Safety and efficacy of selenium-enriched yeast (*Saccharomyces cerevisiae* CNCM I-3399) for all animal species

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Guido Rychen, Gabriele Aquilina, Giovanna Azimonti, Vasileios Bampidis, Maria de Lourdes Bastos, Georges Bories, Andrew Chesson, Pier Sandro Cocconcelli, Gerhard Flachowsky, Jürgen Gropp, Boris Kolar, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Baltasar Mayo, Fernando Ramos, Maria Saarela, Roberto Edoardo Villa, Robert John Wallace, Pieter Wester, Alena Pechova, Gloria López-Gálvez and Alberto Mantovani

Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of selenium-enriched yeast (*Saccharomyces cerevisiae* CNCM I-3399) for all animal species, based on a dossier submitted for the modification of the terms of authorisation of the additive. The additive is currently authorised as selenomethionine produced by *S. cerevisiae* CNCM I-3399 as a nutritional additive (compound of trace elements) with a minimum selenium content of 2,000 mg/kg. The applicant proposed the inclusion of an additional formulation with a minimum content of selenium in the additive of 3,000 mg/kg. Considering (i) that there are no relevant changes in the manufacturing of the product compared to the former application and (ii) that the conditions of use already authorised remain the same, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) stated that the modification requested would not affect the conclusions of the FEEDAP Panel on safety for the consumer, safety for the environment and efficacy of the product made in a previous assessment of a similar product with a lower selenium concentration. Since the capacity of the additive to homogeneously distribute in feed was proven, the tolerance studies already provided for the currently authorised product could be used to conclude on the safety of the additive for the target animals. Selenium is hazardous upon inhalation; owing to the dusting potential and the selenium content of dust, persons handling the additive are at risk. The additive should be considered as a respiratory sensitiser. The additive is not an irritant for eyes and skin. No conclusions can be reached on the dermal sensitising properties of the additive.

Keywords: nutritional additive, compounds of trace elements, selenium, selenium-enriched yeast, SELSAF, safety

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Correspondence: feedap@efsa.europa.eu
Panel members: Gabriele Aquilina, Giovanna Azimonti, Vasileios Bampidis, Maria de Lourdes Bastos, Georges Bories, Andrew Chesson, Pier Sandro Cocconcelli, Gerhard Flachowsky, Jürgen Gropp, Boris Kolar, Maryline Koubà, Marta López-Alonso, Secundino López Puente, Alberto Mantovani, Baltasar Mayo, Fernando Ramos, Guido Rychen, Maria Saarela, Roberto Edoardo Villa, Robert John Wallace and Pieter Wester.

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Summary

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of selenium-enriched yeast (*Saccharomyces cerevisiae* CNCM I-3399) for all animal species. The additive is currently authorised as selenomethionine produced by *S. cerevisiae* CNCM I-3399 as a nutritional additive (compound of trace elements) with a minimum selenium content of 2,000 mg/kg. The applicant proposed to modify the minimum content of selenium in the additive by including also the option of 3,000 mg/kg.

The applicant provided data of 10 batches which supported specifications for (i) selenium content of minimum 3,000 mg/kg and (ii) selenomethionine content of minimum 63% of the organic selenium.

Considering (i) that there are no relevant changes in the manufacturing of the product compared to the former application and (ii) that the conditions of use already authorised remain the same, the FEEDAP Panel stated that the modification requested would not affect the conclusions of the FEEDAP Panel on safety for the consumer, safety for the environment and efficacy of the product made in a previous assessment of a similar product with a lower selenium concentration. Since the capacity of the additive to homogeneously distribute in feed was proven, the tolerance studies already provided for the currently authorised product could be used to conclude on the safety of the additive for the target animals.

Selenium is hazardous upon inhalation; owing to the dusting potential and the selenium content of dust, persons handling the additive are at risk. The additive should be considered as a respiratory sensitiser. The additive is not an irritant for eyes and skin. No conclusions can be reached on the dermal sensitising properties of the additive.

The FEEDAP Panel gave some recommendations regarding (i) the denomination of the additive under assessment as 'selenium in the form of organic compounds produced by the selenium-enriched yeast *Saccharomyces cerevisiae* CNCM I-3399', (ii) the consideration of a maximum selenium guaranteed specification, (iii) the minimum content of selenomethionine and (iv) the inclusion of a selenocysteine content in the characterisation of the product.
1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003\(^1\) establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 13(3) of that Regulation lays down that if the holder of an authorisation proposes changing the terms of the authorisation by submitting an application to the Commission, accompanied by the relevant data supporting the request for the change, the Authority shall transmit its opinion on the proposal to the Commission and the Member States.

The European Commission received a request from Phileo, a division of S.I. Lesaffre\(^2\) for modification of the terms of authorisation of the product selenium-enriched yeast \(\textit{Saccharomyces cerevisiae} \) CNCM I-3399, when used as a feed additive for all animal species (category: nutritional additives; functional group: compounds of trace elements).

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 13(3) (modification of the authorisation of a feed additive). The particulars and documents in support of the application were considered valid by EFSA as of 28 June 2016.

According to Article 8 of Regulation (EC) No 1831/2003, 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. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment, and on the efficacy of the product selenium-enriched yeast \(\textit{Saccharomyces cerevisiae} \) CNCM I-3399, when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The additive is based on selenium-enriched yeast from \(\textit{S. cerevisiae} \) CNCM I-3399. The FEEDAP Panel adopted in 2009 an opinion on the safety and efficacy of this additive for all species (EFSA FEEDAP Panel, 2009). The respective authorisation of selenomethionine (Se-Met) produced by \(\textit{S. cerevisiae} \) CNCM I-3399 was granted in 2009 by Commission Regulation (EC) No 900/2009\(^3\). The authorisation was later modified by Commission Regulation Implementing Regulation (EU) No 427/2013\(^4\) limiting the maximum selenium supplementation rate from organic selenium sources to 0.2 mg/kg complete feed.

The application is for the modification of the current authorisation to introduce a new formulation of the additive with a higher selenium concentration (minimum 3,000 mg Se/kg; trade name: SELSAF 3000), with the same conditions of use as for the authorised product.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier\(^5\) in support of the request of modification of the authorisation for selenium-enriched yeast \(\textit{Saccharomyces cerevisiae} \) CNCM I-3399 as a feed additive. The technical dossier was prepared following the provisions of Article 13 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008\(^6\) and the applicable EFSA guidance documents.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies to deliver the present output.

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\(^1\) 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.

\(^2\) Phileo - Division of S.I. Lesaffre. Rue Gabriel Peri - 137 - BP 3029. 59703 Marcq en Baroeul. France.

\(^3\) Commission Regulation (EC) No 900/2009 of 25 September 2009 concerning the authorisation of selenomethionine produced by \textit{Saccharomyces cerevisiae} I-3399 as a feed additive. OJ L 256, 29.9.2009, p. 12.

\(^4\) Commission Implementing Regulation (EU) No 427/2013 of 8 May 2013 concerning the authorisation of selenomethionine produced by \textit{Saccharomyces cerevisiae} NCYC R646 as a feed additive for all animal species and amending Regulations (EC) No 1750/2006, (EC) No 634/2007 and (EC) No 900/2009 as regards the maximum supplementation with selenised yeast. OJ L 127, 9.5.2013, p. 20.

\(^5\) FEED dossier reference: FAD-2016-0028.

\(^6\) 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.
The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment are valid and applicable for the current application.\(^7\)

### 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of selenium-enriched yeast \((\textit{Saccharomyces cerevisiae} \text{ CNCM I-3399})\) is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on nutritional additives (EFSA FEEDAP Panel, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011) and Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b).

### 3. Assessment

The additive selenium-enriched yeast \((\textit{Saccharomyces cerevisiae} \text{ CNCM I-3399})\) is authorised as a nutritional additive, functional group ‘Compounds of trace elements’, to be used in feedingstuffs for all animal species up to the maximum content in complete feeds authorised in the European Union (EU). The specifications of the currently authorised additive are organic selenium mainly Se-Met (63%) content of 2,000–2,400 mg Se/kg (97–99% of organic selenium).

The FEEDAP Panel has already assessed the safety and efficacy of selenium-enriched yeast \((\textit{Saccharomyces cerevisiae} \text{ CNCM I-3399})\) in a previous opinion (EFSA FEEDAP Panel, 2009). It considered that the proposed modification (see Section 1.2) would not substantially affect the previous assessment as related to the safety of the consumers and the environment and the efficacy of the product. Consequently, the FEEDAP Panel focused this assessment on the characterisation of the product and relevant safety aspects (target animals and users).

#### 3.1. Characterisation

##### 3.1.1. Manufacturing Process\(^8\)

The manufacturing process is fully described in the technical dossier; it is, in essence, identical to that of the previously submitted and authorised product. No new components of concern have been introduced.

##### 3.1.2. Characterisation of the additive

The product consists of inactivated whole cell yeast containing a minimum of 3,000 mg/kg of total selenium and a maximum of 2% of residual inorganic selenium. At least 60% of the total organic selenium is in the form of Se-Met.

The additive does not have a chemical name according to International Union of Pure and Applied Chemistry (IUPAC) nomenclature or a Chemical Abstracts Service (CAS) number.

The analyses of five batches for the content of total selenium and selenium from Se-Met gave values between 3,031 and 3,226 mg/kg (mean: 3,109), and 1,949 and 2,099 mg Se/kg (mean: 2,042), respectively.\(^9\) The percentage of dry matter ranged between 94.8% and 96.9% (specification ≥ 94%). Three of these five batches were also analysed for Se(IV) (range: 2.1–3.9 mg/kg) and Se(VI) (< 1 mg/kg).\(^10\)

The applicant provided the analysis of another set of five batches of the additive for further characterisation of the selenium species in the additive.\(^11\) The mean values were for total selenium 3,087 mg/kg (range: 2,947–3,224), for selenium from Se-Met 2,070 mg/kg (range: 1,950–2,290), for selenium from selenocysteine (Se-Cys) 404 mg/kg (range: 340–466), for selenium from water-soluble selenometabolites 397 mg/kg (range: 359–435), for selenium from selenium unknown species 221 mg/kg (range: 172–287). It is noted that the soluble selenium fraction is likely to contain some amount of unidentified organic selenium sources. In summary, the mean values would indicate that total selenium of

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\(^7\) The report linked to the previous dossier (related to EFSA-Q-2008-381) is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2008-0005.pdf

\(^8\) This section has been amended following the confidentiality claims made by the applicant.

\(^9\) Technical dossier/Section II/Annex II_1_10.

\(^10\) Technical dossier/Supplementary information/Appendix II-1-17.
the additive consists of approximately 67% Se-Met, 13% Se-Cys, 13% of water-soluble selenometabolites, 7% of unknown selenium species and < 1% of inorganic Se(IV) and Se(VI).

Heavy metals, fluorine and arsenic in the manufactured additive were analysed in three batches. The results were: lead < 0.5, cadmium < 0.1, mercury < 0.01, fluorine < 20 and arsenic < 0.5 mg/kg.

Dioxins and the sum of dioxins plus dioxin-like PCBs in the additive were analysed in three batches; the results showed an average of 0.042 ng WHO-PCDD/F-TEQ per kg and 0.064 ng WHO-PCDD/F-PCB-TEQ per kg. The applicant also provided analytical data on mycotoxins in three batches. The mycotoxin levels, except aflatoxin B1 (0.13–0.30 µg/kg), were below the limit of quantification. All the reported values are within limits set in the Directive 2002/32/EC on undesirable substances in animal feed for feed additives belonging to the functional group compounds of trace elements or, where no specific limit is mentioned, do not represent a safety concern.

The applicant provided analytical data from five batches on wild yeast and moulds, Escherichia coli, and Salmonella. The results showed average contents of (in CFU/g): yeast and moulds < 100, E. coli < 1; Salmonella was absent in 25 g product.

3.1.2.1. Physical state of the product

The additive is light beige, fine powder with typical yeast odour. Its bulk density is 540 kg/m³ (range: 520–560) and the tapped density of 0.66 g/cm³ (range: 0.64–0.67) (average of three batches each).

Particle size distribution was measured by laser diffraction in three batches. The particle fractions below 10, 50 and 100 µm were 3.0–3.8%, 20.7–24.3% and 51.1–57.0% (v/v), respectively. A comparison of these data with those provided in the previous dossier was possible for the particles below 10 and 100 µm in which those were 6.7% and 62%, respectively, thus showing the same dimension.

The dusting potential of three batches of the additive was analysed by the Stauber–Heubach method; further to that the dust was submitted to selenium analysis. The emitted dust was around 0.72 g/m³ (0.61–0.88 g/m³). The selenium content of the dust varies between 2,316 and 2,481 mg/kg (overall mean of 2,420 mg/kg).

3.1.3. Stability and homogeneity

For compounds of trace elements stability studies are generally not required.

The applicant presented a study to demonstrate the homogenous distribution of the additive in complete feedingstuffs. For this, piglet feed (selenium background level not considered) was supplemented with the additive (SELSAF 3000) to a level of 0.2 mg Se/kg; eight samples were analysed. The mean value of total selenium was 0.27 mg/kg, with a coefficient of variation (CV) of 7.6%. Although the selenium background level is not known, the small CV indicates the capacity of the additive to homogenously distribute in feed.

3.1.4. Conditions of use

The additive is intended to be used as a nutritional feed additive for all animal species. The maximum supplementation of feed with the additive should not exceed 0.2 mg Se/kg of complete feed with a moisture content of 12%, and should respect the maximum total content of selenium authorised (0.5 mg Se/kg complete feed).

3.2. Safety

In its previous opinion (EFSA FEEDAP Panel, 2009), the FEEDAP Panel concluded that the additive was safe at the maximum total dose of selenium at the time of the assessment. Safety for consumer and environment of the product would not be affected by the proposed modification to the terms of authorisation, considering (i) that there are no relevant changes in the manufacturing of the product compared to the former application, and (ii) that the application does not interfere with Commission
Implementing Regulation (EU) No 427/2013 establishing a maximum supplementation of selenium from all organic sources (0.2 mg/kg).

3.2.1. Safety for the target species

The only aspect which would require a reconsideration of the previous assessment of the safety for target species could be derived from the higher selenium concentration of the additive and the subsequent potential of a less homogeneous distribution in feed. However, homogeneity data (see Section 3.1.3) could demonstrate that there is no concern. The active compound/s in the additive is/are the same as in the previously assessed selenised yeast (EFSA FEEDAP Panel, 2009); consequently, the additive is considered safe for all animal species.

3.2.2. Safety for the users

3.2.2.1. Effects on the respiratory system

In the previous assessment, the FEEDAP Panel concluded that 'SELSAF® 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 sensitisation, any precautions appropriate to protect the user from the sensitising properties would be sufficient to protect against any potential inhalation toxicity’ (EFSA FEEDAP Panel, 2009).

Owing to the higher concentration of selenium in the additive proposed within the current application, safety for the user could be impacted, and therefore, the FEEDAP Panel considers it necessary to reconsider its former assessment. The highest measured dusting potential of the additive was 0.88 g/m³. The selenium concentration in the dust varied between 2,316 and 2,481 mg Se/kg (average = 2,420 mg/kg). Thus, up to 2.2 mg Se/m³ (0.88 g dust/m³ × 2,481 mg Se/kg dust) could be released by the dust when handling the additive. The corresponding value in the previous dossier submitted in 2008, for which an EFSA opinion was delivered in 2009, has a similar magnitude (2.3 mg Se/m³) (EFSA FEEDAP Panel, 2009).

The maximum respirable fraction in the additive was 3.8%, the maximum fraction of particle size < 50 µm was 24.3%. Assuming that the dust consists only of particles < 50 µm, its respirable fraction could be estimated to be 15.6% (3.8 of 24.3). The selenium concentration in the respirable dust would then be 0.33 mg/m³ (0.156 × 2.1 mg Se/m³).

Selenium compounds are recognised as highly toxic by inhalation. Concerning threshold limit values (TLV) for selenium compounds, air concentrations between 0.02 and 0.2 mg Se/m³ have been set by different organisations (e.g. German Maximale Arbeitsplatz Konzentration List (DGF, 2012), Occupational Safety and Health Administration, National European Authorities). Consequently, and considering the above estimate of selenium from the dust of the additive, its handling is a risk to users.

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

3.2.2.2. Effects on the eyes and skin

The FEEDAP Panel, in its previous opinion, concluded that ‘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, as well as that ‘SELSAF proved to have no significant potential for eye irritation as only moderate and transient chemosis and conjunctival redness were elicited by the application of 100 mg SELSAF in the conjunctival sac’ (EFSA FEEDAP Panel, 2009). However, the FEEDAP Panel notes that selenium in high concentrations is recognised to be an irritant to eyes and skin (ATSDR, 2003).

No studies on dermal sensitisation were provided by the applicant.

3.2.2.3. Conclusions on safety for the user

Selenium is hazardous upon inhalation; owing to the dusting potential and the selenium content of dust, persons handling the additive are at risk. The additive should be considered as a respiratory sensitiser. The additive is not an irritant for eyes and skin. No conclusions can be reached on the dermal sensitising properties of the additive.

3.3. Efficacy for all animal species

As already stated in the introductory paragraph under Assessment (Section 3), the efficacy of the product would not be affected by the proposed modification of the terms of authorisation.
3.4. 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\(^\text{20}\) and Good Manufacturing Practice.

4. Conclusions

The modification of the terms of authorisation of the selenium-enriched yeast, consisting of an introduction of a new formulation with a minimum selenium content of 3,000 mg Se/kg additive does not affect the conclusions of the FEEDAP Panel on safety for the target animals, consumer, safety for the environment and efficacy of the product made in a previous assessment of a similar product with a lower selenium concentration.

The additive is not an irritant for the eyes and skin, but should be considered as a respiratory sensitisier. No conclusion can be reached on its dermal sensitising properties. Selenium is hazardous upon inhalation; owing to the high dusting potential, persons handling the additive are at risk.

5. Recommendations

In accordance with the more recent relevant opinions on selenium from selenised yeasts, the FEEDAP Panel recommends the denomination of the additive under assessment as 'selenium in the form of organic compounds produced by the selenium-enriched yeast \textit{Saccharomyces cerevisiae} CNCM I-3399'. In the view of the Panel, the denomination of selenised-yeast derived additives as Se-Met could be misleading.

In order to respect the maximum supplementation dose of selenium currently permitted in the EU with selenised yeast (0.2 mg Se/kg), and in agreement with the previous assessment of this selenised yeast (\textit{Saccharomyces cerevisiae} CNCM I-3399), the FEEDAP Panel recommends the potential newly guaranteed minimum selenium content of 3,000 mg/kg additive being supplemented by a maximum guaranteed specification (e.g. up to 3,500 mg/kg).

As in the current authorisation, at least 63% of the total selenium should be in the form of Se-Met. The content of Se-Cys could be included as specification of the additive (at least 10% of total selenium); an analytical method should be validated.

**Documentation provided to EFSA**

1. Dossier Selenium-enriched yeast (\textit{Saccharomyces cerevisiae} CNCM I-3399). March 2016. Submitted by Phileo - Division of S.I. Lesaffre.
2. Dossier Selenium-enriched yeast (\textit{Saccharomyces cerevisiae} CNCM I-3399). Supplementary information. December 2016. Submitted by Phileo - Division of S.I. Lesaffre.
3. Comments from Member States.

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EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012a. Guidance for the preparation of dossiers for nutritional additives. EFSA Journal 2012;10(1):2535, 14 pp. https://doi.org/10.2903/j.efsa.2012.2535

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\(^{20}\) 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.
Abbreviations

CAS   Chemical Abstracts Service
CFU   colony forming unit
CV    coefficient of variation
EUROD  European Union Reference Laboratory
FEEDAP EFSA Panel on Additives and Products or Substances used in Animal Feed
IUPAC International Union of Pure and Applied Chemistry
LOQ   limit of quantification
PCBs  polychlorinated biphenyls
PCDDs polychlorinated dibenzoparadioxins
Se-Cys selenocysteine
Se-Met selenomethionine
TLV   threshold limit value