European Food Safety Authority

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SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of formaldehyde for all animal species based on a dossier submitted by Adiveter S.L.[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The additive formaldehyde is an aqueous solution containing 37 % formaldehyde and 14 % methanol. It is intended for use in all animal species at concentrations between 68 and 680 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 340 and 680 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-2011-00334, adopted on 28 January 2014.

[2] Panel members: Gabriele Aquilina, Vasileios Bampidis, Maria De Lourdes Bastos, Lucio Guido Costa, Gerhard Flachowsky, Mikolaj Antoni Gralak, Christer Hogstrand, Lubomir Leng, Secundino López-Puente, Giovanna Martelli, Baltasar Mayo, Fernando Ramos, Derek Renshaw, Guido Rychen, Maria Saarela, Kristen Sejrsen, Patrick Van Beelen, Robert John Wallace and Johannes Westendorf. Correspondence: FEEDAP@efsa.europa.eu

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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 37 % formaldehyde and 14 % methanol. It is intended for use in all animal species at concentration between 68 and 680 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) 600 mg formaldehyde/kg feed would be safe for chickens and Japanese quail, (ii) 600 mg formaldehyde/kg feed would be safe for piglets with a margin of safety of approximately 2.5. 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 340 and 680 mg/kg feed (compound feed and/or feed material) has the potential to be an efficacious preservative.

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BACKGROUND

Regulation (EC) No 1831/2003[4] 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 Adiveter S.L.[5] for authorisation/re-evaluation of the product formaldehyde , when used as a feed additive for all animal species (category: technological additives; functional group: preservative) under the conditions mentioned in Table 1.

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.[6] 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.

[4] 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.
[5] Adiveter S.L. C/Prudenci Betrana, 5. E-43206, Reus, Spain.
[6] EFSA Dossier reference: FAD-2010-0399.

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

Additive	Formaldehyde			
Registration number/EC No/No (if appropriate)	E 240, 1a			
Category(ies) of additive	Technological			
Functional group(s) of additive	Preservative			
Description				
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)	
Minimum 34% formaldehyde.	**CH2O**	**Complies with Dir. 2003/32 on undesirable substances**	**BS EN ISO 17726-1:2008 (HPLC)**	
Trade name (if appropriate)	Salmocid®			
Name of the holder of authorisation (if appropriate)	Adiveter S.L.			
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 animal species	Within any age	0.2 ml/kg (68 mg formaldehyde/kg)	2.0 ml/kg (680 mg formaldehyde/kg)	Nil
Other provisions and additional requirements for the labelling				
Specific conditions or restrictions for use (if appropriate)	None			
Specific conditions or restrictions for handling (if appropriate)	For user safety, breathing protection, gloves, overalls & safety glasses. Hazard warnings: T – Toxic. F - flammable. R11 – Highly flammable. C – corrosive. R 23/24/25 – Toxic by inhalation, in contact with skin and if swallowed. R 34 – Causes burns. R 40 – Limited evidence of carcinogenic effect. R 43 – May caused sensitization by skin contact. S 1/2 - Keep locked up and out of the reach of children. S 26 – In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S 36/37/39 – Wear suitable protective clothing, gloves & eyes/face protection. S51 – Use only in well-ventilated areas. S 45 – In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Please consult the Material Safety Data Sheet for further information.			
Post-market monitoring (if appropriate)	Full technical support on safe product handling, barrier ventilation systems in bulk feedingstuffs handling areas and feed mills, traceability, product recall capability & monitoring of customer feedback via formal product/service complaint procedures.			
Specific conditions for use in complementary feedingstuffs (if appropriate)	Use q.s. (quantum satis) while assuring that the legal maximum for complete feed is respected.			
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).[7]

In the USA formaldehyde is authorised for use as feed additive at maximum levels of 2.5 g/kg (formaldehyde aqueous solution 37%),[8] as fumigant for the fumigation of eggs in hatcheries,[9] and as a fungicide, pesticide and bactericide in aquaculture.[10] Formaldehyde is also authorised for use in vaccines.[11]

2. Characterisation and identity

The additive formaldehyde is an aqueous solution of formaldehyde (> 34.0 % w/v by specification) and methanol (9.0–14.0 % by specification), with a maximum concentration of formic acid of 0.027 %. The analysis of five batches of the additive showed concentrations of 37.2 to 37.8 % w/v formaldehyde, 13.2 to 14.0 % w/w methanol, and 0.009 to 0.013 % w/w formic acid).[12]

The active substance formaldehyde (Chemical Abstracts Service (CAS) No 50-00-0; EC No 200-001-8) is a gas with a molecular weight of 30.02; its molecular formula is 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 $CH_2(OH)_2$.

The analysis of three batches of the additive showed concentrations of heavy metals as follows: lead, < 0.1 mg/kg; mercury, < 0.01 mg/kg; cadmium, < 0.3 mg/kg; and arsenic, < 0.15 mg/kg.[13] Dioxins were detected at concentrations ≤ 0.09 ng PCDD/F (polychlorinated dibenzo-*p*-dioxin and polychlorinated dibenzofuran) World Health Organization (WHO) toxic equivalent (TEQ))/kg, while

[7] 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).
[8] Available online: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=573.460
[9] Avalable 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
[10] Avalable online: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=529.1030
[11] Avalable online: http://www.gpo.gov/fdsys/pkg/CFR-2003-title9-vol1/pdf/CFR-2003-title9-vol1.pdf
[12] Technical dossier/Section II/Annex_II_1_3.
[13] Technical dossier/Section II/Annex_II_1_4.

the sum of dioxins and dioxin-like polychlorinated biphenyls (PCBs) was ≤ 0.121 ng PCDD/F/PCBs (WHO-TEQ)/kg.[14]

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

The shelf life of the additive has been examined in a study in which three batches were stored in closed polyethylene containers at room temperature for 24 months. No differences in the formaldehyde concentration (initial mean concentration 34.2 % w/v, final mean concentration 34.1 % w/v) have been recorded.[15]

2.2.2. Stability in feedingstuffs

Three sets of stability studies in which 10 subsamples of feedingstuffs were analysed were provided.[16]

The additive was added at 0.5 g/kg mash feed (expected formaldehyde concentration 170 mg/kg). The recovery of formaldehyde after mixing was 60 %, corresponding to 102.2 mg formaldehyde/kg feed.

The subject of the second study was a compound feed with an intended total formaldehyde content of 202 mg/kg feed coming from (i) 44 % soya bean meal treated with the additive at 0.6 g/kg (providing 90 mg formaldehyde/kg feed), (ii) 6 % toasted extruded soya bean meal treated with 0.5 g additive/kg (supplying 10 mg formaldehyde/kg feed) and (iii) the direct addition of 102 mg formaldehyde/kg. The feed was steam pelleted at 60–70 °C. The recovery of formaldehyde was 67 %, corresponding to 135.7 mg formaldehyde/kg feed.

In the third study, soya bean meal was supplemented with 2 g additive/kg (680 mg formaldehyde/kg feed material). The recovery of formaldehyde after mixing was 49 %, corresponding to 333.6 mg formaldehyde/kg feed.

Two subsamples of the mash and pelleted feed and of the soya bean meal used to study the stability during feed processing (102.2, 135.7 and 333.6 mg active substance/kg, respectively) were stored for three months in plastic bags under ambient conditions. Final recovery of formaldehyde was 75.0 % in mash feed, 87 % in pelleted feed and 77 % in soya bean meal.[17]

Considering the initial formaldehyde concentrations, the losses by mixing or pelleting and three months storage sum up to about 40 % to 60 %. It should be noted that formaldehyde is a very reactive chemical which interacts with feedingstuffs, particularly its protein fraction.

[14] Technical dossier/Section II/Annex_II_1_4.
[15] Technical dossier/Section II/Annex II_4_1_1.
[16] Technical dossier/Section II/Annex II_4_1_9.
[17] Technical dossier/Section II/Annex II_4_1_5, Annex_II_4_1_6 and Annex_II_4_1_7.

2.2.3. Homogeneity

The 10 subsamples each of the mash and pelleted feed and of the soya bean meal analysed in the stability studies showed coefficients of variation of 3.6 %, 3.4 % and 4.9 %, respectively.[18]

2.3. Conditions of use

The additive formaldehyde is intended to be used as preservative in feedingstuffs for all animal species, with a minimum content of 68 mg/kg feed and a maximum content of 680 mg formaldehyde (active substance)/kg feed. The applicant further noted that formaldehyde should not be incorporated in feedingstuffs via vitamin and mineral premixtures.

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 Appendix A.

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

[18] Technical dossier/Section II/Annex II_4_2.

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 target animals

The applicant has performed tolerance studies in chickens for fattening and weaned piglets. 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

The applicant provided a tolerance study in chickens for fattening.[19] A total of 280 male Cobb chickens for fattening (average body weight 40 g) were divided into four groups (seven replicates per treatment, 10 broilers per replicate) and fed for 35 days with mash commercial diets supplemented with formaldehyde, at intended levels of 0, 680, 1 020 and 1 360 mg active substance/kg feed. Zootechnical parameters (live weight, feed intake), were recorded per pen at day 0, 7, 14, 21, 28 and 35, and average daily weight gain and feed to gain ratio calculated for the respective intervals. At the end of the experimental period (day 35), blood samples were taken from two birds per pen (14 per treatment) for routine haematology and clinical biochemistry.[20] Seven animals per treatment were sacrificed and subjected to necropsy and histopathology. The results were analysed using ANOVA as the main statistical test. The main results are listed in Table 2.

[19] Supplementary Information December 2012/Annex_III_1_1_1.

[20] Haematology: Erytrocytes, Haemoglobin, Haematocrit, MCV, MCH, MCHC, WBC, Lymphocytes, Monocytes, Neutrophils, Eosinophils, Basophils, Thrombocytes. Biochemistry: Calcium, Phosphorus, Chloride, Trigycerides, Total protein, Proteinogram, Uric acid, Bile acid, CPK, LDH, AST.

Table 2: Results of the tolerance study with formaldehyde in chickens for fattening

	Dietary intended formaldehyde concentration (mg/kg feed)			
	0	680	1020	1360
Formaldehyde analysed values (mg/kg)*	-	601	1017	1362
Initial body weight (g/head)	40	40	39	39
Final body weight (g/head)	2230	2270	2230	2200
Average daily weight gain (g/day)	61	64	61	62
Feed intake (g/day)	98	98	101	101
Feed to gain ratio	1.61	1.54	1.64	1.64
Haematocrit (%)	28.57	28.93	29.93	29.93
Haemoglobin (g/dL)	11.73	12.40	12.03	11.67
Erythrocytes (10^6/µL)	1.50	1.60	1.70	1.60
Thrombocytes (10^9/L)	25.90	23.60	24.30	27.30
Total protein (g/L)	28.97	28.92	28.21	28.22
AST (U/L)	302	281	280	277
CPK (U/L)	17169	13254	14051	13169
LDH (U/L)	4194	3830	3662	4190
Calcium (mg/L)	109.7	109.9	108.6	107.3
Phosphorus (mg/L)	80.2	75.3	79.9	78.8

* Values reported are means of starter and grower feeds.

Zootechnical performances as well as haematological and blood biochemical parameters were not affected by the additive. Mortality was low (1.4%) and apparently not treatment-related. Absolute and relative liver weight was increased ($P<0.05$) in chickens exposed to the maximum recommended dose (601 mg/kg) compared to both controls and birds treated with 1.5 or 2 times such dose; by contrast, a statistical significant decrease in relative spleen weight was recorded in birds dosed with 601 mg formaldehyde/kg feed compared with those exposed to 1 020 mg formaldehyde/kg feed. Aside from a slight reddening of the duodenum recorded in two cases, no other gross lesions were reported in any of the examined tissues, which did not include the testes. Histopathological results were not reported.

3.3.1.2. 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.

3.3.1.3. 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.

3.3.1.4. Summary of the findings in poultry

The tolerance study provided in chickens for fattening did not show negative effects on zootechnical parameters, haematology and clinical biochemistry up to the highest dose tested (1360 mg formaldehyde/kg feed). Nonetheless, absolute and relative liver weight were affected at the lowest tested dose (600 mg/kg), but apparently not at the higher dosages (1020 or 1360 mg/kg) and the results of the histology of the testes (one of the target organs for formaldehyde toxicity) were not provided. 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 effect in female quail was reported up to 1850 mg/kg feed, which affected the morphology of the oviduct. In the other study on male quail, 1850 mg reduced the weight of testis and 930 mg resulted in a smaller diameter of seminiferous tubules.

In summary, four studies were available for the assessment of safety for poultry species. Two of them did not confirm tolerance of the lowest dose tested (930 mg/kg). One study supports the safety of 600 mg formaldehyde/kg feed, corresponding to the intended maximum proposed dose.

3.3.2. Tolerance in weaned piglets

In a study provided by the applicant,,a total of 144 male and female (ACMC x Pietrain) piglets (average body weight 8.1 kg) were divided in four groups (6 replicates per treatment, six piglets per replicate) and fed for 42 days with mash commercial diets supplemented with formaldehyde at intended levels of 0, 680, 1 700 and 3 400 mg active substance/kg feed.[21] Zootechnical parameters (live weight, feed intake), were recorded per pen on day 0, 14 and 42, and average daily weight gain and feed to gain ratio calculated for the respective intervals. Blood samples were taken from 12 random piglets (six male and six female) on day 0, and from 12 piglets (six males and six females) per treatment on day 42 for routine haematology and clinical biochemistry.[22] Six animals per treatment (three males and three females) were sacrificed and subjected to necropsy. The results were analysed using ANOVA as the main statistical test. The main results are listed in Table 3.

[21] Supplementary Information December 2012/Annex_III_1_1_2.

[22] Haematology: Erytrocytes, Haemoglobin, Haematocrit, MCV, MCH, MCHC, WBC, Lymphocytes, Monocytes, Neutrophils, Eosinophils, Basophils, Thrombocytes. Biochemistry: Glucose, Total protein, Albumin, Uric acid, ALT, AST, GGT.

Table 3: Results of the tolerance study with formaldehyde in weaned piglets

	Dietary formaldehyde concentration (mg/kg feed)			
	0	680	1700	3400
Formaldehyde analysed values (mg/kg)*	-	606	1630	3335
Initial body weight (kg/head)	8.2	7.9	8.2	8.2
Final body weight (kg/head)	23.6^{xy}	24.4^{x}	22.8^{xy}	20.7^{y}
Average daily weight gain (g/day)	368^{a}	390^{a}	349^{ab}	299^{b}
Feed intake (g/day)	521	558	507	520
Feed to gain ratio	1.42^{b}	1.43^{b}	1.47^{ab}	1.72^{a}
Haematocrit (%)	37.78	38.36	38.96	37.84
Haemoglobin (g/dL)	10.33	10.64	10.88	10.67
Erythrocytes (10^6/µL)	6.16	6.64	6.46	6.23
MCHC (g/dL)	27.39	27.76	27.95	28.21
Leucocytes (10^3/µL)	23.54	22.34	20.05	24.33
Lymphocytes (%)	52.00	50.17	49.00	59.25
Monocytes (%)	11.83	9.33	6.36	5.00
Total protein (g/dL)	5.41^{a}	5.73^{a}	5.40^{a}	4.90^{b}
Albumin (g/dL)	2.78^{a}	3.01^{a}	2.7^{a}	2.18^{b}
Glucose (mg/dL)	82.88^{ab}	92.08^{a}	74.74^{ab}	69.71^{b}

* Values reported are means of pre-starter and starter feeds.
[a,b] Values in the same row with different superscript are statistically different ($P \leq 0.05$).
[x,y] Values in the same row with different superscript indicate a near-significant trend ($0.05 \leq P \leq 0.10$).

Zootechnical parameters, average daily weight gain and feed to gain ratio, were negatively affected in piglets fed the highest additive dosage (3335 mg/kg feed), which also exhibited a tendency to have a lower body weight at the end of the experimental period. Some treatment-unrelated mortality (n=8) occurred during the whole trial due to diarrhoea and meningitis (*Streptococcus suis*). No significant gross lesions were noticed, results of histopathological results were not provided. Total protein and albumin levels were significantly lower in animals administered with 3335 mg formaldehyde/kg feed. No significant effects were apparent on the tested serum enzymes (AST, ALT, and CK).

3.3.2.1. Summary of the findings in piglets

Zootechnical parameters, haematology, clinical biochemistry and necropsy confirm the safety of 606 mg formaldehyde/kg, corresponding to the intended maximum proposed dose, with a margin of safety of about 2.5.

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 four 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) 600 mg formaldehyde/kg feed would be safe for chickens and Japanese quail, and (ii) 600 mg formaldehyde/kg feed would be safe for piglets with a margin of safety of approximately 2.5.

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).

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).

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 (1 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).[23] 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

A total of five efficacy trials performed with the additive were provided by the applicant. Three of the trials are aimed to identify the minimum inhibitory concentration (MIC) of formaldehyde, against a large number of microorganisms and to compare the effects with those produced by other preservatives, and are therefore not considered suitable for the assessment of efficacy. The other two trials have been designed to evaluate the preservative effect of formaldehyde on feedingstuffs and feed materials and are further described.

In the first trial, a commercial mash poultry feed and a batch of soya bean meal were contaminated with culture collection strains of *Salmonella* Typhimurium (ATCC 14028), *Escherichia coli* (ATCC 25922) and *Enterobacter cloacae* (ATCC 13047), two replicates per feed per microorganism, at the level of approximately 1.0 x 10^5 colony-forming units (CFU)/g.[24] The feeds were treated with the

[23] One ppm equals to about 1.23 mg formaldehyde/m^3 (EC, 2008).
[24] Technical dossier/Section IV/Annex_IV_1_1.

additive at different concentrations (0, 68, 340, 510 and 680 mg formaldehyde/kg feed). Samples were collected and analysed at zero, one, and seven days and one, two and three months after treatment and stored in plastic bags at ambient conditions. The reduction in *Salmonella* Typhimurium was not consistently shown for any formaldehyde dose tested.In contrast, there was a dose dependent reduction of *E. coli* only in mash feed demonstrating efficacy starting from 340 mg/kg already one day after inoculation. Inconsistent results between the feed material and the compound feed were observed for *E. cloacae*.

In the second trial, a commercial mash ruminant compound feed was contaminated (two replicates per microorganism) with approximately 1.0 x 10^4 CFU/g) with *Salmonella* Typhimurium (ATCC 14028), 1.0 x 10^4 CFU/g with *Salmonella* Enteritidis (ATCC13076), 1.0 x 10^6 CFU/g with *Campylobacter jejuni* (ATCC33291) and 1.0 x 10^6 CFU/g with *Escherichia coli* (ATCC 23922).[25] The feeds were treated with the additive at different concentrations (0, 68, 340 and 680 mg formaldehyde/kg feed). A negative control (no contamination, no formaldehyde) was also included in the trial. Samples were collected and analysed at zero, one, and seven days and one, two and three months after treatment and stored in plastic bags at ambient conditions. The reduction in *Salmonella* Typhimurium was not consistently shown for any formaldehyde dose tested. The action against *E. coli* resulted in an effect at doses of 340 mg/kg and 680 mg/kg. Efficacy against *Campylobacter jejuni* could not be demonstrated.

4.1. Conclusions on the efficacy

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 several microorganisms by the additive after artificial inoculation. This study type is also considered as indicative for a preservative effect by the additive. Differences at the end of the observation period were not considered since the contaminated but not treated group showed a significant reduction of microbial contamination. Therefore, the assessment was concentrated on the early appearance of differences between the contaminated untreated and treated groups.

The two *in vitro* studies in which a mash poultry feed, soya bean meal and a ruminant feed were inoculated with different serovars of *Salmonella* and strains of *E. coli*, *E. cloacae* and *C. jejuni* and subsequently treated with the additive demonstrated the efficacy of the additive as preservative against *E. coli* in a dose range of 340-680 mg formaldehyde/kg feed. The lowest proposed dose (68 mg/kg) failed to demonstrate efficacy. No conclusions could be reached on the effects on the other micro-organism considered

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

[25] Supplementary Information December 2012/Annex_IV_1_5.

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 apparently safe concentration can be established for veal calves. It appears that (i) 600 mg formaldehyde/kg feed would be safe for chickens and Japanese quail, (ii) 600 mg formaldehyde/kg feed would be safe for piglets with a margin of safety of approximately 2.5. 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 340 and 680 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 Adiveter S.L.
2. Formaldehyde for all animal species. Supplementary information. December 2012. Adiveter S.L.
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[26]

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.

[26] 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