European Food Safety Authority

EFSA Journal 2014;12(7):3796

SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of copper chelate of L-lysinate-HCl as feed additive for all animal species[1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Copper chelate of L-lysinate-HCl, provided as powder and as granulate, is intended for use as a copper source in animal nutrition. Tolerance studies with chicken for fattening and weaned piglets, allowed the FEEDAP Panel to conclude that copper chelate of L-lysinate-HCl is a safe source of copper for all animal species, provided that the maximum copper contents authorised in feed are respected. The supplementation of feeds with copper from copper chelate of L-lysinate-HCl up to the maximum authorised copper levels is not expected to result in a different copper deposition in edible tissues/products than the standard inorganic source cupric sulphate pentahydrate. No concerns for consumer safety will arise from the use of the additive in animal nutrition, provided that the maximum copper contents authorised in feed are respected. The powder form of copper chelate of L-lysinate-HCl should be considered as a risk by inhalation; exposure by inhalation should be minimised. Neither form of the additive is a dermal irritant but the powder form is an eye irritant. In the absence of data, it is considered prudent to regard both forms as potential skin sensitisers. Copper chelate of L-lysinate-HCl is intended to be a substitute for other authorised copper additives; it will therefore not further increase the environmental burden from copper. Copper chelate of L-lysinate-HCl is an efficacious source of copper in meeting animal requirements. The Panel made some recommendations regarding the *Description and Conditions of use of the additive* and the maximum residue limits established for copper in animal tissues and products.

KEY WORDS

nutritional additive, compounds of trace elements, copper, copper chelate of L-lysinate-HCl, safety, environment, efficacy

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Trace Elements, including Noël Dierick, Jürgen Gropp and Alberto Mantovani, for the preparatory work on this scientific opinion.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2014. Scientific Opinion on the safety and efficacy of copper chelate of L-lysinate-HCl as feed additive for all animal species. EFSA Journal 2014;12(7):3796, 20 pp. doi:10.2903/j.efsa.2014.3796

Available online: www.efsa.europa.eu/efsajournal

SUMMARY

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of copper chelate of L-lysinate-HCl for all animal species. The additive is intended to be marketed in two forms, powder and granules.

Tolerance studies with chicken for fattening and weaned piglets, allowed the FEEDAP Panel to conclude that copper chelate of L-lysinate-HCl is a safe source of copper for all animal species, provided that the maximum copper contents authorised in feed are respected.

The supplementation of feeds with copper from copper chelate of l-lysinate-HCl up to the maximum authorised copper levels is not expected to result in a different copper deposition in edible tissues/products than the standard inorganic source cupric sulphate pentahydrate. No concerns for consumer safety will arise from the use of the additive in animal nutrition, provided that the maximum copper contents authorised in feed are respected.

The powder form of copper chelate of l-lysinate-HCl should be considered as a risk by inhalation; exposure by inhalation should be minimised. Neither form of the additive is a dermal irritant but the powder form is an eye irritant. In the absence of data, it is considered prudent to regard both forms as potential skin sensitisers.

Copper chelate of L-lysinate-HCl is intended to be a substitute for other authorised copper additives; it will therefore not further increase the environmental burden from copper.

In one study in chickens, dietary copper chelate of L-lysinate-HCl increased the copper concentration in liver in a dose-dependent manner comparable to a standard authorised copper source; an additional study in chickens also supported the bioavailability of copper from a copper-lysine complex. It is therefore concluded that copper chelate of L-lysinate-HCl is an effective source of copper for all animal species.

The Panel made some recommendations regarding the *Description and Conditions of use of the additive* and the maximum residue limits established for copper in animal tissues and products.

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BACKGROUND

Regulation (EC) No 1831/2003[4] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from the company Senzyme GmbH[5] for authorisation of the product copper chelate of L-lysinate-HCl, when used as a feed additive for all animal species (category: Nutritional additives; functional group: compounds of trace elements) under the conditions mentioned in Table 1.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive).[6] According to Article 8 of that Regulation, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. The particulars and documents in support of the application were considered valid by EFSA as of 25 September 2013.

The feed additive Aminotrace Copper Bislysinate has not been previously authorised in the European Union (EU). Several other copper compounds are authorised in the EU to be used as nutritional feed additives (trace elements): cupric acetate, monohydrate; basic cupric carbonate, monohydrate; cupric chloride, dihydrate; cupric methionate; cupric oxide; cupric sulphate, pentahydrate; cupric chelate of amino acids hydrate; copperlysine sulphate;[7] cupric chelate of glycine, hydrate;[8] copper chelate of hydroxy analogue of methionine;[9] dicopper chloride trihydroxide.[10]

The Scientific Committee on Animal Nutrition (SCAN) delivered reports on the use of copper methionate for pigs (EC, 1981), copper compounds in feedingstuffs (EC, 1982) and in feedingstuffs for pigs (EC, 1983) and the use of copper in feedingstuffs (EC, 2003a). EFSA issued opinions on the safety of the chelated forms of iron, copper, manganese and zinc with synthetic feed grade glycine (EFSA, 2005), on the safety and efficacy of a copper chelate of hydroxy analogue of methionine (Mintrex®Cu) as feed additive for all animal species (EFSA, 2008; EFSA FEEDAP Panel, 2009), and on the safety and efficacy of di copper chloride tri hydroxide (tribasic copper chloride, TBCC) as feed additive for all animal species (EFSA FEEDAP Panel, 2011). EFSA has issued two opinions concerning the re-evaluation of cupric sulphate pentahydrate (EFSA FEEDAP Panel, 2012) and cupric chelate of amino acids hydrate (EFSA FEEDAP Panel, 2013).

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and the efficacy of copper chelate of L-lysinate-HCl, when used under the conditions described in Table 1.

[4] 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.

[5] Senzyme GmbH, Mülheimer Straße , 26 32, 53840 Troisdorf , Germany.

[6] EFSA Dossier reference: FAD-2013-0003.

[7] Commission Regulation (EC) No 1334/2003 of 25 July 2003 amending the conditions for authorisation of a number of additives in feedingstuffs belonging to the group of trace elements. OJ L 187, 26.7.2003, p. 11.

[8] Commission Regulation (EC) No 479/2006 of 23 March 2006 as regards the authorisation of certain additives belonging to the group compounds of trace elements. OJ L 86, 24.3.2006, p. 4.

[9] Commission Regulation (EU) No 349/2010 of 23 April 2010 concerning the authorisation of copper chelate of hydroxy analogue of methionine as a feed additive for all animal species. OJ L 104, 24.4.2010, p. 31.

[10] Commission Implementing Regulation (EU) No 269/2012 of 26 March 2012 concerning the authorisation of dicopper chloride trihydroxide as feed additive for all animal species. OJ L 89, 27.3.2012, p. 3.

Table 1: Description and conditions of use of the additive as proposed by the applicant

Additive	Aminotrace Copper Bislysinate
Registration number/EC No/No (if appropriate)	-
Category(-ies) of additive	nutritional additive, 3
Functional group(s) of additive	compounds of trace elements, b

Description			
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)
Copper chelate from L-Lysinate HCl	**$Cu(C_6H_{13}N_2O_2)_2$ X HCl**	**in compliance with EU legislation**	**EN 15510:2007 (copper) VDLUFA 4.11.6 (L-Lysinate HCl)**

Trade name (if appropriate)	n.a.
Name of the holder of authorisation (if appropriate)	n.a.

Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg/kg of complete feedingstuffs		
All animal species	-	-	according Commission Regulation (EC) 1334/3002	n.a.

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use (if appropriate)	Feed formulation should be adjusted to account for the lysine activity of L-Lysinate HCl. A level of copper exceeding 10 mg/kg in feed may cause poisoning in certain breeds of sheep. A level of copper less than 20 mg/kg may cause deficiencies in cattle grazing pastures with high contents of molybdenum or sulfur.
Specific conditions or restrictions for handling (if appropriate)	For user safety: use safety glasses and protective gloves. In case of dust formation take appropriate measures for breathing protection. The precautions for handling on the safety data sheet must be observed.
Post-market monitoring (if appropriate)	Senzyme GmbH will conduct post-marketing monitoring in compliance with EU law on feed hygiene, namely by use of HACCP and Traceability systems and formal monitoring of customer feedback through product or service complaints.
Specific conditions for use in complementary feedingstuffs (if appropriate)	To supply copper in final feeds within EU legal limits for each species

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

ASSESSMENT

1. Introduction

Copper is an essential trace element. The biological role of copper, its requirements/recommendations, deficiency and toxicity symptoms in farm animals have been described in a former opinion of the Scientific Committee on Animal Nutrition (SCAN) (EC, 2003a); the maximum levels authorised for total copper in feedingstuffs are derived from that opinion. To the knowledge of the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), there is no additional relevant information that might lead it to modify the SCAN opinion.

The applicant is seeking authorisation for the use of copper chelate of L-lysinate-HCl in feed for all animal species/categories. This copper compound is not currently authorised in the EU as feed additive.

2. Characterisation

For compounds of trace elements, the element itself is considered the active substance.

2.1. Characterisation of copper chelate of L-lysinate-HCl

The additive copper chelate of L-lysinate-HCl is identified with the Chemical Abstracts Service (CAS) No 53383–2–7. Its International Union of Pure and Applied Chemistry (IUPAC) name is copper dichloro-bis(L-lysinate)-complex.[11] Its chemical formula is $Cu(C_6H_{13}N_2O_2)_2 \times 2HCl$, and it has a molecular weight of 426.83 Da. The theoretical content of copper is 14.9 %.

The additive is intended to be marketed in two forms: powder, containing the additive as such, and granulate, with an admixture of approximately 0.5 % binding agents (e.g. carboxymethylcellulose). Both forms of the additive are specified to contain ≥ 14.5 % copper and ≤ 85.5 % L-lysine HCl. The analysis of five batches of each form was submitted. Average values for the powder were 14.6 ± 0.1 % Cu and 85.2 ± 0.2 % lysine HCl[12] and for the granulate were 14.6 ± 0.1 % Cu and 84.9 ± 0.1 % lysine HCl.[13] Absorbance data indicate that the additive consists of only copper chelate of L-lysinate-HCl.[14]

A chemical structure of copper chelate of L-lysinate-HCl is shown in Figure 1.

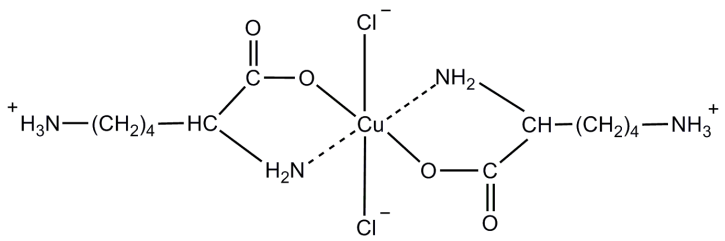

Figure 1: Chemical structure of copper chelate of L-lysinate-HCl according to Duarte et al. (1985)

Heavy metals (Cd, Pb, Hg), fluorine and arsenic, dioxins and dioxin like polychlorinated biphenyls (PCB's) were analysed in three batches of the powder form.[15,16] All values were far below the

[11] Other names: copper chelate from L-Lysinate-HCl, copper lysinate, copper bislysinate, di-Lysinate, dichloro, coppersalt, copper chelate of dichloro-bis-l-lysinate. Trade name: Aminotrace Copper Bislysinate.
[12] Technical Dossier/Section II/Annex II_1_2
[13] Technical Dossier/Section II/Annex II_1_3
[14] Technical Dossier/Supplementary Information/Annex 2
[15] Technical Dossier/Section II/Annex II_1_4.
[16] Technical Dossier/Section II/Annex II_1_5.

thresholds of the directive on undesirable substances.[17] Mercury was below the limit of quantification (0.1 mg/kg additive).

2.1.1. Physical state of the product

The product is dark grey-black in colour, has an unobtrusive odour and comes in two forms: a solid free-flowing powder or granulates. The relative density for the powder is 440 kg/m^3.[18] For the granulate form of the additive, the corresponding value is 400 kg/m^3.[19]

Mean particle size of the powder form was determined in three batches and was in the range of 43–48 µm (v/v).[20] The samples contain 25–27 % of particulate matter with particle sizes < 10 µm; the dust contains 95–97 % particles < 10 µm. The granulate form has a mean particle size of 853–886 µm with virtually no particles below 10 µm.[21]

The dusting potential of the powder form as measured by the Stauber-Heubach method was 4.5–5.1 g/m^3,[22] whereas the corresponding value for the granulate was 0.05 g/m^3.[23]

2.2. Manufacturing process

The starting raw materials used for the production of the additive are cupric oxide and L-lysine HCl. The manufacturing process of the product is fully described in the technical dossier.

The material safety data sheets of the raw materials were provided: L-lysine,[24] cupric oxide[25] and carboxymethylcellulose.[26]

2.3. Stability and homogeneity

Stability data of the additive were not provided by the applicant and are generally not required for compounds of trace elements. A minimum shelf life of one year is proposed by the applicant.

The capacity of both forms of the additive to homogeneously distribute was presented in studies conducted with a premixture for sows and with a variety of compound feedingstuffs (composition not given).[27] Based on the analysis of copper, the coefficients of variation were similar for the powder and granulate forms and ranged from 0.29 to 0.43 % in the premixture and from 0.21 to 6.4 % in feeds. The lowest deviation was obtained with mineral piglet feed and the highest one with pelleted piglet feed.

2.4. Conditions of use

The copper compound under application, copper chelate of L-lysinate-HCl, is intended to supply copper in final feed for all animal species/categories up to a maximum total content of 170 mg Cu/kg in complete feedingstuffs for piglets (up to 12 weeks) and 25 mg Cu/kg for other pigs; 15 mg Cu/kg complete feedingstuffs for bovine before the start of rumination (milk replacers and other complete feedingstuffs) and 35 mg Cu/kg for other bovine; 15 mg Cu/kg complete feedingstuffs for ovine; 50 mg Cu/kg complete feedingstuffs for crustaceans; 25 mg Cu/kg complete feedingstuffs for fish; and 25 mg Cu/kg complete feedingstuffs for other species.

[17] 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.
[18] Technical Dossier/Section II/Annex II_1_6.
[19] Technical Dossier/Section II/Annex II_1_9.
[20] Technical Dossier/Section II/Annex II_1_7.
[21] Technical Dossier/Section II/Annex II_1_10.
[22] Technical Dossier/Section II/Annex II_1_8.
[23] Technical Dossier/Section II/Annex II_1_11.
[24] Technical Dossier/Section II/Annex II_3_6.
[25] Technical Dossier/Section II/Annex II_3_7.
[26] Technical Dossier/Section II/Annex II_3_8.
[27] Technical Dossier/Section II/Annex II_4_1 and Annex II_4_2.

2.5. Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)

EFSA has verified the EURL report as it relates to the methods used for the control of copper chelate of L-lysinate-HCl in animal feed. The Executive Summary of the EURL report can be found in the Appendix.

3. Safety

3.1. Safety for the target species

3.1.1. Tolerance studies for the target species

The applicant has provided studies on calves, chicken for fattening and piglets. The safety demonstration is based on the comparison of the effects of copper chelate of L-lysinate-HCl with a compound of trace elements already authorised (cupric sulphate pentahydrate) at different supplementation levels.

3.1.1.1. Tolerance study in calves

Two 6-week tolerance trials were carried out with 60 Friesian male calves each (trial 1: initial age 18 days, average body weight 51 kg; trial 2: initial age 12 days, average body weight 52 kg).[28] For each of the four treatments, 15 calves were housed together in one pen and fed individually by automatic feeders. A basal diet (milk replacer; background copper: 3 mg/kg feed) was supplemented with 7 and 12 mg Cu/kg from cupric sulphate pentahydrate and copper chelate of L-lysinate-HCl, respectively in the first trial and with 7 and 42 mg Cu/kg in the second trial. The copper concentration in the diets was confirmed by analysis. During the trials, the animals received hay for *ad libitum* intake. Animals were medicated for the first ten days prophylactically against respiratory diseases[29] and diarrhoea,[30] and were vaccinated twice against the bovine respiratory syncytial virus and treated against endo- and ectoparasites.[31] The animals were weighed weekly; feed intake was recorded daily. At the end of the trial, blood samples were taken from four calves per treatment for haematology[32] and clinical chemistry.[33] No statistics were provided.

No conclusions on the safety of the copper compound under assessment can be drawn from this study with calves owing to several weaknesses in the design (absence of values for copper concentration in liver, which is used as a biomarker; absence of statistical evaluation; small number of animals used for haematology and clinical chemistry). It is noted that the feeding regime is considered untypical since no concentrate has been provided.

3.1.1.2. Tolerance study in chickens for fattening

A 35-day tolerance study was performed in three consecutive experiments with a total of 600 two-day-old chickens for fattening (Ross 308, both sexes).[34] In each experiment, 200 chickens for fattening were allocated to two dietary treatments, with four replicates of 25 chicken per treatment. Each experiment was performed with a different copper supplementation level (12.5, 25 and 250 mg total Cu/kg feed). The diets of the control groups were supplemented with cupric sulphate pentahydrate and those of the treatment groups with copper chelate of L-lysinate-HCl. The basal diet, consisting mainly of maize and soybean meal (background copper: 7.5 mg/kg), was given as starter (days 0–10, 20 % crude protein (CP)) and grower (days 11–35, 19 % CP) diets.

[28] Technical Dossier/Section III/Annex III_1_1 and Annex III_1_2.
[29] Non-steroidal anti-inflammatory substances.
[30] An aqueous extract of spruce needles.
[31] Pour-on-solution containing doramectin.
[32] Total leucocytes, erythrocytes, platelets, haemoglobin, haematocrit, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean cell volume (MCV).
[33] Albumin, urea, total protein, uric acid, aspartate transaminase (AST), gamma-glutamyl transferase (γ-GT).
[34] Technical Dossier/Section III/Annex III_1_3.

The intended copper concentration in the feed was confirmed by analysis (see Table 2). The birds were weighed and feed intake was recorded weekly. At the end of each experiment, samples (blood, liver, tibia) from eight broilers per experiment (presumably one broiler per replicate) were taken for haematology,[35] clinical chemistry[36] and copper analysis in liver and tibia. The statistical model applied was not reported.

Mortality was low and not treatment related (Table 2). The zootechnical data are summarised in Table 2. No effects of copper source and copper concentration on body weight and feed-to-gain ratio were observed at dietary copper levels of 12.5 and 25 mg/kg. At the highest copper concentration (250 mg/kg) birds in the copper lysinate group showed a final weight which was about 30 % higher than that of the control (Table 2). It should be noted that the body weight of the birds in experiments 1 and 2 and of the control group in experiment 3 was lower than expected for the broiler breed and the duration of the experiment; this could be related to the low protein content, particularly of the grower diet. The lysine content of the grower diet (three analyses) was 0.83 %, which is considered low. The addition of about 240 mg copper from copper bislysinate would have increased dietary lysine to about 0.98 %, which in turn might have resulted in a higher body weight.

Some significant differences were observed in uric acid and albumin. However, considering the high variability of these parameters, the FEEDAP Panel considers that these observations did not indicate adverse effects. Furthermore, haematological and the other clinical chemical parameters were not affected by treatment. There were no substance-related differences in the copper concentration of liver and tibia. The copper level in liver appeared to be dose-dependent, as indicated by the values of the high-copper groups compared with the lower ones. It should however be mentioned that the standard deviation of the liver copper values in experiment 3 (four animals/treatment) is very high (169 ± 124 and 126 ± 180 mg/kg dry matter (DM)). Thus, no conclusion can be drawn on the differences in the mean values observed in the copper sulphate and copper lysine complex groups.

Table 2: Effect of level and source of copper on performance and copper content in liver and tibia of chickens for fattening

Experiment No.	**1**		**2**		**3**	
Cu source	**CSP**	**CC-Lys**	**CSP**	**CC-Lys**	**CSP**	**CC-Lys**
Intended total copper (mg Cu/kg feed)	**12.5**		**25**		**250**	
Analysed copper (mg Cu/kg feed)	**12.7**	**13.2**	**25.6**	**25.5**	**251.9**	**251.9**
Mortality n (out of 100)	3	2	2	2	3	0
Final body weight (g)	1405	1384	1537	1541	1455	1916*
Feed-to-gain ratio	1.72	1.72	1.58	1.65	1.65	1.53
Copper in liver (mg/kg DM)[1]	12	12	10	11	169	126
Copper in tibia (mg/kg fat-free DM)[1]	16	9	9	7	9	7

(1) Four animals per treatment.
* reported to be significantly different from cupric sulphate 250 mg/kg group ($P \leq 0.05$).
CSP: Cupric sulphate pentahydrate; CC-Lys: Copper chelate of L-lysinate-HCl.

There was no indication of differences between tolerance parameters for the two copper compounds, the chelate of L-lysinate-HCl and the sulphate pentahydrate, by chicken for fattening up to 10-fold the maximum authorised total copper in complete feed. The FEEDAP Panel notes that a small number of animals was used to analyse copper in the liver and tibia, as well as for the haematology and clinical chemistry analyses; therefore only limited conclusions can be drawn from this part of the study.

[35] Total leucocytes, erythrocytes, haematocrit; only in experiments 2 and 3.
[36] Albumin, total protein, uric acid, AST, γ-GT.

3.1.1.3. Tolerance study in weaned piglets

A 42-day tolerance study was performed with 72 weaned piglets (castrated males and females; ♂ Piétrain × ♀ (Euroc × German Landrace); age 25 days, average body weight: 6.7 kg). The piglets were allocated according to weight and sex to six dietary treatments (four replicates per treatment, three animals/replicate).[37] The basal diet (maize, wheat, barley and soybean meal; background copper concentration: 4 mg/kg feed) was supplemented with copper from cupric sulphate or copper chelate of L-lysinate-HCl to obtain diets with 6, 170 and 340 mg total Cu/kg, as confirmed by analysis (see Table 3). The starter diet was fed for two weeks followed by a grower diet until completion of the study. Body weight and feed intake per pen were recorded weekly. At the end of the study, blood samples were taken from all animals for haematology[38] and clinical chemistry.[39] Copper in liver was analysed from six animals per treatment. An analysis of variance (ANOVA) was done with the pen as experimental unit, followed by a Tukey post-hoc test.

There was no mortality. Treatments did not significantly affect animal performance. The addition of approximately 0.1 and 0.2 % Lys to the diets with 170 and 340 mg total Cu/kg could be expected to improve the performance of piglets (lysine in starter and grower diets 1.12/1.03 %). This may be reflected in numerically better feed-to-gain ratios for the copper chelate treatments at the intermediate and high groups (Table 3).

Although some significant differences were observed for neutrophils, mean corpuscular haemoglobin concentration, calcium, chlorine, cholesterol, urea and glucose, they are considered as not biologically relevant and mostly not dose- or substance-related. It should be noted that the means were always within the physiological range for piglets (Kraft and Dürr, 2005). Plasma copper was not affected by dietary copper from either source. No differences were seen in liver copper at 6 and 170 mg total Cu/kg feed, which is in accordance with a previous SCAN opinion (EC, 2003a). As concluded from the same opinion, dietary copper higher than 170 mg would result in increased liver copper concentrations, as shown in the present study from cupric sulphate, but not for the lysinate. It should be noted that the liver copper concentration of 340 mg Cu/kg feed in the cupric sulphate group was approximately half of that reported in the SCAN opinion at a comparable dose.

Table 3: Effect of level and source of copper on performance and copper content in blood and liver of piglets

Cu Source	**CSP**	**CC-Lys**	**CSP**	**CC-Lys**	**CSP**	**CC-Lys**
Intended total copper (mg/kg feed)	**6**		**170**		**340**	
Analysed copper (mg/kg feed)	**6.2**	**6.3**	**180**	**181**	**350**	**350**
Total feed intake (kg/pig)	26.2	25.9	26.7	26.1	27.1	26.0
Final body weight (kg)	20.6	20.9	21.6	22.5	22.7	22.8
Feed-to-gain ratio	1.88	1.83	1.80	1.65	1.71	1.62
Copper in blood (mg Cu/L)[1]	1.6	1.6	1.6	1.6	1.6	1.6
Copper in liver (mg Cu/kg DM)[2]	78[a]	84[a]	88[a]	83[a]	106[b]	82[a]

(1) Twelve animals per treatment.
(2) Six animals per treatment.
a,b: different superscripts within a row indicate statistical differences ($P \leq 0.05$).
CSP: Cupric sulphate pentahydrate; CC-Lys: Copper chelate of L-lysinate-HCl.

[37] Technical Dossier/Section III/Annex III_1_4
[38] Total and differential leucocyte counts, erythrocytes, haematocrit, haemoglobin, MCV, MCH, MCHC.
[39] Electrolytes (Na, K, Cl, Ca, P), cholesterol, triglycerides, bilirubin, urea, glucose, albumin, total protein, AST, alanine aminotransferase (ALT) and alkaline phosphatase (ALP).

The study indicates that copper chelate of L-lysinate-HCl is equally tolerated as the cupric sulphate pentahydrate, by piglets up to 2-fold the maximum authorised total copper in complete feed for this species/category.

3.1.2. Conclusions on the safety for the target species

Tolerance studies with chicken for fattening and weaned piglets, allowed the FEEDAP Panel to conclude that copper chelate of L-lysinate-HCl does not elicit additional or different adverse effects in target species compared with the standard inorganic source cupric sulphate pentahydrate. Therefore, the FEEDAP Panel considers copper chelate of L-lysinate-HCl as a safe source of copper for all animal species, provided that the maximum copper contents authorised in feed are respected.

3.2. Safety for the consumer

3.2.1. Metabolic and residue studies

Copper is absorbed from the diet in the upper jejunum by active and passive processes, stored in the liver and kidney, secreted in the bile and excreted in the faeces. Copper excretion via the kidneys is quantitatively insignificant if complex-forming substances are not administered (thiomolybdate from oral molybdenum in ruminants, dimethyl cysteine). Copper status is not easy to determine, and the homeostatic mechanisms that control copper distribution and metabolism are not completely understood. Copper interacts with other divalent cations, such as calcium, iron and zinc, for gastrointestinal absorption and metabolism. Absorption and availability may be influenced by the carbohydrate content of the diet, with reduced availability by phytate containing diets or those containing fructose (EC, 2003b). The application of copper as a chelate complex with amino acids or other strong binding chelators may also affect absorption and bioavailability.

The SCAN delivered an opinion on copper (EC, 2003a) in which the metabolism and tissue deposition of copper was reviewed. Other reviews are available from McDowell (2003) and Suttle (2010). The distribution of total copper in the body varies with species, age and copper status of the animal. In general, levels in newborn and suckling animals are higher, followed by a steady fall during growth to the time when adult values are reached. The main target organ for copper deposition is the liver. Other edible tissues containing high concentrations of copper are the heart, brain and kidney. Lower levels are found in muscle. Liver and kidney copper concentrations are related to dietary intake, whereas muscle is less affected. Its presence in milk is generally very low and not influenced by dietary supplementation levels. Clearance is higher in poultry than in mammals; the copper concentration in eggs is generally low. In fish, copper is primarily stored in the liver.

For the assessment of copper deposition from the product under application in products of animal origin, only liver data were available. As stated above, the liver is the edible tissue with the highest affinity for copper deposition in food producing animals, as well as with deposition patterns readily influenced by dietary intakes; therefore liver deposition can be taken as a proxy for the overall deposition of copper in edible tissues and products. Copper concentration in the tibia is considered as a reliable indicator of copper bioavailability. Two studies in two different target species (one on chicken for fattening and one on piglets; see sections 3.1.1.2 and 3.1.1.3) submitted by the applicant for tolerance in target species showed that the copper deposition in tibia bone from copper chelate of L-lysinate-HCl was not higher than that from its standard inorganic source cupric sulphate pentahydrate when supplemented in feed up to the maximum authorised copper level in the EU; the similar response suggested an almost equivalent copper bioavailability from the two sources, providing further reassurance that the supplementation with copper chelate of L-lysinate-HCl would not result in an increased deposition of copper compared with inorganic sources. This conclusion is also supported by Jongbloed et al. (2002) who evaluated that copper from copper chelate of L-lysinate-HCl and cupric sulphate pentahydrate have equivalent bioavailabilities in terms of liver copper deposition based on a review of two studies on pigs (Coffey at al., 1994; Apgar et al., 1995), three on poultry (Baker et al., 1991; Aoyagi and Baker, 1993; Pott et al., 1994) and three in ruminants (Pott et al., 1994; Luo et al., 1996; Chase et al., 2000). Although some of these reviewed studies used supplementation levels

leading to feed copper levels considerably higher than those currently authorised, none of them showed a significantly higher copper liver deposition from copper chelate of L-lysinate-HCl than from cupric sulphate pentahydrate.

The amount of lysine as an essential amino acid released from the dissociation of copper chelate of L-lysinate-HCl in the animal body after its oral intake will be limited by the maximum authorised copper level in animal feed and this lysine will be used in protein synthesis and/or metabolised to urea and carbon dioxide without any modification of edible tissues/products.

The FEEDAP Panel considers that copper from copper chelate of L-lysinate-HCl would not result in a different copper deposition in edible tissue/products than the standard inorganic source cupric sulphate pentahydrate when supplemented to feed up to the maximum authorised copper level in the EU.

The supplementation of feeds with copper from copper chelate of L-lysinate-HCl up to the maximum authorised copper levels is not expected to result in a different copper deposition in edible tissues/products than the standard inorganic source cupric sulphate pentahydrate. No concerns for consumer safety will arise from the use of the additive in animal nutrition, provided that the maximum copper contents authorised in feed are respected.

3.2.2. Toxicological studies

No specific toxicological studies with the product under assessment were submitted. The compound copper chelate of L-lysinate-HCl is expected to be dissociated in the target animal and, consequently, only copper is considered for potential toxicological concerns for consumers.

The Scientific Committee on Food (SCF) (EC, 2003b) summarised data on the toxic properties of copper. Tolerance to high intakes of copper varies greatly from one species to another, in relation to the vulnerability of the species and the levels of zinc, iron and molybdenum in the diet. Copper excess causes impaired growth and extensive necrosis of hepatocytes. Susceptibility to copper excess is also influenced by its chemical form. Manifestations of copper toxicity include weakness, tremors, anorexia and jaundice. As tissue copper levels increase, haemolytic crisis may ensue, resulting in liver, kidney and brain damage.

3.2.2.1. Genotoxicity/Mutagenicity/Carcinogenicity

Copper(II) has been reported to be genotoxic *in vitro* and also in some *in vivo* bone marrow micronucleus assays in mice after intraperitoneal injection. As with other essential trace elements (zinc, iron), copper is known to have a genotoxic potential when present at high local concentrations. In particular, copper is a redox-active transition element, potentially able to catalyse the Fenton/Haber–Weiss reaction, with the consequent production of reactive oxygen species. As copper is physiologically present in the intracellular environment at very low levels, a genotoxic concern for the human population is not foreseen, except under conditions of overload, which are not relevant to the use of the additive under evaluation.

The International Agency for Research on Cancer (IARC) allocated copper (II) 8-hydroxyquinoline to Group 3 "Not classifiable as to their carcinogenicity to humans" (IARC, 1987). The SCF (EC, 2003b) concluded that studies on the carcinogenicity of copper compounds in rats and mice have given no indication of carcinogenic potential; however, some degree of uncertainty exists owing to limitations in available studies.

3.2.2.2. Reproduction toxicity

With regard to the influence on reproduction, studies in rodents demonstrated that oral exposure to copper during gestation induced embryo/fetotoxic and developmental effects. Copper(II) sulphate administered at doses of 12 and 80 mg Cu per kg body weight and day was embryolethal to mink and mice, respectively (IPCS, 1998).

3.2.3. Assessment of consumer safety

A tolerable upper intake level (UL) of 5 mg Cu/day for adults and 1 mg/day for toddlers (one to three years of age) was defined by the SCF (EC, 2003b). This figure was derived from an overall no observed adverse effect level (NOAEL) of 10 mg Cu/day identified in the study by Pratt et al. (1985) (daily single dose levels only administered to seven male adult volunteers for 12 weeks, with serum liver markers as endpoints), applying an uncertainty factor of 2 for potential variability in the normal population.

Studies on copper dietary intakes in industrialised countries did provide comparable results. Mean dietary copper intakes by adults in different European countries have been estimated to be within a range of about 1.0-2.0 mg/day (Van Dokkum, 1995; EC, 2003b; Sadhra et al., 2007; Rubio et al., 2009; Turconi et al., 2009). Based on 11 independent, peer-reviewed surveys considering only analytically confirmed copper (n= 849) in Belgium, Canada, the United Kingdom and the USA, the mean copper intakes for men and women were estimated to be 1.48 (2.87 for P95) and 0.92 (2.18 for P95) mg/day, respectively (Klevay, 2011). A recent analytical study of Catalonian diets showed a copper intake of 1.2 mg/day (Domingo et al., 2012). It has been suggested that calculated copper intakes are overestimated when only food composition tables are used in nutrition surveys (Klevay, 2012).

Among edible tissues of animal origin, the highest concentration of copper is found in liver (the main deposition organ), followed by kidney and muscle. Among products of animal origin, milk shows the lowest values.

The supplementation of feeds with copper from copper chelate of L-lysinate-HCl up to maximum authorised copper level is not expected to result in a different copper deposition in edible tissue/products than the standard inorganic source cupric sulphate pentahydrate (section 3.2.1.). Since the supplementation of animal feed with copper-containing compounds has not been essentially changed over the last decade, the use of the additive under application would not modify the consumer exposure to copper. The FEEDAP Panel concludes that copper chelate of L-lysinate-HCl is safe for consumers when used in animal nutrition according to the legislation.

The FEEDAP Panel reconfirms its previous proposal concerning the modification of the current maximum residue limits for copper in animal tissues and products (EFSA FEEDAP Panel, 2012).

3.2.4. Conclusions on the safety for the consumer

The supplementation of feeds with copper from copper chelate of L-lysinate-HCl up to the maximum authorised copper levels is not expected to result in a different copper deposition in edible tissues/products than the standard inorganic source cupric sulphate pentahydrate. No concerns for consumer safety will arise from the use of the additive in animal nutrition, provided that the maximum copper contents authorised in feed are respected.

3.3. Safety for the user

3.3.1. Effects on the respiratory system

The powder form of copper chelate of L-lysinate-HCl shows a significant dusting potential (up to 5.1 g/m^3). About 96 % of particles of the dust have a diameter < 10 µm. Considering the theoretical copper content of the dust (about 14 %), the absence of inhalation toxicity studies and the possible hazardous effects of inhaled copper, in particular for the immune response of the lungs (EFSA FEEDAP Panel, 2012; EC, 2013), handling the product should be considered as a risk by inhalation. Granulating the copper chelate of l-lysinate-HCl reduces the dusting potential of the powder by a factor of 100 (0.05 g/m^3), and because of the virtual absence of particle of respirable size, the risk of exposure by inhalation is minimised.

3.3.2. Effects on the eyes and skin

Good Laboratory Practice-compliant tests were conducted in rabbits for dermal irritation (according to the Organisation for Economic Co-operation and Development (OECD) testing guideline 404)[40] and eye irritation (according to OECD testing guideline 405)[41] with both forms of the additive.

Both forms of the additive produced reversible dermal irritation, which was slight to moderate for the powder form and very slight for the granulate form. Similarly, both forms of the additive produced reversible ocular reactions; for the powder form this reaction was moderate, and, for the granulate form, only a slight irritation was produced.

No skin sensitisation studies were performed. In the absence of specific information, the FEEDAP Panel considers it prudent to regard the additive as a potential skin sensitiser.

3.3.3. Conclusions on the safety for users/workers

The powder form of copper chelate of L-lysinate-HCl should be considered as a risk by inhalation; exposure by inhalation should be minimised. Neither form of the additive is a not dermal irritant but the powder form is an eye irritant. In the absence of data, it is considered prudent to regard both forms as potential skin sensitisers.

3.4. Safety for the environment

The FEEDAP Panel has already considered the potential risks to the environment posed by the use of copper in feedingstuffs up to the maximum authorized contents in the EU (EFSA FEEDAP Panel, 2012). The additive under assessment, copper chelate of L-lysinate-HCl, is intended to be a substitute for other authorised copper additives. It will therefore not further increase the environmental burden of copper.

4. Efficacy

The applicant provided two trials to demonstrate the efficacy of copper chelate of L-lysinate-HCl, one with chicken for fattening and another with weaned piglets. In both experiments the efficacy of copper chelate of L-lysinate-HCl was compared with cupric sulphate pentahydrate. In addition the FEEDAP Panel identified a study with chicken for fattening from the available literature to evaluate the efficacy of the compound under assessment.

4.1. Chickens for fattening

4.1.1. Study 1

The applicant submitted a study with chickens for fattening to support the efficacy of the additive. This study has already been described in section 3.1.1.2. No differences in the liver copper concentration between the two copper sources, the L-lysinate-HCl and the sulphate, were found at the three dietary levels tested.

A considerable increase in liver copper could be seen as a consequence of an increase of dietary copper from 12/25 mg/kg to 250 mg/kg. This increase was observed for both copper sources (Table 2).

4.1.2. Study 2

This study, in which two independent experiments with chicken were performed, was taken from the literature (Guo et al., 2001). The exact composition of the copper lysine complex used was not described. The copper and nitrogen contents were 10.1 and 7.77 %, respectively. The additive in the present opinion has a copper content of 14.5 % and a nitrogen content (calculated from lysine HCl) of

[40] Technical Dossier/Section III/Annex III_3_5 and Annex III_3_7.
[41] Technical Dossier/Section III/Annex III_3_6 and Annex III_3_8 .

13.1 %, with a copper:lysine ratio of 1:2. The corresponding copper:lysine ratio of the copper-lysine complex used by Guo et al. is 1:1.74. Thus, this copper-lysine complex contains other unknown ingredients and an insufficient relative concentration of lysine to form a complete bivalent complex with copper. However, the Cu:Lys ratio is relatively close to 1:2. It can therefore be suggested that the copper predominantly exists in the studied product as a bivalent complex with lysine.

In each of the two experiments, five pens of five-day-old male chicks were used for each of the five dietary treatments. The treatments were 150, 300 and 450 mg Cu supplemental as reagent-grade Cu sulphate/kg or 150 and 300 mg Cu supplemental as copper lysine complex/kg basal diet. The basal maize-soybean meal diet contained 16 and 22 mg Cu/kg DM in experiments 1 and 2, respectively. The concentration of copper in the diets was confirmed by analysis. At the end of the experiment (21 and 20 days in experiments 1 and 2, respectively), body weight and feed consumption were recorded. Copper in liver was analysed in five and three chickens per pen in experiments 1 and 2, respectively. Data were analysed by two-way ANOVA.

Considering the duration of the experiments, both are regarded as bioavailability studies; only the copper deposition in liver is described. Copper supplementation of 150 to 300 mg/kg feed, using cupric sulphate as copper source, resulted in an increase of liver copper from 21 mg/kg DM to 124 mg/kg DM (averages of both experiments). The corresponding figures after the addition of 150 and 300 mg Cu/kg feed from copper lysinate were 29 and 147 mg Cu/kg liver DM, resèctively.

4.2. Weaned piglets

This study has already been described in section 3.1.1.3. Copper concentration of the liver is the best parameter to demonstrate bioavailability of the copper source; however, feed concentration needs to be above the maximum authorised level for demonstration. No dose-dependent increase in liver copper with application of the additive, was observed in this study, even at the highest concentration used (350 mg/kg feed). For cupric sulphate a marginal increase in liver copper was observed at 350 mg/kg feed (see also Table 3). Therefore, the data do not provide clear indication of copper availability for both substances (copper chelate of L-lysinate-HCl and copper sulphate).

4.3. Conclusions on the efficacy for the target species

Liver copper concentration is considered a valid biomarker for the bioavailability of dietary copper. A response in some species (pigs and chickens) can be seen only at high copper levels in feed. In one study in chickens, dietary copper chelate of L-lysinate-HCl increased the copper concentration in liver in a dose-dependent manner comparable to a standard authorised copper source; an additional study in chickens also supported the bioavailability of copper from a copper-lysine complex. Based on the evaluation of liver deposition data in comparison with a standard inorganic copper source, it is therefore concluded that copper chelate of L-lysinate-HCl is efficacious as a source of copper in meeting the requirements of all animal species.

5. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[42] and Good Manufacturing Practice.

[42] 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.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Tolerance studies provided with chickens for fattening and weaned piglets allowed the FEEDAP Panel to conclude that copper chelate of L-lysinate-HCl is a safe source of copper for all species, provided that the maximum copper contents authorised in feed are respected.

The supplementation of feeds with copper from copper chelate of L-lysinate-HCl up to the maximum authorised copper levels is not expected to result in a different copper deposition in edible tissues/products than the standard inorganic source cupric sulphate pentahydrate. No concerns for consumer safety will arise from the use of the additive in animal nutrition, provided that the maximum copper contents authorised in feed are respected.

The powder form of copper chelate of L-lysinate-HCl should be considered as a risk by inhalation; exposure by inhalation should be minimised. Neither form of the additive is a dermal irritant but the powder form is an eye irritant. In the absence of data, it is considered prudent to regard both forms as potential skin sensitisers.

Copper chelate of L-lysinate-HCl, is intended to be a substitute for other authorised copper additives; it will therefore not further increase the environmental burden from copper.

Copper chelate of L-lysinate-HCl is an efficacious source of copper for all animal species.

RECOMMENDATIONS

In order to avoid confusion with other copper chelates of lysine containing 1 mol copper with 1 mol of Lys, the FEEDAP Panel recommends "Copper bislysinate" for the name of the additive under assessment.

The description of the additive should include the copper and lysine contents. The proposed figures are ≥ 14.5 % copper and ≥ 84.0 % lysine, for both forms of the additive.

The complex contains two molecules of hydrochloric acid, and therefore the molecular formula is: $Cu(C_6H_{13}N_2O_2)_2 \times 2HCl$.

The lysine content of the additive should be considered when formulating feed for piglets.

The FEEDAP Panel reiterates its previous proposal concerning the modification of the current maximum residue limits for copper in animal tissues and products (EFSA FEEDAP Panel, 2012).

DOCUMENTATION PROVIDED TO EFSA

1. Dossier Aminotrace Copper Bislysinate. October 2012. Submitted by Senzyme GmbH.
2. Dossier Aminotrace Copper Bislysinate. Supplementary information. February 2014. Submitted by Senzyme GmbH.
3. Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Aminotrace Copper Bislysinate.
4. Comments from Member States received through the ScienceNet.

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APPENDIX

Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Aminotrace Copper Bislysinate[43]

In the current application authorisation is sought under article 4(1) for *Copper Chelate of L-Lysinate-HCl* 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.

Copper Chelate of L-Lysinate-HCl is a dark-grey or black free flowing powder or granules with a minimum content of 14.5 % *total copper*, and a maximum content of 85.5 % *L-Lysine-HCl.*

The *feed additive* is intended to be incorporated into *premixtures* and *feedingstuffs*. The Applicant suggested maximum levels of *total copper* in the *feedingstuffs* complying with the limits set in Regulations (EC) No 1334/2003 and 479/2006, and ranging from 15 to 170 mg/kg, depending of the animal species/category.

For the quantification of *Lysine* content in the *feed additive* the Applicant submitted the ring-trial validated VDLUFA method, based on ion-exchange chromatography coupled with post-column derivatisation and colourimetric or fluorescence detection. However, the EURL identified and recommends instead the recently published ring-trial validated EN ISO 17180 method - based on a similar analytical protocol - for the "determination of *Lysine* […] in commercial amino acid products and premixtures". The following performance characteristics were reported for Lysine contents ranging from 100 to 740 g/kg: - a relative standard deviation for *repeatability* (RSDr) ranging from 0.7 to 1.7 %; and - a relative standard deviation for *reproducibility* (RSDR) ranging from 1.5 to 2.5 %.

For the determination of *total copper* in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted the internationally recognised ring-trial validated method EN 15510 based on inductively coupled plasma atomic emission spectroscopy (ICP-AES). The following performance characteristics were reported for the copper content ranging from 6.8 to 775 mg/kg *premixtures* and *feedingstuffs*: - RSDr ranging from 2.9 to 12 %; RSDR ranging from 8 to 22 %; and - a limit for quantification (LOQ) of 3 mg/kg *feedingstuffs*.

Additionally, the EURL already recommended in the frame of the Copper group evaluation (cf. Final report JRC.D.5/CvH/PRO/ago/ARES(2012)108233) the ring-trial validated EN 15621 method, based on ICP-AES after pressure digestion. The following performance characteristics were reported for a copper content ranging from 7.3 to 470 mg/kg: - RSDr ranging from 2.6 to 6.8 %; - RSDR ranging from 3.8 to 12 %; and - LOQ = 1 mg/kg *feedingstuffs*. Furthermore, a Community method is available for the determination of *total copper* in *feedingstuffs*, but no performance characteristics for the method are available, except an LOQ of 10 mg/kg *feedingstuffs*. However, the UK Food Standards Agency recently published a ring-trial based on the above mentioned Community method and reported precisions (RSDr and RSDR) from 2.4 to 9.2 % for copper contents ranging from 17 to 39 mg/kg *feedingstuffs*. Based on the available information the EURL recommends for official control the two CEN methods (EN 15510 or EN 15621) together with the Community method (Com Reg (EC) No 152/2009 – Annex IV-C) for the determination of *total copper* in the *feed additive*, *premixtures* and *feedingstuffs*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

[43] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2013-0003_Copper_Lysinate.pdf