Safety and efficacy of a feed additive consisting of manganous lysinate sulfate for all animal species (Phytobiotics Futterzusatzstoffe GmbH)

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Abstract
Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of manganous lysinate sulfate as nutritional feed additive for all animal species. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) was assigned to this mandate. Based on the results of a tolerance study, the FEEDAP Panel concluded that manganous lysinate sulfate is safe for chickens for fattening when used up to the current maximum authorised levels of total manganese in feed; this conclusion was extrapolated to all animal species and categories at the respective maximum manganese levels in complete feed authorised in the EU. The FEEDAP Panel concluded that the use of manganous lysinate sulfate in animal nutrition at the maximum manganese levels authorised for the animal species poses no concern to the safety of consumers and the environment. The Panel also concluded that manganous lysinate sulfate poses a risk to users by inhalation, is irritant to eyes and skin and should be considered a dermal sensitiser. Based on the results of a bioequivalence study, the FEEDAP Panel concluded that manganous lysinate sulfate is considered efficacious for chickens for fattening as a nutritional additive. This conclusion was extrapolated to all animal species and categories.

Keywords: nutritional additives, compounds of trace elements, manganese, manganous lysinate sulfate, safety, efficacy

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Declarations of interest: The declarations of interest of all scientific experts active in EFSA’s work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

Acknowledgments: The Panel wishes to thank the following for the support provided to this scientific output (in alphabetical order of the last name): Elisa Pettenati, Anita Radovnikovic, FEEDAP Working Groups on Animal Nutrition and Environmental Risk Assessment.

Legal notice: Relevant information or parts of this scientific output have been blackened in accordance with the confidentiality requests formulated by the applicant pending a decision thereon by the European Commission. The full output has been shared with the European Commission, EU Member States and the applicant. The blackening will be subject to review once the decision on the confidentiality requests is adopted by the European Commission.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Fasmon Durjava M, Koubia M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Dierick N, Tarrés-Call J, Galobart J, Anguita M and Ortuño J, 2022. Scientific Opinion on the safety and efficacy of a feed additive consisting of manganous lysinate sulfate for all animal species (Phytobiotics Futterzusatzstoffe GmbH). EFSA Journal 2022;20(3):7165, 14 pp. https://doi.org/10.2903/j.efsa.2022.7165

ISSN: 1831-4732

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The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.
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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003 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 Phytobiotics Futterzusatzstoffe GmbH¹ for authorisation of the product manganous lysinate sulfate, 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 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. The particulars and documents in support of the application were considered valid by EFSA as of 18 of May 2021.

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 manganous lysinate sulfate, when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The additive manganous lysinate sulfate is intended for use as a source of manganese in all animal species. The additive has not been previously authorised as a feed additive in the European Union (EU).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier² in support of the authorisation request for the use of manganous lysinate sulfate as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003.

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, peer-reviewed scientific papers, other scientific reports and experts’ elicitation knowledge, to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of manganous lysinate sulfate in animal feed. The Executive Summary of the EURL report can be found in Annex A.³

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of manganous lysinate sulfate is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019a).

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¹ Phytobiotics Futterzusatzstoffe GmbH, Wallufer Str. 10a, 65343 Eltville, Germany.
² FEED dossier reference: FAD-2020-0108.
³ The full report is available on the EURL website: https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports/fad-2020-0108
3. Assessment

The additive under assessment is manganous lysinate sulfate. It is intended to be used in feed as a nutritional additive (functional group: compounds of trace elements) as a source of manganese (Mn) in all animal species and categories.

3.1. Characterisation

3.1.1. Characterisation of the additive

The additive consists of manganous lysinate sulfate. The compound contains manganese and the amino acid L-lysine in the molar ratio 1:1 and occurs as a monohydrate. Its chemical formula is C₆H₁₆MnN₂O₇S and it has a molecular weight of 315.20 g/mol. The International Union of Pure and Applied Chemistry (IUPAC) name of the additive is monoaquamonolysinatomanganese (II) sulfate. The compound does not have a Chemical Abstracts Service (CAS) number. The theoretical contents of manganese, lysine and sulfate are 17.4 %, 46.4 % and 30.5 %, respectively.

The specifications of the additive are as follows: 16–18% Mn, 44–47% lysine, 27–31% sulfate (calculated from sulfur), 4–10% moisture and 3% of other mineral components. Analytical data to confirm the specifications were provided for five batches of the additive, showing the following average values:

Three batches of the additive were analysed for chemical and microbiological impurities. The concentrations of the analysed impurities comply with the limits set in Directive 2002/32/EC for compounds of trace elements or, for those not specified for such compounds, do not represent a concern.

3.1.2. Physical properties of the additive

The additive appears as a beige to light brown free flowing granulate with apparent density of 700–900 kg/m³.

Technical dossier/Section II/Annex II_85.
Technical Dossier/Section II/Annex II_57.
Technical Dossier/Section II/Annex II_58.
Technical Dossier/Section II/Annex II_74,75, 76, 77 and 78.
LOQ (in mg/kg additive): cadmium < 0.2, lead < 0.5, mercury < 0.02 and arsenic < 0.5.
LOQ: < 0.3-0.6 µg/kg additive.
Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed.
OJ L 140, 30.5.2002, p. 10.
Technical dossier/Section II/Annex 1.
Technical dossier/Section II/Annex 85.
The dusting potential of three batches of the additive, measured \[14\] The content of manganese in dust (analysis of three batches) \[15\] The particle size of the dust was analysed \[16\]

3.1.3. Manufacturing process

3.1.4. Stability and homogeneity

No data have been provided to support the shelf-life of the additive. The applicant proposed a shelf-life of manganous lysinate sulfate of 24 months when stored in a cool and dry place in its original packaging.\[20\]

The capacity for homogenous distribution of manganese lysinate sulfate in feedingstuffs was tested in complete feed for chickens for fattening \[21\] twelve sub-samples were analysed. with a coefficient of variation of 10.9%.

3.1.5. Conditions of use

Manganous lysinate sulfate is intended to be used in feed for all animal species and categories via premixture. No minimum inclusion level is recommended but the maximum permitted level for the additive should be in compliance with currently authorised levels of total manganese in feed in the EU: 100 mg total Mn/kg complete feed for fish; 150 mg total Mn/kg complete feed for all other species.\[22\]

3.2. Safety

The additive, manganous lysinate sulfate, is a complex which is likely dissociated under physiological conditions into its three main components: manganese (II), lysine and sulfate.

The safety of L-lysine produced by \[18\] has been established and the additive is currently authorised in the EU (\[19\]).

The sulfate present in the additive amounts up to a maximum of 31%. It originates from manganese (II) sulfate monohydrate used as starting material, for which the safety has been assessed (EFSA FEEDAP Panel, 2016b) and is currently authorised as a feed additive in the EU (3b503).

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\[14\] Technical Dossier/Section II/Annex_II_79.
\[15\] Technical Dossier/Section II/Annex_II_80 and 81.
\[16\] Technical Dossier/Section II/Annex_II_83 and 84.
\[17\] Technical dossier/Section II/Annexes_II_66 and 67;
\[18\] Technical dossier/Section II/Annexes_II_68 and 69;
\[19\] Technical dossier/Section II/Annex_II_1.
\[20\] Technical dossier/Section II/Annex_II_97.
\[21\] Technical dossier/Section II/Annexes_II_1.
\[22\] COMMISSION IMPLEMENTING REGULATION (EU) 2017/1490 of 21 August 2017 concerning the authorisation of manganous chloride tetrahydrate, manganese (II) oxide, manganous sulphate monohydrate, manganese chelate of amino acids hydrate, manganese chelate of protein hydrolysates, manganese chelate of glycine hydrate and dimanganese chloride trihydroxide as feed additives for all animal species. OJ L 216 22.8.2017 p.1
3.2.1. Safety for the target species

The maximum tolerable levels of manganese for different animal species have been previously reviewed by the FEEDAP Panel (EFSA FEEDAP Panel, 2016b).

The additional lysine contribution to the diet when using manganese lysinate sulfate at the maximum authorised manganese concentrations (100 mg/kg feed for fish or 150 mg/kg feed for the other animal species) would range from 255 to 300 (fish) or from 383 to 450 mg Lys/kg complete feed (other animal species). Therefore, its contribution should be taken into consideration when formulating diets (as requirements might be as low as 4,500 mg lysine/kg feed in pullets (NRC, 1994).

The inclusion of the maximum authorised manganese concentrations would result in additional 200–291 (150–194 in fish) mg sulfate/kg complete feed. The FEEDAP Panel has assessed the sulfur/sulfate contribution from sulfate-containing additives (EFSA FEEDAP Panel, 2019b) and concluded that the formulation of the complete feed should carefully take into account the maximum tolerable level of total sulfur, as established by the NRC (in ruminant diets at 3 g S/kg dry matter (DM) (diet rich in concentrate) and at 5 g S/kg DM (diet rich in roughage) and in non-ruminant diets at 4 g S/kg DM; NRC, 2005); also, the contribution of sulfur/sulfate present in water for drinking to the total sulfur intake should be considered, especially when the content is high.

Therefore, considering the above, no safety concerns are expected from the sulfur/sulfate and lysine delivered by the additive. Thus, the assessment of safety of the additive for the target species will focus on manganese.

Tolerance study in chickens for fattening

The applicant provided one study in chickens for fattening to support the safety for the target animals.23

A total of 480 one-day-old male chickens for fattening (Ross 308) were distributed in 30 pens in groups of 16 animals and allocated to 3 dietary treatments (10 replicates per treatment). Two basal diets (starter and grower) based on maize, wheat and soybean meal, were either not supplemented (control; manganese basal content of 50 mg/kg complete feed) or supplemented with manganous lysinate sulfate to obtain the intended dietary total manganese concentrations of 150 mg/kg complete feed (the maximum authorised level) or 200 mg/kg complete feed (overdose level). The manganese content of the experimental diets was confirmed by analysis (Table 1). Diets were offered in mash form for 35 days.
Conclusions on safety for the target species

Based on the results of a tolerance study, the FEEDAP Panel concludes that manganous lysinate sulfate is safe for chickens for fattening when used up to the current maximum authorised level of total manganese in complete feed; this conclusion can be extrapolated to all animal species and categories at the respective maximum authorised manganese levels in complete feed.

3.2.2. Safety for the consumer

Since the L-lysine and sulfate of the additive do not represent a safety concern (see above, under Section 3.2 Safety), in the context of this scientific opinion the FEEDAP Panel focuses on manganese in the assessment of safety for consumers.

Deposition study

The applicant submitted a bioequivalence study in chickens for fattening which included data on manganese deposition in edible tissues and organs.28

Table 1: Effect of manganese lysinate sulfate level on performance parameters in chickens for fattening after 35 days

| Level (mg/kg) | Feed | Gain | Feed conversion | Protein | Lipid |
|--------------|-----|------|-----------------|--------|-------|
| 0            |     |      |                 |        |       |
| 50           |     |      |                 |        |       |
| 100          |     |      |                 |        |       |
| 150          |     |      |                 |        |       |

28 Technical dossier/Section IV/Annex 1.
29
Metabolism and deposition of manganese have been previously reviewed in FEEDAP opinions (EFSA FEEDAP Panel, 2016b, 2021). The Panel concluded that the use of the manganese compounds under assessment in animal nutrition is of no concern for the safety of consumers, provided that the current maximum total contents of manganese authorised in feed are respected (100 mg total Mn/kg for fish and 150 mg total Mn/kg for other species). To the knowledge of the FEEDAP Panel, no new evidence is available that could modify this previous evaluation. Therefore, the previous conclusion still applies.

Based on a residue study in chickens for fattening, the use of manganous lysinate sulfate up to the maximum authorised content of manganese in complete feed is not expected to increase the manganese content of edible tissues and products, hence, to increase the exposure of consumers to manganese. Therefore, the FEEDAP Panel considers that the use of manganous lysinate sulfate in animal nutrition at the proposed conditions of use would not be of concern to the safety of consumers.

3.2.3. Safety for the user

3.2.3.1. Effect on respiratory system

The threshold limit value (TLV) for manganese is 0.02 mg/m$^3$ (ACGIH, 2008); therefore, the TLV is exceeded.

The occupational exposure limit (OEL) for the inhalable fraction of water-soluble nickel is 0.03 mg Ni/m$^3$ (ECHA, 2018). Therefore, the OEL for nickel is exceeded.

Exposure to dust of the additive is considered a risk for the users.

3.2.3.2. Effect on eyes and skin

The skin irritancy potential of manganous lysinate sulfate was tested in a Good Laboratory Practice (GLP) test conducted following the OECD Test Guideline (TG) 439. The result demonstrated irritant potential, thus manganous lysinate sulfate should be considered as a dermal irritant.

The eye irritancy potential of the additive was tested in a GLP test conducted following the OECD TG 492. Based on the results obtained, manganous lysinate sulfate should be considered as an irritant to eyes.

Moreover, nickel, which is also present in the additive, is a recognised dermal sensitiser that may induce contact dermatitis (EFSA FEEDAP Panel, 2016a,b).

Conclusions on safety for the user

The FEEDAP Panel concludes that manganous lysinate sulfate poses a risk to users by inhalation, is irritant to skin and eyes and should be considered a dermal sensitisier.

Table 2: Effect of the manganese level and source in the Mn content in edible tissues and bile (results in mg/kg fresh matter)

| Manganese Level | Source | Edible Tissues | Bile |
|-----------------|--------|----------------|------|
| Low             | Fish   | 0.01           | 0.02 |
| High            | Fish   | 0.05           | 0.07 |
| Low             | Poultry| 0.02           | 0.03 |
| High            | Poultry| 0.06           | 0.08 |

References

30 Technical dossier/Section III/Annex III_44.
31 Technical dossier/Section III/Annex III_47.
3.2.4. Safety for the environment

The additive under assessment, manganous lysinate sulfate, contains manganese, the amino acid L-lysine and sulfate. L-Lysine, as amino acid, is a physiological and natural component in animals and plants. Sulfur content, in its various forms, in topsoil, is in the range of 50–112,000 mg/kg; it is not expected that the use of manganous lysinate sulfate in feed could significantly change the background concentration of sulfur in the environment.

Manganese is the second most abundant (after iron) transition element in the Earth’s crust, with an estimated global average in soil of 437 mg/kg ranging from 40 to 900 mg/kg (FOREGS, 2005). The median concentration of manganese in 845 samples of topsoil collected throughout Europe for the FOREGS survey was 504 mg/kg Mn mg/kg. Concentrations of dissolved manganese in European stream waters from the FOREGS survey range from 0.05 to 698 µg/L, with a median value of 15.9 µg/L (N = 804). The median concentration of manganese in 850 samples of stream sediment collected throughout Europe for the FOREGS survey was 612 mg Mn/kg.

**Environmental safety from use in feeds for terrestrial farm animals**

The worst-case PEC<sub>soil</sub> calculated using the additive up to the maximum authorised level of manganese in feed is around 3 mg/kg. As the median content of manganese in European soil is over two orders of magnitude higher than this value, the use of manganese in animal feeds up to the current maximum authorised inclusion levels is not expected to pose a risk to the soil compartment.

**Environmental safety from use in aquaculture feeds**

The PEC<sub>sed</sub> was calculated to be 220.5 mg/kg wet weight, lower than the background concentration in sediments.

The PEC<sub>swaq</sub> was calculated to be 0.12–0.25 µg/L, depending on the target species, and this concentration is 2 orders of magnitude lower than the median concentration of manganese in European freshwaters.

According to these data, the use of manganous lysinate sulfate in animal feeds would not substantially increase the background concentrations of Mn in the environment. Furthermore, the additive under assessment, manganous lysinate sulfate, is intended to be a substitute for other authorised manganese additives and will not further increase the environmental burden of manganese. Therefore, the FEEDAP Panel concludes that the use of manganous lysinate sulfate in animal nutrition for all animal species is safe for the environment, provided that the current maximum total contents of manganese authorised in feed are respected.

3.3. Efficacy

The applicant provided a bioequivalence study on chickens for fattening that has been described above (Section 3.2.2.1). The productive performance data is shown in Table 3 and the mineral bone composition in Table 4.

**Table 3:** Effect of manganese source and level on the performance parameters of chickens for fattening fed the additive

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32 Sulfur value calculated from FOREGS database, available online: https://weppi.gtk.fi/publ/foregsatlas/article.php?id=15
Conclusions on efficacy

Based on the outcome of the bioequivalence trial in chickens for fattening, the FEEDAP Panel concludes that manganous lysinate sulfate is considered efficacious for all animal species/categories.

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 and Good Manufacturing Practice.

4. Conclusions

The FEEDAP Panel concludes that manganous lysinate sulfate is safe for all animal species when used up to the respective maximum authorised Mn levels in feed.

The use of manganous lysinate sulfate in animal nutrition up to the maximum Mn content in complete feed authorised in the EU poses no concern to the safety of consumers.

The FEEDAP Panel concludes that manganous lysinate sulfate poses a risk to users by inhalation, it is irritant to eyes and skin and should be considered a dermal sensitiser.

The FEEDAP Panel considers that the use of manganous lysinate sulfate in animal nutrition would not pose a risk for the environment.

Based on the results of a bioequivalence study, the FEEDAP Panel concludes that manganous lysinate sulfate is efficacious as a source of Mn for all animal species/categories.

5. Documentation provided to EFSA/Chronology

| Date       | Event                                                                 |
|------------|-----------------------------------------------------------------------|
| 08/01/2021 | Reception mandate from the European Commission                        |
| 18/02/2021 | Dossier received by EFSA. Plexomin® L-Mn (manganese lysinate sulfate) for all animal species. Submitted by Phytobiotics Futterzusatzstoffe GmbH |
| 18/05/2021 | Application validated by EFSA – Start of the scientific assessment    |
| 25/08/2021 | Comments received from Member States                                  |
| 01/09/2021 | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. Issues: characterisation/safety for the target species/safety for the user |
| 20/09/2021 | Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives |

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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.
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Abbreviations

ADFI average daily feed intake
ADG average daily gain
ADI acceptable daily intake
BW body weight
CAS Chemical Abstracts Service
CD Commission Decision
CFU colony forming unit
CV coefficient of variation
DM dry matter
EINECS European Inventory of Existing Chemical Substances
EURL European Union Reference Laboratory
FAO Food Agricultural Organization
FCR feed conversion ratio
FEEDAP EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FLD fluorescence detection
GLP Good Laboratory Practice
IEC ion-exchange chromatography
IUPAC International Union of Pure and Applied Chemistry
LOD limit of detection
LOQ limit of quantification
MCHC mean corpuscular haemoglobin concentration
MCV mean corpuscular volume
MW molecular weight
NOAEL no observed adverse effect level
NTP National Toxicology Program
OECD Organisation for Economic Co-operation and Development
RH relative humidity
SCAN Scientific Committee on Animal Nutrition
VIS visible
WHO World Health Organization
Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method of the Analysis for manganous lysinate sulfate

In the current application an authorisation is sought under Article 4(1) for Manganese lysinate sulfate (Plexomin® L-Mn) under the category/functional group (3b) “nutritional additives”/“compounds of trace elements”, according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, the authorisation is sought for the use of the feed additive for all animal species.

Manganese lysinate sulfate is a complex of manganese with L-lysine with a content ranging from 15 to 18 % (w/w) for manganese as an active substance, 44 to 47 % (w/w) for lysine and 27 to 31 % (w/w) for sulfate.

The feed additive is intended to be incorporated into feedingstuffs through premixtures. The Applicant proposed maximum levels of total manganese in feedingstuffs complying with the limits set in Regulations (EC) No 1334/2003 and (EU) 2017/1490: 100 mg/kg for fish; and 150 mg/kg for other species.

For the quantification of total manganese in the feed additive, premixtures and feedingstuffs the Applicant proposed several internationally recognised ring-trial validated methods, namely ISO 6869 based on atomic absorption spectrometry (AAS), EN 15621 and EN 15510 based on inductively coupled plasma-atomic emission spectrometry (ICP-AES).

Moreover, the internationally recognised ring-trial validated method EN 17053 based on inductively coupled plasma-mass spectrometry (ICP-MS) and the European Union method based on AAS (Commission Regulation (EC) No 152/2009 – Annex IV-C), which has been further ring-trial validated by the UK Food Standards Agency (FSA), have been proposed by the Applicant for the quantification of total manganese in premixtures and feedingstuffs and feedingstuffs only, respectively. The above mentioned methods were previously evaluated and recommended by the EURL in the frame of several manganese based feed additive dossiers.

Based on the available performance characteristics of the methods, the EURL recommends for official control the five ring-trial validated methods: (i) ISO 6869, EN 15621 and EN 15510 for the quantification of total manganese in the feed additive, premixtures and feedingstuffs; (ii) EN 17053 for the quantification of total manganese in premixtures and feedingstuffs; and (iii) the European Union method (Commission Regulation (EC) No 152/2009 – Annex IV-C) for the quantification of total manganese in feedingstuffs.

For the quantification of lysine in the feed additive the Applicant proposed the ring-trial validated method EN ISO 17180 based on ion-exchange chromatography (IEC) coupled to post-column derivatisation and optical (visible (VIS) or fluorescence (FLD)) detection.

Based on the performance characteristics available, the EURL recommends for official control the above mentioned EN ISO 17180 method based on IEC-VIS/FLD to quantify lysine in the feed additive.

For the identification of the sulfate in the relevant feed additives the EURL has recommended in former evaluations the generic European Pharmacopoeia monograph (Ph. Eur. 01/2008:20301) on identification of ions and functional groups. The EURL recommends for official control the above mentioned the above-mentioned European Pharmacopoeia for the identification of sulfate in the feed additive.

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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.