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**CRL Evaluation Report on the Analytical Methods  
submitted in connection with the Application for the  
Authorisation of a new Feed Additive  
according to Regulation (EC) No 1831/2003**

Dossier related to:	FAD-2009-0044 CRL/090026
Name of Product:	KemTRACE Zn
Active Substance(s):	Zinc Propionate
Rapporteur Laboratory:	Community Reference Laboratory for Feed Additives (CRL-FA)
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Date:	16/04/2010
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Date:	20/04/2010

## EXECUTIVE SUMMARY

In the current application authorisation is sought for *zinc propionate* under the category/functional group 3(b) ‘nutritional additives’/‘compounds of trace elements’ according to the classification system of Regulation (EC) No 1831/2003 – Annex I [1]. The product with the trade name *KemTRACE Zn* consists of bio-available *zinc propionate* (active substance)  $C_6H_{10}O_4Zn$ , containing a total zinc concentration ranging from 27% to 33% [2].

In the current application submitted according to Article 4(1) of Regulation (EC) No 1831/2003, the authorisation is sought to use *zinc propionate* in *premixtures* and *feedingstuffs* for all species. The proposed conditions of use include no minimum nor maximum levels of the active substance in feedingstuffs.

For the determination of total zinc in the *feed additive*, *premixtures* and *feedingstuffs*, the Applicant proposed two international standard methods: (a) the CEN method EN 14084 for the determination of total zinc in foodstuffs by atomic absorption spectrometry (AAS) after microwave digestion and (b) the ISO method 11885 for the determination of total zinc in water by inductively coupled plasma atomic emission spectrometry (ICP-AES). The CRL recommends instead the dedicated CEN method EN 15510 for the determination of total zinc in animal feedingstuffs (and premixtures) by ICP-AES. The performance characteristics of this ring trial validated method (determined for a total zinc concentration ranging from 27 to 3600 mg/kg) are:

- a relative standard deviation for *repeatability* ( $RSD_r$ ) ranging from 1.7 to 3.3 % for *premixtures*; and from 3.5 to 5.2 % for *feedingstuffs*;
- a relative standard deviation for *reproducibility* ( $RSD_R$ ) around 7.5% for *premixtures*; and ranging from 7 to 8.4 % for *feedingstuffs*; and
- a limit of quantification (LOQ) of 3 mg Zn /kg *feedingstuffs*.

Based on these acceptable performance characteristics the CRL recommends for official control the CEN method EN 15510 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 CRL recommends for official control the above mentioned CEN method (EN 15510) and the Community method based on atomic absorption spectrometry (AAS) (Commission Regulation (EC) No 152/2009 – Annex IV-C).

No further testing or validation is required.

## KEYWORDS

zinc propionate, nutritional additive, trace elements, all species.

## 1. BACKGROUND

In the current application authorisation is sought for *zinc propionate* under the category/functional group 3(b) ‘nutritional additives’/‘compounds of trace elements’ according to the classification system of Regulation (EC) No 1831/2003 – Annex I [1]. The product with the trade name *KemTRACE Zn* consists of bio-available *zinc propionate* (active substance)  $C_6H_{10}O_4Zn$ , containing a total zinc concentration ranging from 27% to 33% [2].

In the current application submitted according to Article 4(1) of Regulation (EC) No 1831/2003, the authorisation is sought to use *zinc propionate* in *premixtures* and *feedingstuffs* for all species [3] within the EU legal limits set by the Council Directive 70/524/EEC (i.e. up to 250 mg total Zn /kg *feedingstuffs*). However, no specific conditions of use (i.e. minimum or maximum) were proposed by the Applicant [2].

## 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 Community Reference Laboratory concerning applications for authorisations of feed additives, the CRL is requested to submit a full evaluation report to the European Food Safety Authority for each application. For this particular dossier, the methods of analysis submitted in connection with *KemTRACE Zn*, 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 and dioxins) are available from the respective Community Reference Laboratories [4].

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

*Zinc propionate* is produced by spraying an excess of propionic acid on a zinc oxide powder: ( $ZnO + 2HPr \rightarrow ZnPr_2 + H_2O$ ). The Applicant determined the reaction yield/completeness based on solubility characteristics of zinc oxide and zinc propionate in water (neutral pH) and acid media (1N hydrochloric acid) [3]. Since zinc oxide is insoluble in water, only zinc from *zinc propionate* will be detected in water. In acid however, both zinc oxide and *zinc propionate* are soluble and the total zinc content in KemTrace Zn will be measured. The reaction yield is then determined as the ratio of contents of [*zinc propionate* (total zinc soluble in water)]/[total zinc soluble in acidic solution]. Each KemTrace Zn sample was extracted in di-ionised water or in 1N hydrochloric acid. All extracts obtained were then diluted in 1N HCl before analysis. Each sample was analysed in duplicates by atomic absorption analysis (AAS). The Applicant reported reaction yields ranging from 92 to 98% [3, 10]. Assuming a minimum reaction rate of 90% the Applicant concludes that the product contains a minimum of 88% of dried zinc propionate [ $M_{ZnPr_2}/(M_{ZnO} + 2 M_{HPr} + M_{ZnPr_2}) = 190.4/(8.1+14.8+190.4) = 89\%$ ], corresponding to a minimum zinc content of 30.6% in the product - close to the expected stoichiometric ratio ( $M_{Zn}/M_{ZnPr_2} = 65.4/211.6$ ).

Assuming high reaction yields with negligible ZnO residues, the Applicant proposes - for the determination of *zinc propionate* in the *feed additive* and *feedingstuffs* – various standard analytical methods (i. e. EN 14084 and ISO 11885, discussed later) including a systematic acid digestion of the sample to be analysed. These analytical methods designed to determine total zinc cannot distinguish between zinc oxide and zinc propionate but will only proof the constancy of the total zinc content of 28.5%, throughout the production reaction. The Applicant should have systematically started with a water extraction step to separate the water soluble *zinc propionate* of interest before analysis. As such experimental data were not provided by the Applicant, the CRL cannot recommend any analytical method suitable for official control to determine zinc propionate in the *feed additive per se*.

Nevertheless, the Applicant described the composition of the active substance in the feed additive (27–33% Zn) [2] and recommended maximum contents in *feedingstuffs* to comply with legal requirements (up to 250 mg Zn /kg *feedingstuffs*) [3] as concentrations of total zinc. The CRL will therefore evaluate analytical methods related to the determination of total zinc in the *feed additive, premixtures* and *feedingstuffs*.

For the determination of total zinc in the *feed additive, premixtures* and *feedingstuffs*, the Applicant proposed two international methods: (a) the CEN method EN 14084 for the determination of trace elements (including zinc) in foodstuffs by atomic absorption

spectrometry (AAS) after microwave digestion [5] and (b) the ISO method ISO 11885 for the determination of 33 elements (including zinc) in waters by inductively coupled plasma atomic emission spectrometry (ICP-AES) [6]. The performance characteristics of these methods are summarised in Table 1.

Another dedicated international standard method was developed by CEN – EN 15510 - for the determination of several trace elements (including zinc) in animal *feedingstuffs* (and *premixtures*) by ICP-AES [7]. A test portion of the sample is ashed and dissolved in 1% hydrochloric acid (in the case of organic feedingstuffs) or wet digested with 1% hydrochloric acid (in the case of mineral compounds). The dissolved sample is then filtered and diluted to the appropriate volume with water. The zinc concentration is determined by ICP-AES at 206 or 214 nm, using external calibration or standard addition techniques. The method was ring-trial validated and the relative standard deviation for *repeatability* (RSD<sub>r</sub>) and *reproducibility* (RSD<sub>R</sub>) and listed in Table 1. The limit of quantification (LOQ) is of 3 mg/kg of *feedingstuffs*.

Furthermore, a Community method [8] 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 Food Standards Agency recently reported results of a ring-trial [9] based on the above mentioned Community method, using samples such as dog biscuits, layer pellets, beef nuts, sow rolls or rabbit pellets. The precision data reported (listed in Table 1) are comparable to those reported for the CEN method EN 15510.

<b>Table 1</b>	EN 14084 [5]	ISO 11885 [6]	EN 15510 [7]		COM [8, 9]
Instrumental	AAS	ICP-AES	ICP-AES		AAS
matrix	<i>foodstuffs</i>	<i>water</i>	<i>premix</i>	<i>feed</i>	<i>feed</i>
<b>Concentration range</b> (mg/kg)	4.5 – 182	1.3	3500 – 3800	119 – 169	9.3-199
RSD <sub>r</sub> (%)	1.6 – 4.0	1.8	1.7 - 3.3	3.5 – 5.2	1.0 - 6.1
RSD <sub>R</sub> (%)	2.0 – 9.7	4.5	7.4 – 7.7	7.1 – 8.4	4.1 – 9.5

RSD<sub>r</sub> and RSD<sub>R</sub> = relative standard deviation for *repeatability* and *reproducibility*

Based on the acceptable method performance characteristics the CRL recommends for official control the CEN method EN 15510 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 CRL recommends for official control the above mentioned CEN method (EN 15510) and the Community method based on AAS.

No further testing or validation is required.

#### 4. CONCLUSIONS AND RECOMMENDATIONS

The CRL recommends for official control the CEN method EN 15510 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 CRL recommends for official control the above mentioned CEN method (EN 15510) and the Community method based on AAS.

##### ***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)

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

- EN 15510: Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES)
- Regulation (EC) No 152/2009: Atomic Absorption Spectrometry (AAS)

#### 5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *KemTRACE Zn* have been sent to the Community Reference Laboratory for Feed Additives.

The dossier has been made available to the CRL by EFSA.

#### 6. REFERENCES

- [1] \*Application, Reference SANCO/D/2 Forw. Appl. 1831/034-2009
- [2] \*Application, Proposal for Register Entry – Annex A
- [3] \*Technical dossier, Section II – 2.2. Characterisation of the active substance
- [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 13 – EN 14084
- [6] \*Technical Dossier, Section II, Annex 14 – ISO 11885
- [7] EN 15510 – Animal feedings stuffs – Determination of calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum, arsenic, lead and cadmium by ICP-AES

- [8] 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)
- [9] Food Standards Agency – Information Bulletin on Methods of Analysis and Sampling for Foodstuffs, No 102; March 2010
- [10] \*Technical Dossier, Section II, Annex 2 – Solubility zinc propionate
- \* Refers to Dossier No. FAD-2009-0044

## 7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was Community 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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