

ADOPTED: 28 January 2020

doi: 10.2903/j.efsa.2020.6012

Safety of hexamethylene tetramine for pigs, poultry, bovines, sheep, goats, rabbits and horses

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Maryline Kouba, Mojca Kos Durjava, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Gabriele Aquilina, Georges Bories, Jürgen Gropp, Carlo Nebbia and Matteo Lorenzo Innocenti

Abstract

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 hexamethylene tetramine (HMTA) for pigs, poultry, bovines, sheep, goats, rabbits and horses. In 2015, the FEEDAP Panel delivered an opinion on the safety and efficacy of HMTA. In that opinion, the Panel noted that the residues of HMTA in the silage amounted to about 5% of the inclusion level of 600 mg HMTA/kg fresh ensiling material. Since the HMTA residues in silage were analysed as formaldehyde, it was not possible to quantify the contribution of formaldehyde and HMTA to the residues. In the absence of a qualitative and quantitative characterisation of the residues, the Panel could not conclude on the safety of HMTA for the target species. Based on the results of a new study in which HMTA was measured in silage, the concentration of HMTA dropped below the limit of quantification of 6 mg HMTA/kg silage, which corresponds to ~ 1% of the HMTA inclusion level in the fresh ensiling material. However, the formaldehyde concentration was not analysed in the silage. In the absence of a qualitative and quantitative characterisation of the residues, the Panel cannot conclude on the safety of HMTA for the target species.

© 2020 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

Keywords: Hexamethylene tetramine, technological additive, silage additive, safety

Requestor: European Commission

Question number: EFSA-Q-2017-00080

Correspondence: feedap@efsa.europa.eu

Panel members: Giovanna Azimonti, Vasileios Bampidis Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Kos Durjava, Maryline Kouba Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa and Ruud Woutersen.

Acknowledgements: The Panel wishes to acknowledge the contribution of Jaime Galobart, Orsolya Holczknecht and Paola Manini to this opinion.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Aquilina G, Bories G, Gropp J, Nebbia C and Innocenti ML, 2020. Scientific Opinion on the safety of hexamethylene tetramine for pigs, poultry, bovines, sheep, goats, rabbits and horses. *EFSA Journal* 2020;18(2):6012, 7 pp. <https://doi.org/10.2903/j.efsa.2020.6012>

ISSN: 1831-4732

© 2020 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

This is an open access article under the terms of the [Creative Commons Attribution-NoDerivs](https://creativecommons.org/licenses/by-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited and no modifications or adaptations are made.



The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.



Table of contents

Abstract.....	1
1. Introduction.....	4
1.1. Background and Terms of Reference as provided by the requestor.....	4
1.2. Additional information.....	4
2. Data and methodologies.....	4
2.1. Data.....	4
2.2. Methodologies.....	5
3. Assessment.....	5
3.1. Safety.....	5
3.1.1. Safety for the target species.....	5
3.1.1.1. Residues of HMTA and formaldehyde in silage.....	5
3.1.1.2. Safety of HMTA for the target species.....	6
4. Conclusions.....	6
Documentation as provided to EFSA/Chronology.....	6
References.....	6
Abbreviations.....	7

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1831/2003¹ establishes the rules governing the Community authorisation of additives for use in animal nutrition and in particular, Article 9 defines the terms of such authorisation by the Commission.

The applicant, ADDCON EUROPE GmbH, is seeking the authorisation of hexamethylene tetramine as a silage additive for pigs, poultry, bovines, sheep, goats, rabbits and horses (Table 1).

Table 1: Description of the substances

Category of additive	Technological additive
Functional group of additive	Silage additive
Description	Hexamethylene tetramine
Target animal category	Pigs, poultry, bovines, sheep, goats, rabbits, horses
Applicant	ADDCON EUROPE GmbH
Type of request	New opinion

On 28 February 2015, the Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority ('Authority'), in its opinion on the safety and efficacy of the product, could not conclude on the efficacy and safety of HMTA.

The Commission gave the possibility to the applicant to submit complementary information in order to complete the assessment and to allow a revision of the Authority's opinion. The new data have been received on 17 January 2017.

In view of the above, the Commission asks the Authority to deliver a new opinion on hexamethylene tetramine as a silage additive for pigs, poultry, bovines, sheep, goats, rabbits and horses based on the additional data submitted by the applicant.

1.2. Additional information

Hexamethylene tetramine (HMTA) is presently listed in the EU Register of Feed Additives as a technological additive (functional group: silage additive) for use with feed for bovines, ovines, pigs, poultry, rabbits, horses and goats, and is subject to re-evaluation.

HMTA is authorised as a food preservative (E 239, Council Directive No 95/2/EC7) for ripening provolone cheese with a maximum level of 25 mg/kg residual amount, expressed as formaldehyde.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) issued several opinions on HMTA (JECFA, 1962, 1965, 1967, 1972, 1974) allocating an acceptable daily intake (ADI) of '0.15 mg/kg body weight and day'. The European Food Safety Authority (EFSA) has issued an opinion on the use of HMTA as a food additive (EFSA ANS Panel, 2014). The ANS Panel considered that, 'due to the limitations in the toxicological database (acute, subchronic, chronic, reproductive and developmental toxicity), it was not possible to decide on a critical study to identify a no observed adverse effect level (NOAEL) that could be used for the derivation of an ADI. The consensus of the various bodies is that, based on the available information, HMTA is of very low to moderate toxicity (EFSA ANS Panel, 2014).'

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) adopted in 2015 an opinion on the safety and efficacy of hexamethylene tetramine as technological additives for pigs, poultry, bovines, sheep, goats, rabbits, horses (EFSA FEEDAP Panel, 2015). In that opinion, the FEEDAP Panel could not conclude on the efficacy of HMTA and on the safety of the additive for the target species.

The applicant has submitted additional information related to the residues of HMTA in silage.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of additional information² to previous applications on the same product.³

¹ 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.

² FEED dossier reference: FAD-2017-0008.

³ FEED dossier reference: FAD-2010-0377.

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety of hexamethylene tetramine is in line with the principles laid down in Regulation (EC) No 429/2008⁴ and the relevant guidance documents: Guidance on technological additives (EFSA FEEDAP Panel, 2012), Guidance for the preparation of dossiers for the re-evaluation of certain additives already authorised under Directive 70/524/EEC (EFSA, 2008), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017).

3. Assessment

The additive under assessment is hexamethylene tetramine (HMTA). It is produced by an air oxidation/dehydrogenation of methanol to provide formaldehyde, followed by the condensation of the resulting formaldehyde with ammonia to provide HMTA. The additive contains > 99.7% HMTA.

HMTA is intended to be used as a technological additive (functional group: silage additive) in silage for pigs, poultry, bovines, sheep, goats, rabbits and horses with a maximum content of 600 mg HMTA/kg fresh matter.

The additive was characterised in the previous opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2015) and no new information regarding characterisation of the additive has been provided in the current submission.

In its previous opinion (EFSA FEEDAP Panel, 2015), the Panel noted that: 'HMTA is extensively decomposed during the ensiling process and the residual concentrations of HMTA plus its degradation product, formaldehyde, in silage are significantly reduced to less than 5% of the original concentration of HMTA (600 mg/kg) after at least three months of ensiling (EFSA FEEDAP Panel, 2015).' This concentration corresponds to approximately 30 mg of the sum of HMTA and formaldehyde/kg silage. However, 'The assessment of HMTA residues in silage is limited because the analytical determination of HMTA is based on its conversion to formaldehyde by acidification, not distinguishing the formaldehyde derived from the acid hydrolysis of HMTA from that already present as formaldehyde as such. Therefore, the actual exposure to HMTA of target animals eating silage preserved with the additive was not accurately determined.'

Regarding the safety for the target species, the FEEDAP Panel identified two scenarios. In the first one, it was assumed that all residues in silage are in the form of HMTA. The information available allowed the Panel to conclude that the additive is safe for lambs at the proposed conditions of use but did not allow to draw any conclusion on the safety for the other target species. In the second scenario, it was assumed that all residues are in the form of formaldehyde. In such a case, the Panel was not in the position to conclude on the safety of the additive for any target species. In the absence of a qualitative and quantitative characterisation of the residues, the Panel could not conclude on the safety of HMTA for the target species.

The applicant has submitted new information on the characterisation of the residues in silage. No information has been submitted on the efficacy of the additive.

3.1. Safety

3.1.1. Safety for the target species

3.1.1.1. Residues of HMTA and formaldehyde in silage

The applicant submitted four new studies in which residues of HMTA in silage were measured. In the first study, the decomposition of HMTA into ammonia was studied *in vitro* in a model simulating the acidic liquid phase of a fermented silage (pH 3.8). The degradation of HMTA into formaldehyde and ammonia is well established, and this study did not add useful information for the assessment, and it was therefore no further considered. Other two studies were designed to measure gaseous formaldehyde formation, in order to assess the respiratory exposure of users and animals to gaseous formaldehyde. These two studies are also not considered relevant to assess the safety of HMTA via oral exposure and were no further considered.

⁴ Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

In the fourth study, the fate of HMTA in a silage matrix was followed using an analytical method (Pavitrapak and Williams, 2006) described to determine HMTA in pharmaceutical tablets. An anionic column is used to separate HMTA from the matrix by high-performance liquid chromatography with a spectrophotometric detection at 212 nm. The method was slightly modified and validated for the determination HMTA in a commercial premixture of HMTA and sodium nitrate. No detail was given on the validation of this method applied to the determination of HMTA in silage, namely regarding the ability of the analytical procedure to deal with matrix interferences. Only the linearity of the response to the addition of standard HMTA to a non-spiked silage extract was given between 6 and 600 mg/kg, establishing 6 mg/kg as the limit of quantification (LOQ). HMTA was added to fresh silage in jars at the concentration of 585 mg/kg. Seven samples were taken at day 0 of the experiment and again at days 2, 5, 14, 28 and 45. Subsamples of 30 g each were taken and extracted with water/ethanol (70/30, v/v) then ultra-filtrated; the extracts were analysed for pH and HMTA concentration. The pH dropped rapidly in the first 2 days to 4.1–4.4, and then continuously to 3.8–4.0. HMTA concentration declined rapidly then asymptotically to reach the LOQ after 45 days. This concentration (6 mg/kg) corresponds to approximately 1% of the additive inclusion level in fresh ensiling material. In this study, the formaldehyde concentration in silage was not measured.

3.1.1.2. Safety of HMTA for the target species

In the studies described in the former FEEDAP Panel opinion (EFSA FEEDAP Panel, 2015), residues of ~ 5% (corresponding to about 30 mg/kg) of the original HMTA inclusion level were measured. These residues were analysed as formaldehyde, and it was not possible to distinguish between formaldehyde and HMTA. In the only new study in which HMTA was measured after the ensiling process, HMTA dropped below the limit of quantification (6 mg/kg feed), which correspond to approximately 1% of the initial inclusion level of HMTA in fresh ensiling material. Formaldehyde was not measured in silage in this study. Therefore, an accurate characterisation of the residues of HMTA in silage is not possible.

The additional data provided by the applicant do not clarify whether the residues of HMTA measured in silage are composed of HMTA or formaldehyde, or by a combination of the two substances. However, the determination of both HMTA and formaldehyde (with and without acidification) in samples taken at different time points would be needed to properly characterise the residues of the additive in silage.

The FEEDAP Panel reiterates its previous conclusion that, in the absence of a qualitative and quantitative characterisation of the residues, the Panel cannot conclude on the safety of HMTA for the target species.

4. Conclusions

Based on the results of a new study in which HMTA was measured in silage, after 45 days of ensiling the concentration of HMTA would drop below the limit of quantification of 6 mg HMTA/kg silage, which corresponds to ~ 1% of the HMTA added initially to the fresh ensiling material. However, the formaldehyde concentration was not analysed in silage. In the absence of a qualitative and quantitative characterisation of the residues, the Panel cannot conclude on the safety of HMTA for the target species.

Documentation as provided to EFSA/Chronology

Date	Event
31/01/2016	Dossier received by EFSA. Hexamethylene tetramine for pigs, poultry, bovines, sheep, goats, rabbits and horses. Submitted by ADDCON EUROPE GmbH
31/01/2017	Reception mandate from the European Commission
17/02/2017	Application validated by EFSA – Start of the scientific assessment
28/01/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

References

EFSA (European Food Safety Authority), 2008. revised in 2009. Guidance of the Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) for the preparation of dossiers for the re-evaluation of certain additives already authorised under Directive 70/524/EEC. EFSA Journal 2008;6(9):779, 9 pp. <https://doi.org/10.2903/j.efsa.2008.779>

- EFSA ANS Panel (EFSA Panel Food additives and Nutrient Sources added to Food), 2014. Scientific Opinion on the re-evaluation of hexamethylene tetramine (E 239) as a food additive. EFSA Journal 2014;12(6):3696, 39 pp. <https://doi.org/10.2903/j.efsa.2014.3696>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012. Guidance for the preparation of dossiers for technological additives. EFSA Journal 2012;10(1):2528, 23 pp. <https://doi.org/10.2903/j.efsa.2012.2528>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2015. Scientific Opinion on the safety and efficacy of hexamethylene tetramine as a silage additive for pigs, poultry, bovines, sheep, goats, rabbits and horses. EFSA Journal 2015;13(2):4014, 25 pp. <https://doi.org/10.2903/j.efsa.2015.4014>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2017. Guidance on the assessment of the safety of feed additives for the target species. EFSA Journal 2017;15(10):5021, 19 pp. <https://doi.org/10.2903/j.efsa.2017.5021>
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1962. 6th report. Evaluation of the toxicity of a number of antimicrobials and antioxidants. WHO Technical Reports Series, No 228, 1962.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1965. 8th report. Specification for the identity and purity and toxicological evaluation of some antimicrobials and antioxidant. FAO Nutrition Meetings Report Series, No 38, 1965; WHO Technical Reports Series, No 309, 1965; WHO Food Additives Series No 38A, 1965.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1967. 10th report. Specification for the identity and purity and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances. FAO Nutrition Meetings Report Series, No 43, 1967; WHO Technical Reports Series, No 373, 1967.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1972. 15th report. Toxicological evaluation of some enzymes, modified starches and certain other substances. FAO Nutrition Meetings Report Series, No 50, 1972; WHO Technical Reports Series, No 488, 1972; WHO Food Additives Series No 1, 1972.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1974. 17th report. Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents. Nutrition Meetings Report Series, No 53, 1974; WHO Technical Reports Series, No 539, 1974; WHO Food Additives Series No 5, 1974.
- Pavitrakop C and Williams D, 2006. Determination of methenamine, methenamine mandelate and methenamine hippurate in pharmaceutical preparations using ion-exchange HPLC. Journal of Pharmaceutical and Biomedical Analysis, 40, 1243–1248.

Abbreviations

ADI	acceptable daily intake
bW	body weight
FA	Formaldehyde
FEEDAP	Panel on Additives and Products or Substances used in Animal Feed
HMTA	Hexamethylene tetramine
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LOQ	limit of quantification
NOAEL	no observed adverse effect level
WHO	World Health Organization