European Food Safety Authority

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SCIENTIFIC OPINION

Safety and efficacy of SELSAF (Selenium enriched yeast from *Saccharomyces cerevisiae* CNCM I-3399) as feed additive for all species[1]

Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed

(Question No EFSA-Q-2008-381)

Adopted on 5 March 2009

PANEL MEMBERS

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SUMMARY

Following a request from the European Commission, the European Food Safety Authority (EFSA) was asked to deliver a scientific opinion on the safety and efficacy of the product selenium enriched yeast (*Saccharomyces cerevisiae* CNCM I-3399) (SELSAF) as a feed additive for all species.

SELSAF is a dry preparation intended to be used as a source of the essential trace element selenium (Se). It contains a minimum of 2000 mg Se kg^{-1}. SELSAF has a shelf life of at least 12 months, and a stability of six months in premixes and four months in complete feed when stored under normal conditions. Two inorganic and two organic sources of Se are already authorised as nutritional additives for animal nutrition in the European Union.

Studies on chickens for fattening, laying hens, piglets and dairy cows demonstrate that SELSAF supplementation increases the levels of Se in whole blood, tissues and blood GSH-Px activity. Therefore, SELSAF is considered as a bioavailable Se source for all animal species.

The tolerance studies with Se supplementation from SELSAF at tenfold the maximum authorised content in complete feed for three major species (laying hens, piglets and dairy cows) demonstrate that SELSAF is safe for major species when supplemented up to the maximum authorised content of Se in complete feed. Provided that the maximum authorised Se

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content in complete feed is not exceeded, the use of SELSAF as a Se source is considered to be safe for all animal species.

Based on data from a series of *in vitro* and *in vivo* assays of toxicity and genotoxicity, the Panel on Additives and Products or Substances use in Animal Feed (FEEDAP) considers that SELSAF does not introduce any additional toxicity compared to other sources of Se.

Se intake from products of SELSAF-treated animals would amount to 30 % and 25 % of the UL for young children (four to six years old) and adults, respectively. Taking into account other Se sources, Se exposure of adults would not exceed 50 % of the upper tolerable intake level (UL). Consequently, the FEEDAP Panel concludes that the use of SELSAF at the recommended level in feeds is safe for the consumers.

SELSAF is not a skin or eye irritant. However, the potential for skin or respiratory sensitisation cannot be excluded and would require that protective measures be taken by the users of the product.

The use of SELSAF in feed does not pose an additional risk to the environment compared to other sources of Se for which it will substitute.

The FEEDAP Panel made some recommendations with regards to the Register entry.

Key words: nutritional additive, compound of trace elements, SELSAF, selenium enriched yeast, selenium, selenomethionine, efficacy, safety

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BACKGROUND

Regulation (EC) No 1831/2003[2] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lies down that any person seeking an 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 joint request from the companies Société Industrielle Lesaffre[3] and Lallemand SAS[4] for authorisation of the product selenium enriched yeast (*Saccharomyces cerevisiae* CNCM I-3399) (SELSAF) as feed additive for all species (category: nutritional additive; 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.[5] 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 18 September 2008.

The inorganic selenium is permanently authorised at Community level under Directive 70/524/EEC, as trace element, in the forms of sodium selenite and sodium selenate.[6] Organic forms of selenium produced by *Saccharomyces cerevisiae* CNCM I-3060 and *S. cerevisiae* NCYC R397 are authorised in the EU under Regulation (EC) No 1831/2003.[7, 8] These latter authorisations have been granted following corresponding EFSA opinions (EFSA, 2006a, 2006b).

A technical dossier on Alkosel/SELSAF was provided to EFSA in 2005. The FEEDAP Panel estimated that the mentioned dossier contained the relevant information only for the product Alkosel®;[9] thus the assessment made by the FEEDAP Panel referred only to Alkosel® (EFSA, 2006b). The applicants have now provided a new application and the corresponding dossier concerning the product SELSAF.

[2] OJ L 268, 18.10.2003, p.29
[3] 1, rue du Haut Touquet. FR 59520 Marquette-Lez-Lille. FRANCE
[4] 19, rue des Briquetiers. FR 31702 Blagnac. France
[5] Dossier reference: FAD-2008-0005
[6] OJ C 50, 25.2.2004, p.1
[7] OJ L 330, 28.11.2006, p.9
[8] OJ L 146, 08.06.2007, p.14
[9] In the first instance, the two applicants presented a dossier dealing with two products made with different strains of *Saccharomyces cerevisiae*. Subsequently, a revised dossier was submitted for one product (manufactured with only one strain). The applicants stated that the product would be marketed with two trade names.

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 efficacy and the safety for the target animal(s), user and consumer and the environment, of the product selenium enriched yeast (*Saccharomyces cerevisiae* CNCM I-3399) (SELSAF) when used under the conditions described in Table 1.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group on Trace Elements (subgroup on SELSAF) for the preparation of this opinion.

Table 1. **Register entry as proposed by the applicant**

Additive	Selenium enriched yeast (*Saccharomyces cerevisiae* CNCM I-3399)
Registration number/EC No/No (if appropriate)	
Category(ies) of additive	Nutritional additives
Functional group(s) of additive	Compounds of trace elements

Description			
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)
Preparation of inactivated yeast of *Saccharomyces cerevisiae* CNCM I-3399 containing a minimum of 2000 mg/kg of total selenium with a maximum of 2 % of residual inorganic selenium		Not appropriate	Not appropriate

Trade name (if appropriate)	SELSAF
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^{-1} of complete feedingstuffs		
All species			0.5 mg of selenium per kg of complete feedingstuffs	Not appropriate

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use (if appropriate)	Reserved exclusively to the manufacture of animals feeds Production/expiry dates and batch number printed on the commercial packaging
Specific conditions or restrictions for handling (if appropriate)	Store in a cool and dry place
Post market monitoring (if appropriate)	Not appropriate
Specific conditions for use in complementary feedingstuffs (if appropriate)	Not appropriate

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

ASSESSMENT

1. Introduction

The product SELSAF is an additive consisting of inactivated and dried *Saccharomyces cerevisiae* (CNCM I-3399) enriched with selenium (Se). Two products of this nature have already been evaluated by the FEEDAP Panel for use as feed additives (EFSA, 2006a, 2006b). Selenised yeasts have already been previously evaluated by the Scientific Committee on Food (EC, 2000) for their uses as food supplements, and by the AFC Panel of EFSA (EFSA, 2008) as a source for selenium added for nutritional purposes in foods for particular nutritional uses and foods (including food supplements) for the general population.

SELSAF is intended to be used as a nutritional additive, providing a source of the essential trace element Se for all animal species and categories. Selenomethionine (Se-Met) is the predominant source of Se in SELSAF.

The biological role of Se, its deficiency and toxicity symptoms in farm animals have already been described in a former opinion of the FEEDAP Panel (EFSA, 2006a). Se 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 reconsider the previous opinion.

Since the Se content of grain and forages is generally low in most European countries, livestock is routinely supplied with extra dietary Se in order to avoid the consequences of Se deficiency. Se-Met is the major form of naturally occurring Se in feedingstuffs.

SELSAF, the trade name used by the applicant, is used in this opinion as a synonym for Se-enriched yeast from *Saccharomyces cerevisiae* (CNCM I-3399).

2. Characterisation of the product

2.1. Identity of the additive

SELSAF consists of inactivated and dried *Saccharomyces cerevisiae* enriched with Se. The yeast strain is deposited at the National Collection of Yeast Cultures of France, with accession number CNCM I-3399. The strain is not genetically modified.[10] Identification of the strain by PCR was provided from three batches to ensure conformity with reference profiles.

According to the product specifications, SELSAF is declared to contain a minimum of 2000 mg Se kg^{-1}, with a range between 2000 and 2400 mg Se kg^{-1}.[11] Analysis of eight batches of Se-enriched yeast showed a mean content of total Se of 2147±108 mg kg^{-1}.[12]

The product is routinely monitored for heavy metals (Cd, Hg and Pb), As and biological contaminants (total plate count, yeast, mould, *E. coli* and *Staphylococcus aureus*). These specifications were confirmed by the analysis of three batches,[13] which also included analysis of dioxins and mycotoxins (aflatoxin B1, zearalenone and ochratoxin) to ensure that they comply with the maximum levels permitted.

[10] Technical dossier, Section II, Appendix 2.11
[11] Technical dossier, Section II, Appendixes 2.4 and 2.5
[12] Technical dossier, Section II, Appendix 2.8
[13] Technical dossier, Section II, Appendix 2.10

The final product is a fine, tan to light brown powder with an odour and taste typical to dried baker`s yeast. It has a low dusting potential (0.01 % from 25 g of product, Steuber-Heubach method);[14] particle size distribution showed that 62 % were ≤ 100 µm and 6.7% ≤ 10µm.[15]

2.2. Characterisation of the active substance

Se is considered as the active substance in SELSAF. Se-Met represents 62.7 % of total Se in the product based on the speciation procedure of selenocompounds in three batches (59.9, 65.2 and 63.1). Selenocysteine was found to be the second identified organic species accounting for 2 to 4 % of total Se. Inorganic Se (IV) was found to account for < 1 % of the total Se while that of hexavalent inorganic Se accounted for < 0.02 %. The remaining organic Se compounds (25 to 30 % of total Se) are not further specified.[16]

2.3. Manufacturing process

The Se-enriched yeast (*Sacharomyces cerevisiae* CNCM I-3399) is produced under aerobic fermentation conditions in a sterile medium containing beet or cane molasses (or other carbon medium), nutritional salts, vitamins and food grade sodium selenite (Na_2SeO_3) as Se source. The Se content of the product is standardised prior to drying by mixing the Se yeast cream with standard yeast cream (without Se enrichment). The final yeast mixture is pasteurised and spray/drum dried.

2.4. Physico-chemical and technological properties of the additive

2.4.1. Stability

The applicant proposes a shelf life of three years based on data on humidity changes during a three-year storage period. The mean water content of three batches increased during this period from 4.9 to 5.4 %.[17]

The stability of SELSAF was studied during storage of three batches at either 25 ºC with 60 % RH or 40 °C with 75 % RH.[18] After 12 months of storage, the recoveries of total Se were 96.1 % and 94.9 % for 25 ºC with 60 % RH or 40 °C with 75 % RH, respectively. The respective recovery rates of Se-Met were 89.2 and 87.8 %. The product is expected to be stable to light and oxygen since the product is packed in light-tight sealed bags.

Stability of SELSAF in premixtures[19] and complete feedingstuffs[20] was tested in commercial mixtures for swine, ruminants and poultry feed stored at 25 °C with 60 % humidity for six (premixtures) or four months (complete feed). No further information was provided on the composition of premixtures and complete feed or on inclusion rates of SELSAF. Recovery of Se-Met in three types of premixes and three types of complete feeds varied from 105 to 130 % and from 86 to 93%, respectively. Based on those figures, a stability of six months in premixtures and four months in complete feed, when stored under normal conditions, can be anticipated.

[14] Technical dossier, Section II, Appendix 2.13
[15] Technical dossier, Section II, Appendix 2.12
[16] Technical dossier, Section II, Appendix 2.5
[17] Technical dossier, Section II, Appendix 2.15
[18] Technical dossier, Section II, Appendixes 2.16 and 2.17
[19] Technical dossier, Section II, Appendix 2.18
[20] Technical dossier, Section II, Appendix 2.19

Pelleting of a ruminant and a monogastric compound feed (0.31 and 0.35 mg Se kg^{-1}, respectively)[21] did not affect total Se or Se-Met. No information on pelleting conditions was provided by the applicant.

2.4.2. Homogeneity

SELSAF was analysed for homogeneous distribution in premixtures for ruminants and piglets (eight samples each). The coefficient of variation (CV) for total Se was 15 % for the ruminants premixture and 5 % for the piglets premixture (for Se-Met, 10 % and 15 %, respectively).[22] The homogeneity was also studied in pelleted feed for ruminants and piglets. The CV for ruminant and piglet feed was 10 % and 9 % for total Se and 13 % and 4 % for Se-Met, respectively.[23]

2.4.3. Incompatibilities

According to the current knowledge, no incompatibilities or adverse interactions –with feed components, carriers, other approved additives or medical products– are to be expected other than those widely recognised for inorganic Se in animal nutrition.

2.5. Conditions of use

The applicant proposes a supplementation of Se from SELSAF at amounts of 0.3 mg kg^{-1} complete feed for ruminants, pigs and poultry, corresponding to 150 mg product kg^{-1}. This would allow compliance with the maximum authorised Se content of 0.5 mg kg^{-1} feed.

Considering the range of Se content in the products (up to 2400 mg kg^{-1}), the FEEDAP Panel notes that the maximum authorised content of Se in complete feed could be exceeded in case of a feed manufacturer calculating the Se supplementation on the basis of the minimum guaranteed content of Se in SELSAF (2000 mg kg^{-1}).

2.6. Evaluation of the analytical methods by the Community Reference Laboratory (CRL)

EFSA has verified the report submitted by the Community Reference Laboratory (CRL) concerning the analytical method(s) for SELSAF. The Executive Summary of the report is attached in the Appendix.

3. Studies provided on target animals

In total, four studies on efficacy were provided, all of them containing tissue deposition data; three of them can be used as tolerance studies.

The study design is described in this section and the results described in the subsequent sections (efficacy, safety for the target animals, tissue deposition).

3.1. Chickens for fattening

A 40-day study involving 120 chickens for fattening (40 animals per treatment, ten cages with four birds each) was performed.[24] The experimental design consisted of three treatments: a group fed a control diet without any Se supplementation and two groups with supplementation of 0.3 mg Se kg^{-1} complete feed, from either sodium selenite or from SELSAF. The feeding regime included two diet types: a starter (0 to 21 days) and a grower diets (21 to 40 days). The

[21] Technical dossier, Section II, Appendixes 2.24 and 2.25
[22] Technical dossier, Section II, Appendixes 2.20 and 2.21
[23] Technical dossier, Section II, Appendixes 2.22 and 2.23
[24] Technical Dossier, Section III, Appendix 3.3

parameters measured were: body weight, daily weight gain, daily feed intake, feed to gain ratio, blood parameters (Se content and erythrocyte GSH-Px activity), liver, muscle and kidney Se content and health status of the animals. All the data were statistically analysed using the General Linear Models (GLM) procedure.

3.2. Laying hens

After a pre-treatment period of five weeks with a Se-unsupplemented diet, 96 laying hens (Isa-Brown-Warren, 22 weeks of age) were allotted to six treatment groups (eight replicates of two hens per treatment).[25, 26] The experimental treatments resulted from the supplementation of a control diet (based on corn and soy bean meal, without Se supplementation) with 0.4 mg kg^{-1} Se from sodium selenite, SELSAF or another Se-enriched yeast, or with 5 mg kg^{-1} Se from SELSAF or another Se-enriched yeast. The trial lasted for eight weeks. The intended Se content of the diets was confirmed by analysis. Health status, mortality and sanitary treatments were evaluated daily by a veterinarian. After eight weeks of treatment, eight hens per group were slaughtered and blood samples were collected for analysis of glucose, total protein, albumin, total bilirubin, cholesterol and enzyme activity (AST, ALT and ALP). Two hens (sodium selenite group) died during the trial.

3.3. Piglets

A total of 108 piglets (Landrace, 36 days of age, 10.4 kg bw) was allotted to four treatment groups (nine pens of three piglets each per group).[27] The feeding regime included three diet types: a pre-starter (main constituents: wheat, soybean meal, whey and barley), a starter (main constituents: soybean meal, corn, wheat and barley) and a pre-grower (main constituents: corn, wheat, soybean meal); they were fed for consecutive periods of two weeks each. The four experimental treatments resulted from the supplementation of a control diet (without Se supplementation) with 0.3 mg Se kg^{-1} complete feed from either sodium selenite or from SELSAF, and with 5 mg Se kg^{-1} from SELSAF. The trial lasted for six weeks. The intended Se content of the diets was analytically confirmed (mean of three diet types: $\leq$ 0.05 for the unsupplemented diets, 0.30, 0.31 and 5.8 mg kg^{-1} diet supplemented with sodium selenite, SELSAF at the highest recommended level and SELSAF at a tenfold overdose, respectively). The health status of the animals was recorded. The parameters measured were, for all piglets: body weight, daily weight gain, daily feed intake and feed to gain ratio. At the end of the study, blood samples were collected from nine piglets per treatment to determine haematology, including blood Se and erythrocyte GSH-Px activity, and routine blood chemistry (glucose, total protein, albumin, urea, cholesterol, total and conjugated bilirubin, AST, ALT and γ-GT). At the end of the experiment, six piglets per treatment were euthanised and gross pathology performed[28] prior to the collection of liver, muscle (*Longissimus dorsi* and *Psoas major*) and kidney for determination of Se content. All the data were statistically analysed using the GLM procedure.

3.4. Dairy cows

A 17-week study involving 35 Holstein crossbreed cows (from five weeks pre-partum to 12 weeks post-partum) was performed.[29] The regular Se supplementation (sodium selenite) of the diet was withdrawn one month before the start of the trial (depletion period). The animals were randomised in four groups, taking into account parity (mean 3.4) and previous lactation

[25] Technical Dossier, Section III, Appendix 3.1
[26] Technical Dossier, Section IV, Appendix 4.1
[27] Technical Dossier, Section IV, Appendix 3.2.
[28] Technical Dossier, Section IV, Appendix 4.2.
[29] Technical Dossier, Section III, Appendix 3.4.

performance (mean 9670 kg). The experimental design consisted of four dietary treatments: an unsupplemented control group (nine cows) and two groups with an intended supplementation of 0.3 mg Se kg^{-1} complete feed DM from sodium selenite (nine cows) or from SELSAF (eight cows) and of 5 mg Se kg^{-1} from SELSAF (nine cows). The cows were fed a TMR based on silage (in summer partly replaced by fresh forage) and grain. Four diet types were applied: a diet for dry cows (0.09 mg Se kg^{-1} DM), a pre-calving (0.16 mg Se kg^{-1} DM), a start-up (0.21 mg Se kg^{-1} DM) and a lactation diet (0.22 mg Se kg^{-1} DM), increasing in protein and energy content. Dietary Se was added as top-dressing, which was fully consumed. The Se source at normal supplementation rate (0.3 mg Se kg^{-1} DM) was incorporated in a daily dose of 60 g pre-mixture with wheat flour and wheat bran (sodium selenite pre-mixture: 114 mg Se kg^{-1} DM, SELSAF pre-mixture: 113 mg Se kg^{-1} DM). The high Se group received 50 g SELSAF (minimum guaranteed 2000 mg Se kg^{-1}) day^{-1}.

The parameters measured were: milk production, milk parameters (fat and protein content), blood parameters (GSH-Px, AST, γ-GT and CK, total protein and bilirubin), at start, one week before calving, at calving and four, eight and 12 weeks after calving; Se in blood, one week pre-partum, at calving, four, eight and 12 weeks after calving; colostrum and milk, four, eight and 12 weeks after calving, and; health status of the animals. However, the analytical methods applied were not described. All the data were statistically analysed either by mixed repeated procedure or by ANOVA.

The Se supplementation rate (intended levels: 0.3 and 5 mg kg^{-1} complete feed DM) was achieved by a daily consumption of fixed amounts of premixtures (6.78 mg from the SELSAF premixture and 6.84 mg from the sodium selenite premixture) and SELSAF (100 mg). Those daily doses could be related to the total feed intake of the cows. Data on feed intake are not given in the report (which only states that 'DM intake in cows in the first lactation phase was 23 kg $head^{-1}day^{-1}$, corresponding to a Se supplementation of 0.3 mg kg^{-1} complete diet DM for the recommended dose and of 4.3 mg kg^{-1} for the tenfold overdose). However, because (i) the 'first lactation period' is not quantitatively defined in terms of a period, (ii) the DM intake was likely different in other periods, (iii) the Se content of SELSAF was not analysed and (iv) the Se content of the basal diets during lactation appears higher than marginal, the results are difficult to interpret.

4. Efficacy

Evidence of *in vivo* bioavailability is required to support efficacy for compounds of trace elements not already authorised as feed additives. A single trial in a single animal species, including laboratory animals, is considered sufficient. As already established in previous opinions (EFSA, 2006a, 2006b), the efficacy of a selenised yeast product as a nutritional additive in terms of providing the essential trace element Se may be demonstrated with the following parameters of Se status:

- concentrations of Se in plasma/serum or whole blood;
- GSH-Px activity in plasma or whole blood;
- contents of Se in liver.

Those indicators should be measured in animals supplied with SELSAF in comparison with a negative control group (animals without any Se supplemented to feed), and possibly also with an animal group given equivalent amounts of Se in any currently authorised form as positive control.

4.1. Results

The results of the study on chickens for fattening are summarised in Table 2. The erythrocyte GSH-Px activity was largely increased in the Se-supplemented groups, without differences between the two Se treatments. The blood Se content also increased in both supplemented groups, being significantly higher in the SELSAF group. Liver deposition also responded to the two different selenium sources.

Table 2. **Effect of selenium supplementation (from two sources) on blood parameters of chickens for fattening**

Parameter	Control	Na_2SeO_3	SELSAF
Intended level (mg kg^{-1} feed)	**0**	**0.3**	**0.3**
Analysed level (mg kg^{-1} feed) (starter and grower diets)	**< 0.05**	**0.34/0.31**	**0.28/0.25**
Erythrocyte GSH-Px activity (U mL^{-1})	$< 1.6^{a}$	15.6^{b}	15.4^{b}
Se content (mg kg^{-1} whole blood DM)	$< 0.20^{a}$	1.16^{b}	1.46^{c}

a-c: Values in a row not sharing a common superscripts are significantly different ($P < 0.05$)

Serum Se and GSH-Px in laying hens after Se supplementation of two different sources is given in Table 3.

Table 3. **Effect of selenium supplementation (from two sources) on blood parameters of laying hens**

Parameter	Control	Na_2SeO_3	SELSAF
Intended level (mg kg^{-1} feed)	**0**	**0.4**	**0.4**
Analysed level (mg kg^{-1} feed)	**0.11**	**0.46**	**0.49**
Serum Se (mg kg^{-1})	0.2^{a}	0.2^{a}	0.3^{b}
GSH-Px[1] (U/ml)	375.3^{a}	1309.4^{b}	993.3^{b}

[1] measured in serum. Figures as reported by the applicant.
a-c: Values in a row not sharing a common superscripts are significantly different ($P < 0.05$)

Serum Se content, as well as GSH-Px activity, reflected the increasing dietary Se levels. Liver deposition also responded significantly to SELSAF supplementation.

The results of the piglets study are listed in Table 4.

Table 4. **Effect of selenium supplementation (from two sources) on blood parameters of piglets**

Parameter	None	Na_2SeO_3	SELSAF
Intended level (mg kg^{-1} feed)	**0**	**0.3**	**0.3**
Analysed level (mg kg^{-1} feed)	**≤0.05**	**0.30**	**0.31**
Blood Se (mg L^{-1})	0.054^{a}	0.195^{b}	0.185^{b}
GSH-Px (U g^{-1} haemoglobin)	61^{a}	421^{b}	352^{b}

a-c: Values in a row not sharing a common superscripts are significantly different ($P < 0.05$)

Blood Se and GSH-Px increased significantly in the selenium supplemented groups, without showing differences between the two different Se sources. Liver deposition also showed a significant increase in both selenium supplemented groups compared to the control.

The results of the dairy cows study are described in Table 5.

Table 5. **Effect of selenium supplementation (from two sources) on blood parameters of dairy cows**

Parameter	None	Na_2SeO_3	SELSAF
Intended level (mg kg^{-1} feed DM)	**0**	**0.3**	**0.3**
Blood Se (mg L^{-1})	0.068^a	0.099^b	0.1178^c
Whole blood GSH-Px (µkat L^{-1})	723^a	796^b	947^c

a-c: Values in a row not sharing a common superscripts are significantly different ($P < 0.05$)

Se supplementation to dairy cows after a depletion period resulted in a significant increase of blood Se concentration and GSH-Px activity.

4.2. Effects on the quality of animal products

An increase in Se levels in edible tissues, eggs and milk of animals fed diets supplemented with SELSAF has been observed (see details in Section 6.1 on tissue deposition).[30] Based on the literature available, adverse effects on sensory or organoleptic properties of food produced from animals treated with SELSAF are not to be expected.

4.3. Conclusions

All the studies are conclusive for the bioavailability of Se from SELSAF, as shown by an increase of Se concentrations in blood and liver as well as of GSH-Px activity.

Besides an increase of Se in tissues and products, an effect of SELSAF on the quality of animal products is not to be expected.

The FEEDAP Panel concludes that SELSAF is an effective source of Se for all animal species.

5. Safety for the target animals

Where the application is for all animal species/categories, tolerance data may be limited to one study in one target species or laboratory animal (the most sensitive in each case). The NRC (2005) published as tolerance limits: 5 mg Se kg^{-1} for cattle, 4 mg Se kg^{-1} pigs, 3 mg Se kg^{-1} for poultry. Growth depression is generally considered as the first (although unspecific) sign of intolerance.

5.1. Tolerance studies

The zootechnical parameters of laying hens taken together (unsupplemented control group: 88 % laying rate, egg weight about 58 g) are not considered indicative of any influence of high selenium supplementation from SELSAF (5.7 mg Se kg^{-1}).

No differences were observed between treatments for any of the parameters analysed, with the exception of non-esterified fatty acids (NEFA) which were significantly lower in all supplemented groups (mean 0.4 mmol) compared to the negative control group (0.8 mmol).

In the tolerance study on piglets, 5 mg supplemental Se from SELSAF did not alter either the zootechnical performance compared to 0.3 mg supplemental Se from SELSAF and to an unsupplemented group (daily body weight gain 593 g, feed to gain ratio 1.62). Haematology

[30] Technical Dossier, Section III

and routine blood biochemistry as well as necropsy were not indicative of any adverse effect of the high selenium level from SELSAF. Alopecia, a characteristic sign of selenosis, was not observed.

In dairy cows, the first hundred-day milk yield in the unsupplemented control group amounted to 4185 L, in the group with the intended Se supplementation from SELSAF of 0.3 mg kg^{-1} complete feed DM to 4159 L and in the overdose group (5 mg supplemental Se kg^{-1} DM) to 4005 L. This small decline in the milk yield of the overdose group is not considered significant; the absolute data should also be considered in relation to the group with 0.3 mg supplemental Se from sodium selenite (3692 L). Fat and protein content in milk (3.7 % and 3.1 %, respectively, in the control group) were not influenced by Se supplementation from SELSAF.

Routine blood biochemistry measurements did not reveal any differences between the unsupplemented control group and the two SELSAF groups, with the exception of CK which showed a slight decline with increasing supplemental Se from SELSAF.

5.2. Microbial studies

The production strain *S. cerevisiae* is recognised by EFSA as qualifying for QPS status. Safety studies relating to the yeast are therefore not required. No antimicrobial effects of selenium added to feeds are currently recognised.

5.3. Conclusions

A tenfold overdose of Se from SELSAF did not result in adverse effects on production parameters or selected biochemical markers in laying hens, piglets and dairy cows. The FEEDAP Panel concludes that Se supplementation from SELSAF at levels between 0.3 and 0.4 mg kg^{-1} complete diet is safe for three major species.

The product strain is recognised by EFSA as qualifying for QPS status and consequently no safety concern from that yeast would arise.

Provided that the maximum authorised Se content in complete feed is not exceeded, the use of SELSAF as a Se source is considered safe for all animal species.

6. Safety for the consumer

6.1. Tissue deposition in animals

An overview of the data reported on tissue deposition (from studies already described under Section 3) is presented in Table 6.

Table 6. **Selenium in edible tissues and products (mg kg^{-1} wet tissue/product)**

Species	**Chickens for fattening**		**Laying hens**		**Piglets**	
Se source (1)	**Na_2SeO_3**	**SELSAF**	**Na_2SeO_3**	**SELSAF**	**Na_2SeO_3**	**SELSAF**
Analysed Se (mg kg^{-1} feed)	**0.33**	**0.27**	**0.46**	**0.49**	**0.30**	**0.31**
Liver	0.51	0.63	0.51	0.72	0.40	0.48
Kidney	0.83	0.92	0.44	0.39	1.02	1.06
Muscle	0.09	0.30	0.11	0.25	0.10	0.24
Skin			0.14	0.18		
Eggs			0.21	0.33		

(1) Source of supplemental Se.

Se contents in milk (intended dietary concentration: 0.5 mg Se kg^{-1} complete feed DM) were 17 µg L^{-1} and 20 µg L^{-1} for supplementation with sodium selenite and SELSAF, respectively.[31]

Only in the study on laying hens and presumably in the study on dairy cows the maximum proposed Se dose in feed was administered. Se in liver and kidney was only marginally affected by the source of dietary Se supplementation, whereas Se in muscle showed a significant increase (by a factor of 2 to 3) in all animal categories studied after application of SELSAF, compared to the inorganic Se source. Se contents in eggs were also significantly increased (~50 %) by supplementation of Se from SELSAF.

6.2. Toxicological studies

S. cerevisiae is considered by EFSA to qualify for QPS status (EFSA, 2007), therefore studies on laboratory animals should be focused on the potential toxicity of Se from SELSAF.

6.2.1. Acute toxicity

No studies on SELSAF were performed. A published study (Vinson and Bose, 1987) on rats treated orally with single doses of Se-enriched yeast and sodium selenite showed a lower acute oral toxicity with the organic Se source: 47.1 mg Se kg^{-1} bw lethality was 40 % whereas sodium selenite elicited a 50 % lethality at 12.8 mg Se kg^{-1} bw.

6.2.2. Genotoxicity/Mutagenicity

SELSAF was tested in an appropriate set of genotoxicity studies, namely:

- Reverse mutation assay on five *S. typhimurium* strains (TA98, TA100, TA102, TA1535 and TA1537) with and without S-9 mix metabolic activation (OECD guideline 471).[32]
- *In vitro* mutation assay on mouse lymphoma L5178Y cells with and without S-9 mix metabolic activation (OECD 476).[33]
- *In vivo* mouse micronucleus in Swiss mice (5/sex/group) treated orally with single doses of 500, 1000 and 2000 mg kg^{-1} bw SELSAF (OECD 474).[34]

No findings indicative of a mutagenic and/or genotoxic potential were observed.

6.2.3. Repeated-dose toxicity studies

In a 28-day oral study, Wistar rats (ten rats per group, five of each sex) were exposed orally through diet to amounts of Se, from either sodium selenite or SELSAF, equivalent to 0.05, 0.25 and 1.0 mg kg^{-1} bw day^{-1}.[35] Reduced weight gain, biochemical markers of liver, kidney and haematopoiesis disturbances (increased bilirubin, alanine aminotransferase activity and albumin, reduced platelets), histological signs of liver toxicity, such as vacuolisation, and increased necrotic foci were seen at top-dose levels for both SELSAF and sodium selenite. An increase of liver histological alterations was seen in mid- and low-dose levels of both Se forms. No significant differences in the amount and type of treatment-related changes were observed between SELSAF and sodium selenite.

In a 90-day oral study, Wistar rats (ten rats per group, five of each sex) were exposed orally through diet to amounts of Se, from either sodium selenite or SELSAF, equivalent to 0.01, 0.05

[31] Technical Dossier, Section III, Appendix 3.4.
[32] Technical Dossier, Section IV, Appendix 4.9.
[33] Technical Dossier, Section IV, Appendix 4.10.
[34] Technical Dossier, Section IV, Appendix 4.11.
[35] Technical Dossier, Section IV, Appendix 4.6.

and 0.20 mg kg^{-1} bw day^{-1}.[36] A trend towards increased weight gain was observed in male rats for both SELSAF and sodium selenite. Aspartate aminotransferase activity in serum increased in female rats only, at all dose levels for SELSAF and at ≥ 0.05 mg kg^{-1} bw for sodium selenite. An increase of necrotic foci and hepatocyte vacuolisation was observed in liver at top-dose level for both SELSAF and sodium selenite.

6.2.4. Conclusions on genotoxicity/toxicity studies

SELSAF has been shown to be non-mutagenic and non-genotoxic. Any toxic effects observed in a repeated-dose study could be ascribed to Se and were independent of the source.

6.3. Consumer exposure

The tolerable upper intake level (UL) established by the EU Scientific Committee on Food (EC, 2000) is 300 µg Se day^{-1} in the adult, with age-related ULs adjusted on a body weight basis (e.g. 90 µg day^{-1} in a child of four to six years old). According to the SCF assessment, a daily background intake of 60 µg Se is assumed in the adult individual in the EU.

The worst case exposure model given by Regulation (EC) No 429/2008[37] can be followed taking into account the caveat that tissue values from chickens for fattening and piglets are obtained with Se concentrations in the diet below the maximum authorised level. There are no data on Se deposition in fat. However, there is data on skin Se deposition for laying hens. Since the Se content in fat is negligible, skin deposition in poultry is used to estimate the potential consumer exposure. In the model calculation, the highest exposure originating from a tissue resulting from a certain animal species is taken. Table 7 summarises the calculation.

Table 7. **Worst case exposure of the consumer to selenium**

Tissue/product	Intake (g)	Se from Na_2SeO_3 (mg day^{-1})	Se from SELSAF (mg day^{-1})	Origin
Liver	100	0.051	0.063	[38]
Kidney	50	0.051	0.053	[39]
Muscle	300	0.027	0.090	[40]
Eggs	100	0.021	0.033	[41]
Skin	90	0.012	0.016	[42]
Milk	1500	0.026	0.030	[43]
Sum		**0.188**	**0.285**	

The use of SELSAF as Se source would result in an increase of the worst case exposure through the consumption of products from treated animals by about 0.1 mg day^{-1} compared to the inorganic Se, which would be near to the UL. Taking into account other sources of Se intake, the UL would be exceeded.

As Se is a natural constituent of food, it appears reasonable to refine the daily intake by more realistic data (Table 8). As a reference, the same SCOOP data (EC, 2004) will be used as in the assessment of other Se-enriched yeasts (EFSA, 2006a, 2006b).

[36] Technical Dossier, Section IV, Appendix 4.4.
[37] OJ L 133, 22.5.2008, p.1
[38] Technical Dossier, Section III, Appendix 3.3
[39] Technical Dossier, Section III, Appendix 3.2
[40] Technical Dossier, Section III, Appendix 3.3
[41] Technical Dossier, Section III, Appendix 3.1
[42] Technical Dossier, Section III, Appendix 3.1
[43] Technical Dossier, Section III, Appendix 3.4

Table 8. **Calculation of the maximum daily selenium intake by adult consumers through the consumption of tissues and products from animals fed SELSAF based on consumption figures corresponding to SCOOP data (EC, 2004)**

Tissue/product	Intake (g)	Se from Na_2SeO_3 (mg day^{-1})	Se from SELSAF (mg day^{-1})	Origin
Liver	35a	0.018	0.022	[44]
Kidney	3.5[a]	0.004	0.004	[45]
Muscle	105[a]	0.010	0.032	[46]
Eggs	36[b]	0.008	0.012	[47]
Milk	280[c]	0.005	0.006	[48]
Sum		**0.045**	**0.076**	

(a) Meat intake calculated with the same proportionas in Regulation (EC) No 429/2008, i.e. 60 % muscle, 20 % liver, 2 % kidney and 18 % skin/fat
(b) Maximum value
(c) Mean value

The resulting consumption figure of 0.076 mg day^{-1} for SELSAF is considerably below the Se UL for adults (0.300 mg day^{-1}). Assuming a background Se intake from other dietary sources (e.g. vegetables, fish) of 0.060 mg day^{-1} (EC, 2000), the overall Se intake would be of 0.136 mg day^{-1} (about 45 % of adult UL). This is in agreement with published data on the use of selenised yeast, suggesting that the total exposure from food of animals treated with Se-enriched yeast would not exceed 0.2 mg day^{-1} (Mahan and Parrett, 1996).

The SCOOP data do not provide information on fat (mammals) or skin + fat (avian species) consumption figures. However, even assuming a highly unlikely daily consumption of 90 g of skin + fat from SELSAF-exposed poultry, this would increase the overall daily Se intake to 0.152 mg, i.e. ~50 % of the UL.

The consumption of food commodities from SELSAF-treated animals will not exceed either the recommended ULs of Se in the adolescent age groups (0.200 and 0.250 mg day^{-1}, for the 11-14 years and 15-17 years age groups, respectively), even taking into account the Se background intake.

Exceeding the UL of younger age groups would require the same consumption figures as for adults, which is extremely unlikely. The FEEDAP Panel has assessed the potential exposure of children (four to six years old) adopting the same approach as that taken in the assessment of consumer exposure to other Se-enriched yeasts (EFSA, 2006a, 2006b) (Table 9).

Table 9. **Calculation of the maximum daily selenium intake (mg day-1) by young children (four to six years old)**

Tissue/product	Intake (g)	Na_2SeO_3 as feed additive	SELSAF as feed additive
Muscle	52.5	0.005	0.016
Eggs	18	0.004	0.006
Milk	250	0.004	0.005
Sum		**0.013**	**0.027**

[44] Technical Dossier, Section III, Appendix 3.3
[45] Technical Dossier, Section III, Appendix 3.2
[46] Technical Dossier, Section III, Appendix 3.3
[47] Technical Dossier, Section III, Appendix 3.1
[48] Technical Dossier, Section III, Appendix 3.4

In young children (four to six years old), the consumption of offal is assumed to be insignificant and the consumption of skin + fat is also taken as very low. Thus, the exposure of small children to Se from animals fed SELSAF would come close to the age-specific UL (90 μg day^{-1}) only with the highly unlikely assumption that Se background intake is the same as in adults.

6.4. Conclusions

Based on the age-specific ULs established by the SCF for Se and on realistic consumption figures from SCOOP, already utilised to assess consumer exposure to other Se-enriched yeasts, the FEEDAP Panel considers that the use of SELSAF at the recommended levels in feeds is safe for the consumer.

7. Safety for the user/worker

7.1. Skin irritation

In an acute dermal irritation test on NZW rabbits, performed according to OECD guideline 404, SELSAF proved to have no significant potential for skin irritation as only transient slight to moderate erythema was elicited by the direct application of 500 mg SELSAF.[49]

7.2. Eye irritation

In an acute eye irritation test on NZW rabbits, performed according to OECD guideline 405, SELSAF proved to have no significant potential for eye irritation as only moderate and transient chemosis and conjuctival redness were elicited by the application of 100 mg SELSAF in the conjunctival sac.[50]

7.3. Acute inhalation toxicity

No acute inhalation studies were provided. The inhalable fraction of SELSAF dust particles is significant: 62 % of particles showing a diameter ≤ 100 μm and 6.7 % of particles ≤ 10 μm. However, the dusting potential is low: 0.01 %.

7.4. Conclusions

SELSAF proved to have no significant potential for skin or eye irritation. It is presumed, as with all proteinaceous products, to be a potential respiratory sensitiser. Although no studies are provided on the effects of inhalation exposure or on skin sensitation, any precautions appropriate to protect the user from the sensitising properties would be sufficient to protect against any potential inhalation toxicity and direct skin contact.

8. Safety for the environment

Selenium is a natural element that is essential for life and is widely spread in the environment. The FEEDAP Panel considers that the use of SELSAF in feed does not represent additional risks to the environment, compared to other sources of Se for which it will substitute, as long as the maximum authorised content in feedingstuffs is not exceeded.

[49] Technical Dossier, Section IV, Appendix 4.7.
[50] Technical Dossier, Section IV, Appendix 4.8.

9. Post-market monitoring

No particular risks associated with the use of the product are foreseen. It is considered that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[51] and Good Manufacturing Practice.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Studies on chickens for fattening, laying hens, piglets and dairy cows demonstrated that SELSAF supplementation increased the levels of Se in whole blood, tissues and blood GSH-Px activity. The FEEDAP Panel concludes that SELSAF can be considered as a bioavailable Se source, comparable to other authorised sources of Se, for all animal species.

The tolerance studies with Se supplementation at tenfold the maximum authorised content in complete feed from SELSAF for three major species (laying hens, piglets and dairy cows) demonstrate that SELSAF is safe for major species when supplemented up to the maximum authorised content of Se in complete feed. Provided that the maximum authorised Se content in complete feed is not exceeded, the use of SELSAF as a Se source is considered by the FEEDAP Panel to be safe for all animal species.

Based on data from a series of *in vitro* and *in vivo* assays of toxicity and genotoxicity, the FEEDAP Panel concludes that SELSAF does not introduce any additional toxicity compared to other authorised sources of Se.

Se intake from products of SELSAF-treated animals would amount to 30 % and 25 % of the UL for young children (four to six years old) and adults, respectively. Taking into account other Se sources, Se exposure of adults would not exceed 50 % of the UL. Consequently, the FEEDAP Panel concludes that the use of SELSAF at the recommended level in feeds is safe for the consumer.

SELSAF is not a skin or eye irritant. However, the potential for skin or respiratory sensitisation cannot be excluded and would require protective measures to be taken by users of the product.

The use of SELSAF in feed does not pose an additional risk to the environment compared to other sources of Se for which it will substitute.

RECOMMENDATIONS

In order to respect the maximum dose of Se currently authorised in the EU for complete feedingstuffs, including the background levels of Se in feeds, the guarantee of the applicants for a minimum Se content of 2000 mg kg^{-1} in SELSAF should be supplemented by a maximum guarantee specification, e.g. up to 2400 mg.

The FEEDAP Panel recommends that a method capable of measuring Se-Met in feedingstuffs should be developed in order to distinguish between organic and inorganic sources of Se. This would facilitate the setting of different maximum levels which would take into account bioavailability.

Due to the proteinaceous nature of the product, the FEEDAP Panel recommends the labelling of SELSAF as a respiratory sensitiser.

[51] OJ L 35, 8.2.2005, p.1

DOCUMENTATION PROVIDED TO EFSA

1. SELSAF (Selenium enriched yeast - *Saccharomyces cerevisiae*). Nutritional additive (compounds of trace elements for all species). February 2008. Submitted by Société Industrielle Lesaffre and Lallemand SAS.
2. Evaluation report of the Community Reference Laboratory feed additives authorisation on the methods(s) of analysis for SELSAF. December 2008.
3. Comments from the Member States received through the ScienceNet.

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APPENDIX

Executive Summary of the Evaluation Report of the Community Reference Laboratory Feed Additives Authorisation on the Method(s) of Analysis for SELSAF

In the current application authorisation is sought for SELSAF under the category/functional group (3/b), nutritional additives/compounds of trace elements, according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought to use SELSAF as a source of selenium for all animal species. SELSAF is an inactivated Selenium enriched yeast (Saccharomyces cerevisiae CNCM I-3399) product, containing high levels of the essential trace element selenium. The inactivated and dried Selenium enriched yeast product is blended with non viable dehydrated yeast (Saccharomyces cerevisiae CNCM I-3399) to adjust the selenium content. The final product is an inactivated whole cell yeast containing minimum 2000 mg/kg of total selenium with a maximum of 2% of residual inorganic selenium. At least 60% of the total organic selenium is in the form of seleno-methionine. SELSAF is added to the feedingstuffs to obtain a concentration of total Se up to 0.5 mg/kg.

The active substance is measured as total selenium regardless of its chemical form, i.e. independently of whether it is present as organically-bound or inorganic Se.

For the determination of the active substance in SELSAF either flame atomic absorption spectrometry (FAAS) or inductively coupled plasma atomic emission spectrometry (ICP-AES) methods are proposed by the applicant. Since both methods are based on well known principles, they are considered suitable for the determination of selenium in the feed additive.

For the determination of the active substance (total selenium) in premixtures and feedingstuffs the same two methods (FAAS and ICP-AES) are proposed. Since information on a complete validation study performed on the target feed was not provided, the suitability of these methods for official control purposes cannot be evaluated.

However, for official control regarding the determination of the active substance in premixtures and feedingstuffs, the CRL recommends an analytical method that has been ring-trial validated in the relevant matrices at the relevant concentrations of the active substance. The method and the results from the related inter-laboratory study are presented in the method collection of the "Association of German Agricultural Analytical and Research Institutes" (VDLUFA, Germany). The method for the determination of selenium by hydride generation atomic absorption spectrometry (HGAAS) after microwave digestion – based on the extraction with 65% nitric acid and 30% H2O2. The following method performance characteristics are reported: - a reproducibility relative standard deviation (RSDR) of 7.3 % for a pre-mixture containing 112 mg/kg of Se; RSDR = 7.4 % for a feedingstuffs containing 0.48 mg/kg of Se and the limits of quantification are clearly below the legal limit of 0.5 mg Se /kg feed and therefore acceptable for the purpose of analysis. This VDLUFA method is currently being adopted as a CEN standard.

No further testing or validation is required.