European Food Safety Authority

EFSA Journal 2012;10(7):2778

SCIENTIFIC OPINION

Scientific Opinion on safety and efficacy of selenium in the form of organic compounds produced by the selenium-enriched yeast *Saccharomyces cerevisiae* NCYC R646 (Selemax 1000/2000) as feed additive for all species[1, 2]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[3, 4]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The additive Selemax consists of selenium-containing inactivated yeast (*Saccharomyces cerevisiae* NCYC R646), enriched during the fermentation process with organic selenocompounds, and is intended to be used as a nutritional additive, providing a source of the essential trace element selenium for all animal species. Based on data from a tolerance study in chickens for fattening, the use of Selemax as a selenium source is considered to be safe for all animal species. The FEEDAP Panel reiterates its former conclusion that the use of any selenised yeast would result in similar selenium deposition in tissues and products. To ensure consumer safety from consumption of tissues and products of animals treated with Selemax, the FEEDAP Panel concludes that dietary selenium supplementation from Selemax, as for other selenised yeasts, should not exceed a maximum of 0.2 mg Se/kg complete feed. In the absence of specific data, the product is considered as a potential irritant to skin and eyes and sensitiser to skin. Owing to its proteinaceous nature, the additive is considered a potential respiratory sensitiser. The FEEDAP Panel considers that the use of Selemax 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 liver glutathione peroxidase activity and the liver/plasma concentration of selenium, the FEEDAP Panel considers Selemax an effective source of selenium for all species. Selemax does not modify the quality of meat as measured by physical parameters.

KEY WORDS

Nutritional additive, compound of trace elements, selenium enriched yeast, selenium, Selemax 1000/2000, selenomethionine, safety, efficacy

[1] On request from the European Commission, Question No EFSA-Q-2010-01029, adopted on 15 June 2012.

[2] This scientific opinion has been adited following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2033. The modified sections are indicated in the text.

[3] Panel members: Gabriele Aquilina, Georges Bories, Andrew Chesson, Pier Sandro Cocconcelli, Joop de Knecht, Noël Albert Dierick, Mikolaj Antoni Gralak, Jürgen Gropp, Ingrid Halle, Christer Hogstrand, the late Reinhard Kroker, Lubomir Leng, Secundino López Puente, Anne-Katrine Lundebye Haldorsen, Alberto Mantovani, Giovanna Martelli, Miklós Mézes, Derek Renshaw, Maria Saarela, Kristen Sejrsen and Johannes Westendorf. Correspondence: FEEDAP@efsa.europa.eu

[4] Acknowledgement: The Panel wishes to thank the members of the Working Group on Trace Elements for the preparatory work on this scientific opinion.

Suggested citation: EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP); Scientific Opinion on safety and efficacy of selenium in the form of organic compounds produced by the selenium-enriched yeast *Saccharomyces cerevisiae* NCYC R646 (Selemax 1000/2000) as feed additive for all species. EFSA Journal 2012;10(7):2778. [17 pp.] doi:10.2903/j.efsa.2012.2778. Available online: www.efsa.europa.eu/efsajournal

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 Selemax, a source of selenium in the form of organic compounds produced by a selenium-enriched yeast when used as a nutritional additive for all animal species.

Selemax consists of selenium-containing inactivated yeasts based on *Saccharomyces cerevisiae* enriched during the fermentation process with selenium. The additive is intended to be marketed in two forms, Selemax 1000 and Selemax 2000, which differ only in the final selenium concentration.

A tolerance study in chickens for fattening, one of the most sensitive species, at 6-fold the maximum authorised content in complete feed demonstrated that Selemax is safe when supplemented up to the maximum authorised content of selenium in complete feed. A margin of safety could not be derived from the data submitted, but it is unlikely to be different from that of other selenised yeasts. Provided that the maximum authorised selenium content in complete feed is not exceeded, the use of Selemax as selenium source is considered to be safe for all animal species.

The FEEDAP Panel reiterates its former conclusion that the use of any selenised yeast would result in similar selenium deposition in animal tissues and products. To ensure consumer safety from consumption of tissues and products of animals treated with Selemax, the FEEDAP Panel concludes that dietary selenium supplementation from Selemax, as for other selenised yeasts, should not exceed a maximum of 0.2 mg Se/kg complete feed.

In the absence of specific data, the product should be considered as a potential irritant to skin and eyes, and a skin sensitiser. As the use of Selemax may result in exposure by inhalation, in the absence of toxicity data, the additive should be considered as hazardous by inhalation. Owing to its proteinaceous nature, the additive should be considered as a respiratory sensitiser.

The FEEDAP Panel considers that the use of Selemax 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 selenium content in feedingstuffs is not exceeded.

Based on the response of liver glutathione peroxidase activity and the selenium level in plasma and liver of piglets to dietary supplementation of selenium from Selemax, the FEEDAP Panel considers this additive an effective source of selenium for all species. Selemax does not modify the quality of meat as measured by physical parameters.

TABLE OF CONTENTS

BACKGROUND

Regulation (EC) No 1831/2003[5] 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 Biorigin Europe N.V.[6] for authorisation of selenium in the form of organic compounds, mainly selenomethionine, produced by the selenium-enriched yeast *Saccharomyces cerevisiae* NCYC R646 (Selemax 1000/2000) when used as a feed additive for all species (category: nutritional additives; functional group: compounds of trace elements) under the conditions mentioned in Table 1.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application.[7] 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 6 December 2010.

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.[8] Organic forms of selenium produced by *Saccharomyces cerevisiae* CNCM I-3060, *S. cerevisiae* NCYC R397 and *Saccharomyces cerevisiae* CNCM I-3399 are authorised in the EU as trace element under Regulation (EC) No 1831/2003.[9,10,11] These latter authorisations have been granted following corresponding EFSA opinions (EFSA 2006a, 2006b, 2009). An opinion on the safety and efficacy of selenium in the form of organic compounds produced by the selenium-enriched yeast *Saccharomyces cerevisiae* NCYC R645 for all animal species was delivered by the FEEDAP Panel (EFSA, 2011a). 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 selenium in the form of organic compounds, mainly selenomethionine, produced by the selenium-enriched yeast *Saccharomyces cerevisiae* NCYC R646 (Selemax 1000/2000) when used under the conditions described in Table 1.

[5] 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.
[6] Biorigin Europe N.V. Vosseschijnstraat 59. B-2030 Antwerpen. Belgium.
[7] EFSA Dossier reference: FAD-2010-0044.
[8] List of the authorised additives in feedingstuffs published in application of Article 9t (b) of Council Directive 70/524/EEC concerning additives in feedingstuff. OJ C 050, 25.02.2004, p. 1.
[9] 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.
[10] 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.
[11] 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	Selenomethionine
Registration number/EC No/No (if appropriate)	
Category(-ies) of additive	3. Nutritional additives
Functional group(s) of additive	(b) Compounds of trace elements

Description			
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)
Selenomethionine **Selemax 1000 minimal 1000 ppm selenium** **Selemax 2000 minimal 2000 ppm selenium**	**$C_5H_{11}NO_2Se$**	-	-

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

Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg/kg of complete feedingstuffs		
All species	-	-	Total Se in complete feedingstuffs may not exceed 0.5 ppm Se	-

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use (if appropriate)	The additive shall be incorporated in compound feedingstuffs via the use 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)	-
Specific conditions for use in complementary feedingstuffs (if appropriate)	Total Se in complete feedingstuffs may not exceed 0.5 ppm Se

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

ASSESSMENT

1. Introduction

The additive Selemax consists of inactivated yeast enriched during fermentation with selenium, and produced by *Saccharomyces cerevisiae* NCYC R646. It is intended to be used as nutritional additive, providing a source of the essential trace element selenium for all animal species and categories. The additive is intended to be marketed in two forms Selemax 1000 and Selemax 2000, differing in their selenium content. In the text, the additive is referred to simply as "Selemax". According to the documentation provided by applicant, the additive is registered and used in animal nutrition in several countries including the Unites States, Brazil, Taiwan, Australia, Argentina, South Korea.

Selenised yeast-based additives produced by other strains of *S. cerevisiae* have been evaluated by the FEEDAP Panel and are currently authorised in the EU as feed additives (see Background section). Selenised yeasts have been evaluated by the Scientific Committee on Food for their use as food supplements (EC, 2000). The EFSA's Panel on food additives, flavourings, processing aids and materials in contact with food (AFC Panel) assessed selenium enriched yeast as a source of selenium for particular nutritional uses and foods (including food supplements) for the general population (EFSA, 2008), and concluded that it was safe at the proposed intake levels. Selenium-enriched yeast is authorised in the EU as a mineral substance which may be used in the manufacture of food supplements and which may be added to food by Commission Regulation (EC) No 1170/2009.[12] Selenium-enriched yeast from *S. cerevisiae* is approved for use as a feed ingredient in the United States by the Association of American Feed Control Officials (AAFCO, 2010; IDC, 2010).

In principle, all selenised yeasts contain a mixture of organic selenocompounds with selenomethionine (Se-Met) as a predominant source of selenium. In general, such products contain up to 36 % of total selenium in the form of unspecified Se-compounds. The nature and amount of these unspecified selenium compounds may vary between different sources of selenised yeasts (Ruiz Encinar et al., 2003).

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 essential for vertebrates, 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 opinions.

As 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. Se-Met is the major form of naturally occurring selenium in feedingstuffs.

2. Characterisation

2.1. Identity of the additive

The products Selemax 1000 and Selemax 2000 consist of selenium-enriched inactivated yeast (*S. cerevisiae*) and untreated *Saccharomyces* yeast, differing in the selenium concentration.

The production strain of yeast used for selenium enrichment is deposited at the National Collection of Yeast Cultures of United Kingdom with the accession number NCYC R646 (YSC 11111). The strain is not genetically modified; its identity is determined by DNA sequencing of the D1/D2 region of the 26S ribosomal RNA gene. *S. cerevisiae* qualifies *per se* for Qualified Presumption of Safety (QPS)

[12] 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.

approach (EFSA 2007, 2010); however, the FEEDAP Panel retains that, in this case, the mixture of selenocompounds in the additive is the subject of the evaluation, therefore the QPS approach does not apply to this aspect of the assessment.

According to the specification, Selemax 1000 and Selemax 2000 contain a minimum selenium content of 1000 and 2000 mg Se/kg, respectively, with at least 70 % of total selenium in the form of Se-Met and less than 2 % of residual inorganic selenium from total selenium in both preparations. No maximum selenium content is specified. Analysis of five batches of Selemax 1000[13] and seven batches of Selemax 2000[14] showed a mean selenium content of 1074 (range: 1001–1200) and 2334 mg/kg (range: 2107–2650), respectively. In one batch of Selemax 1000, selenium in the form of Se-Met constituted 82.0 % of the total selenium; in three batches of Selemax 2000 it was 75.1 % (from 69.7 to 84.2). Analysis of one batch of each product showed that selenocysteine (Se-Cys) contributed 1.4–2.2 % of total selenium, whereas both residual inorganic Se(IV) and Se(VI) were below the limit of detection (5 mg/kg). However, a considerable portion of organic selenocompounds in the additive (13.6–28.9 %) remained unidentified.

As both products, Selemax 1000 and Selemax 2000, consist of the same yeast, only in different proportions, the data submitted for each product on impurities, shelf-life, stability in premixtures/feeds and physical properties are considered together.

2.2. Impurities

Six batches showed bacterial total plate counts ≤ 4100 CFU/g and moulds plus yeasts ≤ 80 CFU/g, whereas total coliforms, *Escherichia coli* (both per gram) and *Salmonella* (per 25 g) were absent.[15] Analytical data on heavy metals and arsenic from two batches raised no safety concerns (Pb≤ 0.3, Cd≤ 0.04, Hg≤ 0.1 and As≤ 0.2 mg/kg). Dioxins and dioxin-like PCBs were 0.06 ng PCDD/F WHO-TEQ/kg, and 0.1 ng PCDD/F/PCB WHO-TEQ/kg, respectively. Aflatoxins B1, B2, G1, G2 and ochratoxin A were below the detection limits of 1.5, 0.5, 1.5, 0.5 and 1.7 µg/kg, respectively.[16] All impurities complied with the thresholds set by Directive 2002/32/EC.[17] Control methods are in place.

2.3. Physical state of the product

Selemax 1000 and Selemax 2000 appear as a light cream, fine free-flowing powder with a density of 450 and 420 kg/m^3, respectively. Particle size analysis of four batches from each preparation was carried out by laser diffraction method[18] and indicated a particle size distribution of: 1 % < 10µm, 25–40 % < 50µm and 75–85 % < 100 µm for both products. Dusting potential measured by the Stauber-Heubach method in three batches of Selemax 2000 was in the range of 0.36–1.7 g/m^3.[19] No analytical data on the selenium content of the dust were provided.

2.4. Characterisation of the active substance

Selenium is considered to be the active substance of Selemax. Since the analysed residual inorganic selenium as Se(IV) and Se(VI) did not exceed 0.5 % of total selenium content, organic selenium is considered to account for > 99 % of total selenium content in both preparations.

2.5. Manufacturing process[20]

The manufacturing process of the product is fully described in the technical dossier.

[13] Technical Dossier/Section II/Annexes: 2.1.3.a, 2.1.3.c, 2.1.3.d and 2.1.3.e.
[14] Technical Dossier/Section II/Annexes: 2.1.3.b, 2.1.3.f, 2.1.3.g, 2.1.3.h and 2.4.1.e.
[15] Technical Dossier/Section II/Annexes: 2.1.4.a and 2.1.4.b.
[16] Supplementary Information/Annex 1.
[17] 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.
[18] Technical Dossier/Section II/Annexes: 2.1.5.a and 2.1.5.b.
[19] Technical Dossier/Section II/Annex 2.1.5.c. Supplementary Information/Annex 2.
[20] This section has been adited following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2033.

2.6. Physico-chemical and technological properties of the additive

2.6.1. Stability

One batch of Selemax 2000 was stored for six months at both standard (25ºC) and accelerated conditions (40°C);[21] the contents of total selenium, Se-Met and Se-Cys were not essentially affected under either storage condition. Two additional batches of Selemax 2000 stored for two years at room temperature (22-25ºC) showed mean recovery rates of 110, 94 and 141 % for total selenium, Se-Met and Se-Cys, respectively.[22]

In a vitamin-mineral premixture, the stability of one batch of Selemax 2000 in closed package at standard (25ºC) and accelerated conditions (40°C) was studied for six months.[23] The results from storage under standard conditions showed a recovery of total selenium, Se-Met and Se-Cys of 114, 123 and 94 %, respectively. Under accelerated conditions, the corresponding figures were 139, 115 and 106 %. The stability of two additional batches of Selemax 2000 was examined in a mineral premixture under standard conditions (25ºC) for six months.[24] Mean recoveries were 111, 100 and 108 % for total selenium, Se-Met and Se-Cys, respectively.

The stability of Selemax 2000 (one batch) in a starter diet (based on maize and soybean meal) for chickens for fattening under both standard (25ºC) and accelerated (40°C) conditions was studied for six months.[25] Under standard conditions the recovery of both total selenium and Se-Met was 109 %, and it was in the same order under accelerated conditions. The stability of three additional batches of Selemax 2000 was tested also in mash and pelleted complementary feed for cattle (based mainly on maize meal, soybean meal, maize gluten meal and wheat bran) at standard conditions for three months.[26] In both types of feed, total selenium and Se-Met were fully recovered after three months. Feed processing by pelleting (steaming for up to 50 seconds, temperature 65–75 ºC) did not affect the content of total selenium and Se-Met.

2.6.2. Homogeneity

To demonstrate the capacity of the additive to homogeneously distribute in feed, the applicant submitted a theoretical calculation (according to Jansen, 1992). Supplementing complete feed for chickens for fattening with Selemax 2000 resulted in a coefficient of variation of < 1 % for the selenium concentration in feed.[27] However, the FEEDAP Panel notes that this method has been developed to test the working accuracy of mixing equipment.

2.7. Physico-chemical incompatibilities in feed

According to the current knowledge, no incompatibilities – with feed components, carriers, other approved additives or medical products – are to be expected.

2.8. Conditions of use

The applicant proposes the use of Selemax 1000 and Selemax 2000 as sources of selenium for all animal species up to a maximum content of 0.5 mg of total selenium per kg complete feed. No limitations on the age of the animals or the administration period are proposed.

The proposal of the applicant would correspond to a maximum supplementation rate of 300-400 mg Selemax 1000, or 150-200 mg Selemax 2000 per kg complete feed, respecting the maximum authorised content and assuming background levels between 0.1 and 0.2 mg Se/kg.

[21] Technical Dossier/Section II/Annex 2.4.1.a.
[22] Supplementary information/Annex 3.
[23] Technical Dossier/Section II/Annex 2.4.1.a.
[24] Supplementary information/Annex 3.
[25] Technical Dossier/Section II/Annex 2.4.1.a.
[26] Supplementary information/Annex 3.
[27] Technical Dossier/Section II/Annexes 2.4.2.a and 2.4.2.b.

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 Selemax 1000/2000 in animal feed. The Executive Summary of the EURL report is in the Appendix.

3. Safety

3.1. Safety for the target species

3.1.1. Tolerance studies

A total of 1280 1-day old male chickens for fattening (Cobb 500) were fed a maize and soybean meal-based mash diet for 42 days (starter 1–21 days, grower 22–35 days, finisher 36–42 days). Four groups (eight replicates, each with 40 birds) were given diets without or with Selemax 2000 to provide supplemental selenium at 0, 0.3, 3.3 or 30.1 mg Se/kg diet.[28] Selenised yeast was substituted at the expenses of a non-selenised inactivated yeast. Zootechnical parameters (body weight and feed consumption) were measured at weekly intervals. At the end of the study, blood samples were taken from eight birds/group for standard haematology and clinical biochemistry parameters (plasma glucose, triglycerides, cholesterol, calcium, phosphorous, sodium, potassium, urea, creatinine, AP, AST, ALT, LDH, pH, pO_2, bicarbonate, TCO_2, oxygen saturation). The data were statistically tested for normality and homoscedasticity and after confirmation subjected to an analysis of variance followed by Tukey test (Duncan test for haematological and clinical blood parameters) for group differences.

Dietary selenium was analysed in the control starter diet (<0.01 mg/kg) and the low- and intermediate-selenium supplementation rate was analysed in the starter, grower and finisher diets. The intended concentrations were confirmed.

After the first week of treatment, body weight (bw) and feed intake (FI) in the high selenium group were significantly reduced (to 45 and 36 % of the control values, respectively). Owing to high mortality, this treatment was not continued. At the end of the trial, body weight and feed intake were significantly increased in the low- and intermediate-selenium groups (by about 8 % each) compared with the selenium deficient control group (2777 g bw, 4.6 kg FI, 1.68 feed to gain ratio). The feed to gain ratio was not affected by the treatment.

The intermediate selenium supplementation affected the white blood cell count (WBC) by significantly increasing the numbers of leucocytes, eosinophils, basophils, monocytes compared with the control group and the low selenium supplementation. There were some significant changes in blood biochemistry parameters. However the physiological meaning of these findings is unclear, as a dose-response relationship could not be established. The only physiologically relevant finding could be a decrease of plasma calcium in the intermediate selenium group compared with the low-selenium group.

3.1.2. Conclusions on the safety for target species

The fact that the study was designed without a conventional control group (fed a nutritionally fully balanced diet with authorised additives), but instead with a control group fed a selenium-deficient diet, accounts for a greater level of uncertainty concerning the conclusions on the safety of Selemax 2000. Supplementation with selenium from Selemax 2000 at 0.3 mg Se/kg diet improved weight gain and feed intake compared with the selenium-deficient control group. Supplementation of a selenium-deficient diet with selenium from Selemax 2000 at 3.3 mg Se/kg diet did not affect the growth rate and feed intake of chickens for fattening over six weeks compared with 0.3 mg/kg supplemental selenium from the same source. However, some changes in white blood cell count and the fall in plasma

[28] Supplementary Information/Annexes 4a to 4d.

calcium could indicate that 3.3 mg supplemental selenium from Selemax 2000 is not free from adverse effects compared with the lower dose.

It is concluded that the supplementation of selenium from Selemax 2000 up to the highest selenium concentration authorised for complete feed is safe for chickens for fattening. Since chickens for fattening are considered one of the most sensitive species, Selemax is considered to be safe for all animal species. A margin of safety could not be numerically derived from the data submitted but is unlikely to be different from that of other selenised yeasts.

3.2. Safety for the consumer

The few data submitted for tissue and product deposition of selenium from Selemax 2000[29] generally confirm (muscle and milk) the findings described in previous FEEDAP opinions that feed supplementation with selenium from selenised yeasts results in similarly higher concentrations in edible tissues and products than from inorganic selenium. At equal supplementation to dairy feed (0.25 mg Se/kg DM) from Selemax and an inorganic selenium source, the selenium concentration of milk was 4-fold higher (0.032 vs. 0.008 mg/kg milk). For selenium supplementation levels of 0.15, 0.3 or 0.45 mg Se/kg feed for chickens for fattening from both sources, the selenium content of breast muscle was in an average about 4–5 times higher when Selemax was used than when sodium selenite was used.

Therefore, the conclusion of the FEEDAP Panel made in a former opinion on a selenised yeast (EFSA, 2011a) also applies to Selemax: "The FEEDAP Panel notes that there are likely no principal differences in the metabolic behaviour of selenium from different selenised yeasts (mainly Se-Met) when fed to animals. Selenium deposition from the use of these selenised yeasts as feed additives would therefore result in similar selenium tissue and product concentrations."

Up to 2010, the FEEDAP Panel assessed consumer exposure to selenium from edible tissues and products from animals given feed supplemented with selenium sources according to consumption data from Regulation (EC) No 429/2008[30] and/or data from the SCOOP project (EC, 2004). In its recent opinion on a selenised yeast (EFSA, 2011b) the FEEDAP Panel reconsidered consumer safety based on the modified food basket (consumers only, 95th percentile) derived from the Comprehensive European Food Consumption Database (EFSA, 2011c). Based on different scenarios (graded selenium supplementation levels, total exposure including background from food of non-animal origin) with toddlers (tolerable upper intake level 60 µg/day), the Panel concluded that selenium supplementation of feed with selenised yeasts should be limited to a maximum of 0.2 mg/kg feed to ensure consumer safety. The same approach should apply to Selemax 1000 and Selemax 2000.

No other safety concerns for the consumer resulting from the use of Selemax 1000 or Selemax 2000 in animal nutrition are envisaged (see also EFSA, 2008).

3.3. Safety for the user

In the absence of specific data on skin/eye irritation and skin sensitisation, the additive should be considered as a potential irritant to skin and eyes and as a skin sensitiser.

The proportion of particles below 50 µm amounted to 25-40 % in both products, and the highest dusting potential measured in Selemax 2000 was 1.7 g/m^3, which may result in exposure by inhalation. In the absence of inhalation toxicity data, the products should be considered as hazardous by inhalation.

Owing to its proteinaceous nature, the additive should be considered as a respiratory sensitiser.

[29] Technical Dossier/Section IV/Annex 4.2.3.a.

[30] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

3.4. Safety for the environment

The FEEDAP Panel considered that the use of Selemax 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 as support for the efficacy for compounds of 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, 2006b and 2009), the bioavailability of selenised yeasts as nutritional additives is considered to be demonstrated if several specific endpoints (e.g. glutathione peroxidase (GSH-Px) activity in plasma or blood or tissues, selenium concentration in plasma/serum or whole blood, selenium content in liver) are significantly influenced by the test item. Those indicators should be measured in animals supplied with the additive under assessment in comparison with a negative control group (animals without any selenium supplementation), and possibly also with an animal group given equivalent amounts of selenium in any currently authorised form as a positive control.

The tissue deposition of selenium from organic sources reflects directly only the unspecific incorporation of Se-Met into body proteins. As the utilisation of selenium from Se-Met in body proteins for specific selenium functions is well known, selenium deposition in tissues can be taken only as indirect proof of selenium bioavailability from organic selenium sources.

For the assessment of efficacy, only one (weaned piglets) out of five studies submitted could be considered. The other four studies (three in chickens for fattening and one in dairy cows) had inadequate experimental designs and/or inappropriate endpoints.

4.1. Piglets

The study conducted on 126 TOPIG piglets of both genders (initial body weight 7.2 kg, 28 days of age) lasted for 35 days (final body weight 22.8 kg).[31] Animals were randomly assigned to seven groups (group size: six pens, three piglets each) and fed diets (based on maize, soybean meal and micronised soybeans) without selenium supplementation or supplemented with selenium at 0.3 or 0.6 mg/kg from sodium selenite or with 0.15, 0.3, 0.45 or 0.6 mg/kg from Selemax 2000. Supplementation of dietary selenium was analytically confirmed. At the end of the study, piglets were slaughtered and samples of blood and liver were collected for analysis of selenium and GSH-Px activity. Statistical evaluation of the results was done by analysis of variance using the GLM procedure.

As the trial was treated as a bioavailability study, zootechnical parameters were not considered. The selenium levels in plasma and liver, as well as GSH-Px activity in liver tissue, were increased by Selemax 2000 compared with unsupplemented animals (Table 2). This response was dose-related for liver selenium and GSH-Px activity. In the view of the FEEDAP Panel, this data confirmed that Selemax is utilised in the body as a source of selenium.

[31] Technical Dossier/Section IV/Annexes 4.2.1.a. and Annexes 4.2.1.a1 to a7.

Table 2: Effect of supplementation with selenium from sodium selenite or Selemax 2000 on plasma and liver selenium levels and GSH-Px activity in liver tissue.

Source of Se	Control	Sodium selenite		Selemax			
Se supplemented (mg/kg feed)	-	0.30	0.60*	0.15	0.30	0.45	0.60*
Se analysed (mg/kg feed)	0.10	0.29	0.65	0.18	0.30	0.49	0.68
Plasma Se (mg/L)	0.04[a]	0.13[b]	0.13[b]	0.06[a]	0.12[b]	0.12[b]	0.15[b]
Liver Se (mg/kg)	0.03[a]	0.81[b]	1.19[b]	0.42[b]	0.88[b]	1.06[b]	1.25[b]
Liver GSH-Px (µmol NADPH/min)	13.6[a]	15.8[b]	18.6[b]	13.7[a]	14.1[b]	14.4[b]	16.1[b]

[a, b:] Values in row not sharing a common letter in superscripts are significantly different from control ($P < 0.05$)
[(*)] Dose exceeding the maximum selenium content in complete feedingstuffs authorised in the EU

4.2. Effects on the quality of meat

Breast muscle (*M. pectoralis major*) from chickens for fattening fed for 45 days diets with two levels of selenium supplementation (0.15 and 0.30 mg/kg feed) from an inorganic source and from Selemax 2000 was analysed for selected meat quality parameters (pH, cooking loss, shear force, and colour L, a, b). Sample size was 16 chickens per group. No significant differences were found. The FEEDAP Panel concludes that Selemax does not modify the quality of meat as measured by physical parameters.

4.3. Conclusion on efficacy

Based on data obtained from a study with piglets, it is concluded that Selemax is utilised in the body of all animal species as a source of selenium. Any modification in the physical properties of meat from animals fed Selemax supplemented diets is not expected.

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[32] and Good Manufacturing Practice.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Supplementation of selenium from Selemax up to the highest selenium concentration authorised for complete feed is safe for chickens for fattening. Since chickens for fattening is considered one of the most sensitive species, Selemax is considered to be safe for all animal species. A margin of safety could not be numerically derived from the data submitted but is unlikely to be different from that of other selenised yeasts.

The FEEDAP Panel reiterates its former conclusion that the use of any selenised yeast would result in similar selenium deposition in animal tissues and products. To ensure consumer safety from consumption of tissues and products of animals treated with Selemax, the FEEDAP Panel concludes that dietary selenium supplementation from the additive as from other selenised yeasts, should not exceed a maximum of 0.2 mg Se/kg complete feed.

In the absence of specific data, the additive should be considered as a potential irritant to skin and eyes, and a skin sensitiser. As the use of Selemax may result in exposure by inhalation, in the absence of toxicity data, the additive should be considered as hazardous by inhalation. Owing to its proteinaceous nature, the additive should be considered as a respiratory sensitiser.

[32] 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.

The FEEDAP Panel considers that the use of Selemax 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 selenium content in feedingstuffs is not exceeded.

Based on data obtained from a study with piglets, it is concluded that Selemax is utilised in the body of all animal species as a source of selenium. Selemax does not modify the quality of meat as measured by physical parameters.

RECOMMENDATIONS

The additive should be called selenised yeast (*Saccharomyces cerevisiae* NCYC R646). Consequently, a chemical formula does not apply.

The specifications for both products, considering the minimum content of selenium, should be amended by a maximum content to avoid overdosing. The FEEDAP Panel proposes the range of 1000-1200 mg Se/kg Selemax 1000 and 2000-2400 mg Se/kg Selemax 2000.

GENERAL REMARK

The FEEDAP Panel repeats a remark, which since 2007 has been an element of all opinions of the Panel related to organic forms of trace elements: the FEEDAP Panel stresses the need for analytical methods to detect those organic compounds in feed (in the case of Selemax: selenomethionine), independent of the trace element background.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier: Selemax. June 2010. Submitted by Biorigin Europe N.V.
2. Dossier: Selemax. Supplementary information. March 2012. Submitted by Biorigin Europe N.V.
3. Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods(s) of analysis for Selemax 1000/2000.
4. Comments from Member States received through the ScienceNet.

REFERENCES

AAFCO (Association of American Feed Control Officials), 2010. Selenium Yeast. Page 374 in Feed Terms and Ingredient Definitions. 57. Mineral Products. Tentative T57.163. Official publication. Association of American Feed Control Officials Inc., Olympia, WA.

EC (European Commission), 2000, online. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Selenium. Available from http://ec.europa.eu/food/fs/sc/scf/out80g_en.pdf

EC (European Commission), 2004, online. Reports on Tasks for Scientific Cooperation (SCOOP). Task 3.2.11. Assessment of the dietary exposures to arsenic, cadmium, lead and mercury of the population of the EU Member States Directorate General Health and Consumer Protection. Available from http://ec.europa.eu/food/food/chemicalsafety/contaminants/scoop_3-2-11_heavy_metals_report_en.pdf

EFSA (European Food Safety Authority), 2006a. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on the safety and efficacy of the product Sel-Plex®2000 as a feed additive according to Regulation (EC) No 1831/2003. The EFSA Journal, 348, 1–40.

EFSA (European Food Safety Authority), 2006b. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on the safety and efficacy of the product Selenium

enriched yeast (*Saccharomyces cerevisiae* NCYC R397) as a feed additive for all species in accordance with Regulation (EC) No 1831/2003. The EFSA Journal 430, 1–23.

EFSA (European Food Safety Authority), 2007. Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. Opinion of the Scientific Committee. The EFSA Journal, 587, 1–16.

EFSA (European Food Safety Authority), 2008. Selenium-enriched yeast as source for selenium added for nutritional purposes in foods for particular nutritional uses and foods (including food supplements) for the general population. The EFSA Journal, 766, 1–42.

EFSA (European Food Safety Authority), 2009. Safety and efficacy of SELSAF (Selenium enriched yeast from *Saccharomyces cerevisiae* CNCM I-3399) as feed additive for all species. The EFSA Journal 992, 1–24.

EFSA (European Food Safety Authority), 2010. Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2010 update). EFSA Journal, 8(12):1944.

EFSA (European Food Safety Authority), 2011a. Scientific Opinion on the safety and efficacy of selenium in the form of organic compounds produced by the selenium-enriched yeast *Saccharomyces cerevisiae* NCYC R645 (SelenoSource AF 2000) for all species. EFSA Journal 9(6):2279.

EFSA (European Food Safety Authority), 2011b. Scientific Opinion on Safety and efficacy of Sel-Plex® (organic form of selenium produced by *Saccharomyces cerevisiae* CNCM I-3060) for all species. EFSA Journal 9(4):2110.

EFSA (European Food Safety Authority), 2011c, online. The EFSA Comprehensive European Food Consumption Database . Available from: http://www.efsa.europa.eu/en/datex/datexfooddb.htm

IDC (Ingredient Definition Committee), 2010, online. Minutes of meeting August 1, 2010. Portland, Oregon. Available from http://www.aafco.org/Portals/0/minutes/idc_aug_2010.pdf

Jansen HD, 1992. Mischtechnik im Futtermittelbetrieb. Anforderungen an Mischenlage, Arbeits- und Mischgenauigkeit. Die Mühle+ Mischfuttertechnik 129, 265–270.

Ruiz Encinar J, Sliwka-Kaszynska M, Połatajko A, Vacchina V and Szpunar J, 2003. Methodological advances for selenium speciation analysis in yeast. Analytica Chimica Acta 500, 171–183.

APPENDIX

Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Selemax 1000/2000[1, 2]

In the current applications authorisation is sought for Sel-Plex 2000 (FAD-2009-29) and *selenomethionine* (FAD-2010-44) under Article 4(1),

- under the category of 'zootechnical' functional group 4(d) 'other zootechnical additives' (for FAD-2009-0029); and

- under 'nutritional additives' functional group 3(b), 'compounds of trace elements' (for FAD-2010-0044),

according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of *selenomethionine* for all animal species and categories.

The product with the trade name Sel-Plex 2000 (FAD-2009-29) is a selenium enriched inactivated yeast (*Saccharomyces cerevisiae* CNCM I-3060*)* containing 2000 to 2400 mg *total selenium* / kg with a maximum of 3% of residual inorganic selenium. At least 63% of the total organic selenium in Sel-Plex 2000 is *selenomethionine*. The product related to application FAD-2010-44 and with the trade name Selemax 1000 and 2000 is a selenium enriched inactivated yeast (S*accharomyces cerevisiae* NCYC R646*)* containing a minimum of 1000 and 2000 mg *total selenium* / kg, respectively, with a maximum of 2% of residual inorganic selenium. At least 70% of the total organic selenium in Selemax is *selenomethionine*.

Both products are intended to be incorporated in the form of *premixtures* to obtain a maximum dosage of 0.5 mg total selenium /kg in *complete feedingstuffs*, to comply with legal requirements. None of the Applicants proposed minimum doses.

Both Applicants submitted a single laboratory validated and further verified methods developed by the same laboratory, internationally reputed in the field of selenium speciation.

For the determination of *selenomethionine* in the *feed additives* the Applicants proposed a triple proteolytic digestion/extraction followed by anion-exchange high performance chromatography coupled to ICPMS (HPLC-ICPMS). The following performance characteristics were presented:

- a *recovery rate* (R_{rec}) ranging from 94 to 103%;

- a relative standard deviation for *repeatability* (RSD_r) ranging from 1 to 4%; and

- a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 5 to 8%.

Based on the performance characteristics presented, the EURL recommends for official control the single laboratory validated and further verified HPLC-ICPMS method, submitted by the Applicants, to determine *selenomethionine* in the *feed additives*.

For the determination of *total selenium* in the *feed additive*, *premixtures* and *feedingstuffs* the Applicants proposed a microwave digestion using nitric acid and hydrogen peroxide (HNO_3/H_2O_2) followed by inductively coupled plasma mass spectrometry (ICPMS). The following performance characteristics were reported for the *feed additives*: R_{rec} ranging from 94 to 95%; and RSD_{ip} ranging from 2 to 7%.

[1] The EURL produced a combined report for the additives Sel-Plex 2000 and for Selemax 1000/2000

[2] The full report is available on the EURL website http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2009-0029+2010-0044.pdf

Based on the performance characteristics presented, the EURL recommends for official control the single laboratory validated and further verified ICPMS method, submitted by the Applicants, to determine *total selenium* in the *feed additives*.

However, for the determination of *total selenium* in *feedingstuffs*, the EURL investigated the former ring trial validated VDLUFA method, recently adopted as CEN standard prEN 16159:2010, based on by hydride generation atomic absorption spectrometry (HGAAS). The following performance characteristics are reported:

- a relative standard deviation for *repeatability* (RSDr) 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 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.

Based on the performance characteristics presented, the EURL recommends for official control, the ring trial validated CEN method (prEN 16159:2010) for the determination of total selenium in *premixtures* and *feedingstuffs*.

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

AAFCO	Association of American Feed Control Officials
ALT	Alanine transaminase
AP	Alkaline phosphatase
As	Arsenic
AST	Aspartate transaminase
bw	Body weight
Cd	Cadmium
CFU	Colony Forming Units
DM	Dry matter
DNA	Deoxyribonucleic acid
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
EURL	European Union Reference Laboratory
FI	Feed Intake
GLM	General Linear Model
GSH-Px	Glutathione peroxidase
Hg	Mercury
IDC	Ingredient Definition Committee
LDH	Lactate dehydrogenase
NADPH	Nicotinamide adenine dinucleotide phosphate
MRL	Maximum Residue Limit
Pb	Lead
PCDDs	Polychlorinated dibenzo-*para*-dioxins
PCDFs	Polychlorinated dibenzofurans
pH	Potential Hydrogen
pO_2	Partial pressure of oxygen
QPS	Qualified Presumption of Safety
RNA	Ribonucleic acid
SCOOP	Scientific Cooperation
Se	Selenium
Se-Cys	Selenocysteine
Se-Met	Selenomethionine
TCO_2	Total CO_2
TEQ	Toxic Equivalent Factor
WBC	White Blood Cell