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

Dossier related to: **FAD-2011-0028 - CRL/110005**

Name of Feed Additive: **L-Selenomethionine**

Active Agent (s): **Selenomethionine**

Rapporteur Laboratory: **European Union Reference Laboratory for Feed Additives (EURL-FA)
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Date: **23/05/2012**

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for *L-Selenomethionine*, under the category/functional group 3(b) 'nutritional additives'/ 'compounds of trace elements', according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use for all animal species and categories.

The *feed additive* is produced by a chemical synthesis with a minimum purity of 97 % *selenomethionine*, and contains a minimum of 35 % *total selenium*. It is intended to be incorporated into *water* or compound *feedingstuffs* through *premixtures* to obtain a maximum *total selenium* dosage of 0.5 mg/kg *complete feedingstuffs*, thus complying with legal requirements. The Applicant proposed no minimum dose, but suggested a maximum *total selenium* dosage of 0.2 mg/L *water*.

For the determination of *selenomethionine* in the *feed additive* the Applicant submitted the chromatography method described in the US Pharmacopoeia method. The EURL recommends instead for official control the previously evaluated single laboratory validated and further verified method (FAD-2010-0044), based on triple proteolytic digestion followed by HPLC-ICPMS method.

For the determination of *total selenium* in the *feed additive* the Applicant submitted the US Pharmacopoeia method based on spectrophotometry at 380 nm. The EURL recommends instead for official control two alternative validated and further verified methods, based either on (i) inductively coupled plasma atomic absorption spectrometry (ICP-AES) (cf. FAD-2009-0010); or (ii) inductively coupled plasma mass spectrometry (ICP-MS) (cf. FAD-2010-0044).

For the determination of *total selenium* in *premixtures* and *feedingstuffs* the Applicant listed several analytical methods, including Flame Atomic Absorption Spectrometry (FAAS) and ICP-AES. The EURL recommends instead for official control the ring trial validated CEN standard method (EN 16159:2012) based on Hydride Generation Atomic Absorption Spectrometry (HGAAS).

For the determination of *total selenium* in *water* the Applicant submitted the method approved by the National Institute for Occupational Safety and Health (NIOSH) and described in their Manual of Analytical Methods (NMAM), based on ICP-AES. The EURL recommends this method for official control to determine *total selenium* 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

Selenomethionine, *total selenium*, nutritional additives, all species, trace elements

1. BACKGROUND

In the current application authorisation is sought under article 4(1) for *L-Selenomethionine*, under the category/functional group 3(b) ‘nutritional additives’/‘compounds of trace elements’ according to the classification system of Regulation (EC) No 1831/2003 [1]. Specifically, authorisation is sought for the use of *selenomethionine* for all animal species and categories [1].

The *feed additive* resulting from the chemical reaction between L-methionine, Selenium and dimethyl sulfate contains at least 97 % *selenomethionine*, which corresponds to a minimum of 35 % *total selenium* [2].

The product is intended to be incorporated into *premixtures*, compound *feedingstuffs* or *water* to obtain a maximum *total selenium* dosage of 0.2 mg/L *water* [2] or 0.5 mg/kg *feedingstuffs* [3] thus complying with legal requirements; no minimum dose was proposed by the Applicant.

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 (EFSA) for each application or group of applications. The methods of analysis submitted in connection with *selenomethionine* and their suitability to be used for official controls in the frame of the authorisation were evaluated for the two dossiers of concern.

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, salmonella, mycotoxins 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, feedingstuffs and water

Selenomethionine

For the determination of *selenomethionine* in the *feed additive* the Applicant submitted the chromatography method described in the US Pharmacopoeia method [5]. The EURL recommends instead for official control the previously evaluated single laboratory validated and further verified method, based on triple proteolytic digestion followed by HPLC-ICPMS method, for which the following performance characteristics were reported (FAD-2010-0044 [6]):

- a *recovery rate* (R_{rec}) ranging from 94 to 103 %;
- a relative standard deviation for *repeatability* (RSD_r) ranging from 1 to 4 %; and
- a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 5 to 8 %.

Total selenium

For the determination of *total selenium* in the *feed additive* the Applicant submitted the US Pharmacopoeia method [7] based on spectrophotometry at 380 nm. The EURL recommends instead for official control two previously evaluated validated and further verified methods, based either on:

- (i) inductively coupled plasma atomic absorption spectrometry (ICP-AES) for which the following performance characteristics were reported (cf. FAD-2009-0010 [8]): - R_{rec} ranging from 99 to 105 %; - RSD_r ranging from 1.1 to 2.7 %; and - RSD_{ip} ranging from 1.5 to 2.5 %; or
- (ii) microwave digestion using nitric acid and hydrogen peroxide ($\text{HNO}_3/\text{H}_2\text{O}_2$) followed by inductively coupled plasma mass spectrometry (ICP-MS), for which the following performance characteristics were reported [6]: - R_{rec} ranging from 94 to 95 %; and - RSD_{ip} ranging from 1.5 to 2.5 %.

For the determination of *total selenium* in *premixtures* and *feedingstuffs* the Applicant listed some analytical methods, including Flame Atomic Absorption Spectrometry (FAAS) and

ICP-AES. The EURL recommends instead for official control the former ring trial validated method developed by the “Association of German Agricultural Analytical and Research Institutes” (VDLUFA, Germany) [9], recently adopted as CEN standard EN 16159:2012 [10]. The method for the determination of *total selenium* is based on Hydride Generation Atomic Absorption Spectrometry (HGAAS) after microwave digestion with HNO₃/H₂O₂. The following performance characteristics are reported for feed samples:

- RSD_r ranging from 3.4 to 10 %;
- a relative standard deviation for *reproducibility* (RSD_R) ranging from 15 to 23 %; and
- a limit of quantification of 0.125 mg/kg, clearly below the maximum legal limit of 0.5 mg Se /kg feed.

For the determination of *total selenium* in *premixtures*, the EURL suggests diluting the *premixtures* samples with ground cereal feed and applying the abovementioned HGAAS method.

For the determination of *total selenium* in *water* the Applicant submitted the method approved by the National Institute for Occupational Safety and Health (NIOSH) and described in their Manual of Analytical Methods (NMAM) [11], based on ICP-AES, for which a limit of detection of 0.02 mg/L is reported. The EURL recommends for official control this method to determine *total selenium* 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.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control

- the single laboratory validated and further verified method, using high performance liquid chromatography and inductively coupled plasma mass spectrometry (HPLC-ICPMS) after triple proteolytic digestion to determine *selenomethionine* in the *feed additive*;
- two alternative laboratory validated and further verified methods, based on (i) inductively coupled plasma atomic absorption spectrometry (ICP-AES) or (ii) inductively coupled plasma mass spectrometry (ICPMS) to determine *total selenium* in the *feed additive*;

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- the CEN ring trial validated method (EN 16159:2012), using hydride generation atomic absorption spectrometry (HGAAS) to determine *total selenium* in *premixtures* and *feedingstuffs*; and
 - the NIOSH method based on ICP-AES for the determination of *total selenium* in *water*.

Recommended text for the register entry (analytical method)

For the determination of *selenomethionine* in the *feed additive*:

- high performance liquid chromatography and inductively coupled plasma mass spectrometry (HPLC-ICPMS) after triple proteolytic digestion

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

- inductively coupled plasma mass spectrometry (ICPMS), or
- inductively coupled plasma atomic absorption spectrometry (ICP-AES)

For the determination of *total selenium* in *premixtures* and *feedingstuffs*:

- hydride generation atomic absorption spectrometry (HGAAS) after microwave digestion (EN 16159:2012)

For the determination of *total selenium* in *water*:

- inductively coupled plasma atomic absorption spectrometry (ICP-AES)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Selenomethionine* 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/Ref:SANCO/D/2:Forw.Appl.1831/0082-2011
- [2] *Technical dossier, Section II – Identity
- [3] *Application, Annex A
- [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_II_35
- [6] #FAD-2010-0044 - JRC.DG.D.6/CvH/PRO/AG/ARES(2011)255176
- [7] * Technical dossier, Section II – Annex_II_32
- [8] #FAD-2009-0010 - JRC.DDG.D.6/CvH/PRO/MDS/ARES(2010)175099
- [9] VDLUFA Methodenbuch III (2003), 11.6.1 - Selen
- [10] EN 16159:2012 - "Animal feeding stuffs: - Determination of selenium by hydride generation atomic absorption spectrometry (HGAAS) after microwave digestion (digestion with 65% nitric acid and 30% hydrogen peroxide)"
- [11] * Technical dossier, Section II – Annex_II_34

*Refers to Dossier no: FAD-2011-0028

#http://irmm.jrc.ec.europa.eu/EURLs/EURL_feed_additives/authorisation/evaluation_reports/

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)
- RIKILT-Instituut voor Voedselveiligheid, Wageningen (NL)
- Skúšobné laboratórium – Oddelenie analýzy krmív, Ústredný kontrolný a skúšobný ústav poľnohospodársky, Bratislava (SK)
- Fødevarestyrelsen, Ringsted (DK)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen, Jena (DE)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)