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SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of L-selenomethionine as feed additive for all animal species[1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Selenium is a trace element that is essential for vertebrates and involved in series of vital metabolic functions. Considering the purity of the L-selenomethionine (L-SeMet) under application and the metabolic pathways of SeMet, the FEEDAP Panel considers the use of L-SeMet as safe for all animal species, provided that the maximum total selenium level authorised in feed is respected. The use of in animal nutrition is expected to result in a similar increase in selenium deposition in animal tissues/products as that resulting from other sources of SeMet. To ensure consumer safety from consumption of food originating from animals fed L-SeMet, the FEEDAP Panel concludes that dietary selenium supplementation from the additive should not exceed a maximum of 0.2 mg Se/kg complete feed. In the absence of specific data, the additive should be considered as an irritant to skin and eyes, as a skin sensitiser and as potentially harmful by inhalation. The FEEDAP Panel considers that the use of L-SeMet in feed does not pose an additional risk to the environment, compared with other sources of selenium for which it will substitute, as long as the maximum authorised content in feedingstuffs is not exceeded. L-SeMet is an efficient source of selenium for all species. This conclusion is derived from studies with laying hens and pigs for fattening and, in the case of ruminants, from literature describing the microbial incorporation of selenium from organic sources in the rumen. The FEEDAP Panel made some recommendations concerning (i) the specification, (ii) the use of the compound in premixtures, (iii) the use in water for drinking and (iv) risk reduction when handling the additive.

KEY WORDS

Nutritional additive, compounds of trace elements, selenium, L-selenomethionine, safety, efficacy

[1] On request from European Commission, Question No EFSA-Q-2011-01109, adopted by written procedure on 2 May 2013.

[2] Panel members: Gabriele Aquilina, Alex Bach, Vasileios Bampidis, Maria De Lourdes Bastos, Lucio Guido Costa, Gerhard Flachowsky, Mikolaj Antoni Gralak, Christer Hogstrand, Lubomir Leng, Secundino López-Puente, Giovanna Martelli, Baltasar Mayo, Fernando Ramos, Derek Renshaw, Guido Rychen, Maria Saarela, Kristen Sejrsen, Patrick Van Beelen, Robert John Wallace and Johannes Westendorf. Correspondence: FEEDAP@efsa.europa.eu

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SUMMARY

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of L-selenomethionine (L-SeMet) as feed additive for all animal species.

Selenium is a trace element that is essential for vertebrates and is involved in series of vital metabolic functions (e.g. prevention of oxidative stress, proper thyroid function, maintenance of cellular redox status, immunocompetence, detoxification of heavy metals and xenobiotics).

Considering the purity of the L-SeMet under application and the metabolic pathways of SeMet, the FEEDAP Panel considers the use of L-SeMet as safe for all animal species, provided that the maximum total selenium level authorised in feed is respected.

The use of L-SeMet in animal nutrition is expected to result in a similar increase in selenium deposition in animal tissues/products as that resulting from other sources of SeMet. To ensure consumer safety from consumption of food originating from animals fed L-SeMet, the FEEDAP Panel concludes that dietary selenium supplementation from the additive should not exceed a maximum of 0.2 mg Se/kg complete feed.

In the absence of specific data, the additive should be considered as an irritant to skin and eyes, as a skin sensitiser and as potentially harmful by inhalation.

The FEEDAP Panel considers that the use of L-SeMet in feed does not pose an additional risk to the environment, compared with other sources of selenium for which it will substitute, as long as the maximum authorised content in feedingstuffs is not exceeded.

L-SeMet is an efficient source of selenium for all species. This conclusion is derived from studies with laying hens and pigs for fattening and, in the case of ruminants, from literature describing the microbial incorporation of selenium from organic sources in the rumen.

The FEEDAP Panel made some recommendations concerning (i) the specification of the additive, (ii) the use of the compound in premixtures, (iii) the use in water for drinking and (iv) risk reduction when handling the additive.

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BACKGROUND

Regulation (EC) No 1831/2003[4] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from the company Excentials IP BV,[5] for authorisation of the product L-selenomethionine when used as a feed additive for all species (category: nutritional additives; functional group: compounds of trace elements) under the conditions mentioned in Table 1.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application.[6] According to Article 8 of that Regulation, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. The particulars and documents in support of the application were considered valid by EFSA as of 30 January 2012.

Two forms of inorganic selenium, sodium selenite and sodium selenate, are authorised in the European Union (EU) as sources of the essential trace element selenium, under Directive 70/524/EEC.[7] Organic forms of selenium produced by *Saccharomyces cerevisiae* strains CNCM I-3060, NCYC R397 and CNCM I-3399 are authorised in the EU as trace element under Regulation (EC) No 1831/2003.[8,9,10] These latter authorisations have been granted following corresponding EFSA opinions (EFSA 2006a, 2006b, 2009a). Additional opinions on the safety and efficacy of selenium sources (two selenium-enriched yeasts based on *Saccharomyces cerevisiae* strains NCYC R645 (EFSA, 2011a) and NCYC R646 (EFSA, 2012), and one on synthetic hydroxy-analogue of selenomethionine (EFSA, 2013)) for all animal species have been delivered by the FEEDAP Panel. Another opinion on the safety and efficacy of Sel-Plex® (selenised yeast produced by *Saccharomyces cerevisiae* CNCM I-3060) when used as zootechnical feed additive was adopted by the FEEDAP Panel (EFSA, 2011b).

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and the efficacy of the product L-selenomethionine, when used under the conditions described in Table 1.

[4] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

[5] On 19 October 2012, EFSA was informed by the applicant that the applicant company had changed to Excentials B.V., Vierlinghstraat 51, 4251 LC Werkendam, The Netherlands.

[6] EFSA Dossier reference: FAD-2011-0028.

[7] List of the authorised additives in feedingstuffs published in application of Article 9t (b) of Council Directive 70/524/EEC concerning additives in feedingstuffs. OJ C 50, 25.2.2004, p. 1.

[8] Commission Regulation (EC) No 1750/2006 of 27 November 2006 concerning the authorisation of selenomethionine as a feed additive. OJ L 330, 28.11.2006, p. 9.

[9] Commission Regulation (EC) No 634/2007 of 7 June 2007 concerning the authorisation of selenomethionine produced by *Saccharomyces cerevisiae* NCYC R397 as a feed additive. OJ L 146, 08.06.2007, p. 14.

[10] Commission Regulation (EC) No 900/2009 of 25 September 2009 concerning the authorisation of selenomethionine produced by *Saccharomyces cerevisiae* CNCM I-3399 as a feed additive. OJ L 256, 29.09.2009, p. 12.

Table 1: Description and conditions of use of the additive as proposed by the applicant

Additive	L-selenomethionine
Registration number/EC No/No (if appropriate)	--
Category(-ies) of additive	Nutritional additives
Functional group(s) of additive	Compounds of trace elements

Description			
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)
L-selenomethionine	**$C_5H_{11}NO_2Se$**	**Min. 35.0% of the element Se**	**current USP method**
Loss on drying	**NA**	**Max. 3.0%**	**NA**

Trade name (if appropriate)	--
Name of the holder of authorisation (if appropriate)	--

Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg/kg of complete feedingstuffs		
All animal species and categories	--	--	0.50 mg of selenium (total) per kg of complete feedingstuffs	--

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use (if appropriate)	Only for manufacture of animal feed. Applicable in premix, feed & water. The additive may be incorporated in feed as such or in the form of a preparation or premixture. Direct incorporation of the additive necessitates specific equipment in the production site, in order to ensure proper and homogeneous mixing.
Specific conditions or restrictions for handling (if appropriate)	--
Post-market monitoring (if appropriate)	No specific requirements other than the traceability and complaint system implemented in compliance with the requirements of Regulation No 183/2005.
Specific conditions for use in complementary feedingstuffs (if appropriate)	--

Maximum Residue Limit (MRL) (if appropriate)			
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues
--	--	--	--

ASSESSMENT

1. Introduction

The application under assessment is for the use of L-selenomethionine (L-SeMet) in feed and water for drinking for all animal species as source of the essential trace element selenium. The additive consists of highly purified (> 99 %) synthetic L-SeMet.

The biological role of selenium, its deficiency and toxicity symptoms in farm animals were described in a previous opinion of the FEEDAP Panel (EFSA, 2006a). Selenium is a trace element that is essential for vertebrates and is involved in series of vital metabolic functions (e.g. prevention of oxidative stress, proper thyroid function, maintenance of cellular redox status, immunocompetence, detoxification of heavy metals and xenobiotics). To the knowledge of the FEEDAP Panel, there is no additional relevant information that may lead to the reconsideration of its previous opinion.

Since the selenium content of grain and forages is generally low in most European countries, livestock are routinely supplied with extra dietary selenium in order to avoid the consequences of selenium deficiency.

Synthetic L-SeMet is authorised by Commission Regulation (EC) No 1170/2009[11] as mineral substance which may be used in the manufacture of food supplements. The opinion on its use in food supplements was adopted by the EFSA's Panel on Food Additives and Nutrient Sources added to Food (EFSA, 2009b).

2. Characterisation

2.1. Characterisation of the L-selenomethionine

L-SeMet (IUPAC name (S2)-2-amino-4-methylselenylbutanoic acid) is identified with the CAS number 3211-76-5. It has a molecular weight of 196.11 daltons and its molecular formula is $C_5H_{11}NO_2Se$. The theoretical content of selenium is 40.26 %. The substance under application contains by specification at least 97 % L-SeMet, at least 39 % selenium, and a maximum of 20 mg heavy metals (expressed as Pb)/kg; maximum loss due to drying is 0.5 %.[12]

The analysis of five batches of the compound showed a mean L-SeMet content of 99.4±0.3 % (range: 99.1–99.9 %; analysis by high-performance liquid chromatography (HPLC)),[13] a mean selenium content of 39.5±0.6 % (range: 39.0–40.6 %; analysis by atomic absorption spectrometry (AAS)).[14] The mean loss on drying is 0.41±0.06 % (range: 0.32–0.48 %) and the mean sulphated ash content is 1.08±0.7 % (range: 0.47–1.95 %).[15]

The identity of $C_5H_{11}NO_2Se$ was confirmed by nuclear magnetic resonance data and mass spectrometry. The specific optical rotation of five product batches ranged from +17.7 to +18.4°.[16] The molecular structure is shown in Figure 1.

[11] Commission Regulation (EC) No 1170/2009 of 30 November 2009 amending Directive 2002/46/EC of the European Parliament and of Council and Regulation (EC) No 1925/2006 of the European Parliament and of the Council as regards the lists of vitamin and minerals and their forms that can be added to foods, including food supplements. OJ L 314, 1.12.2009, p. 36.
[12] Technical Dossier/Section II/Annex_II_3.
[13] Supplementary Information September 2012.
[14] Supplementary Information September 2012.
[15] Technical Dossier/Section II/Annex_II_2.
[16] Technical Dossier/Section II/Annex_II_2.

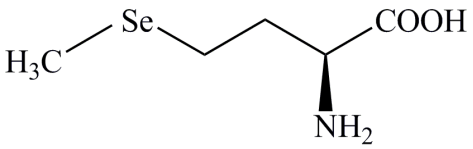

Figure 1: Molecular structure of L-selenomethionine

For feed supplementation, the substance L-SeMet may be blended with limestone and silica, to reach concentrations between 1000 and 2000 mg Se/kg formulated additive.

2.1.1. Impurities

Lead, cadmium, mercury, arsenic, chromium (analysed by inductively coupled plasma optical emission spectrometry) and fluorine (analysed by ion selective electrode) contents, measured in three batches, were below the detection limit (1 mg/kg).[17]

As an impurity resulting from the synthesis process L-methionine is specified with a maximum content of 0.5 %. This specification is confirmed by analysis of five batches.[18] Residual solvents monitored by gas chromatography showed concentrations of methanol ≤ 150 mg/kg and acetone ≤ 21 mg/kg, both being in compliance with the relevant Veterinary International Cooperation on Harmonization (VICH) guideline (VICH, 2011); *n*-butanol, isopropyl alcohol and dimethyl formamide were below detection limit (not given).[19]

Three batches of the formulated additive—based on silica and limestone (1 500 mg Se/kg)—were analysed for heavy metals, arsenic and fluorine,[20] dioxins and dioxin-like polychlorinated biphenyls (PCBs).[21] All values were well below the thresholds set by the EU Directive on undesirable substances.[22]

2.1.2. Physical state of the product

L-SeMet is a white to off-white crystalline powder with a bulk density of 0.63 g/cm^3 and a tapped density of 0.85 g/cm^3. Its solubility in water (temperature not specified) is 36.2 g/L (average of three batches).[23]

Analysis of particle size distribution by laser diffraction in one batch of L-SeMet showed that 1.2 % of the particles (v/v) were < 10 µm, 30.4 % < 50 µm and 55.6 % < 100 µm in diameter; [24] analysis of further three batches showed that 2.5 % of the particles (v/v) were < 10 µm, 25.8 % < 50 µm and 46.1 % < 100 µm.[25] The dusting potential, measured by the Stauber-Heubach method, was 9.7 mg/50 g sample in one batch;[26] in further three batches it ranged from 0.5 to 4.1 g/m^3,[27] corresponding to a calculated content of about 0.2–1.6 g Se/m^3.

[17] Supplementary Information September 2012/Annex A.
[18] Technical Dossier/Section II/Annex_II_2.
[19] Supplementary Information September 2012.
[20] Supplementary Information September 2012/Annex B.
[21] Supplementary Information September 2012/Annex C.
[22] Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. OJ L 140, 30.5.2002, p. 10.
[23] Supplementary Information September 2012.
[24] Technical Dossier/Section II/Annex_II_4.
[25] Supplementary Information September 2012/Annex E-bis.
[26] Technical Dossier/Section II/Annex_II_30.
[27] Supplementary Information September 2012/Annex F-bis.

A formulated additive (1500 mg Se/kg) showed a bulk density of 0.93 g/cm^3 and tapped density of 1.102 g/cm^3 (average of three batches); it is practically insoluble in water.[28] Analysis of particle size distribution by laser diffraction in one batch showed that 34 % of the particles (v/v) were < 10 µm, 82 % < 50 µm and 89 % < 100 µm in diameter;[29] analysis of further three batches showed that 36.7 % of the particles (v/v) were < 10 µm, 82.3 % < 50 µm and 90.6 % < 100 µm.[30] Dusting potential, measured by the Stauber-Heubach method, ranged from 6.5 to 7.5 g/m^3 in three batches.[31] The selenium content of the dust ranged from 3.0 to 7.3 mg/m^3.[32]

2.2. Manufacturing process

The production process of the compound and of the additive are fully described in the technical dossier.

Material safety data sheets (MSDS) of all materials used in the manufacture and purification process are provided.[33] The MSDS for the final product is also provided.[34]

2.3. Stability and homogeneity

2.3.1. Shelf life

The shelf life of the compound L-SeMet stored in high density polyethylene (HDPE) containers was studied by HPLC analysis.[35] Batches stored at 25 °C for nine months (three batches) or one year (two batches)[36,37] or at 40 °C (five other batches) for three months were examined.[38] No losses were observed.

2.3.2. Stability in premixtures and feedingstuffs

L-SeMet from the formulated additive (1288 mg Se/kg) was added to a vitamin-trace elements premixture for laying hens. The premixture was stored for nine months at room temperature. SeMet recovery after three, six and nine months was 55, 54 and 37 %, respectively.[39] In a further stability study with a vitamin premixture without compounds of trace elements, SeMet was fully recovered after three months (storage conditions not specified).[40] The data do not support the stability of SeMet in premixtures containing compounds of trace elements.

The stability of L-SeMet when added to a complete feed for laying hens (from the formulated additive of 1288 mg Se/kg; final supplementation in feed 50 mg Se/kg) and stored for six months at room temperature, in closed bags, was studied.[41] The recovery of SeMet after three and six months was 90 and 74 %, respectively. The 6-month sample was subsequently analysed in another laboratory resulting in 96 % recovery of the initial value analysed in the first laboratory. The study cannot be

[28] Supplementary Information September 2012/Annex D.
[29] Technical Dossier/Section II/Annex_II_5.
[30] Supplementary Information September 2012/Annex E.
[31] Technical Dossier/Section II/Annex_II_30 and Supplementary Information September 2012/Annex F.
[32] Supplementary Information September 2012.
[33] Technical Dossier/Section II/Annex_II_8 to II_22.
[34] Technical Dossier/Section II/Annex_II_24 and II_25.
[35] A method based in HPLC technique, specific for the analysis of SeMet, was used by the applicant.
[36] Technical Dossier/Section II/Annex_II_26.
[37] Supplementary Information September 2012/Annex J.
[38] Technical Dossier/Section II/Annex_II_27.
[39] Supplementary Information February 2013/Annex E.
[40] Supplementary Information February 2013/Annex F.
[41] Supplementary Information September 2012/Annex H and Supplementary Information February 2013/Annex B.

considered as relevant for complete feed since the selenium content exceeds the EU maximum selenium concentration in feed by a factor of 100.

Stability during feed processing was examined by adding L-SeMet to a complementary feed (190 mg Se/kg, which would allow a daily intake of that feed of about 40–50 g only) for dairy cows and subsequent pelleting (surface temperature measured after sample collection: 46–50 °C).[42] SeMet was fully recovered in pellets. The study appears insufficient for a full conclusion on the resistance of the substance to heat which is released during pelleting under practical conditions.

The stability of L-SeMet in water (0.5 mg/L; one batch) when stored for 49 hours at room temperature in darkness was studied.[43] No losses of SeMe were noted.

2.3.3. Homogeneity

The ability of L-SeMet (one batch) to distribute homogeneously was tested in compound feed for laying hens supplemented with 0.5 mg Se/kg via a formulated additive (1300 mg Se/kg).[44] The selenium concentrations in 10 feed subsamples showed a coefficient of variation of 7.6 %.

Since the solubility in water of L-SeMet is two orders of magnitude higher than the amount to be supplemented to water for drinking, confirmation of the additive's ability to distribute homogeneously in water for drinking is not required.

2.4. Conditions of use

L-SeMet is proposed to be used as source of essential trace element selenium for all animal species/categories up to maximum total authorised level 0.5 mg Se/kg complete feed. According to the applicant, L-SeMet can be added directly to feedingstuffs or via a premixture. However, the applicant recommends the use of a formulated additive with selenium contents between 1000 and 2000 mg/kg for supplementation of premixtures and complete/complementary feeds.

The additive L-SeMet is proposed also for the use in water for drinking up to a maximum level 0.2 mg Se/L. Whenever the additive is used in water for drinking, the selenium content in solid feed should be taken into account in order to avoid and prevent the dose achieved exceeding the maximum authorised total selenium level in complete feed alone.

2.5. Physico-chemical incompatibilities in feed

The applicant reports no incompatibilities with feed components, carriers or other approved additives known for the product. However, the findings on stability of L-SeMet in vitamin-mineral containing premixtures are not in accordance with that statement.

2.6. Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)

EFSA has verified the EURL report as it relates to the methods used for the control of L-SeMet in animal feed. The Executive Summary of the EURL report can be found in the Appendix.

[42] Supplementary Information February 2013/Annex G.
[43] Supplementary Information September 2012/Annex I.
[44] Technical Dossier/Section II.

3. Safety

3.1. Safety for the target species

L-SeMet is a chemically well defined compound of high purity. The components of the additive, which could theoretically affect its safety for target animals are selenium and L-Met. Safe levels of selenium for all animal species are determined by European Feed Law with a maximum of 0.5 mg total Se/kg feed. The FEEDAP Panel does not see a need to revise this maximum content. L-Met can be used for amino acid supplementation of compound feed. It is known that the L-Met is somewhat more toxic than the DL-Met because of the metabolic fate of the D-enantiomer of Met (Baker, 2006); however, the concentrations of Met in feed at which Met toxicity would play a role are at least four orders of magnitude higher than the amount of L-SeMet supplemented. Therefore, when L-SeMet is used as a source of the trace element selenium, any potential intolerance to Met in target animals is of no concern. The interaction between selenium and L-Met should be considered as a further safety aspect. However, the available literature describes unequivocally a higher tolerance to selenium from SeMet, than to selenium from sodium selenite (Schrauzer, 2000; Surai, 2006). Significant amounts of SeMet would, after intestinal absorption, escape the metabolic conversion into dihydrogen selenide (H_2Se) since a part of it is non-specifically incorporated into general proteins.

Considering the purity of the L-SeMet under application and the metabolic pathways of SeMet, the FEEDAP Panel considers the use of L-SeMet as safe for all animal species, provided that the maximum total selenium level authorised in feed is respected.

3.2. Safety for the consumer

Some of the absorbed selenium from SeMet is metabolised to dihydrogen selenide to be utilised in selenium pathways, whereas another portion is non-specifically incorporated into the general body proteins as a substitute for the common amino acid Met (Schrauzer, 2000). Consequently, it is well established from a number of experimental studies that edible tissues and animal products—particularly meat, eggs and milk—from animals fed diets supplemented with selenium sources based on SeMet as the predominant selenocompound contain significantly more selenium than those from animals given inorganic sources of selenium (Mahan and Parrett, 1996; Knowles et al., 1999; Ševčíková et al., 2006; Skřivan et al., 2006, 2010).

No toxicological studies have been provided by the applicant. However, an extensive evaluation of toxicity data has previously been made by the ANS Panel of EFSA when assessing the same compound for human use (EFSA, 2009b). The ANS Panel considered L-SeMet as a safe source of selenium for humans up to the intake of 100 µg Se/adult person per day; this daily dose was derived based on calculation of its contribution to total daily intake of selenium and the upper limit (UL). The UL for selenium has been set by the Scientific Committee on Food (EC, 2000) (adults: 300 µg/day; toddlers: 60 µg/day), and used by the FEEDAP Panel in previous assessments of the consumer safety for different selenium compounds.

The FEEDAP Panel has several times expressed its view that all SeMet sources would result in similar selenium deposition (EFSA 2011a,b, 2012); this view was later extended to the hydroxy-analogue of SeMet (HMSeBA) (EFSA, 2013). Since L-SeMet is the predominant selenocompound incorporated into proteins of selenised yeast, there is no reason to assume that free L-SeMet would result in an essentially different deposition pattern. Limited data on selenium tissue deposition provided by the applicant from L-SeMet efficacy studies (one for laying hens and one for pigs for fattening) support previous conclusions on the amount of selenium deposited. The Panel has previously concluded that the selenium supplementation of feed by selenised yeast or by HMSeBA should be limited to a maximum of 0.2 mg/kg feed. Therefore with respect to consumer safety, the

FEEDAP Panel concludes that supplemental selenium from L-SeMet should be limited to a maximum of 0.2 mg/kg feed.

No other safety concerns for the consumer resulting from the use of synthetic L-SeMet in animal nutrition are envisaged.

3.3. Safety for the user

Selenium is a recognised occupational hazard, inhalation representing the main route of exposure. The commonly accepted threshold limit value (TLV) for inorganic selenium in air is 0.2 mg/m^3 (EFSA, 2006a). No specific inhalation toxicity data have been provided. The dust generated in a Stauber-Heubach test on the formulated additive contained 3-7 mg selenium/m^3. Thus, the exposure by inhalation to selenium from L-SeMet is potentially above the TLV for inorganic selenium, with a high proportion of respirable particles in both L-SeMet and the formulated product. In the absence of information on the inhalation toxicity of L-SeMet, it would be prudent to take measures to minimise the inhalation exposure of users to the additive.

Exposure to high concentrations of selenium can cause skin rash and irritation of the eyes and the mucosae. Direct contact with concentrated materials could be of importance, particularly when cutaneous absorption might be facilitated by the local irritation and skin damage. In the absence of specific data on skin/eye irritation and skin sensitisation, the additive should be considered as an irritant to skin and eyes and as a skin sensitiser.

3.4. Safety for the environment

There is no information suggesting that selenium, when provided to animals as L-SeMet, would be more harmful to the environment than selenium from other sources already authorised as feed additives. It is well documented in the scientific literature that, owing to the specific metabolic fate of SeMet, significantly more selenium from organic sources than from inorganic sources is retained in the animal body.

Therefore, the FEEDAP Panel considers that the use of L-SeMet in feed does not pose an additional risk to the environment, compared with other sources of selenium for which it will substitute, as long as the maximum authorised content in feedingstuffs is not exceeded.

4. Efficacy

Evidence of *in vivo* bioavailability can be taken to support efficacy of compounds of essential trace elements. One trial in a single animal species, including laboratory animals, is considered sufficient. As already established in previous opinions of the FEEDAP Panel (EFSA 2006a,b, 2009a, 2011a, 2012, 2013), the bioavailability of a source of selenium as nutritional additive is considered to be demonstrated if one of the specific endpoints (glutathione peroxidase (GSH-Px) activity in plasma or whole blood, selenium concentration in plasma/serum or whole blood, selenium content in liver) is significantly influenced by the test item.

The tissue deposition of selenium from feed additives that are sources of SeMet was considered to reflect directly only this non-specific incorporation of SeMet into general body proteins (EFSA, 2006a). Since utilisation of selenium from SeMet released from body proteins for specific selenium functions is well known, the selenium deposition in tissues is taken as indirect proof of selenium bioavailability from each additive with a mode of action based on enriching the SeMet body pool.

To demonstrate efficacy of L-SeMet, the applicant provided three studies. The study with laying hens was performed with L-SeMet from the applicant. In the study with pigs for fattening, L-SeMet (purity

≥ 98 %) from another supplier was used; however, considering purity criteria, the FEEDAP Panel assumes product identity. A third study on sows and their offspring could not be considered because the experimental design was inadequate and because not L-SeMet, but DL-SeMet, was used.

4.1. Laying hens

A study was conducted in Lohman Brown laying hens.[45] Birds were divided into groups and housed in cages. After a pre-period during which the hens received a mash diet without selenium supplementation, groups were given diets supplemented with increasing concentrations of selenium from different sources. The control group received the unsupplemented diet. Selenium supplementation was analytically confirmed. Diets were offered *ad libitum*. At the end of the trial, blood samples were collected.

Selenium concentrations in eggs increased in a dose-dependent way. L-SeMet is an efficient source of selenium for laying hens.

4.2. Pigs for fattening

In a published study, pigs fed a diet containing L-SeMet were compared with a group fed a diet containing sodium selenite and an unsupplemented group (Zhan et al., 2007). A total 108 barrows (Duroc × (Landrace × Yorkshire)) with a mean initial body weight of 60 kg were equally allotted to three treatments, each replicated three times with 12 pigs. The control diet was based on maize, soybean meal and animal fat (background selenium content: 0.05 mg/kg). The supplementation rate of selenium was 0.3 mg/kg feed (selenium supplementation confirmed by analysis; however, data are not given). Diets were fed *ad libitum* for 40 days. At the end of the study, six animals per treatment (two pigs per pen) were slaughtered and samples of blood, liver, kidney, muscle and pancreas were collected. No data on zootechnical performance were given.

The selenium level in serum and tissues and GSH-Px activity in liver and muscle were significantly increased by feed supplementation of selenium from either sodium selenite or L-SeMet (Table 2). The parameters for carcases and meat quality measured (malondialdehyde content, pH, myoglobin, crude fat and protein, drip loss and Hunter value in muscle) were essentially not affected by either treatment.

Table 2: Effect of feed supplementation (for 40 days) with selenium either from sodium selenite or L-SeMet on selenium levels in serum and tissues, and GSH-Px activity in tissues of pigs for fattening

Source of Se	Control	Sodium selenite	L-SeMet
Se supplemented (mg/kg feed)	-	0.3	0.3
Selenium level			
Serum (mg/L)	0.06^{a}	0.15^{b}	0.16^{b}
Liver (mg/kg)	0.3^{a}	0.5^{b}	0.7^{c}
Kidney (mg/kg)	1.7^{a}	2.3^{b}	2.6^{b}
Muscle (mg/kg)	0.10^{a}	0.14^{b}	0.35^{c}
Pancreas(mg/kg)	0.3^{a}	0.4^{b}	0.58^{c}
GSH-Px activity			
Liver (U/mg)	46^{a}	69^{b}	74^{b}
Muscle (U/mg)	5^{a}	7^{b}	8^{b}

a, b, c: Means with different superscript within a row are significantly different (P<0.05).

[45] Technical Dossier/Section IV/Annexes IV_1 and IV_2.

4.3. Ruminants

The applicant provided no studies on supplementation of ruminants with free L-SeMet as a source of the trace element selenium and none could be found in the available published literature. It is clear that L-SeMet, when added as free amino acid to feed for ruminants, would be fully exposed to degradation processes by ruminal microbiota. Considering the maximum currently authorised total selenium level in complete feed (0.5 mg/kg), the natural background of this trace element (up to 0.1 mg/kg feed) and selenium content of SeMet, the daily dose of L-SeMet as a source of selenium for dairy cows would not exceed 20 mg/animal/day.

Literature data show that the fate of dietary selenium in ruminants is influenced not only by the chemical form of selenium supplemented to feed, but also by diet composition, rumen microbiota profile and population, and the selenium status of animals (Koenig et al., 1997; van Ryssen and Schroeder, 2003). It has been shown that ruminal bacteria as well as protozoa are able to incorporate selenium from both inorganic and organic sources into their own proteins (Hidiroglou et al., 1968; Hidiroglou and Zarkadas, 1976; Hudmann and Glenn, 1984; Fujihara et al., 2004) and also into wall components of microbial cells (Koenig et al., 1997). There is evidence to indicate that the metabolism of selenium in the rumen is dependent on the prevalence of particular species of bacteria. It was found that pure cultures of *Selenomonas ruminantium* and *Butyrivibrio fibrisolvens* incorporated selenium into selenoamino acids (mainly selenocysteine (SeCys)) while *Bacteroides ruminicola* reduced selenocompounds to elemental selenium only (Hudman and Glen, 1984, 1985).

Selenised yeast containing SeMet as the predominant selenocompound (90 % of total selenium (Schrauzer, 2006)), which is incorporated into the protein/peptide chains of yeast, has been shown unequivocally to be an efficient source of selenium in ruminants (Juniper et al., 2008; Pechova et al., 2012). Using stable selenium isotopes for labelling of sodium selenite and selenised yeast in an *in vivo* study on dairy cows, it was found that both selenium sources resulted in selenium uptake by ruminal microbiota and that uptake of selenium was significantly higher from the organic source (Maiville et al., 2009). Higher uptake of selenium by rumen microbiota from an organic than from an inorganic source was recently confirmed also in sheep fed diets supplemented with selenium from sodium selenite or selenised yeast (Panev et al., 2013). Such a selenium uptake by rumen microbiota is assumed to be mediated by direct uptake of released SeMet from yeast as well as being a result of utilisation of selenium metabolites and/or elemental selenium from degraded SeMet by rumen bacteria in a synthesis of selenoamino acids, mainly SeCys, and subsequent microbial protein synthesis.

Based on the information stated above, the FEEDAP Panel considers L-SeMet to be equivalent to other selenium sources based on SeMet and used in ruminant nutrition.

4.4. Conclusions on efficacy

L-SeMet is an efficient source of selenium for all species. This conclusion is derived from studies with laying hens and pigs for fattening and, in the case of ruminants, from literature describing the microbial incorporation of selenium from organic sources in the rumen. The endpoints used in the experiments with target animals were specific markers for selenium efficacy as nutritional additive.

5. Post-market monitoring

No risks associated with the use of the product are foreseen. It is considered that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[46] and Good Manufacturing Practice.

[46] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Considering the purity of the L-SeMet under application and the metabolic pathways of SeMet, the FEEDAP Panel considers the use of L-SeMet as safe for all animal species, provided that the maximum total selenium level authorised in feed is respected.

The use of L-SeMet in animal nutrition is expected to result in a similar increase in selenium deposition in animal tissues/products as that resulting from other sources of SeMet. To ensure consumer safety from consumption of food originating from animals fed L-SeMet, the FEEDAP Panel concludes that dietary selenium supplementation from the additive should not exceed a maximum of 0.2 mg Se/kg complete feed.[47]

In the absence of specific data, the additive should be considered as an irritant to skin and eyes, as a skin sensitiser and as potentially harmful by inhalation.

The FEEDAP Panel considers that the use of L-SeMet in feed does not pose an additional risk to the environment, compared with other sources of selenium for which it will substitute, as long as the maximum authorised content in feedingstuffs is not exceeded.

L-SeMet is an efficient source of selenium for all species. This conclusion is derived from studies with laying hens and pigs for fattening and, in the case of ruminants, from literature describing the microbial incorporation of selenium from organic sources in the rumen.

RECOMMENDATIONS

The "Description and conditions of use of the additive" as proposed by the applicant should be amended as follows:

- The description of the additive, as proposed by the applicant should be modified to adapt to the analytical data. The minimum content of selenium in L-SeMet should be increased to 39 %.

- To list under *Other Provisions*:
  "The incorporation of the additive into feed should be made via premixtures only"
  "The additive should not be incorporated in premixtures containing compounds of trace elements"

The maximum content for total selenium in feed is set by legislation. The conclusions of the FEEDAP Panel on the safety of L-SeMet for the target animals, the consumer and the environment are valid only if these maximum contents are strictly considered in feed formulation and feeding practices. Since selenium is routinely supplemented to feed, only a small amount, if any, could be administered additionally via water for drinking. Exact dosing in water for drinking can be achieved only if the total dietary selenium content and its availability are known, which is normally not the case. The FEEDAP Panel therefore recommends not to introduce the use of L-SeMet via water for drinking.

Exposure of users by inhalation should be avoided. Protection measures are recommended. Furthermore, the FEEDAP Panel strongly recommends that L-SeMet should only be placed on the market (to premixture compounders only) in a formulation with reduced selenium content (i.e. ≤40 000 mg Se/kg) and reduced selenium dusting potential.

[47] The maximum total selenium level in animal feeds in the EU is set to 0.5 mg Se/kg complete feed, of which L-SeMet can contribute up to 0.2 mg Se/kg complete feed.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier L-Selenomethionine. July 2011. Submitted by Excentials IP BV.
2. Dossier L-Selenomethionine. Supplementary information. September 2012. Submitted by Excentials IP BV.
3. Dossier L-Selenomethionine. Supplementary information. February 2013. Submitted by Excentials IP BV.
4. Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for L-Selenomethionine.
5. Comments from Member States received through the ScienceNet.

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APPENDIX

Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for L-Selenomethionine[48]

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.

[48] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2011-0028.pdf