EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate F – Health and Food
Food and Feed Compliance

European Union Reference Laboratory
Feed Additives

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

Subject: addendum of the EURL evaluation report

References:

FAD-2011-0007 – Tetra basic zinc chloride (JRC.DG.D.6/CvH/GB/ag/ARES(2011)\648498, Ares(2012)835116)

Upon a recent publication of multi-analyte, ring-trial validated method EN 17053 [1], dedicated for the determination of various trace elements (including *zinc*) 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 its corrigendum [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 *zinc* in animal feedingstuffs. In the current addendum, the EURL also re-iterates the X-ray diffraction (XRD) method, which has been previously recommended [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 *zinc* is detected by inductively coupled plasma-mass spectrometry (ICP-MS) at mass-to-charge (m/z) of 66 and/or 68. 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 (213.8 nm). The quantification is performed using an external standard calibration [2].

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

	EN 17053	ISO 6869
Method	ICP-MS	AAS
Mass fraction (mg/kg)	55 – 6286(*)	29 – 14600
RSD_r (%)	2.3 – 5.5	1.7 – 7.6
RSD_R (%)	5.2 – 11.3	3.3 – 15.0
LOQ (mg/kg)	5	5
Reference	[1]	[2]

RSD_r and RSD_R: relative standard deviation for *repeatability and reproducibility; LOQ: a limit of quantification;* (*) based on dry weight.

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 *zinc* in the *feed additive, premixtures* and *compound feed*; and (ii) the EN 17053 method for the determination of total *zinc* in *premixtures* and *compound feed.*

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

For the identification of zinc chloride hydroxide crystal form in the *feed additive*:

- X-ray diffraction (XRD)

For the determination of total *zinc* 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 *zinc* 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 *zinc* 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-2011-0007, Tetra-basic zinc chloride (TBZC) (JRC.DG.D.6/CvH/GB/ag/ARES(2011)\648498)

[4] Corrigendum to EURL Report – FAD-2010-0046, Tetra-basic zinc chloride (Ares(2012)835116)

[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

Addendum

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

European Union Reference Laboratory
Feed Additives

Corrigendum to EURL report

FAD-2011-0007 (Tetra Basic zinc chloride)

Ref. JRC.DG.D.6/CvH/GB/ag/ARES(2011)\648498 [1]

Geel, 09/07/2012

Section 3 Evaluation – Identification/characterisation of the feed additive states:

> For the identification of TBZC in the feed additive, the Applicant submitted an X-ray diffraction (XRD) method described by the European Pharmacopoeia

Therefore, the following sentences are to be included

- in the Executive Summary:

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

and

- in the Recommended text for register entry (analytical method):

> For the identification of Tetra Basic zinc chloride crystal form in the feed additive:
> - X-ray diffraction (XRD)

[1] http://irmm.jrc.ec.europa.eu/EURLs/EURL_feed_additives/authorisation/evaluation_reports/

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European Union Reference Laboratory for Feed Additives

JRC.DG.D.6/CvH/GB/ag/ARES(2011)648498

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-2011-0007 CRL/100346
Product Name:	Tetra-basic zinc chloride (TBZC)
Active Substance(s):	Zinc chloride hydroxide hydrate; crystal form simonkolleite
Rapporteur Laboratory:	European Union Reference Laboratory for Feed Additives (EURL-FA) Geel, Belgium
Report prepared by:	Gerhard Buttinger (EURL-FA)
Report revised by: Date:	Piotr Robouch (EURL-FA) 16/06/2011
Report approved by: Date:	Christoph von Holst 16/06/2011

EXECUTIVE SUMMARY

In the current application authorisation is sought under articles 4(1) for *Zinc chloride hydroxide hydrate* 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.

Zinc chloride hydroxide hydrate ($Zn_5(OH)_8Cl_2(H_2O)$, also called *tetra-basic zinc chloride* – TBZC) has minimum content of total zinc of 54 %. The *feed additive* is intended to be incorporated into *premixtures* and *feedingstuffs*. The Applicant suggested the following maximum levels of total zinc in the *feedingstuffs* ranging from 150 to 250 mg/kg depending on the species of interest.

For the *determination* of total zinc 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* (RSD_r) ranging from 1.7 to 8.8 %;
- a relative standard deviation for *reproducibility* (RSD_R) ranging from 5.0 to 19 %; 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 zinc in the *feed additive*, *premixtures* and *feedingstuffs*. The total zinc 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 zinc content ranged from 26.6 to 3618 mg/kg: - RSD_r ranging from 1.5 to 5.4 %; - RSD_R ranging from 2.7 to 22 %; and - LOQ = 1 mg/kg *feedingstuffs*.

Furthermore, a Community method is available for the determination of total zinc in *feedingstuffs*, but limited 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 1.0 to 9.5 %.

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 zinc content

by ICP-AES in the *feed additive* and *premixtures.* As for the determination of total zinc 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

Zinc chloride hydroxide hydrate, 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 *Zinc chloride hydroxide hydrate* 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].

Zinc chloride hydroxide hydrate ($Zn_5(OH)_8Cl_2(H_2O)$, also called *tetra-basic zinc chloride* – TBZC) is a hybrid between zinc chloride and zinc hydroxide. The product is insoluble in water. At first the Applicant specified a minimum purity of 98 % and a content of total zinc of 54 % [3]. The Applicant corrected later the value of minimum purity to be 90 % [12].

The *feed additive* is intended to be incorporated into *premixtures* and *feedingstuffs* [3]. The Applicant suggested the following maximum levels of total zinc in the *feedingstuffs* [2]: 250 mg/kg for pet animals; 200 mg/kg for milk replacer and fish, and 150 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 *Zinc chloride hydroxide hydrate*, 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

For the *identification* of *TBZC* in the *feed additive*, the Applicant submitted an X-ray diffraction (XRD) method described by the European Pharmacopoeia [10]. The sample is ground, seaved 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 [11] is deconvoluted and compared to standard chromatograms JPDS files.

Qualitative and quantitative composition of impurities in the additive

For the determination of fluoride impurity in the *feed additive*, the Applicant submitted an ion chromatography method from the U.S. Environmental Protection Agency (EPA) for the determination of inorganic anions [5].

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 [4].

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

For the determination of chloride content in the *feed additive*, the Applicant submitted an ion chromatography method from EPA for the determination of inorganic anions [5].

For the *determination* of total zinc in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted the internationally recognised ring trial validated method EN 15510 [6], based on inductively coupled plasma atomic emission spectroscopy (ICP-AES). For the determination of total zinc, 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 zinc content ranged from 27.4 to 3826 mg/kg:

- a relative standard deviation of *repeatability* (RSD_r) ranging from 1.7 to 8.8 %(*)
- a relative standard deviation for *reproducibility* (RSD_R) ranging from 5.0 to 19 %(*);
- a limit of quantification 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 zinc in the *feed additive*, *premixtures* and *feedingstuffs*. The total zinc concentration is determined using external calibration or standard addition technique. The following performance characteristics were reported [9] for a feed for pigs, and for sheep, a rock phosphate, a mineral premix and a mineral mix, where the total zinc content ranged from 26.6 to 3618 mg/kg:

- RSD_r ranging from 1.5 to 5.4 %
- RSD_R ranging from 2.7 to 22 %; and
- LOQ = 1 mg/kg *feedingstuffs*, suitable for low total zinc contents.

Furthermore, a Community method [7] is available for the determination of total zinc in *feedingstuffs*. The sample is brought into solution in hydrochloric acid after destruction of organic matter, if any. Zinc is then determined after appropriate dilution by AAS. No method performance characteristics are reported in the Regulation, except an LOQ of 20 mg/kg *feedingstuffs*. However, the UK Food Standards Agency recently reported results of a ring-trial [8] 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 1.0 to 9.5 % were reported for samples containing total zinc levels ranging from 93 to 199 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 zinc content by ICP-AES in the *feed additive* and *premixtures.* As for the determination of total zinc 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 zinc content by ICP-AES in the *feed additive* and *premixtures.* As for the determination of total zinc content in *feedingstuffs*, the EURL recommends for official control the Community method based on AAS and the above mentioned CEN method (EN 15510 or CEN/TS 15621).

Recommended text for the register entry (analytical method)

For the determination of total zinc 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 zinc 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 *Zinc chloride hydroxide hydrate* 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. 1831/0007-2011

[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] 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 – Annex 2.6.3.b (Method 300.0)

[6] EN 15510:2007 – *Animal feeding stuffs – Determination of calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganes, coblat, molybdenum, arsenic, lead and caldmium by ICP-AES*

[7] 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)

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

[9] 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*

[10] European Pharmacopeia Monograph 20933

[11] *Technical dossier, Section II – Annex 2.1.3.c (XRD CoA)

[12] *Supplementary Information 'Annex A Revised- Tables O110608'

* Refers to Dossier No. FAD-2011-0007

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)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)