SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of formaldehyde for all animal species based on a dossier submitted by Regal BV[1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 15 October 2014, replaces the earlier version published on 18 February 2014.[4]

ABSTRACT

The additive formaldehyde is an aqueous solution containing 35 % formaldehyde and 14 % methanol. It is intended for use in all animal species at concentrations between 200 and 1000 mg active substance/kg complete feed. Free and reversibly bound formaldehyde is readily absorbed in the gastrointestinal tract and joins the pool of endogenous formaldehyde. It is rapidly oxidised to formic acid further on to carbon dioxide and water. Formaldehyde is a carcinogen by inhalation. While local irritation is expected to strongly promote carcinogenesis, lower local concentrations of formaldehyde are known to produce DNA adducts. Therefore, the FEEDAP Panel deems it prudent not to consider the exposure to non-irritant concentration as totally riskless. Moreover, on the basis of the present knowledge, a causal association between formaldehyde exposure and leukaemia cannot be ruled out. The FEEDAP Panel estimated the oral intake of formaldehyde of consumers from food of animal origin to be 4 mg per person per day. A reliable additional exposure of consumers to formaldehyde from supplementing feedingstuffs cannot be calculated. However, the FEEDAP Panel considers that the proposed use of formaldehyde as a feed additive would not increase consumer exposure and consequently would not pose an additional risk for the consumer. A safe feed concentration for all animal species and categories could not be determined. Formaldehyde is a strong irritant, a potent skin and respiratory sensitiser. Measures should be taken to ensure that the respiratory tract, skin and eyes of any person handling the product are not exposed to any dust, mist or vapour generated by the use of formaldehyde. The use of formaldehyde in animal nutrition is not expected to pose a risk for the environment. Formaldehyde in concentrations between 200 and 1000 mg/kg feed (compound feed and/or feed material) has the potential to be an efficacious preservative.

KEY WORDS

technological additive, preservative, formaldehyde, safety, efficacy

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[1] On request from the European Commission, Question No EFSA-Q-2011-00333, adopted on 28 January 2014.

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Technological Additives, including Jurgen Gropp, Georges Bories, Carlo Nebbia and Anne-Katrine Lundeby, for the preparatory work on this scientific opinion.

[4] An editorial amendment was carried out that does not materially affect the contents or outcome of this Scientific Opinion. To avoid confusion, the original version has been removed from the EFSA Journal, but is available on request, as is a version showing all the changes made.

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SUMMARY

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of formaldehyde as preservative in feedingstuffs for all animal species.

The additive formaldehyde is an aqueous solution containing 35 % formaldehyde and 14 % methanol. It is intended for use in all animal species at concentration between 200 and 1 000 mg formaldehyde (active substance)/kg complete feed.

Free and reversibly bound formaldehyde, when ingested, is readily absorbed in the gastrointestinal tract and joins the pool of endogenous formaldehyde. It is rapidly oxidised to formic acid, which enters the one-carbon pool of the body and is further oxidised to carbon dioxide and water. The additive contains also methanol, which is oxidised to formaldehyde.

Formaldehyde is a carcinogen by inhalation. While local irritation is expected to strongly promote carcinogenesis, lower local concentrations of formaldehyde are known to produce DNA adducts. Therefore, the FEEDAP Panel deems it prudent not to consider the exposure to non-irritant concentration as totally riskless. Moreover, on the basis of the present knowledge, a causal association between formaldehyde exposure and leukaemia cannot be ruled out.

The FEEDAP Panel estimated the oral intake of formaldehyde of consumers from food of animal origin to be 4 mg per person per day. A reliable additional exposure of consumers to formaldehyde from supplementing feedingstuffs cannot be calculated. However, the highest values found in the few available deposition studies are much lower than those reported in the available literature, and are therefore already included in the exposure scenario. Therefore, the FEEDAP Panel considered that the proposed use of formaldehyde as a feed additive would not increase consumer exposure and consequently would not pose an additional risk for the consumer.

No apparently safe concentration can be established for veal calves. It appears that (i) 470 mg formaldehyde/kg feed would be safe for chickens for fattening, laying hens and Japanese quail and (ii) 630 mg formaldehyde/kg feed would be safe for piglet, a margin of safety could not be identified considering the shortcomings of the study. However, adverse effects on reproductive organs were seen at 930 mg/kg feed for male poultry and at 1 850 mg/kg feed for female Japanese quail. Since these endpoints are not specifically addressed in tolerance studies, a formaldehyde concentration safe for reproduction cannot be derived. In conclusion, a safe level for all animal species and categories, including all poultry and all pigs, could not be determined.

Formaldehyde is a toxic substance, a strong irritant, a potent skin and respiratory sensitiser (including occupational asthma) and a proven human carcinogen by the respiratory route. No safe level of exposure of the skin, eyes or the respiratory system to formaldehyde could be identified. Therefore, measures should be taken to ensure that the respiratory tract, as well as skin and eyes, of any person handling the product are not exposed to any dust, mist or vapour generated by the use of formaldehyde. The FEEDAP Panel recommended that consideration should be given to whether the strict protection measures, once established, would effectively protect users at the level of feed compounders and farmers.

Formaldehyde will not accumulate in the environment and its use in animal nutrition is not expected to pose a risk for the environment.

Formaldehyde in concentrations between 200 and 1 000 mg/kg feed (compound feed and/or feed material) has the potential to be an efficacious preservative.

TABLE OF CONTENTS

BACKGROUND

Regulation (EC) No 1831/2003[5] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7 and Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of this Regulation for additives authorised without time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from the company Regal BV[6] for authorisation/re-evaluation of the product formaldehyde , when used as a feed additive for all animal species (category: technological additives; functional group: preservatives, silage additives) under the conditions mentioned in Table 1. During the course of the assessment, the applicant requested to limit the application to the functional group preservatives.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive) and under Article 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application.[7] According to Article 8 of that Regulation, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. The particulars and documents in support of the application were considered valid by EFSA as of 06 June 2011.

The additive is aqueous solution of formaldehyde. The active substance is currently authorised for use as silage additive for all species and categories of animals, with no maximum feed inclusion limit, and without a time limit and for use as preservative for skimmed milk intended for use in pigs up to 6 months of age, with a maximum content of 600 mg/kg.

The Scientific Committee on Animal Nutrition (SCAN) issued several opinions on the use of formaldehyde in feedingstuffs for piglets (EC, 1983) and on the use of formaldehyde as preserving agent for animal feedingstuff (EC, 1995; EC, 1999; EC, 2002). The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued an opinion on the safety of formaldehyde for poultry (EFSA, 2004). The Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) issued an opinion on the use of formaldehyde as a preservative during the manufacture and preparation of food additives (EFSA, 2006).

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animal(s), consumer, user and the environment and the efficacy of the product formaldehyde, when used under the conditions described in Table 1.

[5] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[6] Regal BV, Wilhelminalaan 90, 6042, Roermond, The Netherlands.
[7] EFSA Dossier reference: FAD-2010-0222.

Table 1: Description and conditions of use of the additive as proposed by the applicant

Additive	formaldehyde			
Registration number/EC No/No (if appropriate)	E240			
Category of additive	1. Technological additives			
Functional group(s) of additive	(a) Preservatives			
Description				
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)	
formaldehyde in solution	CH2O	-	ISO 2227	
Trade name (if appropriate)	-			
Name of the holder of authorisation (if appropriate)	-			
Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg or Units of activity or CFU kg^{-1} of complete feedingstuffs (select what applicable)		
All species	-	200	1000	-
Other provisions and additional requirements for the labelling				
Specific conditions or restrictions for use (if appropriate)	All species, no restrictions			
Specific conditions or restrictions for handling (if appropriate)	Avoid contact with skin and eyes. Do not ingest. Handle/weigh this product under conditions of good local exhaust ventilation to avoid breathing fumes or aerosol. Use personal protective equipment			
Post-market monitoring (if appropriate)				
Specific conditions for use in complementary feedingstuffs (if appropriate)				
Maximum Residue Limit (MRL) (if appropriate)				
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues	
-				

ASSESSMENT

This opinion is based on data provided by a company involved in the production of formaldehyde. The FEEDAP Panel has sought to use the data provided together with data from other sources to deliver an opinion.

1. Introduction

Formaldehyde is currently authorised for use as silage additive for all species and categories of animals, with no maximum feed inclusion limit and without a time limit, and for use as preservative for skimmed milk intended for use in pigs up to six months of age, with a maximum content of 600 mg/kg. Both uses are foreseen for re-evaluation according to the provisions set out in Regulation (EC) No 1831/2003. No other feed or food uses of formaldehyde are authorised in Europe.

The applicant is seeking authorisation/re-evaluation for formaldehyde as technological additive (functional group preservative) in feed for all animal species.

Formaldehyde is authorised in the EU as a preservative in cosmetics (0.2 % in all cosmetics, 0.1 % in products for oral hygiene, expressed as free formaldehyde, and 0.5 % in nail hardeners).[8]

In the USA formaldehyde is authorised for use as feed additive at maximum levels of 2.5 g/kg (formaldehyde aqueous solution 37%),[9] as fumigant for the fumigation of eggs in hatcheries,[10] and as a fungicide, pesticide and bactericide in aquaculture.[11] Formaldehyde is also authorised for use in vaccines.[12]

2. Characterisation and identity

The additive formaldehyde is an aqueous solution of formaldehyde (34.9-35.1 % w/w by specification) and methanol (13.8-14.2 % w/w by specification), with a maximum concentration of formic acid of 0.05 % and maximum iron content of 2.0 mg/kg. The analysis of five batches of the additive showed that it complies with the specifications (formaldehyde 35.0 to 35.1 % w/w, methanol 13.9 to 14.2 % w/w, formic acid 0.015 to 0.023 % w/w).[13] No information has been provided on iron concentration.

The active substance formaldehyde (Chemical Abstracts Service (CAS) no 50-00-0; EC no. 200-001-8) is a gas with molecular weight of 30.02 and molecular formula HCHO.

Formaldehyde is chemically synthesised using methanol as starting material and diluted in water to reach the specified concentration. Methanol is added to the aqueous solution to avoid the formation and precipitation of polymers during storage at temperatures < 20 °C (Walker, 1964; Reuss et al., 2005). In aqueous solution, most of formaldehyde (99.9 %) is in the hydrated form, gem-diol $CH_2(OH)_2$.

A premixture containing 90 % of the additive formaldehyde was analysed for heavy metals and arsenic. Mean values of the analysis of five batches were < 0.5 mg lead/L , < 0.5 mg cadmium/L, < 0.02 mg mercury/L and < 0.05 mg arsenic/L.[14] Assuming that all measured concentrations for heavy metals and arsenic come from formaldehyde, the concentration in the additive would not be of concern.

[8] Council Directive of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products (76/768/EEC). OJ L 262, 27.9.1976, p. 169.
[9] Available online: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=573.460
[10] Available online: http://www.ecfr.gov/cgi-bin/retrieveECFR?gp=&SID=abb09b2cbcba5d684a6c0a6776d7b040&n=9y1.0.1.7.64.3&r=SUBPART&ty=HTML#9:1.0.1.7.64.3.82.5
[11] Available online: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=529.1030
[12] Available online: http://www.gpo.gov/fdsys/pkg/CFR-2003-title9-vol1/pdf/CFR-2003-title9-vol1.pdf
[13] Technical dossier/Section II/Annex 2.1.1c.
[14] Technical dossier/Supplementary Information April 2012/Appendix 1.

2.1. Physico-chemical properties

In the presence of feed materials, formaldehyde reacts with primary and secondary amines of proteins and purine and pyrimidine bases to produce methylol groups (R-NH-CH_2OH) or Schiff bases (R_1-N=CH-R_2), in both cases in reversible reactions. The amino groups (α and ε) of proteins react rapidly, whereas those of nucleic acids more slowly. Further irreversible condensation of methylol groups with amines that bridges amino groups (R_1=N-CH_2-N=R_2) takes place intramolecularly, to form cyclic structures, or intermolecularly, to produce aggregates. As a consequence, formaldehyde exists in different forms in formaldehyde-treated feedingstuffs: (i) free HCHO, (ii) reversibly bound and labile under weakly acid conditions and (iii) irreversibly bound (AFSSA, 2004).

2.2. Stability and homogeneity

2.2.1. Shelf life

Three sub-samples from two batches each of formaldehyde were stored in closed polyethylene containers at room temperature for 18 months. No differences in the formaldehyde concentration (initial mean concentration: 36.1 % w/w, final mean concentration 37.3 % w/w) were recorded.[15]

2.2.2. Stability in premixtures and feedingstuffs

The recovery of the active substance (formaldehyde) was studied in three batches of a premixture (90 % of the additive formaldehyde) stored in closed polyethylene containers kept for two years at 25° C, and three batches of a commercial compound feed for poultry supplemented with 630 mg formaldehyde/kg using the same premixture. Final recovery after three months storage was 94.8 %[16] and 84.8 %[17] formaldehyde, respectively. It should be noted that formaldehyde is a very reactive chemical which interacts with feedingstuffs, particularly its protein fraction.

2.2.3. Homogeneity

Three poultry feeds (two mash and one extruded) were treated with formaldehyde (190 mg/kg mash feed (two batches) and about 300 mg/kg extruded feed) incorporated via a premixture (90 % of the additive formaldehyde).[18] Ten subsamples of each feed were analysed for formaldehyde concentration. The coefficient of variation (CV %) ranged from 9.5 % to 12.5 %.

2.3. Conditions of use[19]

The additive formaldehyde is intended to be used as preservative in feedingstuffs for all animal species, with a minimum content of 200 mg/kg feed and a maximum content of 1 000 mg formaldehyde (active substance)/kg feed. The applicant further noted that formaldehyde should not be incorporated to feedingstuffs via vitamin and mineral premixtures.

The additive formaldehyde is intended by the applicant to be incorporated in final feed only via a premixture, containing, among others, propionic acid and an emulsifier.

2.4. Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)

EFSA has verified the EURL report as it relates to the methods used for the control of formaldehyde in animal feed. The Executive Summary of the EURL report can be found in the Appendix.

[15] Technical dossier/Section II/Annex 2.4.1.a.
[16] Technical dossier/Section II/Annex 2.4.1.b.
[17] Technical dossier/Section II/Annex 2.4.1.c.
[18] Technical dossier/Supplementary Information April 2012/Appendix 2.
[19] This section has been edited following the confidentiality claims made by the applicant.

3. Safety

In recent years the kinetics and toxicity of formaldehyde have been described in a series of comprehensive reviews (OECD, 2002; Skrzydlewska, 2003, WHO, 2005; IARC, 2006, 2012; ATSDR 2010; US EPA, 2010; ECHA, 2011;. NRC 2011; NTP, 2011). The majority of toxicological findings originate from inhalation studies.

3.1. Absorption, distribution, metabolism and excretion

In all animal species, formaldehyde is an essential metabolic intermediate in all cells, in which it can be formed from hydroxymethyl groups during enzymatic methylation and demethylation processes. It is also an essential intermediate in the biosynthesis of purines, thymidine and certain amino acids (Neuberger, 1981). Under physiological conditions, the level of endogenous formaldehyde is maintained at a low concentration being regulated by the expression and activity of both formaldehyde-generating and formaldehyde-degrading enzymes (Teng et al., 2001). In humans and experimental animals, blood levels are in the range of 2–3 mg/L, with concentrations in the liver and nasal mucosa of the rat being two to four-fold higher than that found in the blood (Heck et al., 1982). In cows and calves, blood levels were 0.5 mg/kg and 0.65 mg/kg, respectively, while tissue levels in calves were in the range 0.13 to 3.6 mg/kg, with muscle showing the lowest and liver showing the highest concentrations (Buckley et al., 1988).

A Scientific Report of EFSA (EFSA, 2014) attempts to quantify the endogenous synthesis in humans. Based on a constant blood concentration of formaldehyde from endogenous production of 2.5 mg/L (Heck et al., 1985), and assuming an equal distribution in the aqueous compartment of the body and a total of 42 L body water for a 60 kg person, the body store of formaldehyde can be estimated to be 105 mg (EFSA, 2014). Given a half-life of formaldehyde in the body of 1.5 minutes (Clary and Sullivan, 2001), 52.5 mg will be degraded every 1.5 minutes. A 60 kg person would metabolise about 50 g formaldehyde per day. This evaluation confirms former results showing that the liver metabolizes 22 mg formaldehyde/minute (about 50 g of formaldehyde per day) to carbon dioxide (Waydhas et al., 1978).

Free and reversibly bound formaldehyde, when ingested, is readily absorbed in the gastrointestinal tract and joins the pool of endogenous formaldehyde (WHO, 2005). Formaldehyde is rapidly oxidised in blood and liver to formic acid by the NAD-dependent formaldehyde dehydrogenase through a glutathione (GSH)-dependent process. In turn, formic acid partially enters the one-carbon pool of the body or is further oxidised to carbon dioxide and water in the liver and in the erythrocytes. In primates, this reaction occurs more slowly than in dogs or rats. The residual unmetabolised formic acid and other minor metabolites are excreted via urine, faeces or expired air (IARC, 2006). Owing to its chemical reactivity, formaldehyde is essentially present in reversibly and irreversibly bound forms, as free formaldehyde, representing 1 to 2 % of total measurable amounts in tissues, and as formaldehyde irreversibly bound to proteins and nucleic acids, accounting for between 50 % - 80 % of endogenous formaldehyde (Heck et al., 1982).

Inhaled formaldehyde is unlikely to be distributed systemically, a strong interaction and/or biotransformation occurring at the site of contact. Exposure of animals (rats, nonhuman primates) to labelled exogenous formaldehyde resulted in the formation of labelled DNA and protein adducts at the site of contact, not in the bone marrow or liver (Lu et al., 2010; Moeller et al., 2011; Edrissi et al., 2013). In a recent review, the NRC (2011) concluded *"the weight of evidence suggests that is unlikely for formaldehyde to appear in the blood as an intact molecule, except perhaps after exposures that are high enough to overwhelm the metabolic capability of the tissues of the site of entry"*. No similar investigations of oral exposure have been performed. However, the administration to target animals of feed supplemented with formaldehyde at doses similar to those proposed for use resulted in a moderate increase in formaldehyde concentrations in tissues (see section 3.2.2). This would that the metabolic capacity to handle these amounts of exogenous formaldehyde is limited.

The additive formaldehyde also contains methanol (13.2 to 14.0 %), which is a further source of formaldehyde. In fact, methanol undergoes oxidation into carbon dioxide and water in the liver via its intermediate metabolites formaldehyde and formic acid.

3.2. Toxicological profile

Owing to the strongly polarized carbonyl group, formaldehyde easily reacts with the amino and sulphydryl groups in small molecular compounds, including GSH, peptides, proteins (including many enzymes) and nucleic acids. These reaction products have been linked to the alterations of the biological properties of several proteins leading to cytotoxicity as well as direct genetic effects. Damage has been observed principally at sites of contact such as the respiratory tract and the oral and gastrointestinal mucosa.

The US EPA (2010) and WHO (2005) set a Reference Dose (RfD) and a Tolerable Daily Intake (TDI), respectively, on the basis of a no observed adverse effect level (NOAEL) of 15 mg per kg bw per day for bodyweight reduction, stomach irritation and related papillary hyperplasia in rats given formaldehyde in water for drinking for two years (Til et al, 1989).

A meta-analysis of 18 retrospective human studies after inhalatory exposure showed increased risks of spontaneous abortion and of all adverse pregnancy outcomes combined (Duong et al., 2011). No safe level of exposure was identified.

Mutagenicity and genotoxicity investigations *in vitro*, in laboratory animals and in humans have shown that formaldehyde can react directly with DNA (Lu et al., 2009), and can cause gene mutations and chromosomal aberrations.

It has been known for decades that formaldehyde can be genotoxic at the site of contact. The carcinogenicity of formaldehyde has been reviewed by the US Environmental Protection Agency (US EPA, 2010) and IARC (2012), taking account of numerous carcinogenicity studies in laboratory animals and human epidemiological studies, and considering possible mechanisms of action.

US EPA (2010) concluded *"human epidemiological evidence is sufficient to conclude a causal association between formaldehyde exposure and nasopharyngeal cancer, nasal and paranasal cancer, all leukemias, myeloid leukemia and lymphohematopoietic cancers as a group"*. However, the NRC (2011) concluded that the US EPA draft assessment *"did not provide a clear framework for causal association" between formaldehyde exposure and lymphohematopoietic cancer and "the absence of a causal framework for these cancers is problematic given the inconsistencies in the epidemiological data, the weak animal data and the lack of mechanistic data."*

The IARC (2012) discussed the evidence for formaldehyde causing three types of human cancer: nasophryngeal cancer, sinonasal cancer and leukaemia. It concluded that: *"there is sufficient evidence in humans for a causal association of formaldehyde with of the nasopharynx and leukaemia and limited evidence for a causal association of formaldehyde with sinonasal cancer"* (IARC 2012). The conclusions about leukaemia were based on human epidemiological data and on the results of mutagenicity/genotoxicity studies. The experimental evidence, reviewed by IARC (2012), indicates the possibility of a systemic genotoxic effect. However, the validity of one of the key studies showing such effects in humans (Zhang et al. 2010) has been questioned by a critical review (Gentry et al., 2013), and the issue of possible systemic genotoxicity of formaldehyde remains controversial.

Site-of-contact tumours (e.g. nasopharyngeal or paranasal cancers) originate through different modes of action involving multifactorial mechanisms. While local irritation is expected to strongly promote carcinogenesis, lower local concentrations of formaldehyde are known to produce DNA adducts. Therefore, the FEEDAP Panel deems it prudent not to consider the exposure to non-irritant concentration as totally riskless. Moreover, on the basis of the present knowledge, a causal association between formaldehyde exposure and leukaemia cannot be ruled out.

3.3. Safety for the target species

The applicant has performed tolerance studies in chickens for fattening, laying hens and weaned piglets. In the three studies the premixture described under 2.2 was supplemented to complete feed. In the view of the FEEDAP Panel these studies can be considered as tolerance studies performed with the additive under application.

Since the application is for all animal species, the applicant was requested by EFSA to conduct further tolerance studies (in salmonids or ruminants) to enable the FEEDAP Panel to assess the safety for all animal species. The applicant did not provide this data; therefore, EFSA continued the assessment on the basis of the available data, including published studies in poultry and calves.

3.3.1. Tolerance in poultry

3.3.1.1. Chickens for fattening[20]

A total of 480 male Hubbard chickens for fattening were fed for 35 days with pelleted commercial diets supplemented with formaldehyde, at intended levels of 0, 630, 1580 and 6 300 mg active substance/kg feed (confirmed by analysis). Zootechnical parameters, routine haematology and clinical biochemistry were analysed.[21] No differences in mortality were observed between the treatments (average mortality 2.7 %). The body weight data indicate a treatment related effect. This view is supported by a significant curvilinear regression (y (body weight (g) at 35 days) = 2443 - 102.7x - $44.2x^2$, x (mean of three formaldehyde determinations per g/kg diet), n = 24 (replicates), R^2 = 0.98). Already the lowest formaldehyde concentration in feed (630 mg/kg) seemed to reduce final body weight (measured values: 2 333 g vs 2 440 g, although not statistically significant).

A marked decrease in erythrocytes, haemoglobin, haematocrit, neutrophils, lymphocytes and monocytes was recorded in the highest dose. A treatment related reduction seemed to occur for thrombocytes number and was demonstrated for lactate dehydrogenase activity, which was reduced at all doses. No effects were observed in any of the other biochemical parameters measured. Necropsy results did not show differences in organ lesions among treatments.

3.3.1.2. Laying hens[22]

A total of 144 individually caged Lohmann Brown laying hens were fed for 56 days with diets containing intended concentrations of 0, 630, 1 580 and 6 300 mg active substance/kg feed (the analysed levels, 0, 470, 910 and 4 980 mg active substance/kg feed, were about 30 % lower than the intended concentration). Zootechnical parameters, routine haematology and clinical biochemistry were analysed.[23] No mortality was registered in any group. Treatment levels of 470 and 910 mg formaldehyde/kg diet did not affect the zootechnical parameters. In contrast, hens administered 4980 mg formaldehyde/kg feed exhibited a marked reduction of daily feed consumption, body weight, and laying rate, along with remarkable changes in blood parameters (increased thrombocytes and heterophils; reduced linfocytes, cholesterol, triglycerides, uric acid calcium and phosphorus). A numerical increase of thrombocytes and a decrease of plasma calcium was also observed at 910 mg formaldehyde/kg feed (egg shell parameters were not measured). It is conceivable that poor performances and the alteration of blood parameters (e.g. triglycerides) in the 4 980 mg formaldehyde group could be linked to the reduced feed intake. Also in this group, high incidences were found for erosions in the crop mucosa together with a black discolouration and necrotic areas in liver. No differences in necropsy results were observed in the 470 and 910 mg/kg treatments compared to the control.

[20] This section has been edited following the confidentiality claims made by the applicant.
[21] Technical dossier/Section III/Annex 3.1.1.a 0-9
[22] This section has been edited following the confidentiality claims made by the applicant.
[23] Technical dossier/Section III/Annex 3.1.1.c 0-4

3.3.1.3. Cockerels

White Leghorn cockerels (10 weeks old, 15 per treatment) were fed diets containing 0, 930, 1 850, 3 700 mg formaldehyde (active substance)/kg complete feed (intended values) for eight weeks. Different endpoints were reported in separate publications (Khan et al., 2003 and 2006). Even the lowest formaldehyde dose significantly reduced haemoglobin and haematocrit after four and eight weeks; leukocyte counts were significantly reduced at the end of the study. Formaldehyde treatment resulted in a significant increase in serum alanine aminotransferase (ALT), whereas serum alkaline phosphatase was reduced. Formaldehyde treatment for eight weeks reduced serum testosterone concentrations, apparently in a dose-related manner. In all groups administered formalin, the diameters of the seminiferous tubules were significantly smaller than in control animals.

3.3.1.4. Quail

A total of 75 male Japanese quail at 35 days of age were fed diets supplemented with formaldehyde at an intended concentration of 0, 930, 1 850, 3 700 or 7 400 mg formaldehyde (active substance)/kg complete feed for eight weeks (Anwar et al., 2001). Quail fed 3 700 and 7 400 mg active substance/kg feed showed reduced feed intake and body weight. Vacuolation in the germinal epithelial layer of their seminiferous tubules was observed. Formaldehyde concentrations starting from 1 850 mg/kg was associated with decreased weight of testes and even 930 mg/kg feed resulted in a statistically significant smaller diameter of seminiferous tubules.

Seventy-five one-day-old female Japanese quail were divided into five groups and fed diets containing formaldehyde at an intended concentration of 0, 930, 1 850, 3 700 and 7 400 mg formaldehyde (active substance)/kg complete feed for eight weeks (Khan et al., 2005). No clinical signs and pathological alterations were observed in quail fed 930 mg active substance/kg feed. At 1850 mg formaldehyde/kg feed, a reduction in area and folds of different segments of the oviduct were recorded. A degeneration of mucosal glands characterised by vacuolation of nuclei of cells was observed in the oviduct. Feed intake, body weight, egg production and egg weight together with absolute and relative weight of organs, erythrocyte and leukocyte counts, haemoglobin concentration and haematocrit were reduced at the high doses of 3 700 and 7 400 mg formaldehyde active substance/kg feed.

3.3.1.5. Summary of the findings in poultry

The study with chickens for fattening did not confirm the safety of the highest proposed formaldehyde concentration in complete feed for chickens for fattening (1 000 mg/kg feed). The results obtained with the lowest concentration tested (630 mg/kg), albeit not all significantly different from the control figures, could be interpreted as weak initial signs of intolerance in chickens for fattening. Although zootechnical performance of laying hens was not affected by 470 and 910 mg active substance/kg feed, the reduced plasma calcium at 910 mg/kg indicated a negative effect on calcium metabolism, which is expected to exert negative consequences on laying rate with longer duration of the experiment (egg shell parameters were not measured). In a published study with cockerels, the lowest dose tested (930 mg formaldehyde/kg feed) affected haematology, clinical biochemistry and reduced serum testosterone concentrations. Comparable results were found in two published studies with Japanese quail. In one study no effects in female quail were reported at 930 mg/kg, while 1 850 mg/kg feed affected the morphology of the oviduct. In the other study on male quail, 1 850 mg reduced the weight of testes and 930 mg/kg resulted in a smaller diameter of seminiferous tubules.

In summary, five studies were available for the assessment of safety for poultry species. Four of them did not confirm the safety of the highest proposed dose (1 000 mg formaldehyde/kg complete feed).

3.3.2. Tolerance in piglets[24]

A total of 144 weaned female and castrated male piglets were fed for 42 days with a pelleted commercial diet supplemented with 0, 630, 1580 and 6300 mg active substance/kg feed, (confirmed

[24] This section has been edited following the confidentiality claims made by the applicant.

by analysis).[25] Zootechnical parameters, routine haematology and clinical biochemistry were analysed. No mortality was observed in any group. All zootechnical performance parameters were negatively influenced only by the highest level of dietary formaldehyde (6300 mg/kg), which also induced a decrease in both mean corpuscular volume and mean content of haemoglobin/erythrocyte and an increase in both serum urea content and ALT activity. In addition there was a statistically significant linear trend toward the increase in blood thrombocytes number and in the serum urea content in treated *vs* untreated piglets irrespective of the dietary formaldehyde level. The results of necropsy could not be evaluated since a list of the recorded lesions was not provided. No histopathological examination was performed.

3.3.2.1. Summary of the findings in piglets

The study in piglets is poorly reported and allows only limited conclusions. Zootechnical parameters, haematology and clinical biochemistry suggest that 630 mg formaldehyde/kg feed would be safe for piglets, but a margin of safety could not be identified considering the shortcomings of the study.

3.3.3. Tolerance in veal calves

No tolerance studies in cattle were provided. One study was found in literature in which two-week-old calves previously fed whole milk were switched to 0.1 % formalin treated skim milk. Difficulty was experienced in accustoming the calves to the formalin-treated milk and scouring occurred within two days of the changeover. Severe gross- and microscopic lesions of the alimentary tract compatible with clinical symptoms were recorded in calves fed formalin-treated skimmed milk (Preston et al., 1960).

3.3.4. Conclusions on the safety for the target species

The conclusions are based on five tolerance studies in poultry (duration 35 to 56 days), one in piglets (duration 42 days) and one in veal calves. No safe concentration can be established for veal calves. It appears that (i) 470 mg formaldehyde/kg feed would be safe for chickens for fattening, laying hens and Japanese quail, and (ii) 630 mg formaldehyde/kg feed would be safe for piglets, a margin of safety could not be identified considering the shortcomings of the study.

However, adverse effects of formaldehyde on reproductive organs were seen at 930 mg/kg feed for male poultry and at 1850 mg/kg feed for female Japanese quail. Since these endpoints are not specifically addressed in tolerance studies, a formaldehyde concentration safe for reproduction cannot be derived. In conclusion, a safe level for all animal species and categories, including all poultry and all pigs, cannot be determined.

3.4. Safety for the consumer

3.4.1. Background occurrence of formaldehyde

Typical formaldehyde concentrations in foodstuffs are summarised by WHO (1989): fruit and vegetables contain between 3 and 60 mg/kg, milk approximately 1 mg/kg, meat and fish 6–20 mg/kg and shellfish 1–100 mg/kg. Drinking water generally contains < 0.1 mg/L.

Analytical data published between 1996 and 2009 confirm the ranges given by WHO (1989). Formaldehyde concentrations in fruit and vegetables are between 6 and 35 mg/kg, in meat between 2 and10 mg/kg, in liver pâté 12 mg/kg, in sausages 10–21 mg/kg and in milk about 0.8 mg/kg (Trezl et al., 1997; Weng et al., 2009). Much lower concentrations were found by Kaminski et al. (1993) for milk, ranging from 0.013 to 0.057 mg/kg in fresh milk (n = 18) from Holstein cows (morning milking). Concentrations in processed milk (i.e. 2% milk fat, partly skimmed, pasteurised) were higher and ranged from 0.075 to 0.255 mg/kg (n = 12).

[25] Technical dossier/Section III/Annex 3.1.1.b 0-8.

In pig liver, kidney and muscle, formaldehyde levels have been measured at 11.8, 8.8, 6.2 mg/kg, respectively (Retfalvi et al., 1998). In meat products, background levels of formaldehyde range from 2.5 mg/kg in sandwich paste made from poultry meat, through 2.9–4.6 mg/kg in cold meat cuts, ham from poultry and turkey and 10–20.7 mg/kg in sausages up to 224–267 mg/kg in the outer layer of smoked ham (Trezl et al., 1997; Brunn and Klostermeyer, 1984).

Formaldehyde concentrations in fish show higher extreme values: 220–290 mg/kg; however, averages are between 2 and 50 mg/kg (Bianchi et al., 2007; Weng et al., 2009). Formaldehyde is formed post mortem in seafood from the enzymatic reduction of trimethylamine-N-oxide (TMAO) to formaldehyde and dimethylamine; formaldehyde accumulates in frozen fish (Sotelo et al., 1995; Badii and Howell, 2002).

3.4.2. Formaldehyde in tissues after feed supplementation

No specific residue studies have been provided by the applicant concerning the transfer of exogenous formaldehyde to edible tissues/products resulting from the use of formaldehyde as feed additive.

Buckley et al. (1988) measured formaldehyde concentration in morning milk of cows fed whey (75 kg/day) supplemented with 0, 185, 370 or 555 mg formaldehyde active substance/kg whey. The formaldehyde level in milk from control cows was below the limit of detection (< 0.026 mg/kg). Formaldehyde concentrations in the milk of the cows receiving 185, 370 and 555 mg formaldehyde active substance/kg whey were in the range of < 0.026–0.05 mg/kg, 0.05–0.11 mg/kg and 0.18–0.26 mg/kg, respectively. The average blood formaldehyde concentration in cows fed 555 mg formaldehyde active substance/kg whey was greater ($P < 0.01$) than that of control cows at 33 days (0.831 ± 0.132 mg/kg vs. 0.615 ± 0.110 mg/kg).

In a 10-week feeding study with dairy cows administered 5 g formaldehyde/day from formaldehyde-treated soya bean meal, an increase in the formaldehyde concentration of milk from the initial level of 0.023–0.039 mg/L to 0.095–0.114 mg/L after three weeks and 0.25 mg/L after 10 weeks was observed (Pinault, 1989, cited in AFSSA, 2004).

Skimmed milk containing 0.1 % formalin (400 mg formaldehyde/L) was fed to pigs. Formalin-treated milk and tissues from control and experimental animals were analysed for residual formaldehyde, present as free and loosely protein-bound. About 20 % of the formaldehyde added to milk was irrecoverable after seven days of storage, probably due to irreversible binding to proteins. The mean concentrations of formaldehyde in tissues taken from experimental and control pigs were similar (19.7 and 20.2 mg/kg, respectively) (Florence and Milner, 1981).

In another study, goats fed various levels of formaldehyde-treated soya bean oil-meal were found to excrete about 0.02 % of ingested formaldehyde in milk as free formaldehyde a measured with a high-performance liquid chromatography (HPLC) method (Barry and Tomé, 1991).

Buckley et al. (1988) also investigated formaldehyde tissue deposition in Holstein calves administered whey (10 kg/day) containing 0, 185 or 370 mg formaldehyde active substance/kg whey for up to 95 days. Two calves from each treatment group were slaughtered 81, 88 and 95 days after the beginning of the trial, and tissue samples of heart, kidney, liver and muscle (m. longissimus dorsi) were collected and frozen until subjected to formaldehyde analysis, which was also performed on fresh muscle samples. The formaldehyde concentration was significantly higher ($P < 0.05$) in fresh muscle samples from calves consuming whey containing 370 mg formaldehyde active substance/kg than in muscle from control calves (0.256 vs. 0.158 mg/kg, respectively). In no other instances were significant differences in formaldehyde content between treated and control calves recorded.

A long-term feeding study (12 months) in beef cattle administered 1 g formaldehyde/day from formalin-treated soya bean meal found an increase in the formaldehyde content of muscle from 0.065 mg/kg to 0.167 mg/kg (Pinault, 1989, cited in AFSSA, 2004).

At the end of the tolerance studies (see section 3.3), the applicant has analysed formaldehyde concentrations in tissues (liver, kidney, muscle and skin/fat) of six chickens for fattening and piglets per group and in whole eggs of five laying hens per group. No formaldehyde was found in any tissue (HPLC, LOQ 2.5 mg/kg) and eggs (titrimetic analysis, no LOQ indicated) of both the control and the treated animals fed diets supplemented with up to 6300 (chickens for fattening and piglets) or 4980 mg formaldehdye/kg (laying hens).

3.4.3. Conclusions on residues

The few studies found in the literature reporting tissue concentrations of formaldehyde after oral administration of formaldehyde indicate an increase in formaldehyde in tissues and milk. However, the absolute values found for formaldehyde concentrations are low and generally not higher than 0.3 mg/kg milk or meat.

The FEEDAP Panel notes that formaldehyde concentrations found after feed supplementation with formaldehyde are about 10 to 20 times lower in meat and three times lower in milk than those reported in the literature for the same food commodities. The differences observed may be partially explained by the different analytical methods used.

3.4.4. Consumer exposure

A rough approximation from the background data for formaldehyde in food of animal and plant origin (section 3.2.1) may allow the conclusion that the total intake of consumers (one kg food per day) would be unlikely to exceed 100 mg exogenous formaldehyde per day. Average dietary exposure is suggested to be about 11 mg per person per day (AFSSA, 2004); another estimate (EC, 2005) gives a range of 4.35 to 41.9 mg per person per day (calculated with the lowest and highest formaldehyde concentrations reported in literature). Milk, meat and fish contribute 18.3% to the high intake (EC, 2005).

For naturally occurring substances, exposure estimates arising from their use as feed additives should be based on the EFSA Comprehensive European Food Consumption Database (EFSA, 2011) and the derived figures given in the FEEDAP guidance on consumer safety (EFSA FEEDPAP Panel, 2012). Exposure attributable to meat consumption (290 g/day, 10 mg formaldehyde/kg; highest values found by Trezl et al., 1997) would amount to 2.9 mg formaldehyde per day and exposure fish consumption (125 g/day, 23 mg formaldehyde/kg as the mean of all values published for fish except hake by Bianchi et al., 2007) would also amount to 2.9 mg formaldehyde per day. Other food sources would result in consumption of lower amounts (1.5 L milk to 1.2 mg formaldehyde/day and 60 g liver (calculated as liver paste) to 0.72 mg/day; Trezl et al., 1997). Since the likelihood that the same high consumer will be found in more than two high consumer groups at the same time is very low, the intake of consumers should be calculated for all food items and the sum of the two highest values should then be taken as the total intake. This calculation shows that the maximum intake of consumers (high consumers of meat and milk) would be 4.1 mg formaldehyde per person per day.

Four mg of orally ingested formaldehyde per person per day from the consumption of food of animal origin correspond to 0.008 % of the estimated endogenous turnover rate of formaldehyde.

3.4.5. Conclusions on the safety for the consumer

A reliable additional exposure of consumers to formaldehyde from supplementing feedingstuffs cannot be calculated. However, the highest values found in the few available deposition studies are much lower than those reported in the available literature, and are therefore already included in the exposure calculated above. Therefore, the FEEDAP Panel considers that the proposed use of formaldehyde as a feed additive would not increase consumer exposure and consequently would not pose an additional risk for the consumer.

3.5. Safety for the user

As reported in many comprehensive reviews (OECD, 2002; WHO, 2005; IARC, 2006;, 2012; ATSDR 2010; ECHA, 2011; NRC 2011; NTP, 2011) formaldehyde is a toxic substance, a strong irritant, a potent skin and respiratory sensitiser (including occupational asthma) and a proven human carcinogen by the respiratory route. In the European Union (EU), occupational exposure limits for formaldehyde based on irritation have been recommended, with a time-weighted average (TWA (eight hours)) of 0.2 ppm and a short-term exposure limit (STEL (15 minutes)) of 0.4 ppm (EC, 2008).[26] The World Health Organisation (WHO, 2010) set a guideline value of 0.1 mg formaldehyde/m^3 (30-minute average concentration).

No safe level of exposure of the skin, eyes or the respiratory system to formaldehyde could be identified as it is a potent sensitiser and as there is uncertainty about identifying a threshold for its carcinogenicity. Therefore, measures should be taken to ensure that the respiratory tract, as well as skin and eyes, of any person handling the product are not exposed to any dust, mist or vapour generated by the use of formaldehyde.

3.6. Safety for the environment

Formaldehyde occurs naturally in the environment as a result of several biochemical pathways and is a widely produced industrial chemical. Air is the most relevant compartment in the formaldehyde cycle, the half-life of formaldehyde in the air is short, due to photodegradation. Formaldehyde is also biodegraded in water and soil in a relatively short time and does not accumulate in organisms (WHO, 1989).

When used as feed additive, the absorbed fraction of formaldehyde is not excreted as such but mainly as formic acid in urine, carbon dioxide and water (see 3.1). No quantitative data on faecal excretion are available. Released formaldehyde would be distributed in the air and photodegraded; the irreversibly bound formaldehyde would after degradation in the environment not release formaldehyde but carbon dioxide and water. In summary, formaldehyde will not accumulate in the environment (see also WHO, 1989) and its use in animal nutrition is not expected to pose a risk for the environment.

4. Efficacy[27]

A total of 12 efficacy trials were provided by the applicant. Eight of the trials are aimed to identify the minimum inhibitory concentration (MIC) of formaldehyde, pure or in premixtures, against a large number of microorganisms. The applicant has also provided a literature review to support these results.[28] However, considering that only MIC was analysed, they were not considered suitable for the assessment of efficacy. The other four trials were designed to evaluate the preservative effect of formaldehyde on artificially feedingstuffs and are further described. Two of them were performed with a premixture containing, among others, propionic acid and an emulsifier.

In the first trial, a commercial laying hen mash feed sterilised was treated with an aqueous solution of formaldehyde (36.98 % w/w, confirmed by analysis) at different concentrations (0, 222, 336, 504, 759, 1 140 and 1 713 mg formaldehyde/kg feed).[29] Four replicates of each treated feed were contaminated with different serovars of *Salmonella* (i.e. *S.* Enteritidis (ATCC 13076), *S.* Typhimurium ATCC 11876), *S.* Senftenburg (ATCC 8400) and *S.* Montevideo (ATCC 8387)) at varying levels of 2.3×10^6 to 1.1×10^9 colony-forming units CFU/g. Samples were collected and analysed at 1, 8, 24 and 48 hours after treatment. Storage conditions were not described. The results showed that no cultivable *Salmonella* could be detected already one hour after treatment until the end of the study at formaldehyde concentrations of 759 mg formaldehyde/kg and more. The lower formaldehyde concentrations also significantly reduced *Salmonella* counts during the observation time.

[26] One ppm equals to about 1.23 mg formaldehyde/m^3 (EC, 2008).
[27] This section has been edited following the confidentiality claims made by the applicant.
[28] Technical dossier/Section IV/Annex 4.1.a.
[29] Technical dossier/Section IV/Annex 4.1.e.

In the second trial, commercial poultry mash feed sterilised was inoculated at level of approximately 10^3 CFU/g with *Salmonella* Typhimurium (ATCC 14028).[30] Part of the inoculated feed (three replicates) was treated with formaldehyde aqueous solution (31.5 % formaldehyde w/w, confirmed by analysis) applied at the level of 3.2 g/kg (corresponding to 1 000 mg formaldehyde/kg feed). Samples were collected and analysed at 1, 24 and 48 hours after treatment. Storage conditions were not described. The results showed that 1 g formaldehyde/kg feed significantly reduced *Salmonella* contamination after 1 hour. Also after 24 and 48 hours a significant reduction could be measured.

In the third trial,[31] nine samples of a commercial fish meal, one sample of a commercial meat and bone meal and 11 samples of poultry starter mesh feed, naturally or artificially contaminated with different serovars of *Salmonella* (i.e. *S.* Enteritidis (ATCC 13076), *S.* Typhimurium (ATCC 14028), *S.* Agona (ATCC 51957), *S.* Hadar (ATCC 51956), *S.* Worthington (ATCC 9607), *S.* Heidelberg (ATCC 8326), *S.* Senftenburg (ATCC 8400), *S.* Pullorum (ATCC 10398), *S.* Gallinarum (ATCC 9184), *S.* Choleraesuis (ATCC 13312)) at varying concentrations of 1.0×10^2 to 1.8×10^{11} CFU/g. Three replicates of each feed were treated with formaldehyde from a premixture at concentrations of 0, 315, 630 and 1260 mg formaldehyde/kg. Samples were collected and analysed 24 hours after treatment. Storage conditions were not described. The results showed that already concentrations of 315 mg formaldehyde/kg feed resulted in a significant *Salmonella* reduction compared to inoculated or artificially contaminated control samples. Since formaldehyde was incorporated via a premixture containing another preservative (propionic acid), the results may not be attributed to the action of formaldehyde alone.

In the fourth trial,[32] one sample each of four sterilised commercial pig feed (weaner, grower, finisher, and sow breeder diet) and of seven sterilised commercial poultry feed (poultry breeder, broiler starter, grower and finisher, chick starter, pullet grower, layer mash) was artificially contaminated with culture collection strains of *Salmonella* Typhimurium (ATCC 14028) at concentrations of 2.4×10^4 to 5.8×10^4 CFU/g. Three replicates of each feed were treated with formaldehyde from a premixture at a concentration of 660 mg formaldehyde/kg feed. Samples were collected and analysed 24 hours after treatment. Storage conditions were not described. The results showed a significant *Salmonella* reduction compared to inoculated control by formaldehyde. Since formaldehyde was incorporated via a premixture containing another preservative (propionic acid), the results may not be attributed to the action of formaldehyde alone.

4.1. Conclusions on the efficacy of the additive

The FEEDAP Panel notes that efficacy of a preservative should normally be demonstrated by the prevention of natural microbial contamination of feed materials/compound feeds. The two studies performed with the additive under application report a reduction of four *Salmonella* serovars (culture collection strains) by the additive after artificial inoculation. This study type is also considered as indicative for a preventive effect by the additive.

The additive has the potential to be effective as preservative in the dose range proposed by the applicant (200-1 000 mg formaldehyde/kg feed). This conclusion is based on two *in vitro* studies in which sterilised poultry feed was treated with the additive as such and subsequently inoculated with different *Salmonella* serovars.

Two other in vitro studies in which the additive was tested as a component of a premixture containing also another preservative agent (propionic acid) on a broad range of different poultry and pig feed formulations as well as feed materials (of animal origin), naturally or artificially contaminated with different *Salmonella* serovars, support the above conclusion.

[30] Technical dossier/Section IV/Annex 4.1.f.
[31] Technical dossier/Section IV/Annex 4.1.c.
[32] Technical dossier/Section IV/Annex 4.1.d.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Free and reversibly bound formaldehyde, when ingested, is readily absorbed in the gastrointestinal tract and joins the pool of endogenous formaldehyde. It is rapidly oxidised to formic acid, which enters the one-carbon pool of the body and is further oxidised to carbon dioxide and water. The additive contains also methanol, which is oxidised to formaldehyde.

Formaldehyde is a carcinogen by inhalation. While local irritation is expected to strongly promote carcinogenesis, lower local concentrations of formaldehyde are known to produce DNA adducts. Therefore, the FEEDAP Panel deems it prudent not to consider the exposure to non-irritant concentration as totally riskless. Moreover, on the basis of the present knowledge, a causal association between formaldehyde exposure and leukaemia cannot be ruled out.

The FEEDAP Panel estimated the oral intake of formaldehyde of consumers from food of animal origin to be 4 mg per person per day. A reliable additional exposure of consumers to formaldehyde from supplementing feedingstuffs cannot be calculated. However, the highest values found in the few available deposition studies are much lower than those reported in the available literature, and are therefore already included in the exposure scenario. Therefore, the FEEDAP Panel considers that the proposed use of formaldehyde as a feed additive would not increase consumer exposure and consequently would not pose an additional risk for the consumer.

No safe concentration can be established for veal calves. It appears that (i) 470 mg formaldehyde/kg feed would be safe for chickens for fattening, laying hens and Japanese quail, (ii) 630 mg formaldehyde/kg feed would be safe for piglet, a margin of safety could not be identified considering the shortcomings of the study. However, adverse effects on reproductive organs were seen at 930 mg/kg feed for male poultry and at 1 850 mg/kg feed for female Japanese quail. Since these endpoints are not specifically addressed in tolerance studies, a formaldehyde concentration safe for reproduction cannot be derived. In conclusion, a safe level for all animal species and categories, including all poultry and all pigs, cannot be determined.

Formaldehyde is a toxic substance, a strong irritant, a potent skin and respiratory sensitiser (including occupational asthma) and a proven human carcinogen by the respiratory route. No safe level of exposure of the skin, eyes or the respiratory system to formaldehyde could be identified. Therefore, measures should be taken to ensure that the respiratory tract, as well as skin and eyes, of any person handling the product are not exposed to any dust, mist or vapour generated by the use of formaldehyde.

Formaldehyde will not accumulate in the environment and its use in animal nutrition is not expected to pose a risk for the environment.

Formaldehyde in concentrations between 200 and 1 000 mg/kg feed (compound feed and/or feed material) has the potential to be an efficacious preservative.

RECOMMENDATIONS

The FEEDAP Panel recommends that consideration should be given to whether the strict protection measures, once established, would effectively protect users at the level of feed compounders and farmers.

DOCUMENTATION PROVIDED TO EFSA

1. Formaldehyde for all animal species. October 2010. Submitted by Regal BV.

2. Formaldehyde for all animal species. Supplementary information. April 2012. Submitted by Regal BV.

3. Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for formaldehyde.

4. Comments from Member States received through the ScienceNet.

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APPENDIX

Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for formaldehyde[33]

In the current group of applications, authorisation is sought under Article 4(1) and 10(2) for *Formaldehyde*, under the category/functional group 1(a) 'technological additives'/'preservatives' and 1(k) 'technological additives'/'silage additives', according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of *Formaldehyde* for all animal species and categories. The *feed additive* is intended to be mixed in *feedingstuffs* or added to *silage*. The Applicants suggested 68 and 1000 mg/kg as minimum and maximum *Formaldehyde* concentration in *feedingstuffs* and *silage* at similar rate (based on 88 % dry matter).

For the determination of the *active substance* in the *feed additive* one of the Applicants (FAD-2010-0222) submitted an ISO method applicable to *Formaldehyde* solutions (content ranging from 25 to 45 %) based on acidimetric titration using thymolphthalein as indicator. Furthermore the EURL identify a European Pharmacopoeia method for the identification and characterisation of *Formaldehyde*, based on titration with sodium thiosulphate 0.1 M.

Even though no performance characteristics are provided, the EURL considers the two titrimetric methods (ISO 2227-1972 and Eur. Ph. 6.0, method 01/2008:0826) suitable to determine *Formaldehyde* in the *feed additive* within the frame of official control.

For the determination of *Formaldehyde* in *feedingstuffs* one Applicant (FAD-2010-0399) submitted a single laboratory validated and further verified method based on Reversed Phase High Performance Liquid Chromatography coupled to Diode-Array detection (RP-HPLC-DAD). The following performance characteristics were reported:

- a *precision* (*repeatability* and *intermediate precision*) ranging from 1.9 to 4.8 %,

- a *recovery rate* ranging from 97.8 to 100.8 %, and

- a limit of quantification of 1.3 mg/kg.

Based on the performance characteristics presented the EURL recommends for official control the single laboratory validated and further verified RP-HPLC-DAD method, submitted by the Applicant, to determine *Formaldehyde* in *feedingstuffs*.

None of the Applicants provided experimental data for the determination of *Formaldehyde* in *silage*. Therefore the EURL could not evaluate nor recommend a method for official control to determine the *feed additive* in *silage*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

[33] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2010-0222+0399.pdf

ABBREVIATIONS

AFC – EFSA Panel on food additives, flavourings, processing aids and material in contact with food

AFSSA - Agence Francaise de Securite Sanitaire des Aliments

ALT – Alanine transaminase

AST – Aspartate transaminase

ATSDR - Agency for Toxic Substances and Disease Registry

CAS – Chemical Abstract Service

CFU – colony forming unit

CHCM - cell counted hemoglobin concentration

CIIT - Chemical Industry Institute of Toxicology

CK – creatine kinase

CV – Coefficient of variation

EC – European Commission

ECHA – European Chemical Agency

EFSA - European Food Safety Authority

EU – European Union

EURL - European Union Reference Laboratory

FEEDAP - EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)

GSH - glutathione

HPLC - High Performance Liquid Chromatography

IARC - International Agency for Research on Cancer

LDH – lactate dehydrogenase

MIC – Minimum inhibitory concentration

NOAEL – No observed adsverse effect level

NRC - National Research Council

NTP - National Toxicology Program

OECD - Organisation for Economic Co-operation and Development

PCBs - dioxin-like polychlorinated biphenyls

PCDD/F - polychlorinated dibenzo-p-dioxin and polychlorinated dibenzofuran

RfD - Reference Dose

SCAN - Scientific Committee on Animal Nutrition

TDI - Tolerable Daily Intake

TEQ - toxic equivalent

TMAO - trimethylamine-N-oxide

TWA - time-weighted average

US EPA - United States Environmental Protection Agency

USA - United States of America

WBC – White blood cell

WHO - World Health Organization