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CRL Evaluation Report on the Analytical Methods submitted in connection with Section II – 5.2 (Control Methods) of the Application for Authorisation as a Feed Additive according to Regulation (EC) No 1831/2003

Dossier related to:	EFSA-Q-2006-066 FAD-2006-0013
Name of Additive:	Availa® Cr
Active Substance(s):	Chromium-methionine chloride
Rapporteur Laboratory:	Centro di Referenza Nazionale per la Sorveglianza ed il Controllo degli Alimenti per Animali (C.Re.A.A), Torino, Italy
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EXECUTIVE SUMMARY

Availa®Cr is a feed additive for which authorisation is sought 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. *Availa®Cr* contains 3% trivalent chromium (Cr) in the complexed form chromium-methionine chloride, which is the active substance. The concentration of the active substance in the feed additive is 31 %.

In the current application authorisation is sought for the use of *Availa®Cr* for all animal species. The feed additive is intended to be added to the compound feed through premixtures. According to the proposed register entry, the dosage of the feed additive is defined in terms of the concentration of *Availa®Cr* in feedingstuffs, ranging from 13.3 to 52.8 mg/kg depending on the target animal. These limits correspond to a concentration of the active substance (chromium-methionine chloride) in feedingstuffs varying from 4.1 to 16.4 mg/kg. The *theoretical* concentration of chromium introduced via the addition of *Availa®Cr* to feedingstuffs ranges from 0.4 to 1.6 mg/kg. However, the *actual* chromium concentration in feedingstuffs is most likely very different from these values due to the contributions from other chromium sources to the overall chromium concentration.

For the determination of the complexed chromium (i.e. chromium-methionine chloride) in the *feed additive* the applicant proposed a method in which *Availa®Cr* is dissolved in water and the complexed chromium is directly measured from the solution. Prior to the measurement free Cr^{3+} is precipitated and thereby separated from complexed Cr^{3+} by using sodium carbonate as a Cr^{3+} specific precipitating agent. Complexed chromium is assayed in the supernatant filtrate using Inductively Coupled Plasma (ICP), measuring the chromium emission at 357.869 nm. The chromium content is calculated from the standard curve. This method is considered suitable for the intended purposes.

The quantitative analysis of the active substance in *premixtures* and *feed* requires an analytical technique that can distinguish between chromium introduced into the feed via the additive and chromium from other sources already present in the premixture or feedingstuffs sample. Theoretically, the same method employed for the determination of chromium-methionine chloride in the additive could be considered to detect the active substance also in

feed. However, according to the applicant's opinion the determination of the active substance in feedingstuffs is not possible, due to the high and rather varying concentration of non specific chromium in this matrix, which could be up to a factor 1000 higher than the chromium content originating from the added feed additive. Therefore the applicant did not propose a method suitable for the detection of the active substance in premixtures and feedingstuffs.

Based on the submitted documentation, the CRL is therefore unable to recommend an analytical method suitable for the determination of the active substance in *premixtures* and *feedingstuffs* for official control purposes.

For official controls regarding the determination of *total* chromium in *feedingstuffs* the CRL recommends in-house validated methods such as "Determination of chromium in feeds by automated microwave digestion and atomic absorption spectrometry" [Gallo et all., J. AOAC Int. 1997 80 (5): 956-960] or draft methods from the European Committee for Standardisation (CEN).

According to the applicant, chromium residues in animal tissues that are related to feeding the animal with *Availa®Cr* are not anymore linked to methionine. In consequence, chromium measurements in animal tissues do not allow for distinguishing between Cr derived from *Availa®Cr* and Cr naturally present in animal tissues. In addition, no maximum residue limit (MRL) has been proposed by the applicant. For these reasons, the applicant did not submit a method suitable for the analysis of animal tissues.

For quantitative analysis to determine *total* Cr in *animal tissues* the standard method EN 14083 could be applied. This method was developed to detect total Chromium in food by graphite furnace absorption spectrometry (GFAAS) after pressure digestion. According to this European standard the achievable limit of quantification for chromium in foodstuffs is in the range of about 0.04 to 0.16 mg/kg, mainly depending on the specific experimental conditions of the measurements.

KEYWORDS

Availa®Cr, chromium-methionine, nutritional additive, trace element, metabolism regulator, all species.

BACKGROUND

Availa®Cr is a feed additive for which authorisation is sought under the category "nutritional additives", functional group 3(b) "compounds of trace elements", according to Annex I of Regulation (EC) No 1831/2003 [1]. Availa®Cr contains 3% trivalent chromium (Cr) in the complexed form chromium-methionine chloride, which is the active substance. The concentration of the active substance in the feed additive is 31 %. The intended use (*cf.* EFSA-Q-2006-066) of the current application is for all animal species of all ages. The feed additive is intended to be added to all compound feed through premixtures. According to the proposed register entry the dosage of the feed additive is defined in terms of the concentration of the *Availa®Cr* in feedingstuffs, ranging from 13.3 to 52.8 mg/kg depending on the target animal. These limits correspond to a concentration of the active substance (chromium-methionine chloride) in feedingstuffs varying from 4.1 to 16.4 mg/kg. The *theoretical* concentration of chromium introduced via the addition of *Availa®Cr* ranges from 0.4 to 1.6 mg/kg.

TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005 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 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 Availa®Cr dossier (EFSA-Q-2006-066) and their suitability to be used for official controls in the frame of the authorisation were evaluated.

EVALUATION

The numbering system under this point refers to that of Section II of the Annex of Commission Directive 2001/79/EC (2.5 Control methods).

Description of the methods used for the determination of the criteria listed under item 2.5.1 of Commission Directive 2001/79/EC

Determination of purity

Availa®Cr is a dry dark purple product containing maltodextrin (mass content 44 %), chromium-methionine chloride (mass content 31 %) and methionine hydrochloride (mass content 21 %) [2]. The moisture content is 4 % [2]. The purity of the feed additive is determined by the following official methods. Total methionine is determined by reaction with ninhydrin with photometric detection at 570 nm [3]. The chloride content is determinate by Volhard's method [4]. The maltodextrin determination is achieved separating glucose from maltose [5]. Moisture content is determined by gravimetric method [6]. These methods are considered suitable for the intended purpose.

Identification test (qualitative analysis)

The Ultraviolet (UV) spectrum of a solution of chromium-methionine shows two peaks at 399 and 536 nm [7]. The Fourier Transform Infrared (FTIR) spectrum of chromium-methionine chloride shows specific peaks and is different from the corresponding spectrum of methionine hydrochloride [8]. The identity of chromium-methionine can be confirmed by High Performance Liquid Chromatography (HPLC) under stated conditions with Ultraviolet/Visible (UV/VIS) detection at 545 nm [9]. For quantification of the active substance, however, the applicant recommends another method based on the determination of chromium as described below.

These methods are considered suitable for the intended purposes.

Quantitative analysis of active substance (chromium- methionine chloride) in the additive

Determination of complexed chromium (i.e. chromium-methionine chloride) is achieved by determining the complexed chromium directly in solution after first precipitating any free chromium [10]. Separation of free Cr^{3+} from complexed Cr^{3+} is achieved by dissolving Availa®Cr in deionised water and precipitating the free Cr^{3+} from the aqueous solution using a Cr^{3+} specific precipitating agent, which is sodium carbonate. After centrifugation and

filtration of the supernatant, complexed chromium is assayed in the supernatant filtrate using Inductively Coupled Plasma (ICP).
The chromium emission was measured at 357.869 nm and the chromium content was calculated from the standard curve. This method is considered suitable for the intended purposes.

Description of the qualitative and quantitative analytical methods for routine control of the active substance in premixtures and feedingstuffs (2.5.2. of the Guidelines)

For the determination of *total* chromium in premixtures and feed, many methods are available that use techniques such as atomic absorption spectrometry with flame or graphite tube, plasma emission spectrometry. However, quantitative analysis of the active substance (Chromium-methionine chloride) in premixtures and feed requires an analytical technique that can distinguish between trivalent chromium deriving from Availa®Cr and background levels of naturally occurring and contaminating chromium species [11,12]. According to the applicant, the determination and speciation of low levels of chromium presents serious difficulties at all stages of sampling, sample digestion and preparation, analysis and interpretation of the results [13,14]. In consequence, the applicant did not propose any detection method.

Description of the qualitative and quantitative analytical methods for determining the marker residue(s) of the active substance in target tissues and animal products

In animal tissues the Cr is not bound to methionine [15]. Because of the low levels of application of Cr, no deviation from normal levels can be measured in target tissues. Therefore no methods for the qualitative and quantitative determination of Cr in tissues are proposed by the applicant.

CONCLUSIONS AND RECOMMENDATIONS

For the determination of Cr-methionine chloride in the *feed additive* the CRL recommends the assay submitted by the applicant.

For the determination of chromium-methionine chloride in *premixtures* and *feedingstuffs* the same methods used to determine the active substance in the feed additive (i.e. separation of complexed chromium by precipitation of free chromium) could be considered. However the applicant did not provide any experimental data to support such an approach. The CRL is therefore unable to recommend an analytical method suitable for the determination of the active substance in premixtures and feedingstuffs for official control purposes.

For official controls regarding the determination of *total* chromium in *feedingstuffs* the CRL recommends in house-validated methods such as "Determination of chromium in feeds by automated microwave digestion and atomic absorption spectrometry" [Gallo et all., J. AOAC Int. 1997 80 (5): 956-960] or draft methods from the European Committee for Standardisation (CEN) [18].

About the speciation of chromium (III, VI) the literature reports on-line methods [17]. In these methods identification and quantification of Cr forms are carried out in a one-step analytical process, in which the separation system (e.g. ion chromatography) is coupled with the detection system such as ICP. Nevertheless, none of these methods are applied to premixtures or feed. Considering the complexity of feed matrices and the target concentration levels of the active substance in feed, the suitability of these methods for official control is questionable.

For the determination of *total* Cr in *animal tissues* the CRL recommends to use the EN 14083 method, which is already applied in some Member States in order to quantify the occurrence of this element in animal tissues.

DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, samples of Availa®Cr have been sent to the Community Reference Laboratory for feed additives authorisation the 11 April 2006.

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

REFERENCES

[1] Annex III – Proposal of Register entry

[2] Technical dossier, Section II-2

[3] Technical dossier, Section II, Annex VI 2.5
[4] Technical dossier, Section II, Annex VI 2.6
[5] Technical dossier, Section II, Annex VI 2.7
[6] Technical dossier, Section II, Annex VI 2.8
[7] Technical dossier, Section II-3, Figure II - 3
[8] Technical dossier, Section II -3, Figure II - 4
[9] Technical dossier, Section II, 3 (b)
[10] Additional information from the application sent to the CRL-FA by e-mail on 23/01/2007
[11] Technical dossier, Section II, Annex VI 2.35
[12] Technical dossier, Section II, Annex VI 2.36
[13] Technical dossier, Section II, Annex VI 2.37
[14] Technical dossier, Section II, Annex VI 2.38
[15] Technical dossier, Section II-4.
[16] J. AOAC Int. 1997 80 (5): 956-960
[17] Technical dossier, Section II, Annex VI 2.40
[18] PR TS 15621 ICP-OES after pressure digestion; PR EN 15550 ICP-OES after classical digestion

RAPPORTEUR LABORATORY

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