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SCIENTIFIC OPINION

Scientific Opinion on safety and efficacy of hydroxy-analogue of selenomethionine as feed additive for all 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

The additive hydroxy-analogue of selenomethionine consists of synthetic *R,S*-2-hydroxy-4-methylselenobutanoic acid (HMSeBA) and is intended to be used as a source of the essential trace element selenium for all animal species/categories. Based on data from tolerance studies in chickens and turkeys for fattening and piglets, the additive is considered as safe for all species/categories up to the maximum authorised total selenium level in complete feed. After being absorbed, HMSeBA is metabolised to selenomethionine; consequently, no residues of the compound itself occur in animal tissues and products. Compared with inorganic selenium sources, the use of HMSeBA in animal nutrition would result in a similar increase in selenium deposition in animal tissues/products as that resulting from selenised yeast. To ensure consumer safety from consumption of food originating from animals supplemented with HMSeBA, the FEEDAP Panel concluded that selenium supplementation from the additive should not exceed a maximum of 0.2 mg Se/kg complete feed. The additive should be regarded as an eye irritant, but should not be classified as skin irritant or skin sensitiser. Inhalation exposure poses a hazard to users; the FEEDAP Panel concludes, therefore, that the formulation and conditions of use of the solid form of the additive should minimise user exposure by inhalation. The use of HMSeBA in feed does not pose an additional risk to the environment, compared to other sources of selenium for which it will substitute, as long as the maximum authorised content in feedingstuffs is not exceeded. Based on the response of plasma glutathione peroxidase activity and the plasma/liver concentration of selenium in chickens for fattening and pigs for fattening, the FEEDAP Panel considers that HMSeBA is an efficaceous source of selenium for all animal species/categories. HMSeBA does not modify the quality of meat as measured by physico-chemical properties.

KEY WORDS

Nutritional additive, compound of trace elements, hydroxy-analogue of selenomethionine, HMSeBA, selenium, safety, efficacy

[1] On request from the European Commission, Question No EFSA-Q-2012-00058, adopted on 11 December 2012. Revision 1 – 10/04/2013: minor editorial changes.

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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 the hydroxy-analogue of selenomethionine (HMSeBA) as feed additive for all species. The additive is intended to be used as a source of the essential trace element selenium in animal nutrition.

Data from tolerance studies on chickens and turkeys for fattening and piglets showed that the additive HMSeBA is a safe selenium source when used up to the maximum authorised total selenium content in complete feed. A safety margin of ten was identified. Provided the maximum authorised total selenium content in complete feed is not exceeded, the use of HMSeBA as a selenium source is considered to be safe for all animal species/categories.

Based on a toxicology testing package (acute toxicity, genotoxicity *in vitro* and *in vivo*, repeated dose toxicity) it is concluded that HMSeBA does not elicit adverse unexpected effects for a selenium compound. After being absorbed, HMSeBA is metabolised to selenomethionine; consequently, no residues of the compound itself occur in animal tissues and products. Compared with inorganic selenium sources, the use of HMSeBA in animal nutrition would result in a similar increase in selenium deposition in animal tissues/products as that resulting from selenised yeast. To ensure consumer safety from consumption of food originating from animals fed diets containing HMSeBA, the FEEDAP Panel concludes that dietary selenium supplementation from the additive should not exceed a maximum of 0.2 mg Se/kg complete feed.

The additive should be regarded as an eye irritant, but should not be classified as skin irritant or skin sensitiser. Inhalation exposure poses a hazard to users; the FEEDAP Panel concludes, therefore, that the formulation and conditions of use of the solid form of the additive should minimise user exposure by inhalation.

The use of HMSeBA 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.

Studies in chickens for fattening and pigs for fattening showed an increased plasma/liver selenium level and plasma glutathione peroxidase activity in response to feed supplementation with HMSeBA. Therefore, the FEEDAP Panel concludes that HMSeBA is an efficacious source of the essential trace element selenium for animal nutrition. The use of the additive does not modify the quality of meat as measured by physico-chemical properties.

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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 ADISSEO France SAS[5] for authorisation of the product hydroxy-analogue of selenomethionine, when used as a feed additive for all animal 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 21 February 2012.

Two forms of inorganic selenium, sodium selenite and sodium selenate, are authorised in the European Union (EU) as source of the essential trace element selenium, under Directive 70/524/EEC.[7] Organic forms of selenium produced by *Saccharomyces cerevisiae* CNCM I-3060, *S. cerevisiae* NCYC R397 and *S. cerevisiae* 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). Two additional opinions on the safety and efficacy of selenium in the form of organic compounds produced by the selenium-enriched yeast *S. cerevisiae* NCYC R645 (EFSA, 2011a) and NCYC R646 (EFSA, 2012a) for all animal species were delivered by the FEEDAP Panel Another opinion on the safety and efficacy of Sel-Plex® (organic form of selenium 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 animal(s), consumer, user and the environment and the efficacy of the product hydroxy-analogue of 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] ADISSEO France S.A.S. Immeuble Antony Parc II, 10 place du Général de Gaulle. 92160 Anthony. France.
[6] EFSA Dossier reference: FAD-2011-0044.
[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	Selenium mainly represented by R,S-2-hydroxy-4-methylselenobutanoic acid (or HMSeBA) and other Se-based components
Registration number/EC No/No (if appropriate)	3b8.xx
Category(-ies) of additive	3. Nutritional additive
Functional group(s) of additive	b. Compounds of trace elements

Description			
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)
Preparation, solid or liquid, with a maximum of 2.4% of selenium mainly represented by R,S-2-hydroxy-4-methylselenobutanoic acid (or HMSeBA) and other Se-based components (HMSeBA oligomers: dimers) on carrier	Not appropriate	Complies with EU law on undesirable substances	- Zeeman graphite furnace atomic absorption spectrometry (AAS) or hydride AAS - Inductively coupled plasma mass spectrometry (ICP-MS)

Trade name (if appropriate)	Selisseo
Name of the holder of authorisation (if appropriate)	ADISSEO France SAS

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 or categories	Not appropriate	Not appropriate	0.5 mg Se (total)/kg of complete feedingstuffs with a moisture content of 12% or 0.25 mg Se (total)/litre of water of drinking	Not appropriate

Other provisions and additional requirements for the labeling	
Specific conditions or restrictions for use (if appropriate)	The additive shall be incorporated into feed in form of a premixture
Specific conditions or restrictions for handling (if appropriate)	For user safety: breathing protection, safety glasses and gloves should be worn during handling
Post-market monitoring (if appropriate)	ADISSEO France SAS will conduct post-marketing monitoring in compliance with EU law on feed hygiene, namely by use of HACCP and Traceability systems, and formal monitoring of customer feedback through product or service complaints.
Specific conditions for use in complementary feedingstuffs (if appropriate)	- The selenium feed additive is given continuously during animal rearing. - To supply selenium in final feeds within EU legal limits.

Maximum Residue Limit (MRL) (if appropriate)			
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues
Not relevant	Not relevant	Not relevant	Not relevant

ASSESSMENT

1. Introduction

The additive consists of 5 % *R,S*-2-hydroxy-4-methylselenobutanoic acid (HMSeBA, hydroxy-analogue of selenomethionine) and 95 % carrier. The application under assessment is for the use of HMSeBA in feed and water for drinking for all animal species as source of the essential trace element selenium.

The biological role of selenium and 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, which is essential for vertebrates and is involved in a 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 it to reconsider its previous opinion.

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

For a similar product, L-selenomethionine, an opinion for use in food supplements has been produced by the EFSA's Panel on Food Additives and Nutrient Sources added to Food (EFSA, 2009b). L-selenomethionine is authorised by Commission Regulation (EC) No 1170/2009[11] as mineral which may be used in the manufacture of food supplements.

2. Characterisation

2.1. Identity of the additive

The additive is intended to be marketed in two forms: solid and liquid.

The solid form of the additive consists of 5 % HMSeBA and 95 % carriers, containing by specification 1.8 % to 2.4 % selenium. Carboxymethylcellulose, aluminosilicate, colloidal silica, cellulose, calcium carbonate and starch can be used as carriers; these compounds are already authorised as additives or are feed materials in the European Union. The analysis of five batches of the additive, each manufactured with a different HMSeBA lot, showed mean HMSeBA and selenium contents of 5.1 % (range 5.0–5.1 %) and 2.0 % (range: 1.9–2.1 %), respectively.[12]

The liquid form of the additive consists of 5 % HMSeBA diluted in distilled water, with the same specification for selenium content as the solid additive. The mean HMSeBA and selenium contents in five analysed batches were 5.1 % (range 5.0–5.1 %) and 2.1 % (range 1.9–2.4 %), respectively.[13]

2.2. Impurities

Both forms of the additive (three batches each) were analysed for heavy metals, arsenic, fluorine, dioxins and the sum of dioxins and dioxin-like polychlorinated biphenyls (PCBs).[14] All values were compliant with EU legislation.[15] Lithium (three batches of the solid form) concentration was in the

[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 2.1.5. Technical Dossier/Supplementary Information/Annex i-1 and Annex v-4.
[13] Technical Dossier/Supplementary Information/Annex v-1.
[14] Technical Dossier/Section II/Annex 2.1.6. Technical Dossier/Supplementary Information/Annex v-3.
[15] Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed—Council statement. OJ L 140, 30.5.2002, p. 10.

range 3.5–5.0 mg/kg, which is not considered of any concern.[16] Microbial contamination was also studied in three batches of each form of the additive and found to be virtually absent (bacteria, yeasts and moulds < 5 CFU/g solid additive, < 1 CFU/g liquid additive; *Salmonella* absent in 25 g of product).[17] Monitoring of residual solvents originating from the synthesis of HMSeBA shows them to be below the limit of detection (LOD; 3.0 mg/kg) for tetrahydrofuran, diethoxymethane and di-isopropylether, and 35 mg/kg for methylcyclohexane; the Veterinary International Cooperation on Harmonization (VICH) Guideline for residual solvents classifies tetrahydrofuran and methylcyclohexane as "Class II solvents, Solvents to be limited", with concentration limits of 720 and 1180 mg/kg respectively (VICH, 2011). Control methods are in place.

2.3. Physical state of the final formulations

The solid form of additive is a white, odourless powder, with a bulk density of 352 kg/m^3.[18] Analysis of the particle size distribution by laser diffraction in three batches showed that 3.6 % of particles were < 10 µm in diameter (range 3.5–3.7 %), 21.9 % < 50 µm (range 20.1–23.2 %) and 42.5 % < 100 µm (range 40.5–43.1 %); the mean particle diameter ranged from 114 to 123 µm.[19] The dusting potential, measured by the Stauber–Heubach method, ranged from 4.4 to 5.9 g/m^3 for six batches.[20] The selenium concentration in the dust amounted 9–11 mg/g, corresponding to about half of the selenium level in the additive.[21]

The liquid form of the additive is of pale-yellow colour and odourless. HMSeBA is highly soluble in water (> 491 g/L at 20 ºC).[22]

2.4. Characterisation of the hydroxy-analogue of selenomethionine

Selenium is considered to be the active substance of HMSeBA. More than 99 % of total selenium in the additive is in the form of HMSeBA and related selenocompounds.

The principal selenocompound of the additive (providing >98 % of total selenium) is *R,S*-2-hydroxy-4-methylselenobutanoic acid[23] (Chemical Abstracts Service (CAS) number 873660-49-2; molecular weight 197.09 Daltons). The molecular formula is $C_5H_{10}O_3Se$, with a theoretical content of selenium of 40.1 %. The identity of HMSeBA was confirmed by measuring its UV spectrum, which showed maximum absorbance at 220 nm, and by structural analysis of its ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra, infra-red spectrum and mass spectrum. The molecular structure is shown in Figure 1.

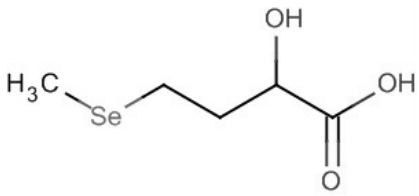

Figure 1: Molecular structure of HMSeBA

[16] Technical Dossier/Supplementary Information/Annex iv-2.
[17] Technical Dossier/Section II/Annex 2.1.8. Technical Dossier/Supplementary Information/Annex v-5.
[18] Technical Dossier/Section II/Annex 2-1-12.
[19] Technical Dossier/Section II/Annex 2-1-11.
[20] Technical Dossier/Section II/Annex 2-1-13.
[21] Technical Dossier/Supplementary Information/Annex viii-2.
[22] Technical Dossier/Section II/Annex 2-2-4.
[23] Other names are hydroxy-analogue of selenomethionine, D,L-2-hydroxy-4-(methylseleno)-butanoic acid, α-hydroxy-γ-methylseleno-butanoic acid, γ-methylseleno-α-hydroxybutanoic acid, *R,S*-2-hydroxy-4-(methylseleno)-butyric acid, D,L-2-hydroxy-4-(methylseleno)-butyric acid, α-hydroxy-γ-methylseleno-butyric acid and γ-methylseleno-α-hydroxybutyric acid.

HMSeBA is a free-flowing, pale yellow odourless powder. Its melting and boiling points are 48.3 and 245.3 °C, respectively and the solubility in water is > 491 g/L at 20 °C.

High-performance liquid chromatographic (HPLC) analysis of six batches of HMSeBA showed a mean HMSeBA content of 98.9 % (range 98.7–99.3 %) with a mean total selenium level of 39.5 % (range 36.4–40.7 %).[24] In another five batches, the mean HMSeBA content was 99.0 % (range 98.8–99.1 %)[25] and the mean total selenium level was 40.0 % (range 38.9–41.2 %).[26] The HMSeBA dimer ($C_{10}H_{18}O_5Se_2$) content was 0.3 % (range 0.2–0.4 %).[27] In five batches, the concentrations of two other selenocompounds, $C_8H_{14}O_6Se$ (molecular weight 286) and $C_8H_{14}O_6Se_2$ (molecular weight 366), were 0.11 % (range 0.02–0.2 %) and 0.17 % (range 0.1–0.2 %),[28] respectively. Inorganic tetravalent and hexavalent selenium were below the limit of quantification (LOQ; 50 mg/kg).[29]

The residual solvents (tetrahydrofuran, diethoxymethane, di-isopropylether and methylcyclohexane) and lithium (used for the synthesis of HMSeBA) were analysed in three batches of HMSeBA.[30] The concentrations found (both tetrahydrofuran and diethoxymethane < LOD 3 mg/kg; di-isopropylether ≤ 2.9 mg/kg; methylcyclohexane ≤ 8.8 mg/kg and Li ≤ 74 mg/kg) do not raise concerns considering the 20-fold dilution in the blended additive. For both tetrahydrofuran and methylcyclohexane, listed in the VICH Guidelines, these analysed values are below the concentration limits set (720 and 1180 mg/kg, respectively) (VICH, 2011).

2.5. Manufacturing process

The synthesis of HMSeBA involves seven steps. It starts with the reaction of methyllithium with selenium in the diethoxymethane and tetrahydrofuran solvents yielding methylselenolithium. After adding *R,S*-2-hydroxybutyrolactone the lithium salt of the 2-hydroxy-4-methylselenobutanoic acid is formed, acidified with sulphuric acid, extracted and concentrated in di-isopropylether solution. In the next step, the *R,S*-2-hydroxy-4-methylselenobutanoic acid is isolated as an off-white solid by crystallisation and precipitated in a mixture of di-isopropylether/methylcyclohexane, filtered and dried. Residual solvents are volatile and eliminated during the drying phase (under vacuum). Control measures are in place and the material safety data sheets of each material used in synthesis of HMSeBA are enclosed to the technical documentation.

The solid form of the additive is manufactured by spraying a 40 % solution of HMSeBA on a carrier (e.g. silica) and mixing; the final product is sealed in aluminium bags with an internal wall of low density polyethylene. The liquid form of additive is manufactured by dissolving solid HMSeBA in distilled water (5 % solution).

Control measures are in place (heavy metals, arsenic, dioxins and dioxin-like PCBs in carrier).

2.6. Physico-chemical and technological properties of additive

2.6.1. Stability

The applicant provided studies based on analysis of HMSeBA and total selenium.

Samples of three batches of the solid form of additive (in total, 15 sealed, low-density polyethylene pouches, each containing 2 g of additive) were stored in the dark and exposed to both normal (20 °C, 60 % relative humidity (RH)) and accelerated conditions (30 °C, 65 % RH) for 12 months; one of the batches was stored for up to 18 months.[31] The recovery of HMSeBA after 12 months at normal and

[24] Technical Dossier/Section II/Annex 2-2-1.
[25] Technical Dossier/Supplementary Information/Annex iii-2.
[26] Technical Dossier/Supplementary Information/Annex iii-3.
[27] Technical Dossier/Supplementary Information/Annex iii-1.
[28] Technical Dossier/Supplementary Information/Annex iii-2.
[29] Technical Dossier/Supplementary Information/Annex iii-3.
[30] Technical Dossier/Supplementary Information/Annex iv-1.
[31] Technical Dossier/Section II/Annex 2-4-3.

accelerated conditions was 91 and 80 %, respectively; the batch stored for 18 months showed recoveries of HMSeBA under normal and accelerated conditions of 89 and 78 %, respectively. In another ongoing study with three batches of the solid form, the available data after storage for up to three months showed recovery of the sum of HMSeBA and other Se-based components (monomer and dimers) under normal and accelerated conditions of 98 and 97 %, respectively.[32]

Stability studies were provided for the solid form of the additive in premixtures (for chickens for fattening, laying hens, turkeys and piglets)[33] and complete feeds (for chickens for fattening and piglets and dairy cows)[34] reporting only total selenium levels. The stability of the additive to pelleting was also addressed only by analytical data on total selenium content.[35]

A study of stability of the liquid form during 48 hours was provided, however only analytical data on total selenium were submitted.[36] Data supporting stability of HMSeBA in water for drinking at the proposed supplementation levels were not provided.

The FEEDAP Panel notes that, in principle, analytical data on total selenium *per se* do not provide full evidence of the stability of an additive based on an organic selenium compound. The Panel also notes that HMSeBA is an analogue of amino acid and, therefore, not expected to dissociate. In dry premixtures/feed the selenium-containing hydroxy-analogue of methionine is expected to behave similarly to the sulphur-containing hydroxy-analogue of methionine, which has an established stability in feed.

2.6.2. Homogeneity

A complete feed for laying hens (based on wheat and soybean meal) was supplemented via a premixture with selenium from the solid form of the additive (one batch) in order to obtain its final intended level of 0.3 mg/kg total feed.[37] The selenium level analysed in ten feed subsamples showed a coefficient of variation (CV) of 10 %.

In complete feed for pigs for fattening supplemented via premixture with 0.3 mg Se/kg from the solid form of the additive (one batch), the analysis of ten subsamples showed a CV for selenium distribution of 6.5 %.[38] In a tolerance trial on piglets, the complete feed was supplemented with 5 mg Se/kg (10-fold higher than the maximum authorised selenium content in feed) from the solid form of the additive (one batch) via a premixture; the analysed selenium levels in ten subsamples of feed showed a CV of 3.8 %.[39]

The selenium analysis of ten subsamples from each mash and pelleted feed for chickens for fattening (supplemented with 0.3 mg Se/kg from the solid form of additive) showed CVs of 7.4 and 5.1 %, respectively. Tests for homogeneous distribution of selenium from the solid form of the additive in 10 subsamples each from pelleted feed for piglets and pelleted complementary feed for dairy cows showed CVs of 4.5 and 5.3 %, respectively, at a selenium supplementation dose of 0.3 mg Se/kg feed.[40]

Since the solubility in water of HMSeBA is > 491 g/L at 20 °C, confirmation of the additive's ability to be homogenously distributed in water for drinking is not required.

[32] Technical Dossier/Section II/Annex 2-4-4.
[33] Technical Dossier/Section II/Annexes 2-4-6 and 2-4-7.
[34] Technical Dossier/Section II/Annex 2-4-8.
[35] Technical Dossier/Section II/Annex 2-4-8.
[36] Technical Dossier/Section II/Annex 2-4-9.
[37] Technical Dossier/Section II/Annex 2-4-10.
[38] Technical Dossier/Section II/Annex 2-4-12.
[39] Technical Dossier/Section II/Annex 2-4-13.
[40] Technical Dossier/Section II/Annex 2-4-8.

2.7. Physico-chemical incompatibilities in feed

No incompatibilities with feed components, carriers, other approved additives or medical products are expected when using HMSeBA as source of selenium in animal nutrition.

2.8. Conditions of use

The conditions of use as described by applicant are as follows. The applicant proposes the use of HMSeBA is feed for all animal species or categories as source of selenium.

When supplementing feed, it is proposed the solid form of additive to be added via premixtures and the liquid form by spraying on feedingstuffs, both up to the maximum authorised total selenium level 0.5 mg Se/kg complete feed.

The liquid form of the additive is also proposed to be used in water for drinking. The applicant proposes a maximum content of selenium of 0.25 mg/L in water for drinking assuming a water to feed intake ratio for poultry, pigs, horses and rabbits of 2. For ruminants, the applicant recommends that the dose of selenium added to water for drinking be adjusted according to the actual daily water intake and/or an approximate water consumption (dairy cows, pregnant heifers 28-114 L/day, cattle for fattening and steers 26-66 L/day, calves up to 26 weeks 9-27 L/day, small ruminants 3-15 L/day). 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.

No limitations on the age of the animals or the administration period are proposed.

2.9. 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 the active substance (HMSeBA) in the feed additive and total selenium in the feed additive and animal feed. The Executive Summary of the EURL report can be found in the Appendix.

3. Safety

The studies were performed using either the pure form of HMSeBA (also called Selest®) or the formulated additives (Selest® 5 % or Selisseo 2 %). These forms are considered bioequivalent based on the metabolic fate of the HMSeBA and its selenium.

3.1. Safety for the target species

According to the Guidance for the preparation of dossiers for nutritional additives, when an application of nutritional additive is intended for all animal species/categories, the tolerance data may be limited to one study on one target species or laboratory animal (the most sensitive in each case) (EFSA, 2012b).

3.1.1. Tolerance studies

The applicant provided seven non-published studies on tolerance to the target species, conducted from 2009 to 2011. Three of those studies were regarded as preliminary trials, aiming to establish a dose regime to apply on the actual tolerance studies.

The first preliminary study was performed in a total of 160 one-day-old chickens (Ross PM3) for nine days (40 cages x 4 birds) allotted into four groups according to dietary selenium supplementation via Selest® (0.3, 0.5, 5.0 or 10 mg Se/kg diet). Analysed dietary selenium concentration was 0.27, 3.0, 5.9 and 11 mg/kg, respectively.[41]

[41] Technical Dossier/Section III/Annex 3-1-1.

The second preliminary study was performed on a total of 40 one-day-old cockerels (Ross PM3) for 34 days. Birds were placed in three pens and fed a diet supplemented with Selest® at a dose of 0 mg Se/kg (20 birds), 10 mg Se/kg (10 birds) or 5.0 mg Se/kg (10 birds). Analysed dietary selenium concentration was 0.06, 0.54 and 5.20 mg/kg, respectively.[42]

The third preliminary study was performed in a total of ten weaned male castrated piglets for 43 days. Piglets with initial body weight (bw) of *ca*. 10 kg were divided into two groups of five piglets each and individually housed. Animals were fed diets supplemented with 0.5 or 5.0 mg Se/kg feed from Selest®.[43]

In all three preliminary experiments, no significant adverse effects from the use of 10-fold selenium overdose from the additive (5.0 mg Se/kg) was observed either in mortality or growth performance.

3.1.1.1. Tolerance study in chickens for fattening (I)

This study was combined to demonstrate both the tolerance of target species and the efficacy of the additive (see Section 4.1.3). A total of 720 one-day-old male chickens (Ross PM3) were allocated to eight groups (six replicates with 15 birds each).[44] The groups were fed maize–soybean-based diets unsupplemented (selenium background: 0.05 mg/kg) or supplemented with 0.3, 0.5, 5.0 or 10.0 mg Se/kg from Selest® or with 0.3, 0.5 or 5.0 mg Se/kg feed from sodium selenite for 42 days. The intended selenium concentration in feed was analytically confirmed.

Body weight was measured at start, after 21 days and at termination of the trial; feed intake was determined at the same time points. At termination, blood (for glutathione peroxidase (GSH-Px) analysis) and tissue samples (liver, muscle for selenium analysis) were taken from 12 birds per treatment (two per replicate) and liver weight was determined. At day 43, blood samples (for haematology and serum clinical biochemistry (aspartate transaminase (AST), alanine transaminase (ALT), γ-glutamyltransferase (GGT), alkaline phosphatase (AP), creatine kinase (CK), glucose, urea, creatine, sodium (Na) and potassium (K)) analyses) and tissue samples (kidney, skin+fat for selenium analysis) from six birds per treatment (one per replicate), were taken.

Mortality varied between 2.2 (10 mg supplemented Se/kg from Selest®) and 7.9 % (5 mg supplemented Se/kg from sodium selenite). At the end of the trial, mean body weight of the control group was 3.27 kg. Mean body weight of the groups supplemented with 0.3 or 0.5 mg Se/kg feed from either sodium selenite or Selest® was not significantly different from the control value. However, body weight was significantly reduced by 5 mg Se/kg from sodium selenite, and by 10 mg selenium from Selest®. Feed intake followed the same pattern. For feed/gain ratio, no significant differences were observed between the supplemented groups and the control group. Absolute and relative liver weight were similar in all groups except a tendency for an increased relative liver weight in the group supplemented with 10 mg Se/kg from Selest®. Haematology and clinical biochemistry (performed in the control group and the groups supplemented with 0.5 and 5.0 mg Se/kg from either sodium selenite or Selest®) did not reveal major differences. Statistical analysis was not performed; however, all values were considered to be in line with the typical data for the breed used.

For the results of the efficacy demonstration (GSH-Px activity in plasma, selenium levels and its speciation in plasma, liver, muscle and skin+fat), see Section 4.1.3.

3.1.1.2. Tolerance study in chickens for fattening (II)

A further study to support tolerance in chickens for fattening was performed on 206 one-day-old chickens of both sexes (Ross PM3) and lasted 35 days.[45] Birds were divided into four groups according to treatment (with 50–55 birds in each) and fed a basal diet (background selenium 0.08–0.10 mg/kg) supplemented with 0, 0.3, 1.5 or 5.0 mg Se/kg feed from Selest®; the intended levels were

[42] Technical Dossier/Section III/Annex 3-1-2.
[43] Technical Dossier/Section III/Annex 3-1-3.
[44] Technical Dossier/Section III/Annex 3-1-5.
[45] Technical Dossier/Section III/Annex 3-1-4.

analytically confirmed. Since the study was designed without replicates, zootechnical performance parameters could not be considered. At the end of the study, blood samples for haematological and biochemical analyses (AST, AP, total bilirubin (tBIL), GGT, total cholesterol, triglycerides, electrolytes, phosphorus, total protein, albumin, glucose, globulin, calcium, creatinine, AP, CK, lactate dehydrogenase (LDH), urea), and liver samples for histopathology examination were collected from eight birds per group. No treatment-related adverse effects were observed regarding mortality and final body weight (2.38, 2.36, 2.44 and 2.20 kg). Necropsy, liver histology, haematology and biochemistry results did not reveal any treatment-related differences between groups.

3.1.1.3. Tolerance study in turkeys

A study carried out in 180 one-day-old turkeys (both sexes of BUT 9) fed a basal diet (background selenium 0.05 mg/kg) supplemented according to treatments with 0, 0.3 or 5.0 mg Se/kg feed from Selest® for 42 days.[46] Analyses showed a selenium content in the respective diets ranging from 0.1 to 0.34 mg Se/kg, from 0.49 to 0.56 and from 4.44 to 4.80 mg Se/kg diet, respectively. There were six replicates per treatment (three per gender), which ten birds in each pen as replicate. At the end of the trial, blood samples for biochemistry (AST, AP, GGT, sodium, potassium, phosphorus, total protein, albumin, glucose, globulin, calcium, CK, LDH, urea and uric acid) and haematology (red blood cells (RBC), white blood cells (WBC), haematocrit (HCT), haemoglobin (Hb), mean corpuscular volume (MCV) and leucogram), as well as liver and kidney samples for histopathology examination, were collected from one bird per replicate. Overall, the growth performance of birds fed diets supplemented with selenium from Selest® did not differ from that of the control unsupplemented group. However, the body weight of the birds supplemented with 0.3 mg Se/kg feed was slightly lower, but in the group supplemented with 5.0 mg Se/kg it was similar to that of control group. Feed to weight gain ratio (1.85, 1.99 and 1.78 kg/kg in the control group, and the groups supplemented with 0.3 and 5.0 mg Se/kg diet, respectively) showed significant differences only between the high-selenium group and low-selenium group, but no differences were observed between the high-selenium group and the unsupplemented birds. These findings are not considered to be treatment-related. The serum biochemistry and haematology parameters were within the expected reference range in all groups and thus there was no indication of any treatment related effect. Gross pathology and histopathological examination did not reveal any adverse effects of the highest dose of selenium from Selest®.

3.1.1.4. Tolerance study in piglets

A 42-day study was performed in 40 weaned piglets (Dutch Landrace, Finnish Landrace and Piétrain) with initial body weight of 8.0–12.5 kg. Animals were divided into four groups (five males and five females per group).[47] Since the study was designed without replicates, zootechnical performance parameters could not be considered. Piglets were fed diets supplemented with 0, 0.3, 1.5 or 5.0 mg Se/kg feed from Selest®. The selenium supplementation was analytically confirmed. At the end of the study blood samples for haematology (RBC, WBC, HCT, Hb, and leucogram) and biochemistry (AST, ALT, GGT, AP, LDH, glutamate dehydrogenase, tBIL, total cholesterol, triglycerides, phospholipids, electrolytes, phosphorus, total protein, albumin, glucose, globulin, calcium, creatinine, urea), and liver samples for histopathology were collected from eight animals per treatment; these animals were subsequently subjected to gross pathology examination. No treatment-related liver gross pathology and histopathology findings were observed at the end of study. Haematological and biochemical parameters were not affected by the treatments; only creatine kinase activity was increased in piglets fed a diet with the highest level of selenium supplementation (5.0 mg/kg). No differences in final body weight within a gender were observed between groups. No mortality occurred.

3.1.2. Conclusions on the safety for target species

Supplementation of selenium from HMSeBA to feed for chickens for fattening up to 10-fold the maximum authorised level did not show adverse effects whereas the highest equivalent dose of sodium selenite led to reduced body weight of chickens for fattening. The absence of significant adverse

[46] Technical Dossier/Section III/Annex 3-1-6.
[47] Technical Dossier/Section III/Annex 3-1-7.

effects at 10-fold overdose of selenium from HMSeBA was also confirmed in further studies in chickens for fattening and other trials in piglets and turkeys. Considering the zootechnical performance, haematological and biochemistal parameters as endpoints, a safety margin of ten was identified. The contribution of the metabolite hydroxy-analogue of methionine from HMSeBA to the animal methionine pool would be negligible considering the limit set by the EU on maximum total selenium level in feed. The additive HMSeBA is considered to be a safe source of selenium for all species/categories when used up to total maximum authorised selenium content in complete feed.

3.2. Safety for the consumer

3.2.1. Metabolic and tissue/product deposition studies

HMSeBA is the DL-form of 2-hydroxy-4-methylselenobutyric acid and is similar to 2-hydroxy-4-methylthiobutanoic acid, well known in animal nutrition as methionine hydroxy-analogue, which is readily converted to methionine *in vivo*. In HMSeBA, the sulphur atom is replaced by a selenium atom. HMSeBA therefore undergoes the same metabolism as the methionine hydroxy-analogue but resulting in selenomethionine (Se-Met), the metabolism of which provides dihydrogen selenide (H_2Se) for the biosynthesis of specific selenoproteins. It is well established that some proportion of Se-Met escapes its metabolism to H_2Se and is non-specifically incorporated into the general body proteins. This metabolic fate of Se-Met is based on the fact that tRNA(Met) in plants, bacteria, birds and mammals does not discriminate between the common amino acid methionine (Met) and Se-Met. Therefore both Met and Se-Met may substitute for each other during incorporation into general proteins (Schrauzer, 2000). Consequently, edible tissues and animal products—particularly meat, eggs and milk—from animals fed diets supplemented with selenium sources based on Se-Met 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; Skřivan et al., 2010).

3.2.1.1. *In vivo* transformation of HMSeBA

In a preliminary study, six rats were dosed with 1 mg HMSeBA/kg bw and sampled at four consecutive time points.[48] HMSeBA, Se-Met, selenocysteine (Se-Cys) and other seleno-metabolites were analysed by HPLC-ICP-MS (HPLC-inductively coupled plasma mass spectrometry) and total selenium by ICP-MS. Within three hours after gavage, HMSeBA concentrations in plasma fell below the LOD (20 µg HMSeBA/L), whereas Se-Met and also Se-Cys were still detected after six hours. In addition, the content of total selenium in plasma and in muscle tissue increased rapidly (within one hour after gavage) and stayed fairly constant in the six-hour time period. Se-Met was detected in muscle tissue, whereas HMSeBA was not. It was concluded that HMSeBA is rapidly transformed into Se-Met and other metabolites.

The effect of selenium source and level on tissue selenium deposition was studied in chickens for fattening.[49] Six groups with six replicates of 17 chickens each were fed different selenium sources and levels from 0 to 21 days of age. Five groups were given a basal diet containing selenium either from sodium selenite (0.1 or 0.3 mg Se/kg feed) or from HMSeBA (0.1, 0.2 or 0.3 mg Se/kg feed), and the control group was fed the basal diet not supplemented with selenium. Growth performance was measured throughout the experimental period and muscle samples (breast muscle) were collected at the end of the experimental period. The comparison of the different selenium sources indicated that muscle selenium concentrations were higher following supplementation with HMSeBA than with sodium selenite. Theoretical selenium levels of 0.1 and 0.3 mg/kg feed resulted in muscle selenium concentrations of 1.63 and 3.88 times higher in the HMSeBA-treated groups than in the sodium selenite-treated groups. HMSeBA was measured in six muscle samples from chickens fed HMSeBA at 0.3 mg Se/kg feed; results indicated a muscle HMSeBA concentration below the detection level (0.01 mg selenium from HMSeBA/kg). Moreover, the same samples, together with six other samples of the

[48] Technical Dossier/Section III/Annex 3-2-1.
[49] Technical Dossier/Section III/Annex 3-2-2.

control group, were analysed for selenium speciation (Se-Met and Se-Cys); results suggested that muscle selenium was mainly present in the form of Se-Met and Se-Cys. The results of this study indicated that (i) HMSeBA is not retained in muscle tissue and (ii) higher muscle selenium would result from the use of HMSeBA compared with the inorganic source, sodium selenite.

3.2.1.2. Tissue deposition study in chickens for fattening

Groups of 12 one-day-old male and 12 one-day-old female chickens for fattening (Ross PM3) received soybean–maize-type diets supplemented with 0.3 or 0.5 mg Se/kg from HMSeBA or 0.5 mg selenium from sodium selenite for 35 days.[50] The supplementation levels in feed were analytically confirmed. At day 36, blood samples were taken from six birds per group; thereafter the birds were slaughtered for tissue collection. No significant differences in body weight and feed intake were observed at the end of the study. The results of plasma and tissue analyses are summarised in Table 2.

Table 2: Selenium levels in plasma and tissues of chickens for fattening after feeding diets supplemented with selenium either from HMSeBA or from sodium selenite for 35 days.

Group	Selenium levels (mg/kg)				
	Plasma	Liver	Kidney	Muscle	Skin+Fat
Sodium selenite (0.5 mg Se/kg)	0.23	0.64	0.77	0.14	0.15
HMSeBA (0.3 mg Se/kg)	0.27	0.84	0.97	0.45	0.21
HMSeBA (0.5 mg Se/kg)	0.29	1.07	1.16	0.62	0.26

The tissue and plasma residue analysis indicated that selenium was present in higher quantities in the HMSeBA-supplemented group than in the sodium selenite-supplemented group (4.4 times the level in muscle at equal selenium supplementation level). GSH-Px activity in plasma was systematically affected by the treatment (range of individual values: 63–102 nmol nicotinamide adenine dinucleotide phosphate (NADPH)/min/mL). HMSeBA could not be detected in tissues.

3.2.1.3. Tissue deposition study in turkeys for fattening

Two groups of 12 one-day-old BUT 9 turkeys (six males and six females in each group; each group housed in cages) were fed soybean–maize-type diets supplemented with 0.5 mg Se/kg either from sodium selenite or from HMSeBA for 84 days.[51] No control unsupplemented group was included. Since the study was designed without replicates, zootechnical performance parameters could not be considered. The selenium supplementation was analytically confirmed. At the end of the study, the birds were sacrificed, and blood and tissue samples from liver, kidney, muscle and skin+fat were taken and analysed for selenium content.

No differences in final body weight within a gender were observed between groups. The results of selenium analysis in plasma and tissue samples are summarised in Table 3.

Table 3: Selenium levels in plasma and tissues of turkeys after feeding diets supplemented with selenium either from HMSeBA or from sodium selenite for 84 days

Group	Selenium levels (mg/kg)				
	Plasma	Liver	Kidney	Muscle	Skin+Fat
Sodium selenite (0.5 mg Se/kg)	0.12	0.71	0.84	0.25	0.16

[50] Technical Dossier/Section III/Annex 3-2-3.
[51] Technical Dossier/Section III/Annex 3-2-4.

HMSeBA (0.5 mg Se/kg)	0.19	1.30	1.07	0.60	0.31

The tissue and plasma selenium analysis indicated that selenium was present in higher quantities in the HMSeBA-supplemented group than in the group given sodium selenite (2.4 times the level in muscle).

3.2.1.4. Egg deposition study in laying hens

Three groups of 20 Lohmann Brown laying hens (24 weeks of age; bw 1723 g) were fed wheat–soybean-type diets supplemented with 0.3 or 0.5 mg Se/kg from HMSeBA or 0.5 mg selenium from sodium selenite for 42 weeks.[52] The average analysed selenium concentration in feed (mean of three samples) was 0.40 and 0.57 mg/kg, respectively, in the groups supplemented with 0.3 and 0.5 mg selenium from HMSeBA and 0.41 mg in the group receiving 0.5 mg/kg supplemental selenium from sodium selenite. All eggs were collected daily; for selenium analysis, ten eggs per group were collected prior to the start, at 56, 112 days and 168 days (cessation) after the start. No relevant differences in feed intake, laying rate and egg weight were observed during the trial. The results of the selenium analyses in eggs are shown in Table 4.

Table 4: Selenium concentration in egg homogenates after feeding diets supplemented with selenium either from sodium selenite or from HMSeBA for 168 days to laying hens.

Group	Selenium levels in egg homogenates (mg/kg)			
	At start	56 days	112 days	168 days
Sodium selenite (0.5 mg Se/kg)	0.28	0.26	0.26	0.23
HMSeBA (0.3 mg Se/kg)	0.29	0.37	0.38	0.36
HMSeBA (0.5 mg Se/kg)	0.26	0.49	0.53	0.48

The selenium content in eggs homogenates appeared to plateau after 56 days of feeding, after which time no further increases were observed. Average values for the three sampling dates were 0.25 mg Se/kg egg homogenate in the sodium selenite group, 0.37 mg Se/kg (1.5 times the level in the sodium selenite group) in the group supplemented with 0.3 mg Se/kg from HMSeBA and 0.50 mg Se/kg (2 times the level in the sodium selenite group) in the group supplemented with 0.5 mg/kg from HMSeBA. No HMSeBA could be detected in egg homogenates (LOQ 100 ng HMSeBA/g).

3.2.1.5. Tissue deposition study in pigs for fattening

Three groups of six pigs for fattening (both genders; commercial pigs: Dutch Landrace, Finnish Landrace and Piétrain; body weight at start between 36.3 and 53.0 kg) were kept in one pen each and received maize–soybean–barley-type diets supplemented with 0.3 or 0.5 mg Se/kg from HMSeBA or 0.5 mg Se/kg from sodium selenite for 84 days.[53] Analysis showed the selenium content of the diets to be 0.47 mg, 0.65 mg and 0.64 mg total Se/kg feed, respectively. Two pigs (receiving 0.5 mg Se/kg from HMSeBA) died during the trial (one from malignant lymphoma, one from gastric ulcer). After cessation of the treatment, the animals were sacrificed, blood and tissue (liver, kidney, muscle (*M. semitendinosus* and *M. semimembranosus*) and skin+fat) samples were collected. Total selenium and HMSeBA and Se-Met (qualitatively) were analysed in all samples, and GSH-Px activity in muscle.

Differences in body weight gain were not observed (mean: 932 g/day). The average data for total selenium in plasma and tissues, as well for GSH-Px activity in muscle tissue, are summarised in Table 5.

[52] Technical Dossier/Section III/Annex 3-2-5.
[53] Technical Dossier/Section III/Annex 3-2-6.

Table 5: Selenium levels in plasma and tissues and GSH-Px activity in muscle of pigs for fattening after feeding diets supplemented with selenium either from HMSeBA or from sodium selenite for 84 days.

Group	GSH-Px activity*	Selenium levels (mg/kg)				
	Muscle	Plasma	Liver	Kidney	Muscle	Skin+Fat
Sodium selenite (0.5 mg Se/kg)	34.6	0.18	0.62	1.53	0.17	0.06
HMSeBA (0.3 mg Se/kg)	44.2	0.22	0.80	1.50	0.38	0.09
HMSeBA (0.5 mg Se/kg)	52.3	0.24	1.02	1.95	0.55	0.13

* nmol NADPH/min/mL

HMSeBA could not be quantified (LOQ= 100 ng/g) in any sample. Se-Met could not be detected in any plasma or muscle tissue samples. In the group supplemented with sodium selenite, the presence of Se-Met was shown in two out of six skin+fat samples, but not in any other samples (kidney and liver). In the group supplemented with the same selenium level from HMSeBA, Se-Met could be detected in all four liver and kidney samples and in three of four skin+fat samples.

At equivalent dietary selenium concentrations, muscle selenium was about 3.2 times higher in the HMSeBA-supplemented group than in the sodium selenite-supplemented group.

3.2.1.6. Milk deposition study in dairy cows

Twenty-four Holstein cows (209 days in milk, on average, at the beginning of the experiment), were fed three different diets for 53 days, which varied only in sources of selenium.[54] All cows were fed maize silage *ad libitum*, and received an additional silage and concentrate (adjusted to individual milk yield) as a total mixed ration. The feed of the negative control group was not supplemented with selenium. Each cow in the other two groups received 2.4 mg Se/day from sodium selenite or HMSeBA via complementary feed. The selenium supplementation was analytically confirmed. There was no significant influence of the different selenium sources on milk production (average: 35.2 kg/day) or milk quality (average 3.16 % protein and 3.64 % fat). Milk selenium concentration, measured on the last three days of the experiment, in the unsupplemented control group and the groups given selenium either from sodium selenite or from HMSeBA was 0.009, 0.014 and 0.017 mg/kg fresh product, respectively. This result suggests that the potency of selenium from HMSeBA in increasing the selenium level in milk is only 1.2 times higher than that of selenium from sodium selenite, being lower than the potency factor identified for poultry and pig muscle and eggs. However, only a relative low dose of selenium from HMSeBA (2.4 mg/head/day) in dairy cows, with a mean dry matter (DM) intake per day of 22.9 kg—thus corresponding to and intake of only 0.1 mg Se/kg DM—was tested.

The FEEDAP Panel notes that organic selenium such as selenised yeast, in which Se-Met is the predominant selenium form (selenised yeasts contains ≥90% selenium in the form of Se-Met (Schrauzer, 2006)), when used as a selenium source in dairy cows, shows 3.6 higher potency to increase milk selenium content compared to sodium selenite (meta-analysis of 33 studies; Ceballos et al., 2009).

Therefore, in the absence of a dose–response study with HMSeBA in dairy cows and considering (i) Se-Met being a resulting metabolite of HMSeBA and (ii) the established knowledge on Se-Met metabolic fate in food-producing animals, the FEEDAP Panel does not expect that the use of HMSeBA as a source of selenium in nutrition of dairy cows would result in a different pattern of milk selenium deposition than that resulting from the use of selenised yeasts.

[54] Technical Dossier/Section III/Annex 3-2-7.

3.2.1.7. Conclusions on metabolic and tissue/product deposition studies

HMSeBA is converted to selenomethionine; consequently, no residues of the compound itself occur in animal tissues and products. Based on data provided and the metabolic fate of HMSeBA, the FFEDAP Panel concludes that the use of HMSeBA as a source of selenium in feed would lead to significantly higher selenium levels in animal tissues/animal products than the standard inorganic selenium source sodium selenite and thus to levels comparable to those resulting from the use of selenised yeasts.

3.2.2. Toxicological studies

3.2.2.1. Acute toxicity

Three acute oral toxicity trials with HMSeBA were performed in rats[55] and mice,[56] according to OECD guideline 423 ("Acute Toxic Class Method").

In rats the LD_{50} ranged between 10 and 25 mg HMSeBA/kg bw, and in mice between 25 and 50 mg HMSeBA/kg bw, indicating that HMSeBA is hazardous by the oral route. A comparative study in rats[57] indicated that the acute oral toxicity of HMSeBA is comparable to that of L-SeMet and is higher than that of sodium selenite (LD_{50} higher than the top dose level tested, 43.6 mg/kg bw).

3.2.2.2. Genotoxicity studies including mutagenicity

The mutagenic potential of HMSeBA was tested in *Salmonella* Typhimurium strains TA1535, TA1537, TA98, TA100 and TA102 in the presence or absence of metabolic activation (S9 fraction from liver of rats pre-treated with Aroclor 1254), in compliance with OECD guideline 471 (rev. 1997).[58] The test item was tested up to cytotoxic levels. No evidence of mutagenicity was seen in any bacterial strain in two independent experiments, while the concurrent positive controls demonstrated the sensitivity of the assay and the metabolising activity of the liver preparations.

HMSeBA was tested for the ability to induce gene mutations at the thymidine kinase (TK) locus in L5178Y mouse lymphoma cells in the presence and in the absence of metabolic activation (S9 fraction from liver of rats pre-treated with Aroclor 1254), in compliance with OECD guideline 476 (rev. 1997).[59] The test item was tested up to cytotoxic levels. Under the experimental conditions of this study, HMSeBA showed mutagenic activity in the mouse lymphoma assay, in the presence of S9 mix, while in the absence of S9 mix the result remained equivocal.

An *in vitro* chromosome aberrations test was conducted in cultured human lymphocytes with HMSeBA, in two independent experiments (OECD guideline 473, rev. 1997).[60] The cells, isolated from pooled blood taken from healthy male donors, were exposed to the test article both in the presence and in the absence of S9-mix derived from the liver of rats pre-treated with Aroclor 1254. In the first experiment, lymphocyte cultures were exposed to the test or control item (with or without S9 mix) for three hours and then rinsed. Cells were harvested 20 hours after the beginning of the treatment, corresponding to approximately 1.5 normal cell cycles. The second experiment was performed without S9 mix, cells being exposed continuously to the test or control items until harvest, or with S9 mix, cells being exposed to the test or control items for three hours and then rinsed. Cells were harvested 20 hours and 44 hours after the beginning of the treatment, corresponding to approximately 1.5 normal cell cycles and 24 hours later, respectively. The test item was tested at levels up to 2500 µg/mL without S9 mix and at levels up to 1500 µg/mL with S9 mix, as higher levels produced excessive cytotoxicity. In the experiments without S9 no effect was seen after three hours, while a statistically significant increase in the frequency of cells with structural chromosomal aberrations was observed after the 20-hour treatment. The first experiment with S9 mix was negative.

[55] Technical Dossier/Section III/Annex 3-2-8.
[56] Technical Dossier/Section III/Annex 3-2-9.
[57] Technical Dossier/Section III/Annex 3-2-10.
[58] Technical Dossier/Section III/Annex 3-2-11.
[59] Technical Dossier/Section III/Annex 3-2-12.
[60] Technical Dossier/Section III/Annex 3-2-13.

In contrast, a dose-related effect was observed in the second experiment for a 20-hour harvest; after 44-hour treatments a slight, non significant effect, was observed both with and without metabolic activation.

The potential of HMSeBA to induce structural or numerical damage in rat bone marrow cells *in vivo* was tested by the micronucleus test (OECD Guideline 474, 1997).[61] Three groups of five male and five female Sprague–Dawley rats received two oral treatments of HMSeBA at dose levels of 3.75, 7.5 or 15 mg/kg bw per day (males) and 2.5, 5 or 10 mg/kg bw per day (females), at 24-hour intervals. The top dose levels were selected on the basis of the systemic toxicity observed in the preliminary test. The animals were sacrificed 24 hours after the last treatment and bone marrow smears were then prepared. For each animal, the number of the micronucleated polychromatic erythrocytes (MPE) was counted in 2000 polychromatic erythrocytes. No statistically significant increase in the frequencies of MPE was found in the treated animals, when compared with the untreated control, while the positive control (cyclophosphamide) produced the expected response. A significant reduction in the polychromatic/ normochromatic erythrocytes ratios of treated animals indicated that the bone marrow cells were effectively exposed to the test item.

3.2.2.3. Subchronic repeated dose oral toxicity studies

A preliminary dietary 28-day study was performed in Sprague–Dawley rats administered HMSeBA as a dose-finding trial for the ensuing subchronic, 90-day study.[62] Dietary concentrations leading to dose levels ≥ 4 mg/kg bw per day were not tolerated, with animals showing poor condition and liver damage; exposure to 0.9 mg/kg bw per day was tolerated, with animals showing prolonged prothrombin time and lower plasma triglycerides (females), as well as hepatocellular hypertrophy (both sexes). Exposure to 0.25 mg/kg bw was the no observed adverse effect level (NOAEL) of the dose-finding study.

In the 90-day study (performed according to OECD guideline 408), four groups of Sprague–Dawley rats (ten of each sex per group) were exposed through the diet to feed concentrations with mean exposure levels of 0 (unexposed), 0.025, 0.125 and 0.625 mg HMSeBA/kg bw per day. The initial concentration was adjusted weekly according to the mean body weight gains and feed consumption of test animals. Blood chemistry, biochemistry and haematological parameters, gross pathology and histopathology were assessed; full pathology examinations were performed in the high-dose and control animal groups, whereas liver was also examined in mid-dose animals. Slight toxicity was observed at the top dose level only, and mainly in females, namely: higher plasma cholesterol and triglyceride concentrations (females), increased relative liver weight (both sexes) and, centrilobular hepatocellular hypertrophy (females). Dietary exposure to 0.125 mg/kg bw per day was the NOAEL.

3.2.2.4. Conclusions on toxicological studies

At the inclusion levels proposed in feed, HMSeBA is not expected to cause acute toxicity.

The compound resulted negative in the bacterial reverse mutation assay, but displayed genotoxic activity in two *in vitro* assays in mammalian cells: chromosomal aberrations in cultured human lymphocytes with and without metabolic activation, and gene mutations in mouse lymphoma cells with metabolic activation (equivocal results were observed without S9 mix). This genotoxic potential was not confirmed *in vivo*; the *in vivo* micronucleus assay was clearly negative under experimental conditions in which evident toxicity was reported in bone marrow, showing that the target cells were sufficiently exposed to the test item. Therefore, on the basis of the experimental results, the test item should not be considered genotoxic *in vivo*. At the trace levels normally found in biological systems, selenium is a pro-antioxidant and a potential antimutagenic agent, whereas at higher concentrations it may work as pro-oxidant and cause DNA damage. This may explain the positive findings for

[61] Technical Dossier/Section III/Annex 3-2-14.
[62] Technical Dossier/Section III/Annex 3-2-15.

genotoxicity in some *in vitro* tests of the HMSeBA and other forms of selenium in which high levels of local exposure are reached.

A 90-day study in rats indicated that dietary exposure to HMSeBA elicits liver effects, more pronounced in female rats, with Lowest Observed Adverse Effect Level (LOAEL) and NOAEL of 0.625 and 0.125 mg/kg bw per day, respectively.[63] No toxic effects unexpected in a selenium compound were observed. When compared to the selenium upper level on body weight basis (0.3 mg/70 kg= 0.004 mg/kg bw), the NOAEL provides an approximate 30-fold safety margin.

3.2.3. Consumer exposure assessment

The data on metabolism and tissue deposition after application of HMSeBA clearly demonstrate that (i) HMSeBA is a source of available selenium, (ii) no residues of the compound itself occur in animal tissues and (iii) HMSeBA is converted to Se-Met.

HMSeBA at 0.5 mg Se/kg feed increased muscle selenium compared with sodium selenite in chickens by a factor of 4.4, in turkeys by 2.4 and in pigs by 3.2. In eggs, this factor for HMSeBA in relation to sodium selenite was 2. Owing to the limitations of the milk study in dairy cows, an appropriate factor for milk selenium deposition from HMSeBA could not be set. However, the FEEDAP Panel concludes that milk selenium deposition from the use of HMSeBA as feed additive would not be different to that from selenised yeast, thus leading to significantly higher selenium milk levels than those derived from use of an inorganic selenium source, and comparable to those resulting from the use of selenised yeasts, as is the case for the other animal tissue/products tested.

When assessing the consumer safety of selenised yeast-based additives (EFSA, 2011a, b, 2012a), the FEEDAP Panel noted that there are likely no principal differences in the metabolic behaviour of selenium from different selenised yeasts (containing Se-Met as the predominant selenium form) when fed to animals. Selenium deposition resulting from the use of these selenised yeasts as feed additives was considered to result in similar selenium tissue deposition and product concentrations, and significantly higher than those resulting from the use of the inorganic selenium source, sodium selenite. The FEEDAP Panel considers that the HMSeBA factors for selenium deposition in animal edible tissues/products are of the same magnitude as those derived for selenised yeasts.

The above conclusion on the similar behaviour of Se-Met from different selenised yeasts in terms of selenium tissue and product deposition, can, therefore, be extended to HMSeBA. A detailed estimate of consumer exposure to food from HMSeBA-supplemented animals is therefore not necessary.

3.2.4. Conclusions on the safety for consumers

Based on a toxicology testing package (acute toxicity, genotoxicity *in vitro* and *in vivo* and repeated dose toxicity) HMSeBA does not elicit any adverse effects not expected in a selenium compound. HMSeBA is converted to Se-Met; consequently, no residues of the compound itself occur in animal tissues and products. Regarding consumer exposure to selenium from the use of HMSeBA in animal nutrition, the FEEDAP Panel extends its former conclusion on the maximum selenium supplementation of feed from selenised yeasts and states that a maximum supplementation level of 0.2 mg Se/kg feed from HMSeBA would be unlikely to result in a health risk tor consumers, including children of one to three years.

3.3. Safety for the user

The dusting potential, the selenium content of the dust (approximately 1 %) and the respirable fraction of about 25 % indicate that users may be exposed by inhalation to HMSeBA when using the solid form of the additive.

[63] Technical Dossier/Section III/Annex 3-2-16.

In an acute inhalation study on rats performed according to the OECD guideline 403, mortality was 0, 40 and 90 % at Selisseo concentrations of 1.1, 2.5 and 5.5 mg/L air, respectively.[64] Dose-related non-lethal effects were observed in all three treated groups, including ruffled fur, tachypnoea and transient weight loss. The results indicate that Selisseo is hazardous by inhalation.

In a series of good laboratory practice (GLP) studies,[65] the additive resulted only in a slight skin irritation and should not be classified as skin irritant. It was found to be an eye irritant in rabbits. It did not show any sensitisation potential in a mouse local lymph node assay.

3.3.1. Conclusions on the safety for the users

The additive should be regarded as an eye irritant, but should not be classified as skin irritant or skin sensitiser. Inhalation exposure poses a hazard to users; the FEEDAP Panel concludes, therefore, that the formulation and conditions of use of the solid form of the additive should minimise user exposure by inhalation.

3.4. Safety for the environment

The FEEDAP Panel considers that the use of HMSeBA 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 for 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, 2012a), 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.

Tissue deposition of selenium from selenised yeast, in which the predominant selonocompound is Se-Met, was considered to reflect directly only the unspecific incorporation of Se-Met into general body proteins (EFSA, 2006a). After being absorbed, HMSeBA is metabolised to Se-Met; consequently, the metabolic fate of its selenium is, in principle, identical to that of Se-Met from a selenised yeast. Since the utilisation of selenium from Se-Met in body proteins for specific selenium functions is well known, selenium deposition in tissues is taken as indirect proof of selenium bioavailability from each additive with a mode of action based on enriching the Se-Met body pool.

Data from seven studies (four in chickens for fattening, two in laying hens and one in pigs for fattening) have been provided to demonstrate the efficacy of HMSeBA as a source of selenium.

4.1. Chickens for fattening

4.1.1. Study 1

A short-term efficacy/selenium digestibility study was conducted in 96 one-day-old male chickens for fattening (Ross PM3), housed in cages for 24 days and allocated to four dietary treatments (each with 24 replicates of one chicken).[66] A maize–soybean meal-containing diet was supplemented with 0.3 mg Se/kg feed from either sodium selenite, HMSeBA, HMSeBA on starch or HMSeBA on silica (selenium contents confirmed by analysis). Diets were fed *ad libitum* in crumble form (days 0–13) or as pellets (days 14–24). To analyse faecal digestibility of selenium, the excreta were collected from 12

[64] Technical Dossier/Section III/Annex 3-3-1.
[65] Technical Dossier/Section III/Annexes 3-3-2, 3-3-3, 3-3-4, 3-3-5 and 3-3-6.
[66] Technical Dossier/Section IV/Annex 4-1-1.

chickens per treatment during the last three days of trial. At the end of study, breast muscle samples from 12 chickens per treatment were collected.

Owing to the short duration of the study, zootechnical performance data could not be considered. The apparent faecal selenium digestibility was increased from about 23 % (sodium selenite) to 50 % (HMSeBA) while no differences were found between various HMSeBA sources. In comparison with the sodium selenite treatment, selenium in muscle was significantly increased in the HMSeBA treatments from about 0.3 to 1.2 mg Se/kg DM.

4.1.2. Study 2

A long-term efficacy study was conducted with 600 one-day-old male chickens for fattening (Ross PM3), housed in floor pens for 42 days and allocated to four dietary treatments (six replicates/treatment, 25 chickens per pen/replicate).[67] A basal maize–soybean meal diet (background selenium content 0.07 mg/kg) was supplemented with 0 or 0.3 mg Se/kg feed from selenite or with 0.1 or 0.3 mg Se/kg feed from HMSeBA. The selenium supplementation was confirmed by feed analysis. Diets were fed *ad libitum* as pellets (as crumbles days 0–5) in two phases (starter: days 0–21; finisher: days 21–42). Animal performance was recorded. At day 42, samples of blood, breast muscle and liver from two birds per pen were collected for analysis of selenium level and speciation of selenocompounds. The quality of meat (breast samples from three birds per pen) stored at 4°C for up to eight days was assessed by measurements of pH, drip loss, thiobarbituric acid-reactive substances (TBARS) and colour.

Mortality ranged between 2.0 and 5.3 %, and was not related to treatments. The statistical analysis did not show any effect on final body weight (mean 3347 g), feed intake (mean 6575 g) or feed/gain ratio (mean 1.97). All groups treated with additional selenium showed a significantly higher selenium concentration in plasma and tissues ($P< 0.05$) than the unsupplemented control. The significantly highest selenium concentrations in plasma and tissue samples were found in birds treated with a higher supplementation level of selenium from HMSeBA. The predominant selenocompound found in plasma and liver was Se-Cys whilst in muscle Se-Met seemed to be the main selenocompound. No significant differences between the treatments were observed on ultimate pH, drip loss, TBARS and meat colour.

4.1.3. Study 3

Data on efficacy are derived from a report on a combined tolerance–efficacy study carried out in chickens for fattening (for description see Section 3.1.1.1).[68] In this study the comparison of birds fed an unsupplemented diet and groups on diets supplemented with 0.3 mg Se/kg feed either from sodium selenite or HMSeBA (Selest®), showed a significant increase in plasma GSH-Px activity in both supplemented groups compared with the control group, but without significant differences between the sources of selenium. Liver and muscle selenium increased with rising dietary selenium from both sources, as did kidney and skin+fat selenium. Supplementation with HMSeBA resulted in considerably and significantly higher tissue selenium concentrations than supplementation with sodium selenite. Selenium speciation (for treatment 0.5 mg supplemental Se/kg from HMSeBA) indicated that Se-Met and Se-Cys were present in similar concentrations in muscle and skin+fat, whereas significantly higher Se-Cys concentrations were found in liver and kidney. The selenium level from both Se-Met and Se-Cys was not statistically different from total selenium, indicating that HMSeBA was effectively converted to Se-Met (and further to Se-Cys).

[67] Technical Dossier/Section IV/Annex 4.1.3.
[68] Technical Dossier/Section IV/Annex 4.1.4.

4.2. Laying hens

4.2.1. Study 1

A study was conducted in 160 ISA Brown laying hens (initial age 40 weeks), housed in pens for 56 days and allocated to four dietary treatments (eight replicates of five hens).[69] The unsupplemented maize and soybean meal-based diet with background selenium content of 0.07 mg/kg (control) was enriched with 0.2 mg Se/kg from sodium selenite or with 0.1 or 0.2 mg Se/kg feed from HMSeBA. Selenium supplementation was confirmed by analysis. Diets were fed *ad libitum*. On the last three days, three eggs per pen were collected for analysis of selenium content. The eggs from hens fed supplemented diets showed significantly higher selenium content than those from control birds on unsupplemented diet ($P< 0.05$). In hens supplemented with the highest HMSeBA dose, the egg selenium content was significantly the highest (1.1 mg /kg DM) whereas eggs from unsupplemented control group showed only 0.3 mg Se/kg DM.

4.2.2. Study 2

A study was conducted in 60 ISA Brown laying hens (initial age 30 weeks), individually housed in pens and allocated to five dietary treatments (12 replicates with one hen in each).[70] A control unsupplemented diet based on maize and soybean meal (background selenium content: 0.07 mg/kg) was supplemented with 0.1 or 0.3 mg Se/kg feed either from sodium selenite or HMSeBA. Selenium supplementation was confirmed by analysis. Diets were fed *ad libitum*. After a 14-day adaptation period, the collection of eggs started at the beginning of experimental phase and continued at two-week intervals for 56 days. Owing to the short duration of the trial, laying performance (97 % laying rate on average) is not further considered. For each collection day, the significantly lowest selenium concentration was observed in eggs from the control unsupplemented group and the highest in eggs from hens supplemented with 0.3 mg Se/kg feed from HMSeBA. In general, the response in egg selenium was dose dependent. However, at the supplementation level of 0.3 mg Se/kg feed, HMSeBA resulted in significantly higher selenium deposition in eggs than did sodium selenite.

4.3. Pigs for fattening

A study was conducted with a total 80 female pigs (Piétrain × [Large white × Landrace], initial age 11 weeks, mean bw 25 kg) in two different blocks/periods, each lasting 36 days. Animals were housed in pens and allocated to five dietary treatments (four replicates of two pigs).[71] A control unsupplemented diet based on barley, wheat, maize, soybeanmeal and canola (background selenium content 0.11 mg/kg) was supplemented with 0, 0.1 or 0.3 mg Se/kg feed either from sodium selenite or HMSeBA (selenium contents confirmed by analysis). Diets were fed *ad libitum*. At the beginning of the trial, three days of progressive intake of supplemented diets were allowed as an adaptation period. After 36 days of feeding experimental diets, pigs were slaughtered and samples of blood for selenium and GSH-Px analysis as well as liver for selenium analysis were collected from all animals.

Although no significant differences were observed in growth performance parameters (mean body weight gain 26 kg; mean feed intake 1600 g/day; mean feed/gain ratio 2.21), owing to the short duration of the study, these performance data are not further considered. Since there were no differences in parameters of selenium metabolism between the two experimental blocks, the cumulated mean values were considered for each parameter. Selenium level and GSH-Px activity in plasma were significantly increased in animals supplemented with HMSeBA. At the highest dose of selenium from the additive, plasma selenium concentration was higher for HMSeBA treatment than for sodium selenite. In liver, selenium concentration was significantly higher in the HMSeBA-treated pigs than in pigs receiving either the sodium selenite or the control treatments (Table 6).

[69] Technical Dossier/Section IV/Annex 4.1.5.
[70] Technical Dossier/Section IV/Annex 4.1.6.
[71] Technical Dossier/Section IV/Annex 4.1.7.

Table 6: Effect of feed supplementation (for 36 days) with selenium from sodium selenite or from HMSeBA on selenium levels in plasma and liver, and GSH-Px activity in blood of pigs for fattening

Source of Se	**Control**	**Sodium selenite**		**HMSeBA**	
Se supplemented (mg/kg feed	**-**	**0.1**	**0.3**	**0.1**	**0.3**
Se analysed (mg/kg feed)					
Block 1	0.11	0.20	0.38	0.21	0.41
Block 2	-	-	-	0.21	0.38
Plasma Se (mg/L)	0.09^{a}	0.12^{b}	0.12^{b}	0.13^{b}	0.16^{c}
Blood GSH-Px activity (U/g Hb)	116^{b}	135^{ab}	152^{a}	146^{a}	138^{a}
Liver Se (mg/kg DM)	1.3^{d}	1.7^{c}	1.8^{c}	2.0^{b}	2.7^{a}

a, b, c, d: Means with different superscript letters within a row are significantly different ($P \leq 0.05$).

4.4. Conclusions on efficacy

The applicant has demonstrated in several studies that HMSeBA is an efficacious source of selenium, showing the response in GSH-Px activity. Therefore, the FEEDAP Panel concludes that HMSeBA is an efficacious source of selenium for all animal species/categories. The use of the additive in animal nutrition does not modify the quality of meat as measured by physico-chemical properties.

5. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[72] and Good Manufacturing Practice

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

The additive hydroxy-analogue of selenomethionine (HMSeBA) is a safe source of selenium for all animal species/categories when used up to maximum authorised total selenium content in complete feed. Considering the zootechnical performance, haematology and biochemistry as endpoints in studies on chickens for fattening, turkeys and piglets, a margin of safety of ten was identified.

Based on a toxicology testing package (acute toxicity, genotoxicity *in vitro* and *in vivo* and repeated dose toxicity) it is concluded that HMSeBA does not elicit adverse unexpected effects for a selenium compound. After being absorbed, HMSeBA is metabolised to Se-Met; consequently, no residues of the compound itself occur in animal tissues and products. Compared with inorganic selenium sources, the use of HMSeBA in animal nutrition would result in a similar increase in selenium deposition in animal tissues/products as that resulting from selenised yeast. To ensure consumer safety from consumption of food originating from animals supplemented with HMSeBA, the FEEDAP Panel concludes that dietary selenium supplementation from the additive should not exceed a maximum of 0.2 mg Se/kg complete feed.[73]

The additive should be regarded as an eye irritant, but should not be classified as skin irritant or skin sensitiser. Inhalation exposure poses a hazard to users; the FEEDAP Panel concludes, therefore, that the formulation and conditions of use of the solid form of the additive should minimise user exposure by inhalation.

[72] 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.

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

The use of HMSeBA 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.

HMSeBA is an efficacious source of selenium for all animal species/categories, as it has been demonstrated in several studies showing the response in GSH-Px activity. The use of the additive in animal nutrition does not modify the quality of meat as measured by physico-chemical properties.

RECOMMENDATIONS

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

- The trivial name of the additive should be: Hydroxy-analogue of selenomethionine.
- The incorporation of the additive into feed should be made via premixtures only; this recommendation concerns both the solid and liquid forms of the product.

The maximum content for total selenium in feed is set by legislation. The conclusions of the FEEDAP Panel on the safety of hydroxy-analogue of selenomethionine 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 only be achieved if the total dietary selenium content is known, which is normally not the case. The FEEDAP Panel therefore recommends not to use the hydroxy-analogue of selenomethionine via water for drinking, also considering the absence of stability data of the additive in water for drinking.

Exposure of users by inhalation should be avoided.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier Selenium: SELISSEO®. April 2011. Submitted by ADISSEO France S.A.S.
2. Dossier Selenium: SELISSEO®. July 2012. Submitted by ADISSEO France S.A.S.
3. Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Hydroxy-Methyl-Seleno-Butanoic-Acid (HMSeBA).
4. 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 Hydroxy-Methyl-Seleno-Butanoic-Acid (HMSeBA)[74]

In the current application authorisation is sought under article 4(1) for *Hydroxy-Methyl-Seleno-Butanoic-Acid* (HMSeBA), under the category/functional group 3(b) 'nutritional additives'/'compounds of trace elements' according to the classification system of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of *HMSeBA* for all animal species and categories.

The *feed additive* is a preparation consisting of a minimum of 5 % R,S-2-hydroxy-4 methylseleno butanoic acid ($C_5H_{10}O_3Se$) and a maximum of 95 % colloidal silica carrier. The organo seleno compounds consist of 99 % HMSeBA monomer and 1% dimers; thus corresponding to 40 % *total selenium* in HMSeBA or to 2 % *total selenium* in the *feed additive*. The preparation, marketed as a liquid or a powder forms, is intended to be incorporated into *premixtures*, compound *feedingstuffs* or *water* to obtain a maximum *total selenium* dosage of 0.25 mg/L *water* or 0.5 mg/kg *feedingstuffs*, thus complying with legal requirements; no minimum dose was proposed by the Applicant.

For the determination of HMSeBA in the active substance (HMSeBA *per se*) and in the *feed additive* (preparation) the Applicant submitted a validated and further verified method, based on High Performance Liquid Chromatography coupled to UV detection at 220 nm (HPLC-UV). The following performance characteristics were reported: - a relative standard deviation for *repeatability* (RSD_r) ranging from 0.21 to 1.3 %; - a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 0.83 to 1.3 %; and – a recovery rate (R_{Rec}) ranging from 89 to 102 %. Based on the experimental evidence provided the EURL recommends for official control the validated and further verified gradient HPCL-UV method for the determination of HMSeBA in the *active substance* and in the *feed additive*.

For the determination of *total selenium* in the *feed additive* and in the *active substance* the Applicant submitted the validated and further verified method developed by the UT2A laboratory - already evaluated and recommended by the EURL - based on microwave digestion using nitric acid and hydrogen peroxide (HNO_3/H_2O_2) followed by inductively coupled plasma mass spectrometry (ICP-MS), for which the following performance characteristics were reported: - R_{rec} ranging from 94 to 95 %; and - RSD_{ip} ranging from 1.5 to 2.5 %. However, an alternative method has already been evaluated and recommended by the EURL, based on inductively coupled plasma atomic emission spectrometry (ICP-AES) for which the following performance characteristics were reported: - 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 %.

For the determination of *total selenium* in *premixtures* and *feedingstuffs* the Applicant submitted the CEN standard method EN 16159:2012 – already recommended in previous EURL reports - based on Hydride Generation Atomic Absorption Spectrometry (HGAAS) after microwave digestion with HNO_3/H_2O_2. 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.

The Applicant did not submit any methods for the determination of *total selenium* in *water* However, the EURL already recommended the method approved by the National Institute for Occupational

[74] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2011-0044.pdf

Safety and Health (NIOSH) and described in their Manual of Analytical Methods (NMAM), based on ICP-AES, for which a limit of detection (LOD) of 0.02 mg/L is reported.

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.

ABBREVIATIONS

AP	alkaline phosphatase
ALT	alanine transaminase
AST	aspartate transaminase
bw	body weight
CFU	colony-forming unit
CNCM	Collection Nationale de Cultures de Microorganismes
CV	coefficient of variation
Cys	cysteine
CK.	creatine kinase
DM	dry matter
DEM	diethoxymethane
DNA	deoxyribonucleic acid
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
GSH-Px	glutathione peroxidase
GGT	γ-glutamyltransferase
GLP	Good Laboratory Practice
HMSeBA	*R,S*-2-hydroxy-4-methylselenobutanoic acid
HPLC	high-performance liquid chromatography
HCT	haematocrit
Hb	haemoglobin
ICP-MS	inductively coupled plasma mass spectrometry
K	potassium
LOAEL	lowest observed adverse effect Level
LOD	limit of detection
LOQ	limit of euantification
LD_{50}	lethal dose for 50 % of the population
LDH	lactate dehydrogenase
MCV	mean corpuscular volume
Met	methionine
MPE	micronucleated polychromatic erythrocyte
Na	sodium
NADPH	nicotinamide adenine dinucleotide phosphate
NE	normochromatic erythrocyte
NMR	nuclear magnetic resonance
NCYC	National Collection of Yeast Cultures
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
PCBs	polychlorinated biphenyl
PE	polychromatic Erythrocyte

RH	relative humidity
RBC	red blood eell
Se	selenium
Se-Cys	selenocysteine
Se-Met	selenomethionine
TBARS	thiobarbituric acid-reactive substances
tBIL	total bilirubin
TMR	total mixed rations
TK	thymidine kinase
UV	vltraviolet
VICH	Veterinary International Cooperation on Harmonization
WBC	white blood cell