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JRC F5/CvH/SB/AS/Ares

Subject: **Addendum to the EURL evaluation report**

Reference: **FAD 2010-0113 - Urea technically pure
(JRC.DG.D.6/CvH/SB/mds/ARES(2011)
301126)**

Urea is a feed additive authorised via Commission Implementing Regulation (EU) No 839/2012, for which an application under Article 14(1) (renewal of the authorisation) has been submitted. This Regulation specifies analytical methods for the determination of (1) total nitrogen in the additive and (2) the biuret contribution to the total nitrogen in the additive. As reference for the method protocols, Regulation (EC) No 2003/2003 is included in the Regulation authorising urea. However, in between Regulation (EC) No 2003/2003 has been repealed and the reference to these analytical methods needs therefore be updated in the annex of the upcoming Regulation authorising urea. For both parameters and analytical methods, EN standards are available

Recommended text for the registry entry (analytical method):

- For the determination of the total nitrogen in the additive: Titrimetry (EN 15478)
- For the determination of the biuret contribution to the total nitrogen in the additive: Spectrophotometry: (EN 15479)



JRC.DG.D.6/CvH/SB/mds/ARES(2011)301126

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-2010-0113
CRL/100028

Name of Feed Additive: Urea technically pure

Active Agent (s): Urea

Rapporteur Laboratory: European Union Reference
Laboratory for Feed Additives
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Date: 18/03/2011

EXECUTIVE SUMMARY

In the current application authorisation is sought for *urea technically pure* under Articles 4(1) (new use of a feed additive in water) and 10(2) (re-evaluation of the already authorised feed additive – Council directive 70/524/EEC), category of 'nutritional additives' functional group 3(d), 'urea and its derivatives' according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additive* for ruminants. According to the Applicant *urea technically pure* is available as a solid and a liquid formulation. The *feed additive* is intended to be mixed to *premixtures*, *feedingstuffs* and *water* at maximum doses of 30 g solid *urea* or 70 g liquid *urea* per 100 kg body weight of adult ruminant per day.

For the determination of *urea* in the *feed additive* the Applicant proposed the ISO 22241-2 method. However, two ring-trial validated Community methods exist for the determination of the *urea* in fertilizers: - the titrimetric method for the determination of the total nitrogen content in the *urea* and - the spectrophotometric method for the determination of the nitrogen content in *biuret*. The content of *urea* is then derived from the total nitrogen in *urea* corrected for the nitrogen content in *biuret*. The performance characteristics of the Community methods are:

(i) For the determination of total nitrogen in the *feed additive*:

- a standard deviation for *repeatability* (RSD_r) of 0.3 %; and
- a standard deviation for *reproducibility* (RSD_R) of 0.6 %.

(ii) For the determination of the nitrogen content in *biuret*:

- RSD_r of 1.7% and
- RSD_R of 8.9%.

Based on the performance characteristics presented, the EURL recommends for official control the two ring-trial validated titrimetric and spectrophotometric Community methods, to determine *urea* in the *feed additive*.

For the determination of the *feed additive* in *feedingstuffs* and *water*, the Applicant suggests the spectrophotometric official Community method specifically designed for the determination of *urea* in *feedingstuffs*.

For the determination of *urea* in *premixtures*, the Applicant did not submit any analytical methods. The EURL suggests diluting the *premixtures* samples with ground cereal feed and apply the abovementioned Community method for the determination of *urea* in *feedingstuffs*.

Based on these considerations the EURL recommends for official control, the spectrophotometric Community method, to determine *urea* in *premixtures*, *feedingstuffs* and *water*.

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

Urea technically pure, Nutritional additives, Urea and its derivatives, Ruminants, Biuret.

1. BACKGROUND

In the current application authorisation is sought for *urea technically pure* under Articles 4(1) (new use of a feed additive in water) and 10(2) (re-evaluation of the already authorised feed additive – Council directive 70/524/EEC), under the category 'nutritional additives' and functional group 3(d), 'urea and its derivatives' according to Annex I of Regulation (EC) No 1831/2003 [1]. Specifically, authorisation is sought for ruminants. According to the Applicant *urea* is available as a solid powder or as a liquid. Solid *urea* is a white spherical powder (prilled) while the liquid is a clear solution. Both forms develop a characteristic odour of ammonia [2]. Based on the Regulation 84/443/EEC requirements - providing minimum urea contents in solid and liquid *urea* formulations of 97% and 42% respectively - the Applicant proposed the following specifications for the *feed additive* [3], [4]:

The *feed additive* is intended to be mixed to *premixtures*, *feedingstuffs* and *water* with maximum doses of 30 g solid urea or 70 g liquid urea per 100 kg body weight of adult ruminant per day [1], [6].

Table 1: Urea content as proposed by the Applicant

Content (%)	Solid	Liquid
Urea	99.3 ± 0.4	43.7 ± 0.5
Nitrogen	46.3 ± 2.2	20.4 ± 0.2
Biuret (*)	0.7 ± 0.3	0.2 ± 0.03

(*) *Biuret* is formed by the condensation of two molecules of *urea*. It is a typical impurity formed during the production processes of the feed additive [5].

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 this dossier, the methods of analysis submitted in connection with *urea technically pure*, 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 [7].

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

For the determination of the *urea* in the *feed additive*, the Applicant proposed the ISO 22241-2 method (cf. Annex B and E - Diesel engines - NO_x reduction agent AUS 32 - Part 2: Test methods) [8], [9]. This ISO method is designed to characterise aqueous urea solution (AUS 32) meant to reduce emissions of oxides of nitrogen freed through the exhausted gases produced by diesel engines vehicles.

The EURL identified an alternative ring-trial validated Community method for the determination of total nitrogen in fertilizers, such as *urea* [10] - [13]. The Applicant indicated that in the *feed additive* nitrogen originates from *urea* and *biuret* (a condensation by-product generated during the production process [5]). The *urea* content in the *feed additive* is then derived from the total nitrogen content in the *feed additive* corrected for the *biuret* nitrogen content.

The following principle is described in the Community method: “*The urea in the feed additive sample is transformed quantitatively into ammonia by boiling in the presence of sulphuric*

acid. The liberated ammonia is distilled in an alkaline medium and collected in an excess of sulphuric acid. The excess acid is titrated by means of a standard alkaline solution.” For the determination of *biuret*, the Community method prescribes the following principle: “In an alkaline medium in the presence of potassium sodium tartrate, *biuret* and bivalent copper form a violet cupric compound. The absorbance of the solution is measured at a wavelength of about 546 nm” [10].

Both methods were ring trial validated [11] and the performance characteristics are presented in Table 2.

Table 2: Method performance characteristics for the determination of *urea* in the *feed additive* applying the official Community and fully ring-trial validated methods [10]

	Determination of total nitrogen in urea [12]	Spectrophotometric determination of <i>biuret</i> in urea [13]
RSD _r (%)	0.3	1.7
RSD _R (%)	0.6	8.9

RSD_r, RSD_R = relative standard deviation for *repeatability* and *reproducibility*, respectively;

Based on the performance characteristics presented, the EURL recommends for official control, the ring trial validated titrimetric and spectrophotometric Community methods, to determine total nitrogen content and the *biuret* nitrogen content to derive the *urea* content in the *feed additive*.

For the determination of the *urea* in *feedingstuffs* and *water*, the Applicant proposed the Community method based on the spectrophotometric determination [14]. However, no performance characteristics were reported in the Community method. The following principle is described: “The sample is suspended in water with a clarifying agent. The suspension is filtered. The *urea* content of the filtrate is determined after the addition of 4-dimethylaminobenzaldehyde (4-DMAB) by measuring the optical density at a wavelength of 420 nm.”

For the determination of the *urea* in *premixtures*, the Applicant did not submit any analytical method. However the EURL suggests diluting the *premixtures* samples containing *urea* with ground cereal feed and applying the abovementioned Community method for *feedingstuffs*.

Based on the abovementioned considerations the EURL recommends for official control, the Community method based on spectrophotometric determination, to determine *urea* in *premixtures*, *feedingstuffs* and *water*.

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 three Community methods to determine *urea* in *feed additive*, *premixtures*, *feedingstuff* and *water*.

Recommended text for the register entry (analytical method)

For the determination of total nitrogen in *urea (feed additive)*:

- Titrimetry, Regulation (EC) No 2003/2003 (Annex IV, Method 2.3.3)

For the determination of the *biuret* contribution to the total nitrogen in *urea*

- Spectrophotometry, Regulation (EC) No 2003/2003 (Annex IV, Method 2.5)

For the determination of *urea* in *premixtures*, *feedingstuff* and *water*:

- Spectrophotometry, Commission Regulation (EC) No 152/2009 (Annex III, D)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *urea technically pure* 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/Ref:SANCO/D/2:Forw.Appl.1831/0061-2010
- [2] * Technical dossier, Section II: 2.1.3.1 Qualitative composition of the feed additive
- [3] Commission Directive of 26 July 1984 amending the Annex to Council Directive 82/471/EEC concerning certain products used in animal nutrition (84/443/EEC)
- [4] *Technical dossier, Section II: 2.1.4.2.2 Specifications of the additive
- [5] *Technical dossier, Section II: 2.3 Manufacturing process, including any specific processing procedures
- [6] *Application, (Annex A), FAD-2010-0113_Conditions of use_ *Urea technically pure*
- [7] 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

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- [8] *Technical dossier, Section II: 2.6.1 Method of analysis for the active substance
- [9] ISO 22241-2:2006 - Diesel engines - NOx reduction agent AUS 32 - Part 2: Test methods
- [10] Regulation (EC) No 2003/2003 of the European Parliament and of the Council of 13 October 2003 relating to fertilisers
- [11] Commission Regulation (EC) No 1020/2009 of 28 October 2009 amending Regulation (EC) No 2003/2003 of the European Parliament and of the Council relating to fertilisers for the purposes of adapting Annexes I, III, IV and V thereto to technical progress
- [12] EN 15478: Fertilisers — Determination of total nitrogen in urea
- [13] EN 15479: Fertilisers — Spectrophotometric determination of biuret in urea
- [14] Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed, O.J. L 54, 26.02.2009
- *Refers to Dossier no: FAD-2010-0113

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

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- 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 (POL)
- Univerza v Ljubljani, Veterinarska fakulteta. Nacionalni veterinarski inštitut, Enota za patologijo prehrane in higieno okolja, Ljubljana (SLO)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)