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EURL Evaluation Report on the Analytical Methods submitted in connection with the Application for the Authorisation of Feed Additives according to Regulation (EC) No 1831/2003

Dossier related to:	FAD-2010-0377 - CRL/100252
Feed additive:	Hexamethylene tetramine
Active Substance(s):	Hexamethylene tetramine
Rapporteur Laboratory:	Agri-Food Laboratory (GENCAT), Cabrils, Spain
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Report approved by: Date:	Christoph von Holst 13/08/2012

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 10(2) for *hexamethylene tetramine* (later referred as HMTA) under the category / functional group 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 the *feed additive* for pigs, bovines, poultry, sheep, goats, rabbits, horses. According to the Applicant, the *feed additive* is a solid substance consisting of *hexamethylene tetramine*, with a minimum purity of 99 %.

The *feed additive* is to be used through *premixtures*, with a maximum content of 164 g HMTA/kg *premixtures*. The *feed additive* is to be marketed as solid or liquid formulations, with concentrations ranging from 69 to 164 g HMTA/kg *premixtures*. The *feed additive* is intended to be used in *silage* with a proposed maximum level of 0.6 g/kg *silage*.

For the determination of *hexamethylene tetramine* in the *feed additive* the Applicant proposed the European Pharmacopoeia Monograph (01/2008:1545) and the FAO JECFA monographs for food additives (Combined Compendium for Food Additive Specifications: Analytical methods Vol. 4 and *hexamethylene tetramine* monograph No. 1, 2006) - as recommended by Commission Directive 2008/84/EC and by Commission Regulation EU/231/2012. The identification is based on: - infrared absorption spectrophotometry; - the reactions for ammonia, ammonium salts and formaldehyde; and - the colour reactions with acetylacetone and potassium iodobismuthate, while quantification is based on a titrimetric methods. Even though no performance characteristics are provided, the EURL recommends for official control the methods mentioned above for the determination of *hexamethylene tetramine* in the *feed additive*.

For the determination of *hexamethylene tetramine* in *premixtures* the Applicant submitted a single-laboratory validated and further verified method, based on High Performance Liquid Chromatography with refractive index detection (HPLC-RI). The following performance characteristics were derived from validation and verification studies performed with premixtures samples containing 69, 71, 107, 112, 120 and 164 g HMTA/kg *premixtures*: - a relative standard deviation of precision, ranging from 0.5 to 4.4 %; and - a recovery rate ranging from 98 to 102%. Based on the performance characteristics presented, the EURL recommends for official control, the single laboratory validated and further verified HPLC-RI method to determine *hexamethylene tetramine* in the *premixtures*.

According to the Applicant *hexamethylene tetramine* is not stable during the fermentation resulting into ammonia and formaldehyde at lower pH values. Furthemore, the Applicant did not provide any data or analytical method for determination of *hexamethylene tetramine* in

silage. As the unambiguous determination of the content of *hexamethylene tetramine* added to *silage* cannot be performed, the EURL cannot recommend any method for official control to determine added *hexamethylene tetramine* 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.

KEYWORDS
Hexamethylene tetramine, technological additives, silage additives, pigs, bovines, poultry, sheep, goats, rabbits, horses

1. BACKGROUND

In the current application authorisation is sought under article 10(2) (re-evaluation of additives already authorised under the provisions of the Council Directive 70/524/EEC) for *hexamethylene tetramine* (later referred as HMTA) under the category / functional group 1(k) 'technological additives'/'silage additives' [1], according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additive* for pigs, bovines, poultry, sheep, goats, rabbits, horses [2].

According to the Applicant, the *feed additive* is a solid substance consisting of *HMTA*, with a minimum purity of 99 % [2, 3].

The *feed additive* is to be used through *premixtures*, with a maximum concentration of 164 g HMTA/kg *premixtures* [2]. The *feed additive* is to be marketed as solid or liquid formulations containing from 69 to 164 g HMTA /kg *premixtures* [3]. The *feed additive* is intended to be used in *silage* with a proposed maximum level of 0.6 g/kg *silage* [2]. According to the Applicant, the treated silage is not to be fed to animals before the completion of a 4 weeks fermentation period [2].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the duties and the tasks of the European Union Reference Laboratory concerning applications for authorisations of feed additives, the EURL is requested to submit a full evaluation report to the European Food Safety Authority for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with *hexamethylene tetramine*, and their suitability to be used for official controls in the frame of the authorisation, were evaluated.

3. EVALUATION

Identification /Characterisation of the feed additive

Qualitative and quantitative composition of impurities in the additive

When required by EU legislation, analytical methods for official control of undesirable substances in the additive (e.g. arsenic, cadmium, lead, mercury, PAHs and dioxins) are available from the respective European Union Reference Laboratories [4].

Description of the analytical methods for the determination of the active substance in feed additive, premixtures and silage

For the determination of *hexamethylene tetramine* in the *feed additive* the Applicant proposed the European Pharmacopoeia Monograph [5] and the internationally recognised FAO JECFA monographs for food additives [6, 7] - as recommended by Commission Directive 2008/84/EC and by Commission Regulation EU/231/2012, where:

- identification is based on: - infrared absorption spectrophotometry; - the reactions for ammonia, ammonium salts and formaldehyde; and - the colour reactions with acetylacetone and potassium iodobismuthate [7]; while
- quantification is based on the titration methods, with 0.1 M perchloric acid [5] or 1 N sulphuric acid [7]

Even though no performance characteristics are provided, the EURL recommends for official control the above mentioned methods recommended by Commission Directive 2008/84/EC and by Commission Regulation EU/231/2012 and described in the FAO JECFA monographs and the European Pharmacopoeia Monograph (01/2008:1545) to determine *hexamethylene tetramine* in the *feed additive*.

For the determination of *hexamethylene tetramine* in *premixtures* the Applicant submitted a single-laboratory validated and further verified method, based on High Performance Liquid Chromatography with refractive index detection (HPLC-RI) [8]. An aliquot of the sample is extracted with water; the solution is then filtered and diluted with the mobile phase before the HPLC measurement. After the chromatography, the analyte is detected by the refractive index and quantified using external standard calibration. The performance characteristics determined using several commercially available *premixtures (e.g. Kofasil)* are presented in Table 1.

Table 1: Method performance characteristics for the determination of *hexamethylene tetramine* in several commercially available *premixtures* (*Kofasil*) [8]

	Liquid		Solid	
	Validation	Verification	Validation	Verification
	Kofasil (Ultra & Liquid)		Kofasil Plus	
Content [g/kg]	69 & 164	71 & 160	107 & 120	112
RSD_r (%)	0.5 - 0.8	0.5 - 1.0	2.0 - 4.4	2.3
RSD_{ip} (%)	0.8	1.0	4.4	2.3
R_{rec} (%)	100	98	102	-
LOQ [g/kg]	-	-	1.4	-

RSD_r, RSD_{ip} - relative standard deviation for *repeatability* and *intermediate precision*, respectively; R_{rec} - *recovery* rate; LOQ – limit of quantification.

Based on the performance characteristics presented, the EURL recommends for official control, the single laboratory validated and further verified method, based on High Performance Liquid Chromatography with refractive index detection (HPLC-RI), to determine *hexamethylene tetramine* in the *premixtures*.

According to the Applicant *hexamethylene tetramine* is not stable during the fermentation resulting into ammonia and formaldehyde at lower pH values [3]. Furthermore, the Applicant did not provide any data or analytical method for determination of *hexamethylene tetramine* in *silage*. Therefore, the unambiguous determination of the content of *hexamethylene tetramine* added to *silage* cannot be performed satisfactorily. Therefore, the EURL cannot recommend any method for official control to determine added *hexamethylene tetramine* 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.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control:

- JECFA monographs (No. 1, Vol. 4) and *hexamethylene tetramine* monograph No. 1 (2006), Combined Compendium for Food Additive Specifications recommended by Commission Directive 2008/84/EC and by Commission Regulation EU/231/2012; or
- European Pharmacopoeia Monograph (01/2008:1545) for the determination of *hexamethylene tetramine* in the *feed additive*

– single laboratory validated and further verified method using High Performance Liquid Chromatography with refraction index detection (HPLC-RI) for the determination of *hexamethylene tetramine* in *premixtures*

As *hexamethylene tetramine* is not stable during the fermentation, the Applicant did not provide any data or analytical method for determination of *hexamethylene tetramine* in *silage*. Therefore, the EURL cannot recommend any method for official control to determine added *hexamethylene tetramine* in *silage*.

Recommended text for the register entry (analytical method)

For the determination of *hexamethylene tetramine* in the *feed additive*:

– Titrimetry - FAO JECFA Combined Compendium for Food Additive Specifications, *hexamethylene tetramine* monograph No. 1 (2006); or

– Monograph 01/2008:1545 of the European Pharmacopoeia

For the determination of *hexamethylene tetramine* in the *premixtures*:

– High performance liquid chromatography with refractive index detection (HPLC-RI)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *hexamethylene tetramine* have been sent to the European Union Reference Laboratory for Feed Additives. The dossier has been made available to the EURL by EFSA.

6. REFERENCES

[1] *Application, Reference SANCO/G1: Forw. Appl. 1831/0026-2012

[2] *Application, Proposal for Register Entry – Annex A

[3] *Technical dossier, Section II: Identity, characterisation and conditions of use; methods of analysis

[4] Commission Regulation (EC) No 776/2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards to Community Reference Laboratories

[5] European Pharmacopoeia Monograph 6.0, 01/2008:1545

[6] FAO JECFA Combined Compendium for Food Additive Specifications - *Analytical methods, test procedures and laboratory solutions used by and referenced in the food additive specifications*, Monographs No. 1, Vol. 4
http://www.fao.org/docrep/009/a0691e/a0691e00.htm (last visited on 13/08/2012)

[7] FAO JECFA Combined Compendium of Food Additive Specifications, *hexamethylene tetramine*, Monograph No. 1 (2006)
http://www.fao.org/ag/agn/jecfa-additives/details.html?id=220
(last visited on 13/08/2012)

[8] *Technical dossier, Section II – Annex_5

* Refers to Dossier No. FAD-2010-0377

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was Agri-Food Laboratory (GENCAT), Cabrils, Spain. This report is in accordance with the opinion of the consortium of National Reference Laboratories as referred to in Article 6(2) of Commission Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009.

8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby (DK)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen, Jena (DE)