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**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**

Copper Chelate of L-Lysinate-HCl
(FAD-2013-0003; CRL/120041)



**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-2013-0003 - CRL/120041**

Name of Product: **Copper Chelate of L-Lysinate-HCl**

Active Agent (s): **Copper Chelate of L-Lysinate-HCl**

Rapporteur Laboratory: **European Union Reference Laboratory for
Feed Additives (EURL-FA)
Geel, Belgium**

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Date: **10/01/2014**

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Date: **24/01/2014**

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for *Copper Chelate of L-Lysinate-HCl* under the category/ functional group (3b) "nutritional additives"/"compounds of trace elements", 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 all categories and species.

Copper Chelate of L-Lysinate-HCl is a dark-grey or black free flowing powder or granules with a minimum content of 14.5 % total copper, and a maximum content of 85.5 % *L-Lysine-HCl*.

The *feed additive* is intended to be incorporated into *premixtures* and *feedingstuffs*. The Applicant suggested maximum levels of total copper in the *feedingstuffs* complying with the limits set in Regulations (EC) No 1334/2003 and 479/2006, and ranging from 15 to 170 mg/kg, depending of the animal species/category.

For the quantification of *Lysine* content in the *feed additive* the Applicant submitted the ring-trial validated VDLUFA method, based on ion-exchange chromatography coupled with post-column derivatisation and colourimetric or fluorescence detection. However, the EURL identified and recommends instead the recently published ring-trial validated EN ISO 17180 method - based on a similar analytical protocol - for the "determination of *Lysine* [...] in commercial amino acid products and premixtures". The following performance characteristics were reported for *Lysine* contents ranging from 100 to 740 g/kg: - a relative standard deviation for *repeatability* (RSD_r) ranging from 0.7 to 1.7 %; and - a relative standard deviation for *reproducibility* (RSD_R) ranging from 1.5 to 2.5 %.

For the determination of total copper in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted the internationally recognised ring-trial validated method EN 15510 based on inductively coupled plasma atomic emission spectroscopy (ICP-AES). The following performance characteristics were reported for the copper content ranging from 6.8 to 775 mg/kg *premixtures* and *feedingstuffs*: - RSD_r ranging from 2.9 to 12 %; RSD_R ranging from 8 to 22 %; and - a limit for quantification (LOQ) of 3 mg/kg *feedingstuffs*. Additionally, the EURL already recommended in the frame of the Copper group evaluation (cf. Final report JRC.D.5/CvH/PRO/ago/ARES(2012)108233) the ring-trial validated EN 15621 method, based on ICP-AES after pressure digestion. The following performance characteristics were reported for a copper content ranging from 7.3 to 470 mg/kg: - RSD_r ranging from 2.6 to 6.8 %; - RSD_R ranging from 3.8 to 12 %; and - LOQ = 1 mg/kg *feedingstuffs*. Furthermore, a Community method is available for the determination of total

copper in *feedingstuffs*, but no performance characteristics for the method are available, except an LOQ of 10 mg/kg *feedingstuffs*. However, the UK Food Standards Agency recently published a ring-trial based on the above mentioned Community method and reported precisions (RSD_r and RSD_R) from 2.4 to 9.2 % for copper contents ranging from 17 to 39 mg/kg *feedingstuffs*. Based on the available information the EURL recommends for official control the two CEN methods (EN 15510 or EN 15621) together with the Community method (Com Reg (EC) No 152/2009 – Annex IV-C) for the determination of *total copper* in the *feed additive, premixtures* and *feedingstuffs*.

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

Copper Chelate of L-Lysinate-HCl, nutritional additives, compounds of trace elements, all animal species and categories

1. BACKGROUND

In the current application authorisation is sought under article 4(1) (new feed additive) for *Copper Chelate of L-Lysinate-HCl* under the category/ functional group (3b) "nutritional additives"/"compounds of trace elements" [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 all categories and species [1,2].

Copper Chelate of L-Lysinate-HCl is a dark-grey or black free flowing powder or granules with a minimum content of 14.5 % *total copper* and a maximum content of 85.5 % *L-Lysine-HCl* [3].

The *feed additive* is intended to be incorporated into *premixtures* and *feedingstuffs* [3]. The Applicant suggested maximum levels of *total copper* in the *feedingstuffs* [3] complying to the limits set in Regulations (EC) No 1334/2003 and 479/2006: 170 mg/kg for piglets (up to 12 weeks); 25 mg/kg for other pigs and fish; 15 mg/kg for bovines before the start of rumination and ovine; 35 mg/kg for bovines after the start of rumination; 50 mg/kg for crustaceans and 25 mg/kg for other species.

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 *Copper Chelate of L-Lysinate-HCl* 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 [4].

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

For the quantification of *Lysine* content in the *feed additive* and *premixtures* the Applicant submitted the ring-trial validated VDLUFA method, based on ion-exchange chromatography coupled with post-column derivatisation and colourimetric or fluorescence detection [5].

However, the EURL identified instead the recently published ring-trial validated EN ISO 17180 method - based on a similar analytical protocol - for the "determination of *Lysine* [...] in commercial amino acid products and premixtures" [6].

The method does not distinguish between the salts and the amino acid enantiomers and applies for the products/premixtures containing more than 10 % of amino acid content. *Lysine* is extracted with diluted hydrochloric acid and mixed with sodium citrate buffer. After addition of norleucine as internal standard, the amino acids are separated by an amino acid analyser or High Performance Liquid Chromatography (HPLC) equipment using a cation exchange column and sodium citrate buffer as an eluent. *Lysine* is determined colourimetrically at 440 and 570 nm, after post-column derivatisation with ninhydrine or by fluorescence detection after post column reaction with orthophtaldialdehyde.

The following performance characteristics were reported for *Lysine* content ranging from 100 to 740 g/kg: - a relative standard deviation for *repeatability* (RSD_t) ranging from 0.7 to 1.7 %; and - a relative standard deviation for *reproducibility* (RSD_R) ranging from 1.5 to 2.5 %.

Based on acceptable performance characteristics presented, the EURL recommends for official control the ring-trial validated EN ISO 17180 method based on ion-exchange chromatography coupled with post-column derivatisation and colourimetric or fluorescence detection to determine *Lysine* in the *feed additive*.

For the *determination of total copper* in the *feed additive, premixtures* and *feedingstuffs* the Applicant submitted the internationally recognised ring-trial validated method EN 15510 based on inductively coupled plasma atomic emission spectroscopy (ICP-AES) [7]. For the determination of *total copper*, a test portion of the sample is ashed and dissolved in hydrochloric acid (in the case of organic *feedingstuffs*) or wet digested with hydrochloric acid (in the case of mineral compounds).

Additionally, when evaluating the Copper group dossiers (FAD-2010-0031; FAD-2010-0070; FAD-2010-0331) [8] the EURL identified the ring-trial validated CEN method (EN 15621) [9] based on ICP-AES after pressure digestion for the quantification of *total copper* content in the *feed additive, premixtures* and *feedingstuffs*.

Furthermore, a Community method [10] is available for the determination of *total copper* in *feedingstuffs*. The sample is brought into solution in hydrochloric acid after destruction of organic matter, if any. Copper is then determined after appropriate dilution by atomic absorption spectroscopy (AAS). No method performance characteristics were reported, except an LOQ of 10 mg/kg *feedingstuffs*. However, the UK Food Standards Agency (FSA) recently reported results of a ring-trial [11] based on the above mentioned Community method, using samples such as dog biscuits, layer pellets, beef nuts, sow rolls or rabbit pellets.

The performance characteristics reported by the three methods mentioned above for the determination of *total copper* content are summarised in Table 1.

Table 1: Performance characteristics for the quantification of *total copper* in *premixtures* and *feedingstuffs*

	EN 15510 [7]	EN 15621 [9]	UK FSA [11]
Method	ICP-AES	ICP-AES	AAS
Content (mg/kg)	6.8 – 775	7.3 – 470	17 – 39
RSD _r (%)	2.9 – 12 ^(*)	2.6 – 6.8	2.4 – 9.2
RSD _R (%)	8.0 – 22 ^(*)	3.8 - 12	5.4 - 9.2
LOQ (mg/kg)	3	1	-

RSD_r and RSD_R: relative standard deviation for *repeatability* and *reproducibility*;

LOQ: limit of quantification;

(*) the highest precision values were obtained for mineral mixes.

Based on the acceptable method performance characteristics presented, the EURL recommends for official control the two CEN methods (EN 15510 or EN 15621) for the determination of total copper content the *feed additive*, *premixtures* and *feedingstuffs*, together with the Community method (Com Reg (EC) No 152/2009 – Annex IV-C) for the determination of total copper content in *feedingstuffs*.

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:

- The EN ISO 17180 method based on ion-exchange chromatography combined with post-column derivatisation and colourimetric or fluorescence detection for the quantification of *Lysine* content in the *feed additive*;
- The EN 15510 method based on inductively coupled plasma atomic emission spectroscopy (ICP-AES) or EN 15621 method based on ICP-AES after pressure digestion for the quantification of total copper content in the *feed additive*, *premixtures*, and *feedingstuffs*; or
- The Community method based on atomic absorption spectroscopy (AAS) for the quantification of total copper in *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the quantification of *Lysine* content in the *feed additive*:

- Ion-exchange chromatography combined with post-column derivatisation and colourimetric or fluorescence detection - EN ISO 17180

For the quantification of total copper content in the *feed additive* and *premixtures*:

- Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) - EN 15510;
or
- Inductively Coupled Plasma Atomic Emission Spectrometry after pressure digestion, (ICP-AES) - EN 15621

For the quantification of total copper content in *feedingstuffs*:

- Atomic Absorption Spectrometry (AAS) - Commission Regulation (EC) No 152/2009
or
- Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) - EN 15510;
or
- Inductively Coupled Plasma Atomic Emission Spectrometry after pressure digestion (ICP-AES) - EN 15621

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Copper Chelate of L-Lysinate-HCl* 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.1831/0002-2013
- [2] *Application, Proposal for Register Entry – Annex A
- [3] *Technical dossier, Section II: Identity, characterisation and conditions of use of the feed additive; 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] *Technical dossier, Section II: Identity, characterisation and conditions of use of the feed additive; methods of analysis – Annex II.6-1 (cf. Bestimmung von Lysin, Methionin und Threonin in Aminosäurehandelsprodukten und Vormischungen– 4.11.6, Methodenbuch III, 5. Erg. 2004, VDLUFA – Verlag, Darmstadt)
- [6] EN ISO 17180:2013 – *Animal feeding stuffs - Determination of lysine, methionine and threonine in commercial amino acid products and premixtures*
- [7] EN 15510:2007 – *Animal feeding stuffs – Determination of calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum, arsenic, lead and cadmium by ICP-AES*
- [8] Copper Group – JRC.D.5/CvH/PRO/ago/ARES(2012)108233
http://irmm.jrc.ec.europa.eu/EURLs/EURL_feed_additives/authorisation/evaluation_reports/
- [9] EN 15621:2012 – *Animal feeding stuffs – Determination of cadmium, sodium, phosphorus, magnesium, potassium, sulphur, iron, zinc, copper, manganese, cobalt and molybdenum after pressure digestion by ICP-AES*

[10] Commission Regulation (EC) No 152/2009 laying down the methods of sampling and analysis for official control of feed – Annex IV-C

[11] Supplementary Information - FAD-2010-0046: Food Standards Agency – Information Bulletin on Methods of Analysis and Sampling for Foodstuffs, No 102; March 2010

*Refers to Dossier no: FAD-2013-0003

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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- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft, Labore Landwirtschaft, Freistaat Sachsen, Nossen¹ (DE)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)

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