



EUROPEAN COMMISSION  
DIRECTORATE GENERAL  
JOINT RESEARCH CENTRE  
Directorate F – Health, Consumers and Reference Materials  
European Union Reference Laboratory for Feed Additives

JRC.D.5/CvH/ZE/mds/Ares

**Addendum to the EURL report  
FAD-2010-0031-Copper Group**  
(JRC.D.5/CvH/PRO/ago/ARES(2012)108233)

Upon request from DG SANTE [1], the EURL evaluated the supplementary information provided in the frame of the FAD-2010-0031 dossier [2] for the quantification of copper chelate of protein hydrolysates in the *feed additive*. The Applicant submitted a single-laboratory validated and further verified method based on Fourier Transformed Infrared (FTIR) spectroscopy coupled with Principal Component Regression (PCR) analysis [3].

Powdered samples and calibration standards (5 to 10 mg) are subjected to infrared spectroscopy using attenuated total reflection. The calibration standards of chelated copper are prepared from copper (II) sulphate and hydrolysed soya flour. FTIR spectra are recorded from 1800 to 650  $\text{cm}^{-1}$ . Nine replicate spectra per calibration standard are acquired to build the model together with six replicate spectra per sample. The statistical treatment of pre-processed spectra is performed using PCR analysis to generate the calibration and prediction models for quantification of chelated copper content in the *feed additive* samples [3].

Different batches of a commercial product were analysed and the following performance characteristics were reported in the frame of the validation and verification studies for samples containing from 74.3 to 84.3 % chelated copper [3]: (i) a relative standard deviation for *repeatability* ( $\text{RSD}_r$ ) ranging from 2.8 to 8.8 %; (ii) a relative standard deviation for *intermediate precision* ( $\text{RSD}_{ip}$ ) ranging from 7.0 to 8.8 %; (iii) a *recovery* ( $\text{R}_{rec}$ ) rate of 97 %; and (iv) a limit of quantification (LOQ) of 25.4 % of copper chelate in the *feed additive*.

In addition, batches of similar commercial products from four other producers were analysed using the method mentioned above and satisfactory  $\text{RSD}_r$  values were reported (ranging from 4.7 to 15.4 %) for products containing minimum of 50 % and up to 101 % chelated copper [4-7].

The EURL performed a similar study on copper chelated products provided by the Applicant confirming the applicability of FTIR followed by multivariate regression methods, such as PCR or Partial Least Squares (PLS) to determine the degree of metal chelation. In addition, the EURL already recommended this FTIR-PCR method for the quantification of zinc and manganese chelates of protein hydrolysates [8,9].

Based on the performance characteristics available the EURL recommends for official control the method based on FT mid-IR followed by PCR or PLS algorithms to quantify minimum content of 50 % chelated copper in the different products investigated in the frame of this *feed additive* dossier.

### **Recommended text for the registry entry (analytical method)**

For the determination of chelated copper content in the *feed additive*:

- Fourier Transformed Infrared (FTIR) spectroscopy followed by multivariate regression methods

### **References**

- [1] Supplementary Information – DG SANTE request cf. Chelate method, Ares(2017)2202334
- [2] EURL Evaluation Report – JRC.D.5/CvH/PRO/ago/ARES(2012)108233
- [3] FAD-2010-0031 Supplementary information – Annex A\_Qi\_company(a1)\_COPPER\_Analytical method description, validation and verification reports
- [4] FAD-2010-0031 Supplementary information – Annex B\_Qi\_company(a14)\_COPPER\_Laboratory report
- [5] FAD-2010-0031 Supplementary information – Annex B\_Qi\_company(a2)\_COPPER\_Laboratory report
- [6] FAD-2010-0031 Supplementary information – Annex B\_Qi\_company(a4)\_COPPER\_Chelation\_Laboratory report
- [7] FAD-2010-0031 Supplementary information – Annex B\_Qi\_company(a10)\_COPPER\_Laboratory report
- [8] EURL Evaluation Report – JRC.D.5/CvH/ZE/mds/Ares(2016)99245
- [9] EURL Evaluation Report – JRC.D.5/CvH/ZE/mds/Ares(2016)6786860

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### **Addendum**

- Prepared by Zigmās Ezerskis and Piotr Robouch

- Reviewed and approved by Christoph von Holst (EURL-FA) Geel, 05/05/2017

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JRC.D.5/CvH/PRO/ago/ARES(2012)108233

**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-2010-0031 – CRL/100001  
FAD-2010-0070 – CRL/100041  
FAD-2010-0331 – CRL/100193**

Product Name: **Copper Group**

Active Substance(s): **E 4  
Cupric acetate, monohydrate  
basic Cupric carbonate, monohydrate  
Cupric chloride, dihydrate  
Cupric oxide  
Cupric sulphate, pentahydrate  
Cupric chelate of amino-acids hydrate  
Cupric chelate of glycine hydrate (solid &  
liquid)**

Rapporteur Laboratory: **European Union Reference Laboratory  
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Report prepared by: **Piotr Robouch (EURL-FA)**

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Date: **31/01/2012**

Report approved by: **Christoph von Holst**  
Date: **31/01/2012**

## EXECUTIVE SUMMARY

In the current application authorisation is sought under articles 4(1) and 10(2) for *Cupric acetate, monohydrate; basic Cupric carbonate, monohydrate; Cupric chloride, dihydrate; Cupric oxide; Cupric sulphate, pentahydrate; Cupric chelate of amino-acids hydrate; Cupric chelate of glycine hydrate (solid & liquid)* under the category/functional group 3(b) "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 additives* for all categories and species.

Applicants stated minimum total copper content of: 31% in *Cupric acetate monohydrate*; 55% in *Basic cupric carbonate monohydrate*; 37% in *Cupric chloride dihydrate*; 77% in *Cupric oxide*; 24% in *Cupric sulphate pentahydrate*; 10% in *Cupric chelate of amino-acids hydrate*; and 23% and 6% in solid and liquid *Cupric chelate of glycine hydrate*, respectively.

The *feed additives* are intended to be incorporated into *premixtures, feedingstuffs* and *water*. All Applicants suggested maximum levels of total copper in the *feedingstuffs* complying to 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.

The EURL recommends three European Pharmacopoeia methods: - Ph. Eur. monograph 01/2008:2146 for the identification of *Copper acetate monohydrate*; - Ph. Eur. monograph 01/2008:0894, for the identification of *Copper sulphate pentahydrate*; and - the generic Ph. Eur. monograph 01/2008:20301 for the "identification reactions of ions and functional groups", such as acetates, carbonates, chlorides and sulfates. Additionally crystallographic techniques such as X-Ray diffraction could be used for the characterisation of crystalline structure of *Cupric acetate monohydrate, Cupric chloride dehydrate, Copper oxide* and *Cupric sulphate pentahydrate*.

For the quantification of "amino" content in the amino copper chelates (i.e. *Copper chelate of glycine hydrate* and *Copper chelate amino acids hydrate*), the Applicant proposed - upon request from the EURL - the Community method based on High Performance Liquid Chromatography (HPLC) combined with post-column derivatisation using ninhydrin as derivatisation agent and photometric detection at 570 nm. The EURL considers the Community method suitable for the characterisation of the amino compounds in the frame of official control.

For the *determination* of total copper in the *feed additive, premixtures* and *feedingstuffs* the Applicants submitted internationally recognised ring trial validated methods EN 15510 and CEN/TS 15621. Both methods are based on inductively coupled plasma atomic emission

spectroscopy, with or without pressure digestion. The following performance characteristics were reported for EN 15510:

- a relative standard deviation of *repeatability* ( $RSD_r$ ) ranging from 2.9 to 12 %;
- a relative standard deviation for *reproducibility* ( $RSD_R$ ) ranging from 8 to 22 %; and
- a limit of quantification (LOQ) of 3 mg/kg.

A variety of matrices (i.e. feed for pigs and for sheep, rock phosphate, a mineral premix and a mineral mix) with a total copper content ranging from 7.3 to 470 mg/kg was used in the frame of the CEN/TS 15621 ring-trial. The following performance characteristics were reported: -  $RSD_r$  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 were provided. The UK Food Standards Agency recently reported results of a ring-trial based on the above mentioned Community method, and reported precisions ( $RSD_r$  and  $RSD_R$ ) for *feedingstuffs* ranging from 2.4 to 9.2 %.

Based on these performance characteristics the EURL recommends for official control the CEN methods EN 15510 or CEN/TS 15621 to determine total copper content by ICP-AES in the *feed additive* and *premixtures*. As for the determination of total copper content in *feedingstuffs*, the EURL recommends for official control the Community method based on AAS and the above mentioned CEN methods (EN 15510 or CEN/TS 15621).

Similarly to the "SANCO Zinc group", the EURL recommends the ring-trial validated CEN method EN ISO 11885, based on inductively coupled plasma optical emission spectroscopy (ICP-AES) for the quantification of total copper in *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**

*Cupric acetate, monohydrate; basic Cupric carbonate, monohydrate; Cupric chloride, dihydrate; Cupric oxide; Cupric sulphate, pentahydrate; Cupric chelate of amino-acids hydrate; Cupric chelate of glycine hydrate (solid & liquid); nutritional additive; compounds of trace elements; all animal species and categories.*

## 1. BACKGROUND

In the current application authorisation is sought under articles 4(1) [1] and 10(2) [2,3] for *Cupric acetate, monohydrate; basic Cupric carbonate, monohydrate; Cupric chloride, dihydrate; Cupric oxide; Cupric sulphate, pentahydrate; Cupric chelate of amino-acids hydrate; Cupric chelate of glycine hydrate (solid & liquid)* under the category of "nutritional additives" functional group 3b (compounds of trace elements) [1-3], according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additives* for all categories and species [1-6].

According to the Applicants:

- *Cupric acetate monohydrate* ( $C_4H_6CuO_4 \cdot H_2O$ ) is a dark green solid with a minimum content of 31% total copper [4];
- *basic Cupric carbonate monohydrate* ( $CuCO_3 \cdot Cu(OH)_2$ ) is a green powder with a minimum content of 55% total copper [4];
- *Cupric chloride dihydrate* ( $CuCl_2 \cdot 2H_2O$ ) is a blue powder with a minimum content of 37% total copper [4];
- *Cupric oxide* ( $CuO$ ) is a brown-black amorphous or crystal powder with a minimum content of 77% total copper [4];
- *Cupric sulphate pentahydrate* ( $CuSO_4 \cdot 5H_2O$ ) is a blue powder with a minimum content of 24% total copper [4,6];
- *Cupric chelate of amino-acids hydrate* is a blue to green free flowing powder with a minimum content of 10% total copper [4,5];
- *Cupric chelate of glycine hydrate* is a pale to dark blue powder (*solid*) with a minimum content of 23% total copper [4]; or a transparent *liquid* with a minimum content of 6% total copper [4].

The *feed additives* are intended to be incorporated into *premixtures, feedingstuffs* [7-9] and *water* [7]. All Applicants suggested maximum levels of total copper in the *feedingstuffs* [7-9] complying to the limits set in Regulations (EC) No 1334/2003 and 479/2006: 170 mg/kg for piglets (suckling and weaning); 25 mg/kg for other piglets and fish; 10 mg/kg for breed of sheep; 15 mg/kg for bovines before the start of rumination and ovine; 50 mg/kg for crustaceans and other species; and concentration levels ranging from 20 to 35 mg/kg for bovines after the start of rumination.

## 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 *Cupric acetate, monohydrate; basic Cupric carbonate, monohydrate; Cupric chloride, dehydrate; Cupric oxide; Cupric sulphate, pentahydrate; Cupric chelate of amino-acids hydrate; Cupric chelate of glycine hydrate (solid & liquid)*, and their suitability to be used for official controls in the frame of the authorisation, were evaluated.

## 3. EVALUATION

### *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, dioxins and dioxin like PCBs) are available from the respective European Union Reference Laboratories [12].

### *Identification /Characterisation of the feed additive*

The EURL recommends three European Pharmacopoeia methods:

- Ph. Eur. monograph 01/2008:2146 based on the dissolution of the salt in water with diluted ammonia for the identification of *Cupric acetate monohydrate*;
- Ph. Eur. monograph 01/2008:0894, for the identification of *Cupric sulphate pentahydrate*; and
- the generic Ph. Eur. monograph 01/2008:20301 for the "identification reactions of ions and functional groups", such as acetates, carbonates, chlorides and sulfates;

Additionally the EURL recommends crystallographic techniques, such as X-Ray diffraction for the characterisation of crystalline structure of *Cupric acetate monohydrate, cupric chloride dehydrate, copper oxide* and *cupric sulphate pentahydrate*.

Finally, the presence of *basic Cupric carbonate* (containing Cupric carbonate and Cupric hydroxide) can be verified with the formation/degassing of CO<sub>2</sub>, when diluted hydrochloric acid is added.

Upon request from the EURL, the Applicants confirmed [10] using the Community method of analysis for amino-acids [11] to quantify the “amino acid” content in the amino copper chelates (i.e. *Cupric chelate of glycine hydrate* and *Cupric chelate amino acids hydrate*).

Additionally, all Applicants suggested [7-9] to quantify the total copper content (described in the next section) for the characterisation of the above mentioned products.

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

For the quantification of "amino" content in the amino copper chelates (i.e. *Cupric chelate of glycine hydrate* and *Cupric chelate amino acids hydrate*), the Applicant proposed [10] - upon request from the EURL - the Community method [11] based on High Performance Liquid Chromatography (HPLC) combined with post-column derivatisation using ninhydrin as derivatisation agent and photometric detection at 570 nm. The EURL considers the Community method suitable for the characterisation of the amino compounds in the frame of official control.

For the determination of total copper in the *feed additive, premixtures and feedingstuffs* Applicants (FAD-2010-0031 and FAD-2010-0331) submitted the internationally recognised ring trial validated method EN 15510 [13], based on inductively coupled plasma atomic emission spectroscopy (ICP-AES). 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). The following performance characteristics were reported [13] for a complete feed for pigs, a complete feed for sheep, a rock phosphate, a mineral premix and two different mineral mixtures, where the total copper content ranged from 6.8 to 775 mg/kg:

- a relative standard deviation of *repeatability* (RSD<sub>r</sub>) ranging from 2.9 to 12 %<sup>(\*)</sup>;
- a relative standard deviation for *reproducibility* (RSD<sub>R</sub>) ranging from 8 to 22 %<sup>(\*)</sup>;
- a limit of quantification (LOQ) of 3 mg/kg.

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

An alternative CEN ring-trial validated method (CEN/TS 15621) [14] based on ICP-AES after pressure digestion, was submitted by Applicants (FAD-2010-0070 and FAD-2010-0331)



for the determination of total copper in the *feed additive*, *premixtures* and *feedingstuffs*, by ICP-AES after pressure digestion. The total copper concentration is determined using external calibration or standard addition technique. The following performance characteristics were reported [14] for a feed for pigs, and for sheep, a rock phosphate, a mineral premix and a mineral mix, where the total copper content ranged 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*, suitable for low total copper contents.

Furthermore, a Community method [15] 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 AAS. No method performance characteristics are reported in the Regulation, except an LOQ of 10 mg/kg *feedingstuffs*. However, the UK Food Standards Agency recently reported results of a ring-trial [16] based on the above mentioned Community method, using samples such as dog biscuits, layer pellets, beef nuts, sow rolls or rabbit pellets. Precisions ( $RSD_r$  and  $RSD_R$ ) ranging from 2.4 to 9.2 % were reported for samples containing total copper levels ranging from 17 to 39 mg/kg *feedingstuffs*.

Based on these acceptable method performance characteristics, the EURL recommends for official control the CEN methods (EN 15510 or CEN/TS 15621) to determine total copper content by ICP-AES in the *feed additive* and *premixtures*. As for the determination of total copper content in *feedingstuffs*, the EURL recommends for official control the Community method based on AAS and the above mentioned CEN methods (EN 15510 or CEN/TS 15621).

For the quantification of total copper in *water* the EURL identified the ring trial validated CEN method EN ISO 11885 [17], based on inductively coupled plasma optical emission spectroscopy (ICP-AES) – previously recommended for the SANCO Zinc group. The total copper concentration is determined using external calibration or standard addition technique. The following performance characteristics were reported for a total copper content of 0.67 mg/L: -  $RSD_r$  of 1.8 %; -  $RSD_R$  of 5.1 %; and - LOQ = 1 µg/L.

Based on the method performance characteristics presented, the EURL recommends for official control the ICP-AES CEN method (EN ISO 11885) to quantify total copper content in the *water*.

The EURL is aware of several ring-trial validated methods (Community method [11], ISO [18], AOAC [19] and VDLUFA [20]) dedicated to the determination of "amino acids" in *premixtures* and *feedingstuffs*. However, these methods are not relevant for official control when the monitoring of total copper content is required, as set in previous legislations.

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 several European Pharmacopoeia monographs (01/2008:2146, 0894 and 20301) for the identification of *Cupric acetate monohydrate*, *Cupric sulphate pentahydrate* and functional groups, such as acetate, carbonates, chlorides and sulfates in the *feed additives*. The EURL also recommends X-Ray diffraction for the characterisation of copper crystalline products.

For the quantification of "amino" content in the amino copper chelates (i.e. *Cupric chelate of glycine hydrate* and *Cupric chelate amino acids hydrate*) in the *feed additive*, the EURL recommends the Community method (Com Reg (EC) No 152/2009 – Annex III-F) based on High Performance Liquid Chromatography (HPLC) combined with post-column derivatisation using ninhydrin as derivatisation agent and photometric detection at 570 nm.

Furthermore, the EURL recommends for official control the CEN methods EN 15510 or CEN/TS 15621 for the determination of total copper content by ICP-AES in the *feed additive* and *premixtures*. As for the determination of total copper content in *feedingstuffs*, the EURL recommends for official control the Community method (Com Reg (EC) No 152/2009 – Annex IV-C) based on AAS and the above mentioned CEN methods (EN 15510 or CEN/TS 15621). Finally the EURL recommends for official control the ICP-AES CEN method (EN ISO 11885) to quantify total copper content in the *water*.

#### ***Recommended text for the register entry (analytical method)***

For the identification of *Cupric acetate monohydrate* in *feed additive*:

- European Pharmacopoeia Monograph 2146;

For the identification of *Cupric sulphate pentahydrate* in *feed additive*

- European Pharmacopoeia Monograph 0894;

For the "identification reactions of ions and functional groups", such as acetates, carbonates, chlorides and sulfates in *feed additive*:

- European Pharmacopoeia Monograph 2.3.1;

For the crystallographic characterisation of feed additives, such as *Cupric acetate monohydrate*, *Cupric chloride dehydrate*, *Cupric oxide* and *cupric sulphate pentahydrate*:

- X-Ray diffraction

For the quantification of amino acid content in the *feed additives* (*Cupric chelate of glycine hydrate* and *Cupric chelate amino acids hydrate*)

- Community method (Com Reg (EC) No 152/2009 – Annex III-F) – Ion exchange chromatography using High-Performance Liquid Chromatography (HPLC) coupled to post column derivatisation and photometric detection;

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

- EN 15510 or CEN/TS 15621 - inductively coupled plasma optical emission spectrometry (ICP-AES);

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

- EN 15510 or CEN/TS 15621; or
- Community method (Com Reg (EC) No 152/2009 – Annex IV-C) – atomic absorption spectrometry (AAS)

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

- EN ISO 11885 - inductively coupled plasma optical emission spectrometry (ICP-AES).

## 5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Cupric acetate, monohydrate*; *Basic cupric carbonate, monohydrate*; *Cupric chloride, dihydrate*; *Cupric oxide*; *Cupric sulphate, pentahydrate*; *Cupric chelate of amino-acids hydrate*; *Cupric chelate of glycine hydrate (solid & liquid)* 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] <sup>a</sup> Application, Reference SANCO/D/2 Forw. Appl. 1831/0029-2010  
[2] <sup>b</sup> Application, Reference SANCO/D/2 Forw. Appl. 1831/7008-2010  
[3] <sup>c</sup> Application, Reference SANCO/D/2 Forw. Appl. 1831/7170-2010

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- [4] <sup>a</sup> Application, Proposal for Register Entry – Annex A.  
[5] <sup>b</sup> Application, Proposal for Register Entry – Annex A  
[6] <sup>c</sup> Application, Proposal for Register Entry – Annex A  
[7] <sup>a</sup> Technical dossier, Section II – Identity; Conditions of use of the additive  
[8] <sup>b</sup> Technical dossier, Section II – Identity; Conditions of use of the additive  
[9] <sup>c</sup> Technical dossier, Section II – Identity; Conditions of use of the additive  
[10] Supplementary information – FAD-2010-0142  
[11] Commission Regulation (EC) No 152/2009 laying down the methods of sampling and analysis for official control of feed– Annex III-F  
[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] 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*  
[14] CEN/TS 15621:2007 – *Animal feeding stuffs – Determination of cadmium, sodium, phosphorus, magnesium, potassium, sulphur, iron, zinc, copper, manganese, cobalt and molybdenum after pressure digestion by ICP-AES*  
[15] Commission Regulation (EC) No 152/2009 - Annex IV-C  
[16] Supplementary Information - FAD-2010-0046: Food Standards Agency – Information Bulletin on Methods of Analysis and Sampling for Foodstuffs, No 102; March 2010  
[17] EN ISO 11885:2009 – *Water quality – Determination of selected elements by inductively coupled plasma optical emission spectrometry (ICP-OES – ICP-AES)*  
[18] EN ISO 13903:2005 - *Animal feeding stuffs – Determination of amino acids content*  
[19] AOAC Official Method 999:13 – Lysine, Methionine and Threonine in Feed Grade Amino Acids and Premixes  
[20] Bestimmung von Lysin, Methionin und Threonin in Aminosäurenhandelsprodukten und Vormischungen – 4.11.6, Methodenbuch III, 5. Erg. 2004, VDLUFA – Verlag, Darmstadt.

<sup>a</sup> Refers to Dossier No. FAD-2010-0031

<sup>b</sup> Refers to Dossier No. FAD-2010-0070

<sup>c</sup> Refers to Dossier No. FAD-2010-0331

## **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.

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## 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)
- Univerza v Ljubljani, Veterinarska fakulteta. Nacionalni veterinarski inštitut, Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- RIKILT-Instituut voor Voedselveiligheid, Wageningen (NL)