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

Dossier related to:	FAD-2010-0222 CRL/ 100248 FAD-2010-0399 CRL/ 100263
Name of Product:	Formaldehyde
Active Agent (s):	Formaldehyde
Rapporteur Laboratory:	European Union Reference Laboratory for Feed Additives (EURL-FA) Geel, Belgium
Report prepared by:	Stefano Bellorini (EURL-FA)
Report checked by:	Piotr Robouch, Roberto Molteni (EURL-FA)
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Report approved by:	Christoph von Holst
Date:	05/09/2011

EXECUTIVE SUMMARY

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.

KEYWORDS
Formaldehyde, technological additives, preservatives, silage additives, all animal species and categories.

1. BACKGROUND

Formaldehyde is already authorised as *feed additive* under the category 'technological additives' in the functional groups 'preservatives' and 'silage additives' [1]. 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 [2, 3]. According to both Applicants (FAD-2010-0222 and FAD-2010-0399) the *feed additive* is obtained by chemical synthesis. *Formaldehyde* is commonly manufactured in water solution containing methanol as stabiliser. In this form the *feed additive* is known as formalin (minimum 34% w/v *Formaldehyde*) and it is a clear colourless to pale orange liquid with pungent odour [4-7].

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*, and it is not intended for use in vitamin/trace mineral premixtures. 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) [8-11].

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 (EFSA) for each application or group of applications. For these dossiers, the methods of analysis submitted in connection with *Formaldehyde*, 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, aflatoxin B1 and dioxins) are available from the respective European Union Reference Laboratories [12].

Description of the analytical methods for the determination of the active substances in feed additive, premixtures and feedingstuffs.

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 [13, 14]. Furthermore the EURL identify a European Pharmacopoeia method for the identification and characterisation of *Formaldehyde,* based on titration with sodium thiosulphate 0.1 M [15].

Even thought 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*, the first Applicant (FAD-2010-0222) submitted a spectrophotometric method [13]. The method consists in an extraction of the *active substance* from the *feedingstuff* using diluted phosphoric acid followed by a reaction with acetylacetone. After 10 minutes at 60°C the solution is cooled and the absorbance is read at 412 nm. The concentration is calculated from a curve of standard *Formaldehyde* solutions [16]. No performance characteristics of this method were provided.

The second Applicant (FAD-2010-0399) submitted for the determination of *Formaldehyde* in *feedingstuffs* a single laboratory validated and further verified method, based on Reversed Phase High Performance Liquid Chromatography coupled to Diode-Array detection (RP-HPLC-DAD) [17]. The analytical method consists in an extraction of the *active substance* from the *feedingstuff* using diluted phosphoric acid, heated to 80°C and followed by a reaction with 2,4-dinitrophenylhydrazine (DNPH). The supernatant is clarified, injected into the RP-HPLC system and the *Formaldehyde* is detected *via* DAD at 360 nm. The concentration of *active substance* in the *feedingstuff* is quantified using an external calibration curve [18]. The following performance characteristics were reported for concentrations ranging from 51 to 680 mg/kg [19]:

- 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 second Applicant, to determine *Formaldehyde* in *feedingstuffs*.

The Applicants provided no 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.

4. CONCLUSIONS AND RECOMMENDATIONS

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

- two titration methods to determine *Formaldehyde* in *feed additive*
  - the ISO 2227-1972, Formaldehyde solutions for industrial use - Determination of formaldehyde content, or
  - the European Pharmacopoeia 6.0, method 01/2008:0826;
- a single laboratory validated and further verified method based on Reversed Phase High Performance Liquid Chromatography coupled to Diode-Array detection (RP-HPLC-DAD) to determine *Formaldehyde* in *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the determination of *Formaldehyde* in the *feed additive*:

- Titrimetry, ISO 2227-1972, *Formaldehyde* solutions for industrial use - Determination of *Formaldehyde* content
- Titrimetry, European Pharmacopoeia (Ph. Eur. 6.0, method 01/2008:0826)

For the determination of *Formaldehyde* in *feedingstuffs*:

- Reversed Phase High Performance Liquid Chromatography coupled to Diode-Array detection (RP-HPLC-DAD, 360 nm)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Formaldehyde* 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] Council Directive 70/524/EEC concerning additives in feedingstuffs – List of authorised additives in feedingstuffs (2004/C50/01)
[2] *Application/Ref:SANCO/D/2:Forw.Appl.1831/(00135) (10115)-2010
[3] +Application/Ref:SANCO/D/2:Forw.Appl.1831/(00180) (9841)-2010
[4] *Technical dossier, Section II: 2.1.3 Qualitative and quantitative composition
[5] +Technical dossier, Section II: 2.1.3 Qualitative and quantitative composition
[6] *Technical dossier, Section II: 2.1.5 Physical state of each form of the product
[7] +Technical dossier, Section II: 2.1.5 Physical state of each form of the product
[8] *Technical dossier, Section II: 2.5.1 Proposed mode of use in animal nutrition
[9] +Technical dossier, Section II: 2.5.1 Proposed mode of use in animal nutrition
[10] *Application, Annex A, FAD-2010-0222_Conditions of use_*Formaldehyde*
[11] +Application, Annex A, FAD-2010-0399_Conditions of use_*Formaldehyde*
[12] 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
[13] *Technical dossier, Section II: 2.6.1 Method of analysis for the active substance
[14] *ISO 2227-1972, Formaldehyde solutions for industrial use - Determination of formaldehyde content (Technical dossier, Section II, Annex 2.6.1.a)
[15] European Pharmacopoeia 6.0, method 01/2008:0826
[16] *Technical dossier, Section II, Annex 2.6.1.b
[17] +Technical dossier, Section II: 2.6 Method of analysis and reference samples
[18] +Technical dossier, Section II, Annex_II_6_1_0
[19] +Technical dossier, Section II, Annex_II_6_2
*Refers to Dossier no: FAD-2010-0222
+Refers to Dossier no: FAD-2010-0399

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was European Union Reference Laboratory for Feed Additives, IRMM, Geel, Belgium. 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:

- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby (DK)
- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA) Speyer, Speyer (DE)
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)