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## Safety and efficacy of Manganese chelates of lysine and glutamic acid as feed additive for all animal species

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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 manganese chelates of lysine and glutamic acid (Manganese-LG) 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. The Panel concluded that, owing to safety considerations, the simultaneous use of both feed and water supplemented with Manganese-LG should be avoided. Manganese-LG showed to be safe for chickens for fattening up to the maximum authorised levels in the EU for manganese in feed (150 mg/kg); however, since a margin of safety could not be derived, the conclusions could not be extrapolated/extended to other categories/species. The data showed that Manganese-LG does not increase nor change the patterns of manganese tissue deposition when administered up to the maximum level allowed in the EU; therefore, the Panel concluded that the additive is safe for consumers of tissues and products from animals fed the additive up to the total manganese content in feed authorised in the EU. Owing to the manganese and nickel content of Manganese-LG, the Panel concluded that the handling of the additive poses a risk to users by inhalation and shall be considered as a skin and respiratory sensitiser; the data showed that the additive is irritant to eye and non-irritant to skin. Manganese-LG, intended to substitute other authorised manganese additives, will not further increase the environmental burden of manganese; the Panel considered that the use of the additive in animal nutrition would not pose an additional risk for the environment. The Panel concluded that the additive is efficacious in chickens for fattening; this conclusion can be extrapolated/extended to other categories/species. The FEEDAP Panel posed a recommendation concerning the description of the additive.

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## 1. Introduction

### 1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003<sup>1</sup> 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 Zinpro animal nutrition (Europe)<sup>2</sup> for authorisation of the product manganese chelates of lysine and glutamic acid, 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). The particulars and documents in support of the application were considered valid by EFSA as of 3 May 2018.

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 manganese chelates of lysine and glutamic acid (Manganese-LG complex), when used under the proposed conditions of use (see Section 3.1.5)

### 1.2. Additional information

Manganese chelates of lysine and glutamic acid, are intended for use as a source of manganese in all animal species. The additive has not been previously authorised as feed additive in the European Union (EU).

The applicant holds a patent on the manganese chelates of lysine and glutamic acid, with the title 'Mixed amino acid metal salt complexes'.<sup>3</sup>

## 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<sup>4</sup> in support of the authorisation request for the use of manganese chelates of lysine and glutamic acid as a feed additive.

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 and other scientific reports, 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 the manganese chelates of lysine and glutamic acid in animal feed. The Executive Summary of the EURL report can be found in Annex A.<sup>5</sup>

### 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of manganese chelates of lysine and glutamic acid is in line with the principles laid down in Regulation (EC) No 429/2008<sup>6</sup> 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), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008), Guidance for the preparation of dossiers for additives already authorised for use in food (EFSA FEEDAP Panel, 2012b),

<sup>1</sup> 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.

<sup>2</sup> Zinpro Animal Nutrition (Europe), Inc. Akkerdistel 2E. 5831 PJ. Boxmeer. The Netherlands.

<sup>3</sup> Technical Dossier/Section II/Annex II\_37.

<sup>4</sup> FEED dossier reference: FAD-2018-0009.

<sup>5</sup> The full report is available on the EURL website: <https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2018-0009-mn-lys-glu.pdf>

<sup>6</sup> 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.

Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012c), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012d).

### 3. Assessment

The additive under assessment is 'Manganese chelates of lysine and glutamic acid' (trade name: ProPath Mn). The additive corresponds to the active compound. As abbreviation, the short name of Manganese-LG will be used throughout this opinion to refer to the additive under assessment. It is intended to be used in feed (either mashed or pelleted form) and water (via complementary feed) as a nutritional additive (functional group: compounds of trace elements), as a source of manganese for all animal species and categories.<sup>7</sup>

#### 3.1. Characterisation

##### 3.1.1. Characterisation of the compound

The active substance is manganese in the form of chelates of lysine and glutamic acid in a mixture 1:1.<sup>7</sup> The chemical names according to International Union of Pure and Applied Chemistry (IUPAC) are manganese-2,6-diaminohexanoic acid and manganese-2-aminopentanedioic acid. The compounds are not identified by the Chemical Abstracts Service (CAS) number. The chemical formulas of the two compounds are [REDACTED] and [REDACTED], respectively. The structural formulas are shown in Figure 1 and the corresponding molecular weights are [REDACTED] g/mol for the manganese chelate of lysine, and [REDACTED] g/mol for manganese chelate of glutamic acid. The theoretical content of manganese is 14.9% and 16.3%, respectively.<sup>8</sup>



**Figure 1:** Structural formula of manganese chelates of lysine (a) and glutamic acid (b)

Five batches of the additive were analysed for manganese, lysine, glutamic acid, chloride and sulfur. The average contents were: manganese 16.0% (range: 15.9–16.2%), lysine 20.3% (19.9–21.3%), glutamic acid 22.7% (22.1–23.8%), chloride 4.8% (4.79–4.88), sulfur 9.1% (8.89–9.37%) and water 3.3% (3.0–3.6%).<sup>9</sup> The remaining composition was not identified, but it can be assumed to be represented by the other components of the counter ions [REDACTED]. The FEEDAP Panel notes that about 2% of the additive might be unidentified.

Five batches of Manganese-LG were analysed for undesirable substances.<sup>10</sup> Levels of heavy metals (cadmium Cd, lead Pb and mercury Hg), arsenic (As) and fluorine (F) were provided (As: < 0.19–< 0.21 mg/kg, Cd: < 0.16–0.20 mg/kg, Pb: < 0.95–1.56 mg/kg, Hg: < 0.08–< 0.09 mg/kg and F: 23.3–24.7 mg/kg).<sup>11</sup> The levels of dioxins and the sum of dioxins and dioxin-like-PCBs were 0.021–0.051 ng WHO-PCDD/F-TEQ/kg and 0.023–0.054 ng WHO-PCDD/F-PCB-TEQ/kg, respectively. The concentrations of the undesirable substances analysed comply with those set in Directive 2002/32/EC for compounds of trace elements or, if not mentioned in the Directive, do not represent a concern.<sup>12</sup>

<sup>7</sup> [REDACTED]

<sup>8</sup> Technical Dossier/Spontaneous Information.

<sup>9</sup> Technical dossier/Section II/ Annex II-1 to II-15.

<sup>10</sup> Technical dossier/Section II/2.1.4.

<sup>11</sup> The applicant confirmed that for lead and mercury the values preceded by the by the sign '<' mean the LOQ. It can be assumed that this is also applicable in the other cases.

<sup>12</sup> 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.

Nickel was analysed in three batches of the additive and the values reported were in the range 3.8–4.0 mg/kg.<sup>7</sup>

Analysis of three batches (aged from 10 to 11 months) for potential microbial contamination showed counts of *Enterobacteriaceae* and *E. coli* were below the Limit of Quantification (LOQ) (< 10 cfu/g) and *Salmonella* was not detected in 25 g. Levels of aflatoxin B1 and ochratoxin A, analysed in three batches, were below the LOQ (< 0.1 µg/kg).

The additive is a solid product, soluble in water, with a melting point at 252°C. The bulk density, tested in three batches of the additive, was 0.812 g/cm<sup>3</sup>.<sup>13</sup>

Dusting potential was analysed in three batches by the Stauber–Heubach method (four measurements of each batch); the values reported were in the range of 2.5–2.8 g/m<sup>3</sup> (g airborne dust per m<sup>3</sup> of air).<sup>14</sup> The same batches were submitted for analysis of the particle size by laser diffraction. The results (v/v) showed on average 6.5% (range 6.1–6.8), 37.3% (range 35.6–38.9) and 76.1% (range 75.2–77.0) of the particles below 10 µm, 50 µm and 100 µm, respectively.<sup>14</sup> Manganese content in the dust was provided with analysis of three batches of the additive with an average of 13.4% (range 13.3–13.5).<sup>7</sup>

### 3.1.2. Manufacturing process

[REDACTED]

### 3.1.3. Stability and homogeneity

For compounds of trace elements (including chelates), stability studies are generally not required.

Two stability studies with one batch each stored at 25°C/60% RH and 40°C/70% RH were performed. After 24 months, the manganese recovery was 101.9% at 25°C and 100.6% at 40°C; the total lysine/glutamic acid recovery was 99.7% and 101.3%, respectively.<sup>19</sup>

A stability study of the additive in premixture, mash feed and pellet feed measuring total manganese content was provided. After 6 months of storage, a recovery of 111.6% was reported for mash starter feed, of 106.7% for pellet grower feed and of 129% for mash starter premixture.<sup>20</sup> The content of choline chloride in the premixture was of 5%.<sup>21</sup>

The capacity of the additive to be homogeneously distributed in feed was tested in feed (premixtures and complete feed) for chickens for fattening in 10 subsamples each. The content of manganese in premixtures showed a coefficient of variation (CV) of 7.5% (starter premixture; mean manganese concentration: 18,717 mg/kg) and 6.2% (grower premixture; mean manganese concentration: 18,229 mg/kg). In complete mash feed, the content of manganese showed a CV of 2.9% (mean

<sup>13</sup> Technical dossier/Section II/2.2.2.1.

<sup>14</sup> Technical dossier/Section II/Annex II-36.

[REDACTED]

<sup>19</sup> Technical dossier/Section II/Annex II-41&42.

<sup>20</sup> Technical dossier/Section II/Annex II-43.

<sup>21</sup> Technical Dossier/Supplementary Information/August 2018 and Spontaneous information.

manganese concentration: 104.5 mg/kg), and for the same feed after pelleting the CV was 5.3% (mean manganese concentration: 149.1 mg/kg).<sup>20</sup>

#### 3.1.4. Physico-chemical incompatibilities or interactions

No incompatibilities are expected due to the nature of the product.

#### 3.1.5. Conditions of use

Manganese-LG is intended to be used in all types of feed, either mashed or pelleted form, including premixtures or water (via complementary feed), for all animal species/categories up to the total maximum manganese content authorised in complete feeds in the EU: fish 100 (total) and other species 150 (total) mg/kg feedingstuffs.

The applicant proposed half of the dose in feed for use in water for drinking.<sup>7</sup>

### 3.2. Safety

The additive is a mixture of manganese chelate of lysine and manganese chelate of glutamic acid. The additive will introduce only a minor fraction of the amino acids lysine and glutamic acid, which contributes negligibly to the intake of the animals. Therefore, no relevance for the safety assessment is foreseen for the amino acids delivered by the additive.

The use in water for drinking of several manganese compounds was discussed in depth in a previous opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2016), concluding that the simultaneous use of both feed and water supplemented with manganese should be avoided, and did not recommend the use of manganese compounds via water for drinking. The FEEDAP Panel retains both the conclusion and recommendation as applicable also to Manganese-LG and reiterates its previous statement that compounds of trace elements should generally not be used in water for drinking (EFSA FEEDAP Panel, 2010).

#### 3.2.1. Safety for the target species

Where a feed additive application is made as a nutritional additive for all animal species, tolerance data may be limited to one species. The maximum tolerable levels for manganese have been reviewed by the FEEDAP Panel in previous opinions (e.g. EFSA FEEDAP Panel, 2016).

##### 3.2.1.1. Safety for the target species

The applicant provided a tolerance study with Manganese-LG in chickens for fattening with duration of 37 days.<sup>22</sup>

A total of 504 chickens (male, Cobb 500) were allocated to seven treatments. The birds were randomly housed in 42 pens with 12 animals per pen. The treatments consisted of a control without added manganese (T1), an organic manganese source (the additive under assessment, manganese-LG) in three doses [standard – 100 (T2), maximum authorised – 150 (T3) and tolerance – 1,600 (T4) mg total manganese per kg of feed] and an inorganic manganese source (manganese sulfate monohydrate) in three doses [standard – 100 (T5), maximum authorised – 150 (T6) and tolerance – 1,600 (T7) mg total manganese per kg of feed], see Table 1.

The chickens were fed a complete starter (crumbles) feed from 1 to 21 days of age and a grower feed (pellets) from 22 to 35 days of age and they had ad libitum access to water and feed. The diets were mainly composed of corn, wheat and soybean meal, and contained as a starter 214 g/kg of crude protein and 12.8 MJ/kg of apparent metabolisable energy (AME) and as a grower 195 g/kg of crude protein and 13 MJ/kg of AME. Manganese content and composition of all feeds were confirmed in the mash and pellet feeding phases before commencing the experiment (Table 1); however, samples of grower feed analysed for manganese from the bags used during the study showed markedly higher concentration, especially for T5 group (402 mg Mn/kg).

<sup>22</sup> The additive supplementation lasted for 35 days. Technical Dossier/Section III/Annex III\_3\_1.



**Table 1:** Description of the seven treatment groups

Treatment	Source	Added Mn mg/kg feed	Total Mn mg/kg feed Intended	Total Mn mg/kg feed Analysed	
				Starter	Grower
T1	None <sup>(1)</sup>	0	60	80	58
T2	Manganese-LG	40	100	105	96
T3		90	150	147	149
T4		1,540	1,600	1,587	1,376
T5	Manganese sulfate	40	100	110	94 (402) <sup>(2)</sup>
T6		90	150	159	144 (192) <sup>(2)</sup>
T7		1,540	1,600	1,554	1,519

(1): Since the background manganese concentration of the basal diets was 38 mg Mn/kg, these diets were supplemented with manganese sulfate to achieve a total of 60 mg Mn/kg basal diet. The control diet was used in the rest of the treatments.

(2): Actual analytical check of the grower feed from the T5 and T6 groups, performed after the end of experiment, gave concentrations of manganese higher than those intended, apparently due to an error in the feed preparation. This reanalysis was done following the unexpected results found in the deposition study (see Section 3.2.2.1).

Mortality and general health were monitored throughout the study. Performance was assessed by measuring weekly feed intake and live weight; feed conversion ratio was calculated. After 35 (up to 37) days, two animals per pen (altogether 12 animals per treatment) were killed and blood samples were taken for analysis of haematology<sup>23</sup> and biochemistry,<sup>24</sup> during this procedure also tissue and organ samples were taken for the residue study (see Section 3.2.2.1).

The study was blinded, controlled and randomised. Data were statistically analysed by analysis of variance (ANOVA) and mean groups comparison was done using Tukey test.<sup>7,25</sup> The pen was considered as the statistical unit for the performance parameters, whilst the individual animal was the statistical unit for haematology and biochemical parameters.

Mortality between treatments varied between 2.8 and 8.3% (two to six animals per treatment; total average: 5.0%) with no significant differences among groups.

The applicant reported an episode of colibacillosis which occurred during the study with mortality and abnormal clinical signs peaking over the D15–D21 period and was present in all groups; treatment was given successfully.<sup>26</sup> The colibacillosis suffered by the birds during the study introduced a source of uncertainty. Upon EFSA's request, the applicant submitted information on the potential effects of antimicrobials (including sulfonamides plus trimethoprim) on the absorption of minerals and trace elements (especially manganese).<sup>27</sup> Investigations on potential interactions between minerals/trace elements (including manganese) and trimethoprim and/or sulfonamides, carried out by the applicant, were based on i) data from reference books in pharmacokinetics, including human and veterinary science, ii) structured literature search on Pubmed (National Center for Biotechnology Information, U.S. National Library of Medicine 8600 Rockville Pike, Bethesda MD, 20894 USA), including the following terms: mineral absorption interaction trimethoprim; mineral absorption interaction sulfonamide; mineral absorption interaction sulfonamide trimethoprim; manganese absorption interaction trimethoprim; manganese absorption interaction sulfonamide; manganese absorption interaction trimethoprim sulfonamide. The outcome of these investigations did not reveal any interaction between sulfonamides, trimethoprim and manganese which could impact on the *in vivo* absorption of manganese.

Therefore, taking into consideration that (i) the colibacillosis affected all groups, (ii) the performance parameters appeared not to be impacted (see Table 2), (iii) the performance objectives for the bird strain were met and iv) the outcome of the above investigations showing no impact from the treatment on *in vivo* absorption of manganese, the FEEDAP Panel considers that this deviation had no major influence on the main study's conclusions.

<sup>23</sup> Basophils, eosinophils, haematocrit, haemoglobin, heterophils, lymphocytes, monocytes, WBC.

<sup>24</sup> ALP, ALT, AST, albumin, amylase, CK, calcium, chloride, cholesterol, GGT, GLDH, glucose, LDH, magnesium, phosphorus, potassium, sodium, total bilirubin, total protein, triglycerides, uric acid.

<sup>25</sup> Technical Dossier/Supplementary Information/September 2019.

<sup>26</sup> The animals were given Trisulmix powder (active substances sulfadimethoxin and trimethoprim) for control of a colibacillosis outbreak; the treatment was given to all the birds at 1 g/L drinking water, *ad libitum* from D17 to D22 (6 days).

<sup>27</sup> Technical Dossier/Supplementary Information/July 2019.



At the end of the study, the performance parameters were similar in all groups, with the exception of the highest dose of the group with Manganese-LG (1,600 mg Mn/kg feed) where the values for these parameters overall indicated an adverse effect of performance.

**Table 2:** Effect of Manganese-LG on performance parameters in chickens for fattening after 35 days.

Treatment	Manganese-intended (mg/kg diet)	Body weight (kg)	Feed intake (total kg/bird)	F/G
Control	60	2.46 <sup>b</sup>	3.635 <sup>a</sup>	1.51 <sup>a</sup>
Manganese-LG	100	2.44 <sup>ab</sup>	3.461 <sup>a</sup>	1.45 <sup>a</sup>
	150	2.41 <sup>ab</sup>	3.562 <sup>a</sup>	1.51 <sup>a</sup>
	1,600	2.29 <sup>a</sup>	3.924 <sup>b</sup>	1.76 <sup>b</sup>
Manganese sulfate	100*	2.41 <sup>ab</sup>	3.469 <sup>a</sup>	1.47 <sup>a</sup>
	150	2.41 <sup>ab</sup>	3.443 <sup>a</sup>	1.46 <sup>a</sup>
	1,600	2.34 <sup>ab</sup>	3.448 <sup>a</sup>	1.51 <sup>a</sup>

a,b: Different superscripts within a column indicates significant differences ( $P \leq 0.05$ ).

(\*): A substantial discrepancy between the intended and the analysed doses was identified (see Table 1).

Regarding biochemical parameters, a statistical significant reduction on the triglycerides values of animals supplemented with the tested tolerance dose (T4) vs. groups T1, T3 and T7 was identified. Within the haematological parameters, a statistical significant reduction was detected for the white blood cells in group 4 (tolerance dose of Manganese-LG) vs. group 6 (maximum authorised dose of manganese sulfate).

Necropsy was only performed in the animals that were found dead or culled; on the former the findings were either absence of lesion or lesions of pericarditis, aerosacculitis and/or perihepatitis, all of which are suggestive of colibacillosis.

The tolerance study shows that Manganese-LG is safe up to 150 mg Mn/kg feed; indications of adverse effects were identified with the next dose tested (1,600 mg Mn/kg feed). A margin of safety cannot be derived from this study. Since significant adverse effects on zootechnical performance were observed at the tolerance dose of Manganese-LG compared to the tolerance dose of Manganese sulfate, it is concluded that overdosing with Manganese LG is less tolerated than a comparable overdosing with a standard inorganic source of manganese.

The additive will introduce only a minor fraction of the amino acids lysine and glutamic acid, which contributes negligibly to the intake of the animals; no relevance for target animals safety is foreseen.

### 3.2.1.2. Conclusions on safety for the target species

Based on a tolerance study, the FEEDAP Panel concludes that the additive is safe for chickens for fattening up to the maximum authorised levels in the EU for total manganese in feed (150 mg/kg). However, since a margin of safety could not be derived, the conclusions cannot be extrapolated to other categories of birds or extended to other animal species. Therefore, the FEEDAP Panel cannot conclude on the safety of Manganese-LG for species/categories other than chickens for fattening.

## 3.2.2. Safety for the consumer

The sources used for both amino acids are authorised in the EU and their safety have been established. Therefore, the FEEDAP Panel retains that only manganese is of interest concerning the consumer safety.

### 3.2.2.1. Deposition study

#### *Residues in chickens for fattening*

The applicant submitted a study on tolerance of chickens for fattening (see Section 3.2.1.1). This study provided data on manganese deposition in tissues and organs (breast muscle, skin-fat, kidney, liver and bone (tibia) samples) on chickens for fattening fed the Manganese-LG. Samples were taken at day 35–37, from a total of 84 birds (12 birds/treatment).

No differences in manganese deposition (groups T2, T3) were observed in groups supplemented with tested additive up to maximum level allowed manganese in feed in the EU. Group T5 showed

high manganese concentrations in most of examined tissues; these values can be explained owing to the higher concentration of manganese in grower feed mixture (402 mg/kg instead of 100 mg/kg), which was found by analysis of feed samples taken during the experiment (see Table 1). Therefore, only the groups fed at the highest manganese concentration in feed authorised in the EU were compared: T3 and T6. The concentration of manganese in all tissues and tibia from group T6 did not differ from T3.<sup>28</sup> These data indicated that deposition was not influenced by the manganese source.

**Table 3:** Analytical results manganese content in edible tissues and tibia (results in fresh matter)

Treatment	Source	Manganese-Intended (mg/kg diet)	Skin & Fat (mg Mn/kg)	Muscle (mg Mn/kg)	Liver (mg Mn/kg)	Kidney (mg Mn/kg)	Tibia (mg Mn/kg)
T1	None	60	0.34 <sup>a</sup>	0.10	2.49 <sup>a</sup>	2.19 <sup>a</sup>	9.27 <sup>a</sup>
T2	Manganese-LG	100	0.45 <sup>ab</sup>	0.11	2.66 <sup>ab</sup>	2.19 <sup>a</sup>	9.08 <sup>a</sup>
T3		150	0.66 <sup>ab</sup>	0.11	2.64 <sup>ab</sup>	2.25 <sup>a</sup>	10.96 <sup>a</sup>
T5	Manganese	100*	1.30 <sup>b</sup>	0.13	3.10 <sup>b</sup>	3.01 <sup>b</sup>	14.56 <sup>b</sup>
T6	Sulfate	150	0.76 <sup>ab</sup>	0.12	3.04 <sup>ab</sup>	2.54 <sup>ab</sup>	11.16 <sup>a</sup>

a,b: Different superscripts within a column indicates significant differences ( $P \leq 0.05$ ).

(\*): A substantial discrepancy between the intended and the analysed doses was identified (see Table 1).

### 3.2.2.2. Toxicology of manganese and consumer exposure

In previous scientific opinions, the FEEDAP Panel reviewed the relevant literature and considered several previous toxicological assessments of manganese (see e.g. EFSA FEEDAP Panel, 2016 and references therein). In summary, manganese is a well-known occupational toxicant upon inhalation exposure (see also Section 3.2.3 User safety); however, there are relatively limited data available on oral toxicity in laboratory animals and humans. To the knowledge of the FEEDAP Panel, no new evidence is available that could modify the previous evaluation by the FEEDAP Panel.

Based on study on chickens for fattening, the supplementation with Manganese-LG up to the maximum level of total manganese in feedingstuffs permitted in the EU is unlikely to increase manganese deposition in edible tissues; therefore, the FEEDAP Panel considers that the use of Manganese-LG as a nutritional additive would not significantly influence consumer exposure.

Whereas manganese is an essential trace element and the usual intake levels of dietary manganese do not appear to be associated with any adverse health effects, high oral exposures are associated with severe adverse neurological effects in humans. The putative pathways of neurological damage (e.g. dopamine oxidation) have been identified, but the available evidence is not robust enough to derive a tolerable upper intake level (UL). Male fertility is another potential target of manganese toxicity; a recent paper showed significantly reduced sperm quality in mice orally exposed to  $\geq 0.13$  mg MnCl<sub>2</sub>/kg body weight (bw)/day, with a non-observed adverse effect level (NOAEL) of 0.013 mg/kg bw/day (Souza et al., 2019). Due to the lack of a UL, it is therefore advisable that oral exposure to manganese should not increase over the background dietary intake.

In the EU, the estimated mean manganese intake ranges from 2 to 6 mg/day, with a majority of values around 3 mg/day, for adults and adolescents, and 1.5–3.5 mg/day for children (EFSA NDA Panel, 2013). Foods of vegetable origin and beverages appear, by far, as the main sources of manganese, being foods of animal origin usually minor contributors.<sup>29</sup> Based on the available evidence, the FEEDAP Panel reiterates its previous general consideration that the use of manganese as feed additive should not increase the human oral exposure over the background dietary intake.

### 3.2.2.3. Conclusions on safety for the consumer

Manganese-LG does not increase nor change the patterns of manganese tissue deposition when administered up to the maximum level allowed in the EU. Therefore, the FEEDAP Panel concludes that Manganese-LG is safe for consumers of tissues and products from animals fed the additive up to the total manganese content in feed authorised in the EU.

<sup>28</sup> At higher levels than the maximum authorised in feed (treatments T4 and T7, see Table 1; total of 1,600 mg Mn/kg feed) higher deposition was observed in all tissues, organs and tibia for both sources compared to the control, with no differences between organic and inorganic.

<sup>29</sup> From two Total Dietary studies reported in the EFSA NDA Panel opinion of 2013, food of animal origin would be included in the category "Others" contributing from 18 to 36%.

### 3.2.3. Safety for the user

#### 3.2.3.1. Effects on the respiratory system

No specific studies were provided by the applicant regarding the toxicity of the additive on the respiratory system.

The highest dusting potential analysed in the additive was 2.8 g/m<sup>3</sup> and the manganese maximum concentration in the dust was 13.5% (see Section 3.1.1). Thus, a maximum concentration of 378 mg Mn/m<sup>3</sup> could be released by the dust when handling the additive. Considering that the respirable and the thoracic fractions amounted up to 6.8% and 38.9%, respectively (see Section 3.1.1), based on a conservative approach, the respirable manganese from dust would be about 66 mg/m<sup>3</sup>, assuming (as a worst case) that the dust consists only of particles ≤ 50 µm and its respirable fraction about 17.5% (6.8 of 38.9). Considering a threshold limit value (TLV) for manganese of 0.02 mg/m<sup>3</sup> (ACGIH, 2015) the TLV is exceeded by more than four orders of magnitude. Owing to the manganese content of the additive, the FEEDAP Panel considers that handling of the additive poses a risk to users by inhalation.

The highest nickel content analysed in the additive was 4.0 mg/kg. The dusting potential of the product amounted up to 2.8 g/m<sup>3</sup>, corresponding to about 0.01 mg Ni/m<sup>3</sup>, which coincides with the occupational exposure limit (OEL) proposed for the inhalable fraction of water-soluble nickel (0.01 mg Ni/m<sup>3</sup>; European Commission, 2011). Nevertheless, due to the presence of nickel in the additive, it should be considered as a respiratory sensitiser.

#### 3.2.3.2. Effects on the eyes and skin

The applicant presented two *in vivo* acute irritation studies on rabbits according OECD guidelines No 404 and No 405, respectively, for skin<sup>30</sup> and eye.<sup>30</sup> Under the experimental conditions adopted, the additive was found to be non-irritant for the skin but irritant for the eye of the rabbit.

The nickel content of the additive is up to 4.0 mg/kg; given its well-known sensitisation potential (European Commission, 2011) and, in the absence of skin sensitisation studies, the additive is classified as a skin sensitiser.<sup>7</sup>

The FEEDAP Panel considers the additive as non-irritant to skin, irritant to eye and a skin sensitiser.

#### 3.2.3.3. Conclusions on safety for the user

Owing to the manganese and nickel content of Manganese-LG, the handling of the additive poses a risk to users by inhalation. The additive is considered as a skin and respiratory sensitiser. It is non-irritant to skin but irritant to eye.

### 3.2.4. Safety for the environment

The additive under assessment, Manganese-LG, 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 considers that the use of the additive in animal nutrition would not pose an additional risk for the environment.

## 3.3. Efficacy

For demonstration of the efficacy of nutritional additives, one study in a single animal species or category, including laboratory animals, is considered sufficient (EFSA FEEDAP Panel, 2012a).

#### 3.3.1. Study in chickens for fattening

The applicant provided a combined tolerance/efficacy study in chickens for fattening<sup>31</sup> (see Sections 3.2.1.1 and 3.2.2.1). The experimental groups in the study are shown in Table 1. In this trial, manganese concentration in edible tissues/organs and tibias was measured (Table 3).

Animals receiving the manganese (from either source, inorganic or organic) supplemented diets at the maximum level authorised in the EU showed no differences in manganese deposition in edible tissues/organs and tibia compared to those in the control group; this lack of difference among these experimental groups (both Manganese-LG and inorganic manganese) with the control group may be explained by the already adequate manganese concentration in the diet of that group (60 mg Mn/kg feed).

<sup>30</sup> Technical dossier/Section III/Annex\_III\_3\_2.

<sup>31</sup> Technical Dossier/Section IV/Annex\_IV\_4\_1.

At higher levels than the maximum authorised in feed, higher manganese deposition was observed in all tissues, organs and tibia for both manganese sources compared to the control, with no differences between organic and inorganic.

The FEEDAP Panel notes again that the differences in manganese deposition in tissues and organs identified for the group T5 might be an artefact due to the uncertainties in dosage of this group, and therefore this group was not considered.

### 3.3.2. Conclusions on efficacy for the target species

Based on the deposition of manganese in edible tissues/organs and tibia in chickens for fattening, the FEEDAP Panel concludes that Manganese-LG is an efficacious source of manganese as the standard inorganic manganese source in meeting the birds requirements. This conclusion can be extrapolated to other categories of birds and extended to other animal species.

### 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<sup>32</sup> and Good Manufacturing Practice.

## 4. Conclusions

The FEEDAP Panel concludes that, owing to safety considerations, the simultaneous use of both feed and water supplemented with Manganese-LG should be avoided.

Based on a tolerance study, the FEEDAP Panel concludes that the additive is safe for chickens for fattening up to the maximum authorised levels in the EU for total manganese in feed (150 mg/kg). However, since a margin of safety could not be derived, the conclusions cannot be extrapolated to other categories of birds or extended to other animal species. Therefore, the FEEDAP Panel cannot conclude on the safety of Manganese-LG for species/categories other than chickens for fattening.

Manganese-LG does not increase nor change the patterns of manganese tissue deposition when administered up to the maximum level allowed in the EU. Therefore, the FEEDAP Panel concludes that Manganese-LG is safe for consumers of tissues and products from animals fed the additive up to the total manganese content in feed authorised in the EU.

Owing to the manganese and nickel content of Manganese-LG, the handling of the additive poses a risk to users by inhalation. The additive is considered as a skin and respiratory sensitiser. It is non-irritant to skin but irritant to eye.

The additive under assessment, Manganese-LG, 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 considers that the use of the additive in animal nutrition would not pose an additional risk for the environment.

The FEEDAP Panel concludes that Manganese-LG is an efficacious source of manganese in meeting the animals' requirements.

## 5. Recommendations

The FEEDAP Panel recommends to include the sources of lysine and glutamic acid (including the production strain(s), where applicable) in the description of the additive. The content of lysine and glutamic acid in the additive should be also indicated in the description of the product.

### Documentation provided to EFSA/Chronology

Date	Event
07/03/2018	Dossier received by EFSA. Manganese chelates of lysine and glutamic acid for all animal species. Submitted by Zinpro Animal Nutrition (Europe), Inc.
16/03/2018	Reception mandate from the European Commission
03/05/2018	Application validated by EFSA – Start of the scientific assessment

<sup>32</sup> Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

Date	Event
21/06/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation, safety for target species, and safety for the user</i>
24/07/2018	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
03/08/2018	Comments received from Member States
22/08/2018	Reception of supplementary information from the applicant - Scientific assessment re-started
17/12/2018	Spontaneous submission of information by the applicant. <i>Issues: characterisation</i>
24/01/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended <i>Issue: characterisation</i>
07/03/2019	Clarification teleconference during risk assessment with the applicant according to the “EFSA’s Catalogue of support initiatives during the life-cycle of applications for regulated products”
14/03/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
06/05/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended <i>Issues: safety for target species, safety for the consumers and efficacy</i>
22/07/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
06/09/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended <i>Issues: safety for target species, safety for the consumers and efficacy</i>
20/09/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
10/01/2020	Opinion adopted by the FEEDAP Panel by written procedure. End of the Scientific assessment

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## Abbreviations

AME	apparent metabolisable energy
ANOVA	analysis of variance
bw	body weight
CAS	Chemical Abstracts Service
CV	coefficient of variation
EURL	European Union Reference Laboratory
IUPAC	International Union of Pure and Applied Chemistry
LOQ	Limit of Quantification
NOAEL	non-observed adverse effect level
OEL	occupational exposure limit
TLV	threshold limit value
UL	upper intake level



## Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for manganese chelates of lysine and glutamic acid

In the current application, authorisation is sought under Article 4(1) for *manganese chelates of lysine and glutamic acid* 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, authorisation is sought for the use of the *feed additive* for all categories and species.

*Manganese chelates of lysine and glutamic acid* is a solid preparation with a minimum content of 15% (w/w) of *manganese*, 19% (w/w) of *lysine* and 19% (w/w) of *glutamic acid*.

The *feed additive* is intended to be incorporated into *premixtures* and *feedingstuffs*. The Applicant proposed maximum levels of *total manganese* in *feedingstuffs* ranging from 100 to 150 mg/kg – depending of the animal species/category – and thus complying with the limits set in the Regulations (EC) No 1334/2003 and (EU) 2017/1490.

For the quantification of *total manganese* in the *feed additive*, *premixtures* and *feedingstuffs*, the Applicant submitted the two internationally recognised ring-trial validated CEN methods based on inductively coupled plasma-atomic emission spectrometry (ICP-AES): EN 15510 and EN 15621. These two methods together with the Community method based on atomic absorption spectrometry, which was further ring-trial validated by the UK Food Standards Agency (FSA), were previously evaluated and recommended by the EURL in the frame of the manganese group dossier.

In addition, the EURL is aware of two ring-trial validated methods, namely: ISO 6869 based on atomic absorption spectrometry (AAS) and EN 17053 based on inductively coupled plasma-mass spectrometry (ICP-MS).

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

For the quantification of *lysine* and *glutamic acid* in the *feed additive*, the Applicant submitted the ring-trial validated EN ISO 13903 method based on ion exchange chromatography coupled with post-column derivatisation and photometric detection (IEC-VIS). This standard method is equivalent to the experimental protocol described in the Community method designed for the determination of *free* (synthetic and natural) and of *total* (peptide-bound and free) amino acids including *lysine* and *glutamic acid*, using an amino acid analyser or a High-Performance Liquid Chromatography (HPLC) equipment. This method does not distinguish between the salts and the amino acid enantiomers.

The Applicant applied the above-mentioned IEC-VIS method for the analysis of five batches of the *feed additive* with an averaged content of 20% (w/w) for *lysine* and 23% (w/w) for *glutamic acid*. Relative standard deviations for *repeatability* (RSDr) of 2.5% were obtained for *lysine* and *glutamic acid*. This is in agreement with the precision values reported in the frame of two ring-trial validation studies.

Based on the performance characteristics available, the EURL recommends for official control the method based on IEC-VIS to quantify *lysine* and *glutamic acid* 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.