Previously, the 1974 JECFA evaluation (JECFA, 1974) established an ADI based on this reproduction study where a NOEL value of 15 mg HMT/kg bw was specified for HMT (JECFA, 1974; TemaNord, 2002). The Panel noted that the EPA evaluation considered that there were a number of inconsistencies in the dog data which precluded their use.

Studies on reproductive and developmental toxicity in humans are described briefly below.

Pregnant women (206) who suffered from asymptomatic bacteriuria were studied in a systematic trial over a 2-year period and given doses equivalent to 29 mg methenamine hippurate/kg bw/day (70 patients) or 57 mg methenamine mandelate/kg bw/day (69 patients) or no treatment (67 patients). Mean birth weights and gestation lengths showed no significant difference from the control group. In addition, the number of abortions, intrauterine deaths and fetal abnormalities in the treated groups did not differ from those of the general population (Furness et al., 1974).

In a surveillance study involving 229 101 completed pregnancies, 8 (3.8 %) of the the newborns whose mothers had been treated with HMT during the first trimester had major birth defects (Briggs et al., 1994).

In another study, no congenital abnormalities were observed in the children of 3 women who had taken HMT as well as 5 other drugs (choleinic sidium, phenolphthalein, papaverine HCL, methylhomatropine, and menthol) during the first two weeks of pregnancy (Siffel & Czeisel, 1995).

In conclusion, a large set of data have been described on the reproductive and developmental toxicity of HMT in rats, dogs, and human. Overall, the information available is limited, due to the use of low numbers of animals, limited number of reproductive and developmental parameters recorded, and teratogenicity not properly assessed. However, data available indicated that HMT did not present the potential to induce adverse effects on the fertility in rats. Both the EPA (EPA, 2006) and the BAuA evaluations (BAuA, 2008) considered a NOAEL of 1.5-2.5 g HMT/kg bw/day for reproductive toxicity in rats, based on the study by Della Porta et al. (1970). With regards to developmental toxicity, in both rats and beagle dogs adverse developmental effects observed during the postnatal period were preweaning mortality and postnatal growth retardation. The BAuA evaluation (BAuA, 2008) reported NOAEL values for developmental toxicity for rats (Natvig et al., 1971) and dogs (Hurni and Ohder, 1973) of 100 mg HMT/kg bw/day (the highest dose) and 15 mg HMT/kg bw/day, respectively. However, the EPA (2006) evaluation concluded that there are many inconsistencies on the results of the dog study, since the effects were not consistent with the dose levels, and no details have been provided to clarify these inconstancies. Therefore, the EPA (2006) did not take into account the dog study for their risk assessment (only the rat studies by Della Porta, 1970 and Berglund, 1966). In humans, the study by Furness et al. (1974) showed that no treatment-related abnormalities during the pregnancy or the development of the children had been reported. Negative findings were also found in the study by Siffel and Czeisel (1995). However, in the surveillance study by Briggs et al. (1994), 3.8% (of 209 newborn whose mothers had been treated with HMT during the first trimester) showed birth defects (BAuA, 2008). The BAuA evaluation (2008) concluded that, overall, all the studies reported did not sufficiently meet the requirements for a sound risk assessment evaluation with respect to reproductive and developmental toxicity. Overall, due to the limited value of the animal data BAuA proposed basing a quantitative risk assessment for developmental toxicity on the human data (NOAEL 27 mg HMT/kg bw/day) (BAuA, 2008).

The EFSA Opinion (EFSA, 2006) on formaldehyde reported that formaldehyde does not affect reproduction or gestational developmental parameters (IPCS, 1989; CICAD, 2002).

3.2.6. Hypersensitivity

Guinea pigs exhibited strong skin sensitization in a maximization test with a 50% aqueous of HMT solution (Degussa et al., 1985). In a Local Lymph Node Assay (LLNA) a positive effect concentration (EC3) of 30.6% HMT was derived. A comparably low EC3 for formaldehyde was determined in the same study (De Jong et al., 2007). Thus, it may be concluded also for skin sensitization, that formaldehyde, which may be generated by hydrolysis of HMT in contact to skin, and not HMT, is the main causative agent of sensitization.

The TemaNord evaluation (TemaNord, 2002) reported that there are several studies on the irritating and sensitising effects of HMT on the skin and the respiratory tract after occupational exposure in humans (TemaNord, 2002). HMT was shown to be non-sensitising to guinea pigs (TemaNord, 2002).

The BAuA evaluation (BAuA, 2008) described that a number of cases of allergic symptoms such as wheezing and asthma were reported upon exposure to HMT (Gelfand, 1963). However, in all these cases exposure to other chemicals occurred simultaneously, and therefore the respiratory hypersensitivity could not specifically be attributed to HMT exposure. In another more recent study, respiratory sensitization (occupational asthma) after occupational exposure to HMT could not be demonstrated. However, irritant dermatitis of the hands in subjects with high exposure to HMT was found (Merget et al., 1999).

3.2.7. Other studies

3.2.7.1. Human studies

The BAuA evaluation (BAuA, 2008) reported that HMT has been used in humans for many years as urinary antibacterial-antiseptic drug as well as for long-term prophylaxis of urinary tract infections. The bactericidal effect of HMT has been linked to its pH- and time-dependent hydrolysis in the urine to ammonia and formaldehyde. Therefore, the effectiveness of HMT depends on an adequately maintained urine concentration of formaldehyde, which in turn depends on the pH of the urine (pH <5.5), an increased fluid intake and high urine output, and the duration that urine is retained in the bladder (BAuA, 2008). The combination of HMT with acid salts (hippurate and mandelate) helps to maintain the urinary pH in the desired range (BAuA, 2008).

The BAuA evaluation (BAuA, 2008) described that adverse effects occurred in less than 3.5% of patients receiving HMT or its salts. The most frequent adverse effects noted were nausea, vomiting, diarrhoea, abdominal cramps, and anorexia. Rarely, hypersensitivity reactions such as rash, pruritus, urticaria and stomatitis have occurred. Other less frequently reported side effects were headache, dyspnoea, generalized oedema, tinnitus, muscle cramps, dysuria, and microscopic or gross haematuria. High therapeutic doses of 8 g HMT/day (corresponding to 114 mg/kg bw/day based on a body weight of 70 kg) administered for 3 to 4 weeks induced bladder irritation, painful and frequent micturition, albuminuria and haematuria. Other adverse effects noted were gingivitis, anorexia, headache, and generalized oedema. No complications were observed in patients receiving HMT in the standard treatment for acute cystitis at dose levels of 2 to 4 g/day (corresponding to about 28 to 57 mg/kg bw/day based on a body weight of 70 kg) for a 7- to 10-day course up to four weeks. No adverse effects were observed in patients receiving HMT as an antiseptic at dose level of 4 to 6 g/day for weeks. HMT is also used for long-term suppressive therapy or for prevention of recurrent urinary infections. In long-term treatment with HMT and its salts (6 months or longer) at the usual oral dose of 2-4 g/day (corresponding to 28-57 mg/kg bw/day based on a body weight of 70 kg) no relevant side effects were reported (BAuA, 2008).

Other studies on the effects of HMT on humans following occupational exposure (by inhalation or skin contact) have been reported in the BAuA evaluation (BAuA, 2008). However, toxic effects in humans at the workplace have only been reported after repeated exposure to mixtures of several compounds in addition to HMT such as formaldehyde, ammonia, resorcinol, phenol, furfuryl alcohol, cyanides, and epoxy resins, curing agents. There are also some reports describing lung and bladder

cancer in occupationally exposed workers in the steel foundry, tyre and rubber industries. However, considering the lack of measurements of HMT concentrations in the blood, urine, exhaled breath, or other biological media from exposed workers, the available occupational exposure studies were not adequately designed to make qualitative assessments of the observed effects in relation to HMT exposure alone (BAuA, 2008).

Overall, these available human data did not provide any conclusive information on any potential association between occupational HMT exposure and cancer in humans. With regards to the use of HMT as a drug in humans there is no information available on the formation of tumours in the urinary tract or in other organs or tissues (BAuA, 2008).

4. Discussion

The ANS Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluation, reviews and additional literature that became available since then. An additional source of information was the registration dossier provided by industry for HMT under the REACH Regulation 1907/2006, published by the European Chemicals Agency (ECHA, 2011). The Panel noted that not all original studies on which previous evaluations or reviews were based were available for re-evaluation by the Panel.

Specifications for HMT have been defined in Commission Regulation (EU) No 231/2012 and by JECFA (2006). HMT is described as colourless or white crystalline powder. The purity is specified as not less than 99% anhydrous. Under acidic aqueous conditions HMT can yield formaldehyde and ammonia.

HMT (E 239) is currently only permitted for use in Provolone cheese at a maximum level of 25 mg/kg residual amount, expressed as formaldehyde. The formaldehyde released from HMT under acidic conditions or in cheese can react with proteins.

HMT has been previously evaluated by the Joint Expert Committee on Food Additives (JECFA) in 1962, 1965, 1967, 1972 and 1974 (JECFA, 1962, 1965, 1967, 1972 and 1974). JECFA established an ADI of 0.15 mg/kg bw/day based on a reproductive study with a NOEL of 15 mg/kg bw/day (JECFA, 1974). HMT has not been directly evaluated as a food additive by the Scientific Committee on Food (SCF). In 1977, the SCF referred to HMT during its evaluation of the use of formaldehyde in grana padano cheese, since HMT decomposes to form formaldehyde under acidic conditions or in the presence of proteins (SCF, 1977). More recently, TemaNord in 2002 (TemaNord, 2002), the United States Environmental Protection Agency (EPA) in 2006 (EPA, 2006), the Federal Institute for Occupational Safety and Health (BAuA, Germany) in 2008 (BAuA, 2008) reviewed the safety of HMT.

In humans, about 88% of an oral dose of HMT was absorbed within 12 hours and excreted mostly unchanged (about 82% of recovery) in the urine in 24 hours (BAuA, 2008). The maximum serum concentration (35.2 mg/L) after a single dose was achieved within 1 hour and the mean elimination half-life in blood was reported to be 4.3 hours. Approximately 10-20% of an oral dose of HMT is converted to formaldehyde prior to absorption. HMT can pass the placenta and is detectable in breast milk of breastfeeding women; however, no accumulation was reported. Since, formaldehyde formation from HMT is strongly dependent on acidic pH values, formaldehyde generation is only relevant following oral ingestion as the pH of the stomach is acidic. Further down the gastrointestinal tract, the pH is neutral with nearly no generation of formaldehyde (BAuA, 2008). However when used therapeutically HMT is intended to break down to formaldehyde in the urinary tract and compounds which lower urinary pH are often co-administered. Since it has been shown that under acidic conditions, HMT is converted to formaldehyde, which in turn, is converted into formic acid, toxicokinetic information on formaldehyde and its metabolite formic acid is relevant for the risk characterisation of HMT. Overall, both in animal and human studies formaldehyde is rapidly absorbed and converted to formic acid. The rate of oxidation of formaldehyde to formic acid was comparable in

all animal species, with a half-life of only 1 minute. The elimination half-life of formic acid varies from 55 minutes in animals (mainly studied in rodents) to 90 minutes in humans with excretion via the kidneys or further oxidation to carbon dioxide and water (JECFA 1962, 1965, 1972, 1974; BAuA, 2008).

Results from animal experiments and limited data in humans indicate that HMT is of very low to moderate acute toxicity.

There are limited studies available on the subchronic toxicity of HMT. None of the studies provided data on haematology and clinical chemistry; data on histopathology were limited. However, body weight gain, food consumption, survival, organ weights, gross pathology and histopathology were generally unaffected following exposure to HMT. The only treatment related clinical observation was a yellow staining of the perineal hair in some cases in studies with rats and decreased body weight gain or weight loss in one 15-week study in rabbits (RTECS, 2005 as referenced by EPA, 2006). Spinacine, the most abundant end-product of formaldehyde in cheese (derived from the N-terminal histidine residue in gamma 2-casein) showed a NOAEL of 300 mg/kg bw/day in short term toxicity studies. The BAuA (2008) evaluation reported that the available repeated dose toxicity studies on HMT via oral administration (gavage, diet, drinking water) neither conformed to standard repeated dose toxicity testing protocols nor were performed according to currently accepted test guidelines. However, BAuA evaluation considered the available data were sufficiently acceptable to derive NOAELs for repeated-dose oral toxicity (BAuA, 2008). The Panel agreed with this conclusion.

With regards to the genotoxicity, HMT was weakly positive in bacterial gene mutation assays and in an indicator tests in yeast and at high doses in tests for chromosomal aberrations and sister chromatid exchanges in mammalian cells in vitro (BAuA, 2008). In vivo, negative results were obtained in chromosomal aberration tests in mouse bone marrow by the oral route and in the dominant lethal test in mice by i.p. administration.(BAuA, 2008), indicating that the genotoxic activity elicited by HMT in vitro is not systemically expressed in vivo. The Panel noted that HMT may be partially converted in the stomach into formaldehyde which, at high doses, is genotoxic in vivo at the site of first contact. In this respect the Panel noted that HMT used as food additive breaks down into formaldehyde during cheese-making and storage, and that *in situ* formed formaldehyde largely reacts with amino groups of milk proteins. Thus, the exposure to formaldehyde resulting from the use of HMT as food additive is expected to be negligible, much lower than resulting from other authorized uses or from normal mammalian metabolism (878-1310 mg/kg bw per day assuming a half -life of1-1.5 min; EFSA, 2014). Overall, the Panel concluded that the proposed use of HMT as food additive does not raise concern for genotoxicity.

The Panel noted that human therapeutic use of HMT would result in much higher levels of formaldehyde generated in the stomach and in the urinary tract than from its use as a food additive.

The Panel noted that HMT is limited to a single use in the EU and taking into account the estimated maximum exposure to formaldehyde from this use, considered that the available data were sufficient for this restricted use. However HMT can be converted into formaldehyde at the acidic pH of the stomach. Given the high reactivity of formaldehyde, as highlighted by the information summarized below, it is conceivable that genotoxicity of HMT-generated formaldehyde would only be detectable at the site of contact. Thus, the Panel noted that further in vivo testing in stomach as target tissue would be required to adequately assess the genotoxic potential of orally ingested HMT. The Panel concluded that this would be a priority if the uses or use levels of HMT were to increase but no information is available for local effects on the gastrointestinal tract.

Available studies on the chronic toxicity of HMT are limited. None of the studies provided data on haematology and clinical chemistry. However, body weight gain, food consumption, organ weights, gross pathology and histopathology were unaffected following exposure to HMT. However, survival rates and growth were significantly decreased in a study in CTM mice treated with 12.5 g HMT/kg bw/day HMT for 30 weeks. The only treatment related clinical observation in studies with rats was

occasional yellow staining of the perineal hair. Overall, the data available do not indicate a potential for HMT to be carcinogenic in experimental animals at dosages up to 2.5 g HMT/kg bw/day (Brendel, 1964; Natvig et al., 1971; Della Porta, 1968; Lijinsky and Taylor, 1977). However, the JECFA evaluation (1972) reported benign and malignant tumours in mice fed 1% HMT or 0.15% formaldehyde for 2 years (Kewitz, 1966). For all the studies, the NOAELs for HMT corresponded to the highest dose tested. The BAuA (2008) evaluation reported that the existing long-term/carcinogenicity studies on HMT were not in line with the current guidelines on carcinogenicity and/or combined chronic toxicity/carcinogenicity. However, the data submitted were considered useful in assessing the carcinogenic potential of HMT (BAuA, 2008). In addition, considering the negative results from in vivo genotoxicity testing, BAuA concluded that HMT was not considered as carcinogenic for experimental animals (BAuA, 2008). The BAuA evaluation (2008) also reported that one valid cancer study with formaldehyde administered via drinking water to rats did not show an increased tumour incidence in any organ (Til et al., 1989). Therefore, the BAuA evaluation (2008) concluded that the formation of formaldehyde from HMT should not be of concern with regards to carcinogenicity (BAuA, 2008). The Panel noted that there are other studies on toxicity of formaldehyde discussed in the EFSA opinion (2006).

A large set of data have been described on the reproductive and developmental toxicity of HMT in rats, dogs, and human. Overall, the information available is limited, due to the use of reduced animal numbers, limited number of reproductive and developmental parameters recorded, and developmental parameters not properly assessed. However, data available indicated that HMT did not have the potential to induce adverse effects on the fertility in rats. Both the EPA (EPA, 2006) and the BAuA evaluations (BAuA, 2008) considered a NOAEL of 1 500-2 500 mg HMT/kg bw/day for reproductive toxicity in male and 2000-2 500 mg HMT/kg bw/day in female rats, based on the study by Della Porta et al. (1970). With regards to developmental toxicity, in both rats and beagle dogs adverse developmental effects observed during the postnatal period were preweaning mortality and postnatal growth retardation. The BAuA evaluation (BAuA, 2008) reported NOAELs for developmental toxicity for rats (Natvig et al., 1971) and dogs (Hurni and Ohder, 1973) of 100 mg HMT/kg bw/day (the highest dose) and 15 mg HMT/kg bw/day, respectively. However, the EPA (2006) evaluation concluded that there are many inconsistencies on the results of the dog study, since the effects were not consistent with the dose levels, and no details have been provided to clarify these inconstancies. Therefore, the EPA (2006) did not take into account the dog study for their risk assessment (only the rat studies by Della Porta, 1970 and Berglund, 1966). In addition, the unpublished study by Kewitz (1966) reported that 66.7% of the HMT-treated dogs (30 litters) were considered unusual in having stillborn pups and cannibalism, as well as 5 cases of animals born were reported with malformation (JECFA, 1972). In humans, the study by Furness et al. (1974) showed that no treatment-related abnormalities during the pregnancy or the development of the children had been reported. Negative findings were also found in the study by Siffel and Czeisel (1995). However, in the surveillance study by Briggs et al. (1994), 3.8% of 209 newborn treated with HMT during the first trimester, showed birth defects (BAuA, 2008). The BAuA evaluation (2008) concluded that, overall, all the studies reported do not sufficiently meet the requirements for a sound risk assessment evaluation with respect to reproductive and developmental toxicity. The Panel concluded that despite limitations in the database on reproductive and developmental toxicity, the available data were sufficient for evaluating the single permitted use and use levels.

No adverse effects have been reported in patients receiving HMT for long-term prophylaxis or therapy as urinary antibacterial-antiseptic substance at dose levels of 2 to 4 g/day (corresponding to 28 to 57 mg/kg bw/day) for up to 4 weeks (corresponding to a NOAEL of 57 mg/kg bw/day). However, with a higher dose of 8 g/day (corresponding to 114 mg/kg bw/day) over 3 to 4 weeks clinical symptoms such as bladder irritation, painful and frequent micturition, albuminuria and haematuria were reported in some individuals (BAuA, 2008). With regards to the use of HMT as a drug in humans there is no information available on the formation of tumours in the urinary tract or in other organs or tissues (BAuA, 2008). In humans, skin sensitizing properties of HMT have been reported. Following skin contact acute dermatitis was the main symptom. Other reports described a number of cases in which allergic symptoms of the respiratory system were also reported following HMT exposure. However, in

all cases exposure to other chemicals occurred simultaneously, therefore the induction of specific respiratory hypersensitivity by HMT cannot be clearly demonstrated (BAuA, 2008). Regarding data available on effects of HMT on human following occupational exposure (by inhalation or skin contact), human data available do not provide any conclusive information on the association between HMT exposure and cancer in humans, since toxic effects in humans at the workplace have only been reported after repeated exposure to mixtures of several compounds rather than HMT alone (BAuA, 2008).

Although there are limitations in the toxicological database overall an assessment of the risks can be made. The available database indicates that whilst HMT demonstrates genotoxic potential in vitro, this is not expressed in vivo. In chronic toxicity and carcinogenicity studies in rodents reported NOAELs were generally the highest dose tested, however the studies were old and not performed according to current standards. There is an extensive database on reproductive toxicity but the studies are poorly reported. JECFA based its ADI on the NOAEL of 15 mg/kg bw/day from a developmental toxicity study in dogs. The Panel noted however that more recent evaluations by EPA and BAuA have not considered this study an adequate basis for establishing health based guidance values. The Panel considered that the limitations in the toxicological database meant that it was not possible to clearly identify the critical study and therefore no NOAEL could be identified as relevant Point of Departure (POD) for derivation of an ADI.

In humans no adverse reactions were reported following doses up to 4 g HMT/day for 4 weeks (equivalent to 57 mg HMT/kg bw/day).

At therapeutic doses the majority of the dose is excreted into urine as HMT but is intended to release formaldehyde under acidic conditions in the urinary tract. A significant portion of the dose (10-20%) produces formaldehyde systemically. HMT has a relatively short half-life. It appears that at high doses the rate of conversion to formaldehyde is insufficient to prevent urinary excretion of the bulk of the dose as HMT. However this might not apply at lower doses.

The estimated mean exposure to HMT (expressed as formaldehyde) via consumption of Provolone cheese was low for the total population: on average 0.3 µg formaldehyde/kg bw/day (Table 4). For consumers only of Provolone cheese the mean exposure ranged from 5 µg formaldehyde/kg bw/day in toddlers up to 20 µg formaldehyde/kg bw/day in children (95th percentiles: 18 µg formaldehyde/kg bw/day for adults). If it was assumed that all ripened cheese consumed was Provolone cheese, the highest estimated exposure, using the 95th percentile of consumers only combined with the MPL, equalled 87 µg formaldehyde/kg bw/day in children.

The Panel noted that in acidic conditions HMT broke down with the formation of formaldehyde which would then be metabolised to formic acid. The Panel noted and endorsed the conclusions of the 2006 EFSA evaluation of formaldehyde. The Panel considered that given the current limited permitted use of HMT and assuming formaldehyde was at the maximum residual levels, the highest exposure via the consumption of Provolone cheese would be 20 µg formaldehyde /kg bw/day in children, this exposure is unlikely to represent a safety concern. The Panel is, however, aware that this exposure level is a mean exposure estimate and that the exposure in this age group could potentially be higher. Due to lack of food consumption data this high exposure could however not be calculated for this population group. The high exposure that could be calculated was for the adults and lower than the mean exposure in children (18 µg formaldehyde /kg bw/day). Assuming that all cheese consumed is Provolone cheese a theoretical conservative assumption of intake of HMT expressed as formaldehyde could be calculated of 87 µg/kg bw day. Also this level is however unlikely to represent a safety concern.

The theoretical conservative assumption of intake of HMT in children of 87 µg formaldehyde/kg bw/day would result in a plasma steady state concentration of 0.25 µM and a peak concentration of 1.5 µM for formaldehyde/formaldehyde acetal. The increase in formaldehyde acetal associated with an exposure to HMT at the currently permitted uses and use levels, expressed as formaldehyde residual

levels (and if ingested as a single dose which then formed a formaldehyde acetal with water),, would be less than 0.07 % (for the steady state level) and less than 0.38 % (for the peak level) of the normal intracellular endogenous levels (EFSA ANS Panel, 2013; EFSA, 2014). Such additional burden should be evaluated in the light of the naturally occurring inter-species and intra-species variation in the internal level of methanol, formaldehyde and formaldehyde acetal which by far exceed the difference between internal concentration of these endogenous substances and the additional exposure by oral intake to HMT at the currently permitted uses and use levels. Kleinnijenhuis et al. (2013) using a sensitive and specific method have measured a formaldehyde concentration in blood of 2.25 ± 0.67 mg/L in rats. This corresponds to a coefficient of variation of 30 % in endogenous formaldehyde blood levels in rats. Thus, the Panel concluded that the additional ammonium and formaldehyde arising from HMT at the currently permitted use and use levels (expressed as formaldehyde residual levels) does not constitute a significant additional risk above the risk given by the naturally occurring endogenous ammonium and formaldehyde, even when worst case assumptions are used.

CONCLUSIONS AND RECOMMENDATIONS

The Panel concluded that due to the limitations in the toxicological database a critical study could not be identified and therefore it was not possible to derive an ADI. The exposure to formaldehyde from HMT of high level consumers (95th) of Provolone cheese equalled 18 µg formaldehyde/kg bw/day in adults and could be as high as 87 µg formaldehyde/kg bw/day in 3-9 year old children according to a theoretical conservative assumption that all ripened cheese consumed was Provolone cheese. These exposures were around 1000 fold lower than formaldehyde exposure corresponding to the human therapeutic doses of 57 HMT mg/kg bw/day not associated with adverse effects in humans. Based on the:

- estimated exposures,
- consideration of the overall toxicological database on HMT,
- oral toxicity and toxicokinetic data of formaldehyde,
- the magnitude of the potential effect on intracellular formaldehyde levels arising from this use of HMT

the Panel concluded that the use of HMT in Provolone cheese at the MPL of 25 mg/kg residual amount, expressed as formaldehyde, would not be of safety concern.

However the Panel considered that any increase in the permitted uses of HMT or increases in the MPL of 25 mg /kg residual amount, expressed as formaldehyde would require detailed assessment which might require new toxicity data as well as use levels and/or an evaluation of its impact on formaldehyde levels in vivo.

DOCUMENTATION PROVIDED TO EFSA

1. Pre-evaluation document prepared by EFSA. September 2011.

REFERENCES

AFFSA, 2004. Evaluation des risques liés à l'utilisation du formaldéhyde en alimentation animale. http://www.econologie.info/share/partager2/1292066952xArJ2J.pdf

Allgen LG, Holmberg G, Persson B, Sörbo B, 1979. Biological fate of methenamine in man. Acta Obstetricia et Gynecologica Scandinavica, 58, 287-293.

Auerbach C, 1951. Problems in chemical mutagenesis. Cold Spring Harbor symposia on quantitative biology, 16, 199-213.

Baldermann KH, Rohrborn G, Schroeder TM, 1967. Mutagenicity tests with trypaflavin and hexamethylenetetramine in mammals in vivo and in vitro. Humangenetik, 4(2), 112-26.

BAuA (Bundesanstalt fur Arbeitsschutz und Arbeitsmedizin (BAuA), 2008. Risk assessment report on methenamine [CAS No. 100-97-0].

Berglund, F. (1966) Unpublished report

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

Brendel R, 1964. Studies on rats on the tolerance of hexamethylene tetramine. Arzneimittel-Forsch, 14, 51.

Briggs GG, Freeman RK, Yaffe SJ. Drugs in pregnancy and lactation: a reference guide to fetal and neonatal risk. 4th ed. Baltimore: Williams & Wilkins. (1994).

Cahill PA, Knight AW, Billinton N, Barker MG, Walsh L, Keenan PO, Williams CV, Tweats DJ, Walmsley RM, 2004. The GreenScreen((R)) genotoxicity assay: a screening validation programme. Mutagenesis, 19(2), 105-119.

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

CIR, 1992. Final report on the safety assessment of methenamine. Twentieth report of the Cosmetic Ingredient Review expert panel. Journal of the American College of Toxicology 11(4), 531-558.

Crebelli R, Falcone E, Aquilina G, Carere A, 1984. In vitro mutagenicity of rubber chemicals and their nitrosation products. Toxicology Letters, 23(3), 307-313.

Degussa AG, 1985. Unpublished data.

Della Porta G, Cabral JR, Parmiani G, 1970. Transplacental Toxicity and Carcinogenesis Studies in Rats Treated with Hexamethylenetetramine. Tumori, 56, 325-334.

Della Porta G, Colnaghi MI, Parmiani G, 1968. Non-carcinogenicity of hexamethylenetetramine in mice and rats. Food and Cosmetics Toxicology, 6, 707.

Dooley JF, Blackburn GR, Schreiner CA, Mackerer CR,1985. Inhibition of the mutagenicity of formaldehyde (HCHO) in the L5178 Y TK+/- mouse lymphoma assay by formaldehyde dehydrogenase (FDH) – Application to characterization of mutagenic components. Environmental Mutagenesis, 7, 9-9.

Dumitrescu H, 1975. General analysis of common chemical preservatives. Revista de Igiena, Bacteriologie, Virusologie, Parazitologie, Epidemiologie, Pneumoftiziologie: Igiena, 24(3), 187-191.

EFSA (European Food Safety Authority), 2004. Opinion of the Scientific Panel on additives and products or substances used in animal feed on a request from the Commission on safety of formaldehyde for poultry as feed additive in accordance with Council Directive 70/524/EEC. The EFSA Journal (2004) 96, 1-5.

EFSA (European Food Safety Authority), 2006. Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on the use of formaldehyde as a preservative during the manufacture and preparation of food additives. The EFSA Journal (2006) 415, 1-10

EFSA (European Food Safety Authority), 2011a. Evaluation of the FoodEx, the food classification system applied to the development of the EFSA Comprehensive European Food Consumption Database. The EFSA Journal, 1970, 27 pp.

EFSA (European Food Safety Authority), 2011b. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. The EFSA Journal, 2011, 34 pp.

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), 2013. Scientific Opinion on the re-evaluation of aspartame (E 951) as a food additive. EFSA Journal 2013;11(12):3496, 263 pp. doi:10.2903/j.efsa.2013.3496

European Food Safety Authority, 2014. Endogenous formaldehyde turnover in humans compared with exogenous contribution from food sources. EFSA Journal 2014;12(2):3550, 11 pp. doi:10.2903/j.efsa.2014.3550

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

Engst R, Prahl L, Jarmatz E, 1969. Analysis of preservatives. II. Colorimetric methods for the determination of hexamethylenetetramine (formaldehyde), benzoic acid sorbic acid and p-hydroxybenzoates. Nahrung, 13(5), 417-426.

EPA, 2006. Reassessemnt of two exemptions from the requirement of a tolerance from hexamethylenetetramine (HMTA; CAS Reg. No. 100-97-0).

Health Canada, 1999. List of substances d'intérêt prioritaire; Rapport d' évaluation. Formaldéhyde. http://publications.gc.ca/site/eng/94477/publication.html

Furness MB, McDonald PJ, Beasley NV, 1974. Urinary antiseptics in asymptomatic bacteriuria of pregnancy. New Zealand Medical Journal, 81, 417-419.

Ganatra VR, Sawant RM, Chaudhuri NK, Vaidya VN, 1998. Determination of hexamethylene tetramine in the process solution of sol-gel method for nuclear fuel fabrication. Journal of Radioanalytical and Nuclear Chemistry, 230(1-2), 323-326.

Gandelman A, 1967. Methenamine mandelate: Antimicrobial activity in urine andcorrelation with Formaldehyde levels. Journal of Urology, 97, 533-536.

Gelfand, 1963. Respiratory allergy due to chemical compounds encountered in the rubber, lacquer , shellac, and beauty culture industries. Journal of Allergy, 34, 374-381.

Girmanova I, Chalupa I, Sekerka V, 1991. Genotoxicity of Hexamethylenetetramine. Biologia, 46(11), 1009-1015.

Gleckman R, Alvarez S, Joubert DW, Matthews SJ, 1979. Drug therapy reviews:methenamine mandelate and methenamine hippurate. American Journal of Hospital Pharmacy, 36, 1509-1512.

Gollamudi R, Straughn AB, Meyer MC, 1981. Urinary excretion of methenamine andformaldehyde: evaluation of 10 methenamine products in humans. Journal of Pharmaceutical Sciences, 70, 596-599.

Greenfield W, Berg R, Moore R, 1969. Determination of urinary formaldehyde following administration of methenamine salts. Clinical Chemistry, 15(12), 1180-5.

Haynes WM and Lide DR, 2010. Handbook of chemistry and Physics, 2010-2011, 91st edition. ISBN 978-1-4398-2077-3; pp 3-262 and 16-43.

Hurni H and Ohder H, 1973. Reproduction study with formaldehyde hexamethylenetetramine in beagle dogs. Food and Cosmetics Toxicology, 11, 459-462.

Hutschenreuter. H. (1956) Z. Lebensmitt. - Untersuch., 104, 161

IARC, 2006. Formaldehyde, 2-butoxyethanol and 1-tert-Butox-2-propanol. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 88, 2-9.

IPCS, 1989. Formaldehyde. Geneva, World Health Organization (Environmental Health Criteria 89).

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1962. 6th Report. Evaluation of the toxicity of a number of antimicrobials and antioxidants. WHO Technical Reports Series, No. 228, 1962.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1965. 8th report. Specification for the identity and purity and toxicological evaluation of some antimicrobials and antioxidant. FAO Nutrition Meetings Report Series, No. 38, 1965. WHO Technical Reports Series, No. 309, 1965; WHO Food Additives Series N.38A.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1967. 10th report. Specification for the identity and purity and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances. FAO Nutrition Meetings Report Series, No. 43, 1967. WHO Technical Reports Series, No. 373, 1967.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1970. 13th report. Specification for the identity and purity and their toxicological evaluation: some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances. FAO Nutrition Meetings Report Series, No. 46, 1970. WHO Technical Reports Series, No. 445, 1970.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1972. 15th report. Toxicological evaluation of some enzymes, modified starches and certain other substances. FAO Nutrition Meetings Report Series, No. 50, 1972.WHO Technical Reports Series, No. 488, 1972. WHO Food Additives Series N.1.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1974. 17th report. Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents. Nutrition Meetings Report Series, No. 53, 1974. WHO Technical Reports Series, No. 539, 1974; WHO Food Additives Series N.5.

JECFA, 2004. Report of the 27th session of the Codex Committee on food Additives and Contaminant, Geneva, Switzerland, 2004.

JECFA, 2005. Evaluation of certain food additives. Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 928. Geneva, 8-17 June 2004.

JECFA, 2006. Codex Alimentarius Commission. Report of the 38th session of the Codex Committee on food Additives and Contaminant, Geneva, Switzerland, 2006.

De Jong CT, De Klerk A, Beek MT, Veenman C, Van Loveren H, 2007. Effect of Prolonged Repeated Exposure to Formaldehyde Donors with Doses Below the EC3 Value on Draining Lymph Node Responses. J Immunotoxicol. 2007 Jul;4(3):239-46. doi: 10.1080/15476910701507967.

Kewitz H and Welsch F, 1966. A Yellow Colored Product from Formaldehyde and Kynurenine in Methenamine Treated Rats. Arch. Pharmak. exp. Path, 254, 101-109.

Kewitz H, 1966. Unpublished report.

Klinge E, Männistö P, Mäntylä R, Lamminsivu U, Ottoila P, 1982. Pharmacokinetics of methenamine in healthy volunteers. Journal of Antimicrobial Chemotherapy, 9, 209-216.

Kotter L, Terplan G, Schulz H, 1959. Biological detection of preservatives in foods of animal origin. Archiv fuer Lebensmittelhygiene, 10, 145-152.

Kovac Kralj, A, 2013. Energy-Efficient Hexamine Production Process. Adv. Chem. Eng. Res., Vol. 2 Iss.

Kovacs AS, Denker P, 1962. Identification and determination of food preservatives in foods. Industrielle Obst- und Gemueseverwertung. 47(1), 1-3.

Lijinsky W and Taylor HW, 1977. Feeding tests in rats on mixtures of nitrite with secondary and tertiary-amines of environmental importance. Food and Cosmetics Toxicology, 15(4), 269-274.

Loeper I and Berzins T. Health effects of selected chemicals 3. Hexamethylenetetraamine. 28, 93-114. 1995

Malorny G and Rietbrock N, 1963. Vortrag auf der X. Tagung des Ernährungswissenschaftlichen Beirats der deutschen Fischwirtschaft, Bremen.

Malorny G, 1966. Unpublished report.

Meissner, F, Schwiedessen, E. and Othmer, D.F. Shatyski ODF, (1954). Continuous production of hexamethylenetetramine. Ind. Eng. Chem. 46, 724.

Merget R, Topcu M, Friese K, Vormberg R, Fuchs T, Raulf-Heimsoth M, Breitstadt R, 1999. A cross-sectional study of workers in the chemical industry with occupational exposure to hexamethylenetetramine. International Archives of Occupational and Environmental Health, 72(8), 533-538.

Migliore L, Ventura L, Barale R, Loprieno N, Castellino S, Pulci R, 1989. Micronuclei and nuclear anomalies induced in the gastrointestinal epithelium of rats treated with formaldehyde. Mutagenesis, 4(5), 327-334.

Musher DM, Griffith DP, 1974. Generation of formaldehyde from Methenamine: effect of pH and concentration, and antibacterial effect. Antimicrobial Agents and Chemotherapy, 6, 708-711.

Natvig H, Andersen J, Rasmussen EW, 1971. A contribution to the toxicological evaluation of hexamethylenetetramine. Food and Cosmetics Toxicology, 9(4), 491-500.

Onur F and Acar N, 1992. Simultaneous determination of methylene-blue, hexamethylene tetramine and resorcinol in pharmaceutical formulations by 1st-derivative UV spectrophotometry. International Journal of Pharmaceutics 78(1), 89-91.

Orstavik D and Hongslo JK, 1985. Mutagenicity of endodontic sealers. Biomaterials, 6(2), 129-132.

Pandey CK, Agarwal A, Baronia A, Singh N, 2000. Toxicity of ingested formalin and its management. Human & Experimental Toxicology, 19, 360-366.

Paseiro Losada P, Simal Lozano J, Simal Gandara J, 1989. Study of the identification of food preservatives. Alimentaria, 204, 57-83.

Restani P and Restelli A, 1992. Formaldehyde and hexamethylenetetramine as food additives: chemical interactions and toxicology. Food Additives & Contaminants, 9(5), 597-605.

Restani P, Galli CL, 1991. Oral toxicity of formaldehyde and its derivates, CRC Critical Reviews in Toxicology, 21, 315-328.

RTECS, 2005. NIOSH Registry of Toxic Effects of Chemical Substances #MN4725000. http://www.cdc.gov/niosh/ipcsneng/neng1228.html

SCF (Scientific Committee on Food), 1977. Reports from the Scientific Committee for Food (4th series). Opinion expressed 1977. Food science and techniques, 1977.

Schlede E, 1966. Metabolism and excretion of formaldehyde, formic acid and hexa-methylenetetramine (used as food preservatives; experiments with radioactively labelled compounds). Thesis, Freie Univ., 82 pp.

Selvaag E and Thune P, 1994. Photosensitivity reaction to methenamine hippurate. A case report. Photodermatol Photoimmunol Photomed, 10(6), 259-60.

Shimizu H, Suzuki Y, Takemura N, Goto S, Matsushita H, 1985. The results of microbial mutation test for forty-three industrial chemicals. Sangyo Igaku, 27(6), 400-19.

Siffel C and Czeizel AE, 1995. Study of developmental abnormalities and deaths after human zygote exposure. Mutat Res, 334, 293-300.

Smolin, EM and Rapoport, L. "The chemistry of heterocyclic compounds, S-Triazines and derivatives." Interscience publication, New York, 1959

Strom JG, Jun HW, 1993. Effect of urine pH and ascorbic acid on the rate of conversion of methenamine to formaldehyde. Biopharmaceutics & Drug Disposition, 14, 61-69.

Takahashi A and Ono H, 1993. Mutagenicity assessment in 44 epoxy resin hardeners in Salmonella typhimurium tester strains. Chem. Express, 8(9), 785-788.

Tanaka T, Kitajima K, Naito T, Hurutani S, Ogura S, 1976. A necropsy case of interstitial nephritis probably related to cefazolin and methenamine. Acta Med Okayama, 30(5), 341-8.

Taghdiri M and Zamani, 2013. Hexamine adsorption study on activated carbon from aqueous solutions for application in treatment of hexamine industrial wastewater. International Journal of Environmental Science and Technology January 2013, 10, 19-26

TemaNord, 2002. Food Additives in Europe, 2000. Status of safety assessment of food additives presently permitted in the EU. TemaNord 2002:560.

Tierfarm AG, 1969. Unpublished report.

Til HP, Woutersen RA, Feron VJ, Hollanders VHM, Falke HE, Clary JJ, 1989. 2-year drinking-water study of formaldehyde in rats. Food and Chemical Toxicology, 27(2), 77-87.

Vujosevic, M., Zivkovic, S., Fister, S. (1986): In vivo mammalian bone marrow cytogenetic test (chromosomal analysis) - Result of testing UROTOVETR. Acta Vet (Beograd), 36, 91-94

WHO, 2008. Guidelines for drinking-water quality. Third Edition, .incorporating first and second addenda. World Health Organisation. Geneva. p. 377b. Available at: http://www.who.int/water_sanitation_health/dwq/fulltext.pdf?ua=1

Wickramaratne GAS, 1987. The Chernoff-Kavlock assay: Its validation and application in rats. Teratogenesis, Carcinogenesis, and Mutagenesis, 7, 73-83.

Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K, 1992. Salmonella mutagenicity tests .5. Results from the testing of 311 chemicals. Environmental and Molecular Mutagenesis, 19, 2-141.

ABBREVIATIONS

ADME	Absorption, Distribution, Metabolism and Excretion
ADI	Acceptable Daily Intake
bw	Body weight
CAS	Chemical Abstract Service
Chem Id Plus	A free database of 350000 chemical compounds
EC	The European Committee
EFSA	The European Food Safety Authority
EINECS	European Inventory of Existing Commercial chemical Substances
EU	The European Union
FAO	The Food and Agriculture Organisation of the United Nations
IARC	International Agency for Research on Cancer
JECFA	The FAO/WHO Joint Expert Committee on Food Additives
TemaNord	Nordic Working Group on Food Toxicology and Risk Assessment
NOEL	No Observed Effect Level
SCF	The EU Scientific Committee on Food
US EPA	The US Environmental Protection Agency
WHO	The World Health Organisation