



JRC.F.5/UV/ZE/AS/Ares

**Subject:** 2<sup>nd</sup> addendum of the EURL evaluation report

**References:**

FAD-2010-0046 – Tribasic copper chloride (JRC.DG.D.6/CvH/PRO/ag/Ares(2011)473276, JRC.DG.D6/FSQ/CvH/ag/Ares(2011)920189)

Upon a recent publication of multi-analyte, ring-trial validated method EN 17053 [1], dedicated for the determination of various trace elements (including *copper*) in animal feedingstuffs, the EURL considered appropriate to evaluate its suitability for official control in the frame of the above-mentioned *feed additive* dossier. In addition, another ring-trial validated method EN ISO 6869 [2], which was not included in the list of the recommended methods in the previous EURL report [3] or in its addendum [4], is also evaluated for its fitness-for-purpose in the frame of the current addendum. The other three ring-trial validated methods, namely European Union (EU) method [5], EN 15621 [6] and EN 15510 [7] in their older versions [8,9] have been already recommended for official control in the frame of previous EURL evaluation [3,4] for the determination of *copper* in animal feedingstuffs. In the current addendum, the EURL also re-iterates the X-ray diffraction (XRD) method, which has been previously recommended [3,4] for the identification of crystallographic composition of the *feed additive*.

This addendum aims to provide up-to-date EURL recommendations, including all the available analytical methods complying with the highest requirements as stated in Annex II of Regulation (EC) No 429/2008 [10], which would allow Member States official control laboratory full flexibility regarding the selection of method of analysis.

According to the EN 17053 method, the sample is digested with concentrated nitric acid under pressure. The elemental *copper* is detected by inductively coupled plasma-mass spectrometry (ICP-MS) at mass-to-charge (m/z) of 63 and/or 65. The quantification of the analyte is performed using an external standard calibration or standard additions [1].

According to EN ISO 6869 method, 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 analyte is detected by an air-acetylene flame atomic absorption spectrometry (AAS) at selected specific wavelength (324.8 nm). The quantification is performed using an external standard calibration [2].

**Table 1:** Performance characteristics for the determination of *total copper* in animal feedingstuffs.

	EN 17053	ISO 6869
Method	ICP-MS	AAS
Mass fraction (mg/kg)	2.5 – 1389	6 – 39100
RSD <sub>r</sub> (%)	2.9 – 6.7	1.2 – 14.5
RSD <sub>R</sub> (%)	5.7 – 9.8	2.9 – 23.8 <sup>(*)</sup>
LOQ (mg/kg)	-	5
Reference	[1]	[2]

RSD<sub>r</sub> and RSD<sub>R</sub>: relative standard deviation for *repeatability and reproducibility*; LOQ: *a limit of quantification*; <sup>(\*)</sup> the precision values obtained for mixed feed.

The performance characteristics reported for the two above-mentioned methods are summarised in Table 1.

Based on the acceptable method performance characteristics, the EURL considers fit-for-purpose the two ring-trial validated methods: (i) EN ISO 6869 for the determination of total *copper* in the *feed additive, premixtures* and *compound feed*; and (ii) the EN 17053 method for the determination of total *copper* in *premixtures* and *compound feed*.

**Recommended text for the registry entry (analytical method) (replacing the previous recommendations)**

For the identification of dicopper chloride trihydroxide atacamite/paratacamite crystal forms in the *feed additive*:

- X-ray diffraction (XRD)

For the determination of total *copper* in the *feed additive*:

- Inductively coupled plasma-atomic emission spectrometry, ICP-AES (EN 15621 or EN 15510) or
- Atomic absorption spectrometry, AAS (ISO 6869)

For the determination of total *copper* in *premixtures*:

- Inductively coupled plasma-atomic emission spectrometry, ICP-AES (EN 15621 or EN 15510) or
- Atomic absorption spectrometry, AAS (ISO 6869) or
- Inductively coupled plasma-mass spectrometry, ICP-MS (EN 17053)

For the determination of total *copper in compound feed*:

- Inductively coupled plasma-atomic emission spectrometry, ICP-AES (EN 15621 or EN 15510) or
- Atomic absorption spectrometry, AAS (Commission Regulation (EC) No 152/2009 (Annex IV-C) or ISO 6869) or
- Inductively coupled plasma-mass spectrometry, ICP-MS (EN 17053)

## References

- [1] EN 17053:2018 Animal feeding stuffs: Methods of sampling and analysis – Determination of trace elements, heavy metals and other elements in feed by ICP-MS (multi-method)
- [2] EN ISO 6869:2000 Animal feeding stuffs – Determination of the contents of calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc – Method using atomic absorption spectrometry
- [3] EURL Report – FAD-2010-0046, Tribasic copper chloride (JRC.DG.D.6/CvH/PRO/ag/Ares(2011)473276)
- [4] Amendment to EURL Report – FAD-2010-0046, Tribasic copper chloride (JRC.DG.D6/FSQ/CvH/ag/Ares(2011)920189)
- [5] Commission Regulation (EC) No 152/2009 laying down the methods of sampling and analysis for official control of feed – Annex IV-C
- [6] EN 15621:2017 – Animal feeding stuffs: Methods of sampling and analysis – Determination of calcium, sodium, phosphorus, magnesium, potassium, sulphur, iron, zinc, copper, manganese and cobalt after pressure digestion by ICP-AES
- [7] EN 15510:2017 – Animal feeding stuffs: Methods of sampling and analysis – Determination of calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum and lead by ICP-AES
- [8] CEN/TS 15621:2007 – Animal feeding stuffs - Determination of calcium, sodium, phosphorus, magnesium, potassium, sulphur, iron, zinc, copper, manganese, cobalt and molybdenum after pressure digestion by ICP-AES
- [9] 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
- [10] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisations of feed additives, OJ L 133 22.5.2008, p. 1

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## Addendum

- Prepared by Zigmantas Ezerskis
- Reviewed and approved by María José González de la Huebra and Ursula Vincent (EURL-FA), respectively, Geel, 23/01/2024



EUROPEAN COMMISSION  
JOINT RESEARCH CENTRE

Institute for reference materials and measurements  
European Union Reference Laboratory for Feed Additives



JRC.DG.D6/FSQ/CvH/ag/Ares(2011)  
Geel, 30/08/2011

European Food Safety Authority  
Animal Feed Unit  
Mrs. C. Roncancio-Peña  
Largo N. Palli 5/A  
I-43100 Parma, Italy

Subject: *Amendment to EURL Final Report (JRC.DG.D.6/CvH/PRO/ag/Ares(2011)473276) on the dossier related to FAD-2010-0046 (Tribasic copper chloride)*

Reference: *Your request from 12/08/2011 (Ares(2011)914354)*

Dear Mrs. Roncancio-Peña,

In relation to the dossier evaluation of *Tribasic copper chloride* (FAD 2010-0046) and the corresponding EURL Final Report sent to EFSA on 05/05/2011, EFSA informed the EURL (e-mail from 12/08/2011, Ares(2011)914354) about a communication from the applicant declaring that the composition of the additive has been changed. In consequence EFSA requests a statement from the EURL, whether the conclusions on the analytical methods previously evaluated against the former composition of the additive are still valid for the new formulation.

The attached amendment to EURL Final Report (JRC.DG.D.6/CvH/PRO/ag/Ares(2011)473276) contains the requested information.

Yours sincerely,

Christoph von Holst  
Operating Manager

Cc.: - F. Ulberth (Head of Unit, IRMM Food Safety and Quality)  
- J. Moynagh (Head of Unit, DG SANCO Animal Nutrition)

**Amendment to EURL Final Report (JRC.DG.D.6/CvH/PRO/ag/  
Ares(2011)473276) on the dossier FAD 2010-0046 (Tribasic copper chloride)**

*Background:*

Di copper chloride tri hydroxide ( $\text{Cu}_2\text{Cl}(\text{OH})_3$ , also called tribasic copper chloride – TBCC) is a pure form of crystalline copper (II) chloride hydroxide containing a minimum of 95 % of a defined ratio of the polymorphs atacamite and paratacamite - equivalent to a minimum content of total copper of 58 %. In the above mentioned report the EURL concluded that X-ray diffraction is suitable for the identification of *di copper chloride tri hydroxide* atacamite/paratacamite crystal forms in the *feed additive*, whereas various standard methods are available for the determination of total copper in the *feed additive, premixtures* and *feedingstuffs*.

On 12/08/2011 EFSA informed the EURL that the applicant has changed the composition of the feed additive, namely by adding 5% of starch thereby reducing the copper content in the feed additive accordingly. In consequence EFSA requests a statement from the EURL, whether the conclusions on the analytical methods previously evaluated against the former composition of the additive are still valid for the new formulation.

*Conclusion*

Based on a new evaluation the EURL concludes that the slightly modified composition of the additive does not influence the suitability of the analytical methods as previously stated in the above mentioned EURL Final Report for official control purposes. Therefore the conclusions in this report are also valid for the new formulation.

Evaluation prepared by: **Piotr Robouch (EURL-FA)**



JRC.DG.D.6/CvH/PRO/ag/ARES(2011)473276

**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-2010-0046  
CRL/100051

Product Name: Tribasic copper chloride (TBCC)

Active Substance(s): Di copper chloride tri hydroxide;  
crystal form atacamite/paratacamite

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

Report prepared by: Piotr Robouch (EURL-FA)

Report revised by: Gerhard Buttinger (EURL-FA)  
Date: 02/05/2011

Report approved by: Christoph von Holst  
Date: 02/05/2011

## EXECUTIVE SUMMARY

In the current application authorisation is sought under articles 4(1) for *Di copper chloride tri hydroxide* under the category "nutritional additives", functional group 3(b) "compounds of trace elements", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* for all animal species and categories.

*Di copper chloride tri hydroxide* ( $\text{Cu}_2\text{Cl}(\text{OH})_3$ ), also called *tribasic copper chloride* – TBCC) is a pure form of crystalline copper (II) chloride hydroxide containing a minimum of 95 % of a defined ratio of the polymorphs atacamite and paratacamite - equivalent to a minimum content of total copper of 58 %. The *feed additive* is intended to be incorporated into *premixtures* and *feedingstuffs*. The Applicant suggested the following maximum levels of total copper in the *feedingstuffs* ranging from 10 to 170 mg/kg depending on the species of interest.

For the *identification* of TBCC in the *feed additive*, the Applicant submitted an X-ray diffraction (XRD) method to confirm the crystal forms of TBCC.

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:

- a relative standard deviation of *repeatability* ( $\text{RSD}_T$ ) ranging from 2.9 to 12 %;
- a relative standard deviation for *reproducibility* ( $\text{RSD}_R$ ) ranging from 8 to 22 %; and
- a limit of quantification of 3 mg/kg.

The EURL identified an alternative CEN ring-trial validated method (CEN/TS 15621) based on ICP-AES after pressure digestion, for the determination of total copper in the *feed additive*, *premixtures* and *feedingstuffs*. The total copper concentration is determined using external calibration or standard addition technique. The following performance characteristics were reported 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: -  $\text{RSD}_T$  ranging from 2.6 to 6.8 %; -  $\text{RSD}_R$  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 ( $\text{RSD}_T$  and  $\text{RSD}_R$ ) for *feedingstuffs* ranging from 2.4 to 9.2 %.

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).

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

*Di copper chloride tri hydroxide*, nutritional additive; compounds of trace elements, all animal species and categories.

## 1. BACKGROUND

In the current application authorisation is sought under articles 4(1) for *Di copper chloride tri hydroxide* under the category "nutritional additives", functional group 3(b) "compounds of trace elements" [1], according to the classification system of Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* for all animal species and categories [2].

*Di copper chloride tri hydroxide* ( $\text{Cu}_2\text{Cl}(\text{OH})_3$ ), also called *tribasic copper chloride* – TBCC) is a pure form of crystalline copper (II) chloride hydroxide containing a minimum of 95% [3] of a defined ratio of the polymorphs atacamite (with a orthorhombic structure) [4] and paratacamite (with a rhombohedral structure type); with no botallackite form present [5]. The product is crystallised from mother liquors in a continuous manufacturing process producing a bright green, dense, flowable dry powder, with a minimum content of total copper of 58 % and a chlorine content ranging from 17 to 19 % [3].

The *feed additive* is intended to be incorporated into *premixtures* and *feedingstuffs* [3]. The Applicant suggested the following maximum levels of total copper in the *feedingstuffs* [2]: 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 *Di copper chloride tri hydroxide*, 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 and dioxins) are available from the respective European Union Reference Laboratories [6].

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

For the *identification* of TBCC in the *feed additive*, the Applicant submitted an X-ray diffraction (XRD) method to confirm the crystal forms of TBCC [7]. The sample is ground with a mortar, sieved through a 325 mesh and packed into a holder. The diffraction pattern is collected using an XRD system equipped with Cu X-ray source and monochromator. The diffraction pattern is deconvoluted and compared to standard chromatograms JPDS files deriving the atacamite:paratacamite ratio to range from 1:1 to 1:1.5 [3].

For the *determination* of fluoride impurity and chloride content, the Applicant submitted an ion chromatography method for the determination of inorganic anions [8].

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 [9], 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

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* ( $RSD_r$ ) ranging from 2.9 to 12 %<sup>(\*)</sup>
- a relative standard deviation for *reproducibility* ( $RSD_R$ ) 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.

The EURL identified an alternative CEN ring-trial validated method (CEN/TS 15621) based on ICP-AES after pressure digestion, for the determination of total copper in the *feed additive*, *premixtures* and *feedingstuffs*. The total copper concentration is determined using external calibration or standard addition technique. The following performance characteristics were reported [10] 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 [11] is available for the determination of total copper in *feedingstuffs*, but no performance characteristics of the method were provided. 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 [12] 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).

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 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 based on AAS and the above mentioned CEN methods (EN 15510 or CEN/TS 15621).

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

For the identification of *di copper chloride tri hydroxide* atacamite/paratacamite crystal forms in the *feed additive*:

- X-ray diffraction (XRD)

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

- EN 15510: Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES), and
- CEN/TS 15621: Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES) after pressure digestion.

For the determination of total copper in the *feedingstuffs*:

- Regulation (EC) No 152/2009 - Atomic Absorption Spectrometry (AAS); and
- EN 15510: Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES); and
- CEN/TS 15621: Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES) after pressure digestion.

## 5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Di copper chloride tri hydroxide* 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/D/2 Forw. Appl. 1831/0044-2010
  - [2] \*Application, Proposal for Register Entry – Annex A
  - [3] \*Technical dossier, Section II – Sect\_II\_Identity.pdf: 2.1. Identity of the additives - 2.5. Conditions of use of the additive – 2.6. Method of analysis and reference samples
  - [4] \*Technical dossier, Section II – Annex 2.1.3.d.Wells
  - [5] \*Technical dossier, Section II – Annex 2.1.3.e.Fleet
  - [6] 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
  - [7] \*Technical dossier, Section II – Annex 2.6.3.f.XRD method
  - [8] \*Technical dossier, Section II – Annex 2.6.3.b (Method 300.0)
  - [9] 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*
  - [10] 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*
  - [11] Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed (cf. Annex IV-C)
  - [12] \*Supplementary Information - Food Standards Agency – Information Bulletin on Methods of Analysis and Sampling for Foodstuffs, No 102; March 2010
- \* Refers to Dossier No. FAD-2010-0 046

## 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:

- Skúšobné laboratórium – Oddelenie analýzy krmív, Ústredný kontrolný a skúšobný ústav poľnohospodársky (UKSUP), Bratislava (SLK)
- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby (DK)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)
- Sächsische Landesanstalt für Landwirtschaft, Fachbereich 8 — Landwirtschaftliches Untersuchungswesen, Leipzig (DE)
- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (POL)