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SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of DL-selenomethionine as a 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

DL-Selenomethionine (DL-SeMet) consists of 40 % selenium, an essential trace element. DL-SeMet. It was tolerated by chickens for fattening at up to 1.5 mg selenium supplemented/kg feed; DL-SeMet is therefore safe for chickens for fattening provided total dietary selenium does not exceed 0.5 mg/kg complete feed; this conclusion is extended to all animal species. Based on available toxicity studies and previous assessments of closely related compounds, it is concluded that selenium from DL-SeMet does not elicit any adverse effects not expected in a selenium compound. The use of DL-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 DL-SeMet, dietary selenium supplementation from the additive should not exceed a maximum of 0.2 mg Se/kg complete feed. Although a DL-SeMet-containing additive did not release any measurable dust, the additive is considered as a hazard by inhalation, which requires protection measures for users since the additive is not the subject of authorisation, and selenium is highly toxic. The additive is not an irritant to skin and eyes and is not a dermal sensitiser. The use of DL-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 complete feed is not exceeded. Sufficient evidence is provided that DL-SeMet is an effective source of selenium in chickens for fattening. Since there are no fundamental differences between target animals in the metabolism of SeMet and its use for the specific biological functions of selenium, the FEEDAP Panel extends its conclusion on the efficacy of DL-SeMet to all animal species and categories.

KEY WORDS

nutritional additive, compounds of trace elements, DL-selenomethionine, safety, efficacy

[1] On request from the European Commission, Question No EFSA-Q-2012-00914, adopted on 30 January 2014.

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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 DL-selenomethionine (Mintrex® Se) as feed additive for all animal species.

DL-Selenomethionine (DL-SeMet) consists of 40 % selenium, an essential trace element.

DL-SeMet was tolerated by chickens for fattening at up to 1.5 mg Se supplemented/kg feed; DL-SeMet is therefore safe for chickens for fattening provided total dietary selenium does not exceed 0.5 mg/kg complete feed. The FEEDAP Panel extends this conclusion to all animal species.

Based on available toxicity studies and previous assessments of closely related compounds, the FEEDAP Panel concludes that selenium from DL-SeMet does not elicit any adverse effects not expected in a selenium compound. The use of DL-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 DL-SeMet, dietary selenium supplementation from the additive should not exceed a maximum of 0.2 mg/kg complete feed.

Although a DL-SeMet-containing additive did not release any measurable dust, the additive is considered as a hazard by inhalation, which requires protection measures for users since the additive is not the subject of authorisation, and selenium is highly toxic. The additive is not an irritant to skin and eyes and is not a dermal sensitiser.

The use of DL-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 complete feed is not exceeded.

Sufficient evidence is provided that DL-SeMet is an effective source of selenium in chickens for fattening. Since there are no fundamental differences between target animals in the metabolism of SeMet and its use for the specific biological functions of selenium, the FEEDAP Panel extends its conclusion on the efficacy of DL-SeMet to all animal species and categories.

The FEEDAP Panel made some recommendations concerning the description and characterisation of the additive, and its use in premixtures only.

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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 Novus Europe SA/NV[5] for authorisation of DL-selenomethionine (Mintrex®Se), 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).[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 March 2013.

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. An 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). Other opinions on the safety and efficacy of hydroxy-analogue of selenomethionine as feed additive for all species (EFSA FEEDAP Panel, 2013a) and on the safety and efficacy of L-selenomethionine as feed additive for all animal species (EFSA FEEDAP Panel, 2013b) have been delivered by the FEEDAP Panel. Following opinions of the FEEDAP Panel on organic selenium-based additives, the EC has issued two Regulations[11] in which the supplementation of organic selenium in complete feed is limited to 0.2 mg Se/kg.

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 DL-selenomethionine (Mintrex®Se), 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] Novus Europe SA/NV, Woluwe Atrium, Neerveld 101-103, 1200 Brussels, Belgium.
[6] EFSA Dossier reference: FAD-2012-0042.
[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.
[11] Commission Implementing Regulation (EU) No 427/2013; Commission Implementing Regulation (EU) No 445/2013.

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

Additive	DL-selenomethionine
Registration number/EC No/No (if appropriate)	3b8.xx
Category(-ies) of additive	Nutritional feed additive
Functional group(s) of additive	Compounds of trace elements

Description			
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)
Preparation of DL-selenomethionine (DL-SeMet)	DL-SeMet 0.5% (5,000 mg DL-SeMet/kg supplying 2,000 mg Se/kg)	Complies with EU feed hygiene law	HPLC-UV for DL-SeMet, ICP-MS for Se

Trade name (if appropriate)	Mintrex® Se
Name of the holder of authorisation (if appropriate)	Novus Europe SA/NV

Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg/kg of complete feedingstuffs		
All species and categories	No maximum age	No minimum content	0.5 mg Se/kg feed from all sources (endogenous and added)	Not applicable

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use (if appropriate)	Store in a cool, dry place (< 25ºC), in closed, original packaging
Specific conditions or restrictions for handling (if appropriate)	Avoid contact with eyes or skin. Breathing protection during handling, safety glasses and gloves
Post-market monitoring (if appropriate)	As per EU feed hygiene regulation: traceability, HACCP-based quality control, formal product/service complaints procedure, and product recall capability
Specific conditions for use in complementary feedingstuffs (if appropriate)	Final feed (12% dry matter basis) should contain not more than 0.5 mg Se/kg, from all sources (endogenous and added)

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

ASSESSMENT

1. Introduction

Several selenium compounds are authorised as feed additives in the EU. The total dietary selenium is limited to 0.5 mg/kg and the addition of organic selenium compounds to 0.2 mg Se/kg.[12]

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 which is essential for vertebrates and 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 to reconsideration of its previous opinion.

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

The FEEDAP Panel has recently adopted an opinion on L-selenomethionine as feed additive for all animal species (EFSA FEEDAP Panel, 2013b). The EFSA's Panel on Food Additives and Nutrient Sources added to Food (EFSA, 2009b) delivered an opinion on L-selenomethionine for use in food supplements; L-selenomethionine is authorised by Commission Regulation (EC) No 1170/2009[13] as mineral which may be used in the manufacture of food supplements.

2. Characterisation

For compounds of trace elements, the element itself is considered the active substance.

2.1. Characterisation of the additive

The additive is intended to be marketed in a solid form with a composition of 0.5 % DL-selenomethionine (DL-SeMet) and 99.5 % carrier, the latter corresponding, by specification, to 99 % calcium carbonate and 0.5 % mineral oil. The intended selenium concentration in the finished product is 2000 mg/kg.

During the course of the assessment the applicant changed the formulation of the additive (the calcium carbonate with a changed granulometry). The data submitted by the applicant on this section, with the exception of the second set of data on particle size distribution and dusting potential, were derived from the additive as initially formulated.

The analysis of five batches of the additive[14] showed a mean content of 5297 mg/kg of SeMet (range 5155–5415) and 2146 mg Se/kg (range 2082–2192). The additive is a solid white to tan powder. It is practically insoluble in water (0.013 g/L at 18 °C). The bulk density (three batches) is 1.55–1.60 kg/L.[15]

Heavy metals, arsenic and dioxins and dioxin-like polychlorinated biphenyls (PCBs) were measured in three batches of the additive. The results per kg additive were: 0.98–1.13 mg lead, <0.5 mg cadmium, <0.02 mg mercury, < 0.50–0.52 mg arsenic,[16] 0.09–0.39 ng PCDD/F-WHO-TEQ/kg, 0.029–0.142 ng

[12] Commission Implementing Regulation (EU) No 427/2013; Commission Implementing Regulation (EU) No 445/2013.
[13] 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.
[14] Technical Dossier/Section II /Annex_II_1_3_2.
[15] Technical Dossier/Section II /Annex_II_1_5_2.
[16] Technical Dossier/Section II/ Annex_II_1_4_2_2.

dioxin-like PCBs-WHO-TEQ/kg and 0.119–0.535 ng for the sum of dioxins plus dioxin like PCBs.[17] The cadmium, lead and arsenic contents in the additive, as well as dioxins and the sum of dioxins and dioxin-like PCBs were below the limits set in Directive 2002/32/EC.[18] The concentration of mercury is not of safety concern. The analysis of five batches of the additive for residual solvents resulted in a content of < 3 mg/kg additive;[19] this value is far below that indicated by the European Agency for the Evaluation of Medicinal Products as concentration limits for the relevant single Class 2 solvents (EMEA, 2000).

Microbial contamination was examined in three batches and was shown to be very low (aerobic plate count, 30°C: < 100 CFU/g; anaerobic count, 30 °C: < 10 colony-forming units (CFU)/g; coagulase-positive Staphylococci counts at 27 °C: < 10 CFU/g; *Clostridium perfringens*, yeasts, moulds and *Enterobacteriaceae*: < 10 CFU/g). *Salmonella* spp. were not detected in 25 g.[20]

Particle size was determined by laser diffraction in three lots. Ten percent of the particles (v/v) showed a diameter < 311–384 µm.[21] The dusting potential measured (as determined using the Stauber-Heubach method) in the same three lots amounted to 0.110–0.124 g/m^3.[22] The median particle diameter of the dust was 9 µm.[23] Upon request of EFSA, the applicant provided information on particle size distribution and dusting potential in other two batches of the additive. The data showed that 10 % of the particles (v/v) had a diameter < 549–565 µm;[24] a dusting potential in the same batches could not be detected.[25] These new data were derived from analysis in the modified formulation of the additive mentioned above, as well as the bulk density which was 1.49 kg/L.[26]

The FEEDAP Panel notes that the calcium carbonate used in the new formulation of the additive could have an impact on the product characterisation, notably on the impurities level. The applicant did not submit any new analyses performed with the new formulated additive, however he stated that the manufacturer has in place an appropriate hazard analysis and critical control points (HACCP) system and quality control procedures to guarantee the compliance with the undesirable substances legislation.

2.2. Characterisation of the DL-selenomethionine

The compound of trace element in the additive is DL-SeMet, the racemic mixture of the D and L enantiomers. The molecular formula is $C_5H_{11}NO_2Se$, Chemical Abstracts Service (CAS) number 2578-28-1, the International Union of Pure and Applied Chemistry (IUPAC) name is (RS2)-2-amino-4-methylselenylbutanoic acid and it has a molecular weight of 196.11 g/mol. The theoretical selenium content is 40.26 %. The molecular structure of DL-SeMet is given in Figure 1. The melting point is 267–269 °C; the boiling point is 320.8 °C.

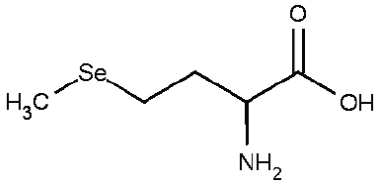

Figure 1: SeMet molecular structure

[17] Technical Dossier/Section II/ Annex_II_1_4_2_3.
[18] 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.
[19] Technical Dossier/Section II/ Annex_II_1_4_2_4
[20] Technical Dossier/Section II/ Annex_II_1_4_2_1.
[21] Technical Dossier/Section II /Annex_II_1_5_1.
[22] Technical Dossier/Section II/ Annex_II_1_5_4.
[23] Technical Dossier/Section II/ Annex_II_1_5_1.
[24] Technical Dossier/Supplementary information/Annex_II_1_5_5.
[25] Technical Dossier/ Supplementary information/Annex_II_1_5_8.
[26] Technical Dossier/Supplementary information/Annex_II_1_5_6.

The purity of the DL-SeMet under assessment is specified as ≥ 97 %. In five batches of DL-SeMet analysed, purity was ≥ 99 %.[27] Analytical data for heavy metals and arsenic, dioxins and dioxin-like PCBs for the compound DL-SeMet were requested but not submitted.

2.3. Manufacturing process[28]

The manufacturing process of the DL-SeMet is fully described in the technical dossier.[29] For the manufacturing of the final additive, DL-SeMet is blended with calcium carbonate (99 %) and mineral oil (0.5 %).[30]

2.4. Stability and homogeneity

2.4.1. Stability

The shelf-life of the additive in metallised polyester bags was tested in three lots under standard (25 °C, 60 % relative humidity (RH)) and accelerated conditions (40 °C, 75 % RH) for three and six months.[31] Recovery rates of SeMet (percentage of the initial value) after six months storage ranged from 97 to 100 % when stored under standard conditions, and from 93 to 100 % when stored under accelerated conditions.

The stability of the additive was tested in a standard vitamin-trace elements premixture (with choline chloride) for chickens for fattening, supplemented with 150 mg DL-SeMet (60 mg Se) per kg; the samples of three lots were stored under standard conditions (25 °C, 60 % RH) up to six months.[32] One sample was frozen for 11 months before analysis, and will not be considered. Recovery of SeMet (two lots) after six months storage at 25 °C and 60 % RH ranged from 70 to 106 % of the expected value.

The stability of the additive (three lots) in feedingstuffs was examined in a complete corn-soybean feed for chickens for fattening for three months. The additive was added via a premixture consisting of the basal feed to provide 0.75 mg DL-SeMet/kg (0.3 mg Se/kg). Stored under standard conditions (25 °C and 60 % RH), the recovery of SeMet in mash feed ranged between 97 and 120 % of the analysed initial value. One lot of the additive in a pelleted sample showed a comparable stability over three months.[33] Data on the stability of SeMet during feed processing were not submitted.

2.4.2. Homogeneity

The capacity of the additive to homogeneously distribute in mash and pelleted feed (unknown composition) was studied by analysing ten samples of each of the different feed types.[34] An average content of 0.596 mg SeMet/kg in mash feed and 0.748 mg SeMet/kg in pelleted feed was found. The coefficients of variation were 9.1 and 11.7 % for mash and pelleted feed, respectively.

Homogeneity tests were conducted with the product in its initial formulation. The FEEDAP Panel cannot exclude the possibility that the change of product formulation (leading to a higher particle size) may have an impact on the homogeneity; however, no new data were submitted.

2.5. Physico-chemical incompatibilities in feed

Based on current knowledge, under practical use conditions, no incompatibilities between DL-SeMet and feed materials, carriers, other approved additives, or medicinal products are expected.

[27] Technical Dossier/Section II/ Annex_II_2_1; Technical Dossier/Supplementary information/Annex_II_2_1_1
[28] This section has been edited following the confidentiality claims made by the applicant.
[29] Technical Dossier/Section II/ Annex_II_3_1_1
[30] Technical Dossier/Section II/ Annex_II_3_2_1
[31] Technical Dossier/Supplementary information/Annex II_4_1_1
[32] Technical Dossier/Supplementary information/Annex II_4_1_2
[33] Technical Dossier/Supplementary information/Annex II_4_1_3
[34] Technical Dossier/Section II/ Annex_II_4_2_1

2.6. Conditions of use

The additive is intended to be used in all animal species and categories as a source of selenium. Total selenium in complete feed should not exceed 0.5 mg/kg.[35]

2.7. 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 selenium from DL-SeMet from Mintrex®Se in animal feed. The executive summary of the EURL report can be found in the Appendix.

3. Safety

3.1. Safety for the target species

3.1.1. Tolerance study

A combined tolerance/efficacy study with DL-SeMet was performed on a total of 672 one-day-old chickens for fattening (Ross 308, equal number of each sex, 40 g initial body weight) for 38 days.[36] The birds were allocated to eight treatments (84 chickens per treatment): a basal diet without selenium supplementation (control, 0.04 mg Se/kg feed); the basal diet supplemented with 0.3 mg Se/kg from sodium selenite (positive inorganic (IO) control); the basal diet supplemented with 0.3 mg Se/kg from a selenised yeast-based additive (positive organic (O) control); and the basal diet supplemented with 0.1, 0.3, 0.6, 0.9 and 1.5 mg Se/kg from DL-SeMet (treatment codes: SeMet-0.1 to SeMet-1.5). A starter diet was fed for the first 21 days followed by a grower feed until completion. Both diet types, fed in mash form *ad libitum*, consisted mainly of corn, soybean meal and soy oil. The starter feed was calculated to contain 21.5 % crude protein (CP) and 11.89 MJ metabolisable energy (ME)/kg, the grower 20.5 % CP and 12.60 MJ ME/kg. The diets were analysed for nutritional homogeneity (confirmed by proximate analysis), and selenium. Group size was six male and six female pens with seven birds per pen. The birds were allocated at random to the pens, and the pens by a random complete block design to the treatments.

Zootechnical parameters[37] were measured on day 36. On day 37, blood samples from one bird per pen were taken for routine haematology and clinical biochemistry,[38] analysis of plasma selenium and plasma glutathione peroxidase (GSH-Px); liver samples also from one bird per pen were analysed for GSH-Px. On day 38, samples of tissues (liver, kidney, muscle, skin/fat) were taken from one bird per pen for determination of selenium concentration (except groups SeMet-0.9 and SeMet-1.5). Necropsy (with particular attention to crop, proventriculus, gizzard, small intestine, caeca, large intestine, liver, kidney, lung, spleen, heart, bone (tibia) and feet) was performed on another two birds from each pen. Analysis of selenium in feed and tissues was carried out by inductively coupled plasma mass spectrometry (ICP-MS) (validated internal method).

Data were subjected to factorial analysis of variance (ANOVA) to examine the main effect of dietary treatment, sex and their interactions using the generalised linear model (GLM) procedure of SAS. The pen was the experimental unit.

The results relevant for assessing the tolerance of chickens for fattening to DL-SeMet are summarised in Table 2. Overall mortality (including culling) was very low (0.89 %). No significant effect of treatment on any of the zootechnical parameters assessed could be detected when analysing male and female broilers together. However, a significant interaction effect between dietary treatment and sex

[35] In recent regulations, the EC has limited the supplementation to feed from other organic sources of selenium to 0.2 mg Se/kg (see footnote 11).
[36] Technical Dossier/Section III/Annex_III_1_1_1.
[37] Average daily weight gain, average feed intake and feed to gain ratio.
[38] Total blood cell count, white blood cell count, haemoglobin, haematocrit, aspartate aminotransferase, alanine aminotransferase, gamma glutamyl transferase, glucose, uric acid, albumin, total protein.

was detected for some parameters. Male chickens of the SeMet-0.1 group gained significantly more weight than male broilers of the SeMet-0.6 group. Additionally, male broilers of the SeMet-1.5 group tended to gain more weight than male broilers of the SeMet-0.6 group ($P = 0.052$). Dietary treatments did not have a significant effect on the performance of female broilers.

Table 2: Zootechnical data (36 days), plasma Se and GSH-Px in plasma and liver, haematology and biochemistry endpoints with significant differences in chickens for fattening (37 days)

Code	Control	IO Control	O Control	SeMet-0.1	SeMet-0.3	SeMet-0.6	SeMet-0.9	SeMet-1.5
Se source	none	Na_2SeO_3	Se-Yeast	DL-SeMet				
Se supplemented (mg/kg)	0	0.3	0.3	0.1	0.3	0.6	0.9	1.5
Se analysed								
- starter (mg/kg)	0.035	0.302	0.328	0.167	0.373	0.737	0.994	1.675
- grower (mg/kg)	0.046	0.303	0.390	0.139	0.353	0.653	0.929	1.760
Mortality (%)	0.0	0.0	0.0	2.4	0.0	1.2	2.4	1.2
Body weight (g)	1643	1664	1660	1713	1685	1628	1706	1711
Feed intake (g/day)	70	70	70	70	70	68	71	71
Feed/gain	1.58	1.55	1.55	1.55	1.54	1.55	1.54	1.53
Plasma Se (µg/kg)	41.2^{f}	136.8^{de}	153.8^{d}	93.6^{e}	184.8^{cd}	226.8^{bc}	270.7^{b}	365.7^{a}
Plasma GSH-Px*	n.d.	344.6^{a}	417.5^{a}	182.8^{b}	374.6^{a}	322.8^{ab}	334.4^{a}	443.2^{a}
Liver GSH-Px*	317.2^{d}	554.8^{a}	514.3^{ab}	318.8^{d}	435.3^{bc}	420.8^{c}	386.3^{cd}	431.1^{bc}
WBC** (10^9/L)	26.6^{ab}	32.3^{a}	26.8^{ab}	27.4^{ab}	22.1^{ab}	20.3^{b}	16.0^{b}	20.8^{ab}
ALT** (U/L)	31.9^{a}	27.5^{ab}	28.7^{ab}	24.6^{ab}	29.8^{a}	22.6^{ab}	23.4^{ab}	18.3^{b}
γGT** (U/L)	20.7^{b}	31.6^{a}	25.6^{ab}	26.2^{ab}	27.3^{ab}	28.4^{ab}	23.5^{ab}	20.8^{b}

* In nmol NADP/mL per min; n.d.= not detectable.
** WBC: White blood cells; ALT: Alanine amino transferase; γGT: Gamma-glutamyltransferase.
a-f: Values with different letter superscripts are significantly different ($P < 0.05$).

The selenium content in plasma was significantly higher in all SeMet groups than in the unsupplemented control group. Plasma selenium concentration in the SeMet groups showed a dose dependent increase. There was no statistically significant difference between the SeMet-0.3 group and the IO and O control groups fed diets with the same level of selenium supplementation (0.3 mg/kg) but different selenium sources (DL-SeMet, sodium selenite, selenium enriched yeast). However, a tendency for higher plasma selenium could be seen in the SeMet-0.3 group compared with the IO control ($P = 0.084$). No significant interaction between sex and treatment was observed. Plasma GSH-Px in the control group was below the detection limit. It increased significantly from 182.8 nmol/mL per min in the SeMet-0.1 group to 374.6 in the SeMet-0.3 group. This value was not significantly different from that in the IO and O control groups and the groups with higher SeMet supplementation. The highest liver GSP-Px activity were found in the IO and O control groups; liver GSH-Px activity in the groups fed 0.3, 0.6, 0.9 and 1.5 mg supplemented selenium from SeMet was not different from each other.

Considering haematology, statistically significant differences among treatments were found in white blood cell counts (WBC) but not for the other parameters (total blood cell counts (2.6–2.7 x 10^{12}/L), haemoglobin (6.4–6.9 mmol/L) and haematocrit (28.9–31.4 %)). WBCs were significantly higher in broilers of the IO control than broilers of the SeMet-0.6 and SeMet-0.9 groups and tended to be higher than in the SeMet-1.5. Despite these few significant differences in WBC, the values found were considered to lie within the physiological range for broilers.

Considering clinical biochemistry, statistically significant differences among treatments were found for alanine amino transferase (ALT) and gamma-glutamyltransferase (γGT) in blood, but not for the other endpoints (aspartate aminotransferase (AST) (213–232 U/L), total protein (35.4–38.3 g/L), albumin (26.9–27.9 g/L), glucose (9.1–10.7 mmol/L), and uric acid (6.1–8.2 mg/dL)). The control and the SeMet-0.3 groups showed a significantly higher ALT value than birds of the SeMet.-1.5 group.

Additionally, birds of the IO group had significantly higher γGT activities compared to control and SeMet-1.5 birds. The ALT and γGT values were all within the physiological range considering the routine laboratory method applied. Although some significant differences in ALT and γGT activity in plasma were observed, no treatment related effect (neither selenium source nor level) could be derived.

At necropsy, liver discoloration (pale) was found in one liver in each of the SeMet-0.3, SeMet-0.6 and SeMet-1.5 groups; heart lesions were found in two animals in each of the control and the IO control groups and one animal in each of the SeMet-0.1, SeMet-0.3, SeMet-0.9 and SeMet-1.5 groups. No other significant lesions were reported. Considering the low rate of occurrence of lesions, and that are not exceptional for a normal healthy broiler population, the FEEDAP Panel concludes that no treatment-related alterations of the digestive tract, the organs and the skeleton were found.

The results of the tissue deposition study are described in Table 3.

Table 3: Selenium content in liver, kidney, muscle and skin/fat (µg Se/kg fresh tissue) in chickens for fattening (38 days)

Code	Control	IO Control	O Control	SeMet-0.1	SeMet-0.3	SeMet-0.6
Se source	none	Na_2SeO_3	Se-yeast	DL-SeMet		
Se supplemented (mg/kg)	0	0.3	0.3	0.1	0.3	0.6
Se analysed						
- starter (mg/kg)	0.035	0.302	0.328	0.167	0.373	0.737
- grower (mg/kg)	0.046	0.303	0.390	0.139	0.353	0.653
Liver (µg/kg)	78^{e}	460^{c}	513^{c}	309^{d}	626^{b}	842^{a}
Kidney (µg/kg)	148^{e}	624^{c}	574^{c}	438^{d}	755^{b}	941^{a}
Muscle (µg/kg)	27^{e}	82^{d}	156^{c}	133^{c}	357^{b}	607^{a}
Skin/fat (µg/kg)	33^{e}	145^{c}	159^{bc}	101^{d}	184^{b}	316^{a}

a-e: Values with different letter superscripts are significantly different ($P < 0.05$).

A significant dose-related response of selenium deposition to dietary selenium was observed in all tissues examined in the SeMet-0.1, SeMet-0.3, and SeMet-0.6 groups. The selenium concentration of all tissues in animals of the SeMet-0.1 group (0.1 mg selenium supplementation from DL-SeMet per kg feed) was significantly higher than in the unsupplemented control group. A significantly higher selenium content was also observed in liver, kidney, muscle and skin/fat of the SeMet-0.3 group than in the IO group (supplemented with the same amount of selenium, but from Na_2SeO_3) and except skin/fat, also in the O-control group (supplemented with Se-yeast). There was no significant interaction between sex and treatment.

The contribution of the DL-SeMet in terms of methionine as an amino acid added to feed would be negligible considering the limits set by the EU for total selenium in feed.

3.1.2. Conclusions on the safety for target species

Selenium from DL-SeMet was tolerated by chickens for fattening at up to 1.5 mg supplemented level/kg feed; DL-SeMet is therefore safe for chickens for fattening provided total dietary selenium does not exceed 0.5 mg/kg complete feed. The FEEDAP Panel extends this conclusion to all animal species.

3.2. Safety for the consumer

3.2.1. Metabolic and residues studies

SeMet from organic selenocompounds can be incorporated nonspecifically into normal body proteins such as albumin and haemoglobin (Hb) in place of methionine. SeMet in body proteins is stored non-functionally but may later be released by catabolism, and then be converted to hydrogen selenide. Alternatively SeMet can be trans-selenated to selenocysteine (SeCys), which is then also converted to hydrogen selenide (Rayman, 2004; Rayman et al., 2008). Selenide is an intermediate compound; which is utilized in the synthesis of selenoenzymes, transformed into methylated metabolites for excretion, as well as oxidised to selenium dioxide, a pathway associated with toxicity, owing to the production of superoxide and other reactive oxygen species (Suzuki et al 2006; Rayman et al., 2008).

The uptake of SeMet in brush border membrane vesicles from rats showed a linear relationship with concentration, whereas selenite showed a curvilinear relationship (Vendeland et al., 1994). In weanling rats fed levels of 0.2–4.0 mg DL-SeMet/kg feed for nine weeks, selenium deposition in liver, muscle and brain was significantly higher than in animals expose to analogous dietary concentrations of selenium from sodium selenite (Deagen et al., 1987). In humans supplemented with SeMet, plasma selenium increased more rapidly and retention (as measured by urine excretion) was higher than with other sources: retentions of SeMet was reflected in blood selenium levels (Robinson et al., 1978).

Concerning the racemic mixture (DL-SeMet), after its absorption in the gastrointestinal tract of animals, part of the SeMet is metabolised to dihydrogen selenide to be utilised in selenium pathways, whereas another portion is non-specifically incorporated into body proteins as a substitute for the common amino acid methionine (Schrauzer, 2000). The D-enantiomer, when absorbed, is converted into the L-enantiomer (Lewis and Baker, 1995), and consequently only L-SeMet is incorporated in the protein of tissues and animal products.

In the combined tolerance/efficacy study submitted by the applicant (for protocol description, see section 3.1.1), the results obtained for selenium deposition in tissues (liver, kidney, muscle, skin/fat) showed a similar pattern for DL-SeMet as for Se-yeast (Table 3), demonstrating that selenium deposition from DL-SeMet is significantly higher than from sodium selenite.

3.2.2. Toxicological studies

The EFSA's Panel on Food Additives and Nutrient Sources added to Food reviewed the toxicological studies of L-SeMet (EFSA, 2009b) and concluded that the toxicity of L-SeMet is comparable to that of other forms of selenium, in terms of equivalent amounts of bioavailable selenium. The applicant has provided two further genotoxicity studies conducted with the DL-SeMet under assessment.

The mutagenic activity of the test item DL-selenomethionine was assessed by means of the Ames test in the five standard *Salmonella* Typhimurium strains (TA1535, TA1537, TA98, TA100 and TA102) tested either in presence or in absence of metabolic activation (S9 mix), in two independent assays, following a preliminary concentration-finding assay; owing to strong toxicity, the resulting highest tested concentrations were rather low (20 to 200 µg/plate).[39] Under the test conditions, no mutagenic activity was revealed.

In an *in vitro* mammalian cell micronucleus test on TK6 lymphoblastoid human cells, cells were treated in presence and in absence of metabolic activation (S9 mix), either with a short (3 hours) or prolonged (27 hours) treatment.[40] DL-SeMet induced statistically significant increases in the number of micronucleated cells both with and without metabolic activation upon 3-hour treatment, and with metabolic activation only upon the prolonged treatment.

[39] Technical Dossier/Section III/ Annex_III_2_2_2_1.
[40] Technical Dossier/Section III/ Annex_III_2_2_2_2.

The FEEDAP Panel considers that the potential to elicit genotoxic effects *in vitro* is consistent with other findings on different selenocompounds indicating DNA damage that may be subsequent to the formation of DNA reactive oxygen radicals by means of a threshold mechanism; this view is in line with that expressed by the Scientific Committee on Food (EC, 2000).

Based on available toxicity studies and previous assessments on closely related compounds, the FEEDAP Panel concludes that selenium from DL-SeMet does not elicit any adverse effects not expected in a selenium compound.

3.2.3. Assessment of safety for consumers

The upper tolerable level (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, 2012a); this view was later extended to the hydroxy-analogue of SeMet (HMSeBA) (EFSA FEEDAP Panel, 2013a) and to L-SeMet (EFSA FEEDAP Panel, 2013b). There is no reason to assume that SeMet from different sources would result in an essentially different deposition pattern in edible tissues/products. This view is supported by data provided by the applicant that showed increased tissue deposition of selenium from DL-SeMet compared with sodium selenite (see Table 3).

The FEEDAP Panel has previously concluded that the selenium supplementation of feed by selenised yeast, by HMSeBA or by L-SeMet should be limited to a maximum of 0.2 mg Se/kg feed. Therefore with respect to consumer safety, the Panel concludes that supplemental selenium from DL-SeMet should be limited to a maximum of 0.2 mg/kg feed.

3.3. Safety for the users/workers

3.3.1. Effects on the respiratory system

The applicant provided data on a DL-SeMet containing additive which did not release any measurable dust in a Stauber-Heubach system. Consequently, there will be no inhalation exposure to DL-SeMet of users/workers. However, as long as the additive is not the subject of authorisation and considering the high toxicity of selenium, it would be prudent to consider DL-SeMet as a hazard by inhalation which requires protection measures.

3.3.2. Effects on eyes and skin

Acute eye irritation of the formulated additive (0.5 % DL-SeMet) was studied following a single ocular instillation in rabbits in accordance with OECD Guideline 405.[41] Three animals were used for the study. A dose of 0.1 g of test item was introduced into the conjunctival sac of the left eye of each of the animals. The untreated right eye served as a control. Any conjunctival, iris and corneal changes were evaluated approximately one hour, 24 hours, 48 hours, 72 hours after instillation. The application of the test item did not induce ocular changes.

Skin irritation was tested in rabbits following a single, semi-occluded application to intact skin in accordance with OECD Guideline 404.[42] Three animals were used for the study. A dose of 0.5 g of the formulated additive (0.5 % DL-SeMet) was applied to the skin. Semi-occlusive dressings held the test item in place for 3 minutes, 1 hour and 4 hours on the skin of the first animal and for 4 hours for the two other animals. Any cutaneous lesion was evaluated approximately one hour, 24, 48 and 72 hours after removal of the dressing. The application of the test item did not induce skin lesions.

[41] Technical Dossier/Section III/Annex III_3_1_2_2.
[42] Technical Dossier/Section III/Annex III_3_1_2_1.

The formulated additive (0.5 % DL-SeMet) was tested for skin sensitising potential (using the Buehler test) in guinea pigs in accordance with OECD Guideline 406.[43] The extent and degree of skin reaction to a challenge exposure at a non irritant dose on day 29, following previous topical exposure (at days 1, 8 and 15) in a near-to-irritant dose (0.5 g) was measured in ten male and ten female guinea pigs. No allergic reaction was induced in any of the treated animals.

3.3.3. Conclusion on the safety for the users/workers

Although a DL-SeMet containing additive did not release any measurable dust, it would be prudent to consider the additive as a hazard by inhalation, which requires protection measures since the additive is not the subject of authorisation, and selenium is highly toxic. The additive is not an irritant to skin and eyes and is not a dermal sensitiser.

3.4. Safety for the environment

There is no information suggesting that selenium, when provided to animals as DL-SeMet, would be more harmful to the environment than selenium from other sources already authorised as feed additives.[44] It is well documented in the scientific literature that, owing to the specific metabolic fate of DL-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 DL-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 complete feed 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 (EFSA, 2012b). As already established in previous opinions of the FEEDAP Panel (e.g. 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 the selenocompound SeMet is considered to reflect directly only the unspecific incorporation of Se-Met into general body proteins (EFSA, 2006a).

The applicant provided two long-term efficacy studies in chickens for fattening, carried out at two different locations.

4.1. Studies in chickens for fattening

The first study provided is a combined tolerance/efficacy trial which has already been described in Section 3.1.1. The zootechnical data did not show any difference among the treatments (see Table 2). The data clearly indicate that selenium from DL-SeMet is absorbed (see plasma selenium concentrations in Table 2), used in the synthesis of selenium-specific enzyme (see GSH-Px in plasma in Table 2) and deposited in edible tissues (see Table 3).

A second study was carried out with a total of 576 one-day old chicks (Ross 308, males), allocated to nine treatments (eight pens of eight broilers/treatment).[45] Five groups were relevant for the assessment of the DL-SeMet efficacy as a source of selenium in chickens: a control group fed a selenium unsupplemented diet (0.23 mg Se/kg), two groups with supplemental 0.15 mg Se/kg and 0.30 mg

[43] Technical Dossier/Section III/Annex III_3_1_2_3.
[44] Inorganic selenium: sodium selenite and sodium selenate. Organic selenium: selenium produced by the selenium-enriched yeasts *Saccharomyces cerevisiae* CNCM I-3060, *S. cerevisiae* NCYC R397, *S. cerevisiae* CNCM I-3399, *S. cerevisiae* NCYC R645 and *S. cerevisiae* NCYC R646; hydroxy-analogue of selenomethionine; L-selenomethionine.
[45] Technical Dossier/Section IV/Annex_III_2_1_2_1.

Se/kg from DL-SeMet and sodium selenite, respectively. Mortality within 35 days was low (3.6 %) and not treatment related. The zootechnical parameters, measured at 27 days, did not significantly differ between the groups. GSH-Px in plasma and liver (at day 28) was also not affected by the treatments. The only difference found was in the selenium concentration in liver (day 35) showing a significant higher concentration for the DL-SeMet group supplemented with 0.3 mg Se/kg than in the unsupplemented control group (640 vs. 519 µg Se/kg liver).

4.2. Efficacy in ruminants

In a previous opinion (EFSA FEEDAP Panel, 2013b), the FEEDAP Panel provided a brief review on the efficacy of free SeMet in ruminants, considering its degradation/incorporation by/into rumen microbiota. Based on the outcome described in the said opinion, the Panel concludes that DL-SeMet would be utilised in ruminants as a selenium source.

4.3. Quality of edible tissues and products

No study on the influence of the DL-SeMet on the physico-chemical properties or sensory quality of edible tissues and products was provided by the applicant. However, one study performed in chickens for fattening with L- and D-SeMet supplemented diets, which examined total antioxidant capability in kidney, pancreas, and breast muscle, malondialdehyde concentration in kidney and breast muscle and drip loss, did not indicate any adverse effect on the quality of edible tissues (Wang et al., 2011). It is concluded that the findings for L- and D-SeMet could be extrapolated to DL-SeMet.

4.4. Conclusions on efficacy

Sufficient evidence that DL-SeMet is an effective source of selenium in chickens for fattening is provided by one study. A second study in the same species failed to reach the same degree of evidence, most probably because of the already high level of selenium in the control diet. Since there are no fundamental differences between target animals in the metabolism of SeMet and its use for the specific selenium biological functions, the FEEDAP Panel extends its conclusion on the efficacy of DL-SeMet to all animal species and categories. It is not expected that the use of DL-SeMet in animal nutrition would negatively affect the quality of edible tissues and products.

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[46] and Good Manufacturing Practice.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Selenium from DL-SeMet was tolerated by chickens for fattening at up to 1.5 mg supplemental level; DL-SeMet is therefore safe for chickens for fattening provided total dietary selenium does not exceed 0.5 mg/kg complete feed. The FEEDAP Panel extends this conclusion to all animal species.

Based on available toxicity studies and previous assessments on closely related compounds, the FEEDAP Panel concludes that selenium from DL-SeMet does not elicit any adverse effects not expected in a selenium compound. The use of DL-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 DL-SeMet, the FEEDAP Panel concludes that dietary selenium supplementation from the additive should not exceed a maximum of 0.2 mg/kg complete feed.

[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.

Although a DL-SeMet containing additive did not release any measurable dust, the additive is considered as a hazard by inhalation, which requires protection measures for users since the additive is not the subject of authorisation, and selenium is highly toxic. The additive is not an irritant to skin and eyes and is not a dermal sensitiser.

The use of DL-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 complete feed is not exceeded.

Sufficient evidence is provided that DL-SeMet is an effective source of selenium in chickens for fattening. Since there are no fundamental differences between target animals in the metabolism of SeMet and its use for the specific biological functions of selenium, the FEEDAP Panel extends its conclusion on the efficacy of DL-SeMet to all animal species and categories.

RECOMMENDATIONS

The "Description and conditions of use of the additive" should refer to the selenium compound and its selenium content. The additive should be incorporated into complete feed via premixtures only.

The FEEDAP Panel strongly recommends that DL-SeMet should be placed on the market only in a formulation with reduced selenium content and low dusting potential (as described in the application by 2000 mg Se/kg additive and no measurable dusting potential).

DOCUMENTATION PROVIDED TO EFSA

1. Dossier DL-selenomethionine. October 2012. Submitted by Novus Europe S.A./N.V.
2. Dossier DL-selenomethionine. Supplementary information. August 2013. Submitted by Novus Europe S.A./N.V.
3. Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods(s) of analysis for DL-Selenomethionine.
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 DL-Selenomethionine[47]

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

The active substance of the product is *DL-Selenomethionine*, produced by chemical synthesis. *Mintrex®Se* is a white to tan powder preparation consisting of 0.5 % of *DL-Selenomethionine*, which corresponds to 0.2 % of selenium; 0.5 % of mineral oil and 99 % of calcium carbonate carrier.

The *feed additive* is intended to be incorporated into *feedingstuffs* through *premixtures*, to obtain a maximum *total selenium* dosage of 0.5 mg/kg *feedingstuffs* thus complying with legal requirements; no minimum dose was proposed by the Applicant.

For the determination of *DL-Selenomethionine* in the *feed additive* and in *feedingstuffs* the Applicant submitted two single laboratory validated and further verified methods based on high performance liquid chromatography with UV detection (HPLC-UV) for *feed additive* samples; and based on high performance liquid chromatography inductively coupled plasma mass spectrometry (HPLC-ICP-MS) for *feedingstuffs* samples.

For the determination of *total selenium* in the *feed additive* the Applicant submitted a single laboratory validated and further verified method based on microwave digestion with nitric acid and hydrogen peroxide (HNO_3/H_2O_2) followed by inductively coupled plasma mass spectrometry (ICP-MS). Furthermore, an alternative single-laboratory validated and further verified method based on inductively coupled plasma atomic emission spectrometry (ICPAES) was previously evaluated and recommended by the EURL for the determination of *total selenium* in the *feed additives* (cf. FAD-2009-0010; FAD-2011-0028 and FAD-2011-0044).

For the determination of *total selenium* in *feedingstuffs* the Applicant applied the abovementioned ICP-MS method. The EURL identified instead for the determination of *total selenium* in *feedingstuffs* the CEN method EN 16159:2012 based on Hydride Generation Atomic Absorption Spectrometry (HGAAS) after microwave digestion with HNO3/H2O2. 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.

Based on the performance characteristics presented the EURL recommends for official control the single-laboratory validated and further verified methods based on HPLC-UV and ICP-MS or ICP-AES for the determination of *DL-Selenomethionine* and *total selenium* in the *feed additive*, respectively. As for the determination of *total selenium* in *premixtures* and *feedingstuffs*, the EURL recommends the ring-trial validated CEN method, based on HGAAS (EN 16159:2012).

Even though the performance characteristics of the HPLC-ICP-MS method for the determination of *DL-Selenomethionine* in *feedingstuffs* are acceptable, the EURL will not recommend this method since no limits for *Selenomethionine* in *feedingstuffs* are included in the proposed Register Entry and the HPLC-ICP-MS equipment required for analysis is not readily available in all official control laboratories.

[47] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2012-0042-DL-MetSe.doc.pdf

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.