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Safety and efficacy of Kelforce® (L-glutamic acid, *N*,*N*-diacetic acid, tetrasodium salt (GLDA-Na_4)) as a feed additive for chickens for fattening

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Abstract

L-Glutamic acid, *N*,*N*-diacetic acid, tetrasodium salt (GLDA-Na_4) (Kelforce®) is sought to be used as a zootechnical feed additive in chickens for fattening to improve the absorption of zinc from feed, reducing zinc emissions through manure and thus, affecting favourably the environment. The product has not been authorised in the European Union as a feed additive. Kelforce® is intended to be marketed as a liquid and solid formulation, containing $\geq$ 47% and $\geq$ 30% of GLDA-Na_4, respectively. Kelforce® is safe for chickens for fattening at the maximum level of 1,000 mg GLDA-Na_4/kg complete feed. Based on the toxicological profile of GLDA-Na_4 and the consumer exposure to GLDA-Na_4 and to nitrilotriacetic acid trisodium salt (NTA-Na_3; an impurity of the additive), the use of Kelforce® at the maximum proposed level in feed of chickens for fattening is of no concern for consumer safety. Due to its low inhalation toxicity, the exposure to GLDA-Na_4 is unlikely to pose a risk by inhalation. However, owing to the high-dusting potential of the solid formulation, a risk from such high level of dust, even if toxicologically inert, cannot be excluded. Kelforce® is not a skin/eye irritant or skin sensitiser. No risks for the terrestrial compartment were identified at the maximum use level of the additive. Risks for the aquatic compartment cannot be excluded based on the secondary effect of the additive on green algae. In the absence of data, the Panel cannot conclude on the safety for the sediment compartment or the possible ground water contamination. The risk of bioaccumulation and secondary poisoning caused by the additive is considered very low. Owing to the inconsistent and conflicting results from the studies assessed, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) cannot conclude on the efficacy of the additive. The Panel made a recommendation regarding the levels of formaldehyde and cyanide in the active substance.

Keywords: Zootechnical additives, substances which favourably affect the environment, other zootechnical additives, Kelforce®, GLDA-Na_4, safety, efficacy

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Summary

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of Kelforce® (L-glutamic acid, *N*,*N*-diacetic acid, tetrasodium salt (GLDA-Na_4)) as a feed additive for chickens for fattening. This product has not been authorised in the European Union as a feed additive.

The additive is intended for use as a zootechnical additive (functional groups: substances which favourably affect the environment and other zootechnical additives) in chickens for fattening. The applicant suggests that it enhances the nutritional availability of dietary zinc in chickens for fattening, by improving solubilisation or preventing precipitation in the gastrointestinal tract. Improving the absorption of zinc from the feed would allow reducing the supplementation of zinc and consequently decrease zinc emission through manure and would, if efficacious, benefit the environment.

The additive will be marketed in two formulations: solid and liquid, with a minimum specified content of GLDA-Na_4 of 30% and 47.4% (w/w), respectively; the remainder in the composition being water. The applicant proposes a maximum use level of the active substance of 1,000 mg GLDA-Na_4/kg feed, equivalent to 2,110 mg/kg for the aqueous preparation and 3,333 mg/kg for the solid formulation of the additive. Typical use ranges are reported to be 100–300 mg GLDA-Na_4/kg feed for the active substance, equivalent to 211–633 mg/kg for the liquid form and 333–1,000 mg/kg for the solid form of the additive.

A tolerance study demonstrated that GLDA-Na_4 was tolerated by chickens for fattening up to a concentration of 3,000 mg/kg complete feed. The maximum recommended use level of 1,000 mg GLDA-Na_4/kg complete feed is therefore safe for chickens for fattening with a margin of safety of about three.

In rats, GLDA-Na_4 is poorly absorbed and is principally excreted as the intact molecule in the faeces, while the limited amount of absorbed, GLDA-Na_4 is eliminated unchanged through the urine. Limited deposition in liver and muscle was observed in chickens fed up to 300 mg/kg feed, but no data were collected from animals given the maximum proposed dose. The higher deposition of GLDA-Na_4 in kidney compared to liver and muscle probably reflects the renal pattern of excretion.

GLDA-Na_4 did not induce any increase of gene mutations in the *in vitro* bacterial (*Salmonella* Typhimurium) reverse and mammalian cell gen (hypoxanthine-guanine phosphoribosyltransferase (HPRT) locus) tests. An *in vitro* chromosome aberration assay on the Chinese hamster lung (CHL) cell line yielded equivocal results, but the outcome of the micronucleus assay in bone marrow of mice indicated that GLDA-Na_4 does not induce chromosome damage *in vivo*. Therefore, based on the weight of evidence, the FEEDAP Panel concludes that GLDA-Na_4 is non-genotoxic. High doses of NTA-Na_3 induce renal tumours in rodents; the available evidence indicates that this compound is not a gene mutagen or a clastogen and that the tumorigenic effect occurs through a thresholded mechanism at high concentrations.

The lowest no observed adverse effect level (NOAEL) found in animal studies with GLDA-Na_4 was 20 mg/kg body weight (bw) per day, which corresponded to dose-related maternal gastrointestinal complications in rabbits. An overall uncertainty factor of 100 is applied to derive an acceptable daily intake (ADI) of 0.20 mg (200 μg) GLDA-Na_4/kg bw.

The consumption of food from chickens fed Kelforce® at 10 times the highest inclusion level of 1,000 mg GLDA-Na_4/kg complete feed would result in an exposure to GLDA-Na_4 28-fold lower than the ADI, and therefore, no concern for consumer safety is envisaged. No other hazards in the additive represent a risk to the consumers.

Due to its low inhalation toxicity, the exposure to GLDA-Na_4 in the additive is unlikely to pose a risk by inhalation. However, the Panel notes that the solid formulation as described has an exceptionally high-dusting potential and a risk from such a high level of dust, even if toxicologically inert, cannot be excluded. Kelforce® has low dermal toxicity and it is not a skin or eye irritant or a skin sensitiser.

No risks for the terrestrial compartment were identified at the maximum use level of 1,000 mg GLDA-Na_4/kg complete feed. Risks for the aquatic compartment cannot be excluded based on the secondary effect of the additive on green algae (reduction of nutrients). In the absence of data, the FEEDAP Panel cannot conclude on the safety for the sediment compartment nor on the possible contamination of the ground water. The risk of bioaccumulation and secondary poisoning caused by the additive is considered very low.

The applicant provided six *in vitro* studies to support efficacy, [redacted]

The applicant also submitted five short-term efficacy studies [redacted] the FEEDAP Panel cannot conclude on the efficacy of the additive either as a *Other zootechnical additive* or as a *Zootechnical additive favourably affecting the environment*.

The FEEDAP Panel recommended to set the levels of both formaldehyde and cyanide in the active substance (aqueous solution of mg GLDA-Na_4) at $\leq$ 10 mg/L.

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person who 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 Selko B.V.[2] for authorisation of the product Kelforce®, L-glutamic acid, *N*,*N*-diacetic acid, tetrasodium salt (GLDA-Na_4), when used as a feed additive for chickens for fattening (category: zootechnical additives; functional groups: substances which favourably affect the environment and other zootechnical additives).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 29 November 2013.

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 Kelforce®, L-glutamic acid, *N*,*N*-diacetic acid, tetrasodium salt (GLDA-Na_4), when used under the proposed conditions of use (see Section 3.1.5).

1.2. Additional information

The additive Kelforce® contains GLDA-Na_4. It is intended for use as a zootechnical additive (functional groups: substances which favourably affect the environment and other zootechnical additives) in chickens for fattening.

This product has not been authorised in the European Union as a feed additive. GLDA-Na_4 is listed under the European Commission Database on Cosmetic Ingredients as a chelating agent.[3]

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[4] in support of the authorisation request for the use of Kelforce®, GLDA-Na_4, as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008[5] and the applicable EFSA guidance documents.

The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) used the data provided by the applicant together with data from other sources, such as 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 Kelforce®, GLDA-Na_4, in animal feed. The Executive Summary of the EURL report can be found in Annex A.[6]

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] Selko B.V. Jellinghausstraat 22, 5048 AZ Tilburg, The Netherlands
[3] EU Cosmetic ingredient database. Available online: https://ec.europa.eu/growth/sectors/cosmetics/cosing_en
[4] FEED dossier reference: FAD-2013-0022.
[5] Commission Regulation (EC) No. 429/2008 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.
[6] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2013-0022-Kelforce-corrected.pdf

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Kelforce®, GLDA-Na_4, is in line with the principles laid down in Regulation (EC) No 429/2008[7] and the relevant guidance documents: Guidance on zootechnical 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 establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012b), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012c).

3. Assessment

Kelforce® (GLDA-Na_4) is intended to be used as a zootechnical feed additive in feed for chickens for fattening. It is intended to enhance the nutritional availability of dietary zinc, by improving solubilisation or preventing precipitation in the gastrointestinal tract. Improving the absorption of zinc from the feed would allow reducing the supplementation of zinc and consequently decrease zinc emission through manure, benefiting the environment.

3.1. Characterisation

3.1.1. Characterisation of the active substance

L-Glutamic acid, *N*,*N*-diacetic acid, tetrasodium salt (International Union of Pure and Applied Chemistry (IUPAC) name: tetrasodium (2*S*)–2–[bis(carboxylatomethyl)amino]pentanedioate; Chemical Abstracts Service (CAS) number: 51981–21–6; European Inventory of Existing Commercial Substances (EINECS) number 257–573–7) has the molecular formula $C_9H_9NO_8 \cdot 4Na$ and a molecular mass of 351.13. The structural formula is shown in Figure 1.

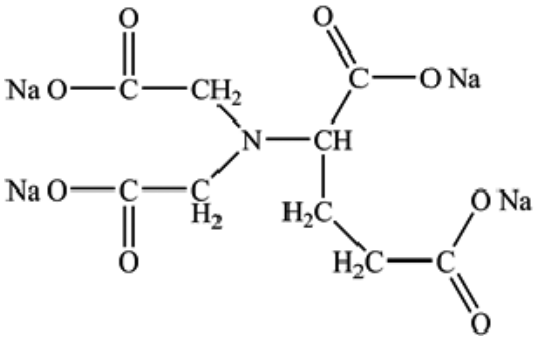


Figure 1: Structural formula of L-glutamic acid, *N*,*N*-diacetic acid, tetrasodium salt

3.1.2. Characterisation of the additive

The additive is presented in two formulations: solid and liquid.

The liquid form of the additive has a minimum specified content of 47.4% (w/w) GLDA-Na_4, the remainder being water; this was confirmed by the analysis of five batches (47.8–48.6% GLDA-Na_4).[8] It is a clear liquid with a slight ammonia odour and a density of 1,420 kg/m^3.

The solid formulation has a minimum specified content of GLDA-Na_4 of 30%, a maximum content of silicic acid of 36% and 35% of water; the content of GLDA-Na_4 was confirmed by the analysis of five batches (30.4–30.9% GLDA-Na_4).[9] It is a white powder with a bulk density of 720 kg/m^3.

Four batches of the liquid form of the additive were analysed for heavy metals (lead (Pb), cadmium (Cd), mercury (Hg)) and arsenic (As), and all values were below the respective limit of detection (LOD) (Pb $\leq$ 0.005 mg/kg; Cd $\leq$ 0.005 mg/kg; Hg $<$ 0.01 mg/kg and As $<$ 0.1 mg/kg).[10] Levels of dioxins

[7] 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.
[8] Technical Dossier/Section II/Annex II_5.
[9] Technical Dossier/Section II/Annex II_6.
[10] Technical Dossier/Section II/Annex II_4.

(0.09 ng WHO PCDD/F-TEQ/kg) and sum of dioxins and dioxins-like polychlorinated biphenyls (PCBs) (0.125 ng WHO PCDD/F-PCB-TEQ/kg) were analysed in four batches of the liquid form.[11] Five batches of the solid form of the additive were analysed for heavy metals (Pb, Cd, Hg), fluorine (F) and As,[12] with values often below the respective LOD (Pb ≤ 0.08 mg/kg, Cd ≤ 0.03 mg/kg, Hg < 0.01 mg/kg, F ≤ 23 mg/kg and As ≤ 0.2 mg/kg).[12] The same batches were analysed for dioxins (0.09 ng WHO PCDD/F-TEQ/kg) and sum of dioxins and dioxins-like PCBs (0.116–0.179 ng WHO PCDD/F-PCB-TEQ/kg).[13] Neither the additive nor the active substance has an specific entry in the Directive on Undesirable substances in animal feed;[14] however, a comparison of the levels of the impurities found in the additive with those allowed in complete feed identified no concerns.

The product is regularly monitored for by-products or residues of raw materials and processing aids that may be present in the aqueous solution of GLDA-Na_4. The analysis of five batches showed that all values (≤ 6 mg formaldehyde/L; ≤ 9 mg cyanide/L; ≤ 15 g sodium hydrochloride/L; ≤ 26 g GLDA-Na_4 linear/L; ≤ 28 g GLDA-Na_4 cyclic/L; ≤ 0.8 g nitrilotriacetic acid trisodium salt (NTA-Na_3)/L; ≤ 50 mg ammonia/L; ≤ 4 g sodium-glycolate /L; ≤ 7 g sodium-formate/L; ≤ 1 g iminodiacetic acid disodium salt/L)[15] were below the specifications set by the applicant.[16] Since both the liquid and solid formulations are obtained from the starting aqueous solution of GLDA-Na_4, the same purity criteria apply.

Particle size was determined in five batches of the solid additive by laser diffraction.[17] The solid formulation has a median particle size (v/v) of 105 μm, with 19.5% of the particles with a diameter < 50 μm and 2.8% of the particles < 10 μm. The mean dusting potential determined in five batches of the additive by the Stauber–Heubach method was 30.6 g/m^3.[18]

3.1.3. Manufacturing process

[redacted]

3.1.4. Stability and homogeneity

The shelf-life of three batches of the aqueous solution was studied during storage in closed containers at 20°C. Losses of GLDA-Na_4 after 2 years were less than 0.4%.[19] Other five batches were stored for 6 months at 40°C. No losses in GLDA-Na_4 were observed.

The shelf-life of the solid formulation (three batches) was studied when stored for 24 months in plastic bags at 25 or 40°C.[20,21] Losses of GLDA-Na_4 amounted to approximately 2% regardless of the temperature.

The stability of the additive in premixtures and feed was studied only for the solid form; the results obtained are also indicative of the stability of the liquid form. GLDA-Na_4 was added at 1% in a vitamin–mineral premix (containing choline chloride) for chickens for fattening (one batch) and stored at room temperature (not specified) up to 6 months.[20] Losses amounted to approximately 4% after 6 months.

The stability of GLDA-Na_4 was monitored in one batch of feed for poultry, both in mash and pelleted form.[20] GLDA-Na_4 was added at 100 mg/kg feed, and part of the feed was pelleted at

[11] Technical Dossier/Section II/Annex II_12.
[12] Technical Dossier/Section II/Annex II_13. Limits of Detection reported: arsenic, 0.10 mg/kg; cadmium, 0.01 mg/kg; fluorine, 20 mg/kg, mercury 0.01 mg/kg; lead, 0.05 mg/kg.
[13] Technical dossier/Section II/Annex II_14.
[14] 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.
[15] Although melamine could be formed from cyanide and ammonia, given the amount of both impurities measured in the additive, melamine will be orders of magnitude lower than the legal limit set for this substance in feed (2.5 mg/kg complete feed).
[16] Specifications indicated in the technical dossier: formaldehyde < 50 mg/L; cyanide < 20 mg/L; free sodium hydrochloride < 19 g/L; L-glutamic acid monoacetic acid sodium salt linear < 30 g/L; L-glutamic acid monoacetic acid sodium salt cyclic < 30 g/L; nitrilotriacetic acid trisodium salt < 1 g/L; ammonia < 50 mg/L; Na-glycolate < 10 g/L; Na-formate < 10 g/L; iminodiacetic acid disodium salt < 5 g/L.
[17] Technical Dossier/Section II/Annex II_18.
[18] Technical Dossier/Section II/Annex II_19.
[19] Technical Dossier/Section II/Annex II_36.
[20] Technical Dossier/Section II/Annex II_37.
[21] Technical Dossier/Supplementary Information June 2014/Annex C.

approximately 80°C. The pelleting process did not cause a decrease in the average content of GLDA-Na_4. No losses were observed either in mash or pelleted feed after 3 months of storage at room temperature (not specified).

The capacity of the additive to homogeneously distribute in feed was studied in 10 subsamples of the mash feed used in the stability study. The coefficient of variation obtained was 6.1%. No data were provided on the homogeneity of the liquid form of Kelforce® in feed, but in general, it might be expected that a liquid additive distribute well in premixtures and complete feeds.

3.1.5. Conditions of use

Both forms of the additive are intended to be used in feed for chickens for fattening, added directly in feed or via complementary feed or premixtures. The applicant proposes a maximum use level of the active substance of 1,000 mg GLDA-Na_4/kg feed, equivalent to 2,110 mg/kg for the aqueous preparation and 3,333 mg/kg for the solid formulation of the additive. Typical use ranges are reported to be 100–300 mg GLDA-Na_4/kg feed for the active substance, equivalent to 211–633 mg/kg for the liquid form and 333–1,000 mg/kg for the solid form of the additive.

3.2. Safety

3.2.1. Safety for the chickens for fattening

A total of 480 one-day-old male Ross 308 chickens were distributed to six treatments (8 replicates of 10 birds per treatment).[22] Maize–soybean meal basal diets (starter and grower) were supplemented with the solid form of the additive to achieve a nominal content of GLDA-Na_4 of 0, 100, 300, 1,000 (1×), 3,000 (3×) and 10,000 (10×) mg/kg complete feed. The recovery of GLDA-Na_4 was 87%, 94%, 95%, 92% and 89% in the starter and 91%, 95%, 91%, 92% and 98% in the grower diets. Diets in pelleted form were fed for 35 days (day 0–10: starter diet; day 11–35 grower diet). A mineral and vitamin premixture was applied across all the experimental diets, aiming to supply 100 mg zinc, 10 mg copper, 100 mg manganese and 120 mg iron per kg diet. Regarding zinc, the analysed content in the starter and grower diets was 96 and 85 mg/kg, respectively.[23]

General health of the animals was monitored daily. Body weight and feed intake per pen were determined at 0, 10 and 35 days of age. Body weight gain and feed to gain ratio were calculated for the different periods 0–10 days, 10–35 days and overall. Blood samples were obtained from three birds per pen on day 35 for haematology and blood biochemistry.[24] At the end of the experiment, one bird per pen was selected for necropsy. In the necropsy, body and organ (liver and kidney) weights were measured. Response parameters were subjected to the analysis of variance (ANOVA) according to a completely randomised block design (pens blocked according to their position in the experimental facility).

Overall mortality was on average 7.4% and not treatment related (Table 1). No significant adverse effects on feed intake and weight gain were observed up to a concentration of GLDA-Na_4 of 3,000 mg/kg feed. A significant reduction in final weight, weight gain and feed intake, with the increase in feed to gain ratio was observed with the 10,000 mg/kg dose (Table 1).

[22] Technical Dossier/Section III/Annexes Sect. III/Annex III-1.

[23] The FEEDAP Panel notes that the analysed and expected zinc concentration in started and grower feed differs considerably (the analysed levels being about 40 mg/kg complete feed lower than the expected).

[24] White blood cells, red blood cells, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin (MCH), differential leucocyte count, albumin, γ-glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, total protein (TP), glucose, alkaline phosphatase (AP), lactate dehydrogenase (LDH), albumin/globulin, creatinine, calcium, phosphorus, chlorine, total bilirubin, sodium and potassium) and levels of zinc, copper, manganese and iron.

Table 1: Feed intake, final body weight, feed to gain ratio and mortality in the tolerance study

GLDA-Na_4 (mg/kg feed)	Total Feed intake (g/bird)	Final body weight (g)	Feed to gain	Mortality (%)
0	3,304^{ab}	2,338^{b}	1.44^{bc}	11.2
100	3,339^{ab}	2,348^{b}	1.45^{c}	3.7
300	3,420^{a}	2,439^{a}	1.43^{ab}	8.9
1000	3,316^{ab}	2,377^{ab}	1.42^{a}	7.6
3000	3,289^{b}	2,374^{ab}	1.41^{a}	7.5
10000	2,961^{c}	2,044^{c}	1.48^{d}	5.3

a,b,c,d: Values in a column with a different superscript are significantly different ($p < 0.05$)

A significant increase in haemoglobin, haematocrit, erythrocyte and heterophil granulocyte counts was observed in the 10,000 mg/kg group, compared to all the rest other treatments. An increase of mean corpuscular haemoglobin was also observed at 3,000 mg/kg, but without a dose-dependent relationship. Total protein, albumin and sodium were significantly increased in the 10,000 mg/kg group. Alkaline phosphatase was reduced and γ-glutamyl transferase was increased in the 10,000 mg/kg group compared with the control. The relative kidney weight of the 10,000 mg/kg group was also significantly increased compared with the control (from 0.68% to 0.79% of live weight).

3.2.1.1. Conclusions on safety for the target species

The study provided demonstrated that GLDA-Na_4 was tolerated by chickens for fattening up to a concentration of 3,000 mg/kg complete feed. The maximum recommended use level of 1,000 mg GLDA-Na_4/kg complete feed is therefore safe for chickens for fattening with a margin of safety of about three. This would correspond to a maximum safe concentration of 2,110 mL liquid Kelforce®/kg feed or 3,333 mg solid Kelforce®/kg feed.

3.2.2. Safety for the consumer

3.2.2.1. Absorption, distribution, metabolism, excretion (ADME) and residues

No studies on absorption, distribution, metabolism and excretion (ADME) in the target animals were provided. The kinetics studies in rats give some information on absorption and excretion. Residue data were obtained from the tolerance study with chickens for fattening.

3.2.2.1.1. Kinetics studies in rats

Excretion of GLDA-Na_4 was studied using 48 Wistar rats of both sexes, receiving either a single oral (gavage) or an intraperitoneal dose of the test item.[25] Urine and faeces samples were taken before dosing, and in the intervals: 0–24, 24–48 and 48–72 h after dosing. Samples were analysed for GLDA-Na_4 by liquid chromatography–mass spectrometry (LC–MS). Four male and four female rats each were given by gavage a single dose of 100, 300 or 1,000 mg GLDA-Na_4/kg body weight (bw). More than 85% of GLDA-Na_4 was recovered intact in the faeces within 24 h and 93–99% after 72 h. The remaining fraction (0.9–7.3%) was detected in the urine. Total recoveries of GLDA-Na_4 from the faeces and urine over the 72-h period ranged from 96% to 103%.

The other 24 rats (four rats of each sex per dose) received an intraperitoneal injection of 5, 15 or 50 mg GLDA-Na_4/kg bw, representing 5% of the oral doses. In 20 of these 24 rats, GLDA-Na_4 was excreted mainly in urine (66–100% of total excretion), whereas only in the other four rats, GLDA-Na_4 excretion in faeces was greater than that in urine. The percentages of faecal or urinary GLDA-Na_4 excretion were not dose related. The total GLDA-Na_4 recovery ranged between 75% and 103%.

Overall, this experiment indicates that (a) GLDA-Na_4 is poorly absorbed (< 5% of the oral dose) and mostly excreted unchanged, and (b) of the limited amount of GLDA-Na_4 absorbed on average > 85% is excreted within 24 h via urine/faeces, with an overall recovery of 75–103% reported after 72 h.

The low urinary excretion of GLDA-Na_4 was confirmed in a subchronic repeated-dose 90-day oral toxicity study (Section 3.2.2.2.2) with Wistar rats using the same GLDA oral doses as above.[26]

[25] Technical Dossier/Supplementary Information June 2014/Annex A.
[26] Technical Dossier/Section III/Reference 3.2.01

3.2.2.1.2. Residue study in chickens for fattening

At the end of the 35-day tolerance study with chickens for fattening (described in Section 3.2.1), birds fed diets with 100, 300 or 10,000 mg GLDA-Na_4/kg were randomly selected (one bird per pen, n = 8 per dose level) and samples of breast muscle, kidney and liver were collected from each animal for analysis of GLDA-Na_4 and NTA-Na_3 residues.[27] The concentrations in feed corresponded to the proposed normal inclusion levels of 100–300 mg/kg and 10-fold the proposed maximum inclusion level. GLDA-Na_4 and NTA were analysed in tissue extracts using LC–MS.[28]

NTA-Na_3 is an impurity that may be present in GLDA-Na_4 containing products and was detected in the analytical data provided for five batches of Kelforce® with a mean value of 0.48 g/L additive (Section 3.1.2)

The concentrations of GLDA-Na_4 were higher in kidney than in liver or muscle, that showed comparable concentrations at 100 or 300 mg/kg; a sharp increase of residues was observed at the 10-fold overdosing (Table 2). The concentration of NTA was found to be below the limit of quantification (LOQ) (0.06 mg/kg) in all tested tissues. No data are available on the residue deposition of GLDA-Na_4 in fat and skin; the FEEDAP Panel considers that given the high water solubility of this compound (at least 65,000 mg/L; see Table 3), deposition in skin and fat is expected to be low.

Table 2: Concentration of GLDA-Na_4 in edible tissues from chickens for fattening

Tissue	GLDA-Na_4 inclusion level in feed (mg/kg)	Tissue GLDA-Na_4 concentration (mg/kg)
Breast meat	100	0.008 ± 0.003
	300	0.021 ± 0.008
	10,000	0.378 ± 0.102
Liver	100	0.010 ± 0.002
	300	0.018 ± 0.004
	10,000	1.35 ± 0.012
Kidney	100	0.100 ± 0.019
	300	0.131 ± 0.018
	10,000	3.99 ± 0.37

3.2.2.1.3. Conclusions

In rats, GLDA-Na_4 is poorly absorbed and is principally excreted as the intact molecule in the faeces, while the limited amount of absorbed GLDA-Na_4 is eliminated unchanged through the urine. Limited deposition in liver and muscle was observed in chickens fed up to 300 mg/kg feed, but no data were collected from animals given the maximum proposed dose. The higher deposition of GLDA-Na_4 in kidney compared to liver and muscle probably reflects the renal pattern of excretion.

3.2.2.2. Toxicological studies

3.2.2.2.1. Genotoxicity studies

The test substance used in the four genotoxicity studies was a preparation containing 71% GLDA-Na_4.

GLDA-Na_4 was tested in *Salmonella* Typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 by the Ames plate incorporation method at five dose levels, in triplicate, both with and without the addition of a rat liver homogenate metabolising system (S9 mix).[29] Two independent experiments were performed up to a maximum concentration of 5,000 μg/plate in compliance with the Organisation for Economic Cooperation and Development (OECD) Guideline No 471 (version 1983). No significant increase in the numbers of revertant colonies was recorded for any of the bacterial strains with any dose of the test item, either with or without metabolic activation, while positive control chemicals produced marked increases in the number of revertant colonies.

[27] NTA-Na_3 is an impurity that may be present in GLDA-Na_4 containing products and was detected in the analytical data provided for five batches of Kelforce® with a mean value of 0.48 g/L additive (Section 3.1.2). It is also a compound of toxicological concern (see Section 3.2.2.2.1).
[28] Technical Dossier/Supplementary Information June 2014/Annex B.
[29] Technical Dossier/Section III/References Sect. III/Ref 3.2.09.

The potential mutagenicity of GLDA-Na_4 was tested on the hypoxanthine-guanine phosphoribosyl-transferase (HPRT) locus of Chinese hamster ovary cells in the presence and absence of an S9 metabolic activation system, in compliance with the OECD Guidelines No. 476 (version 1984).[30] The cells were treated with the test material at five dose levels, in duplicate, up to a maximum concentration of 3,650 μg/mL, corresponding to 10 mM. The entire experiment was repeated to confirm the result. No significant increases in mutant frequency were observed at any dose level of the test substance, either with or without metabolic activation, in either the first or second experiment. Significant increases in the mutant frequency were observed in the positive controls, indicating the satisfactory performance of the test and of the metabolising system.

The potential of GLDA-Na_4 to induce chromosomal aberrations was tested on the metaphase chromosomes of the CHL cell line, according to the OECD Guidleline 473.[31] The test material was evaluated at dose levels of 456.25, 912.5, 1,825 and 3,650 μg/mL. In Experiment 1, cells were harvested at a single time point (12 h), both with and without metabolic activation (S9 mix). In Experiment 2, six treatment regimens were used: 6 h exposure both with and without S9 mix followed by 18 h treatment-free incubation; 4 h exposure with the addition S9 mix followed by 8 h treatment-free incubation; 12 h continuous exposure; 24 h continuous exposure and 48 h continuous exposure without S9 (top exposure tested 1,825 μg/mL). The test material induced no concentration-related or statistically significant increases in the frequency of cells with aberrations in Experiment 1, whereas two positive control materials (mitomycin-C (MMC) and cyclophosphamide (CP)) induced significant increases of aberrations as expected. In Experiment 2, small, but statistically significant, increases in the frequency of cells with aberrations were observed at the highest concentration tested (3,650 μg/mL) in the 6-h exposure group, both with and without S9 activation; both positive controls (MMC and CP) performed as expected. For the 12 h continuous exposure group, there were no concentration-related or statistically significant increases in the number of cell with aberrations, while positive controls (MMC and CP) responded as expected. No effect was evident for the 24 h continuous exposure group at any concentration tested, with the positive control (MMC) behaving as expected. On the other hand, in the 48-h continuous exposure group, a small but statistically significant increase in the numbers of cells with aberrations was found at the highest concentration tested (1,825 μg/mL); in this treatment, cells were exposed only without S-9 and there was no corresponding positive control. All of the increases in aberrant cell frequency seen in this study were small and restricted to the maximum dose levels at which some cytotoxicity was observed. Indeed, 3,650 μg/mL (top concentration tested in the 6 h and 12 h groups) corresponds to 10 mM, usually considered the limit concentration that should not be exceeded in genotoxicity studies, because an osmotic effect can affect the integrity of cell membranes. Overall, the results of this study should be considered as positive but only at a limit concentration for osmotic effects; therefore, its biological relevance is questionable.

A micronucleus test in mice was performed in compliance with the OECD Guideline No 474 (version 1981).[32] Groups of 10 mice (five males and five females) received the test substance via the intraperitoneal route at 100, 200 and 400 mg/kg bw; further groups were given the vehicle alone or cyclophosphamide (positive control), respectively. Animals from all treatments were killed at 24 h post dosing. Additionally, four groups of 10 mice (five males and five females) received the top dose (400 mg/kg bw) or the vehicle only and were killed at 48 or 72 h post dosing, respectively. The bone marrow from all animals was examined. Polychromatic erythrocyte (PCE) and normochromatic erythrocyte (NCE) were scored for the presence of micronuclei. Two female rats died at the top dose level in the 72-h group. Increased activity, decreased respiratory rate, laboured respiration and ataxia were seen in the 400 mg/kg treated group. Only increased activity was observed in the 200 mg/kg group and no clinical signs were observed in the 100 mg/kg group. No significant change in the PCE/NCE ratio was observed after any treatment. There was a small but statistically significant increase in the incidence of micronucleated PCE in mice dosed with 400 mg/kg of the test item in the 72-h harvest group when compared to the concurrent vehicle control group. The positive control group showed a significant increase in the incidence of micronucleated PCE, hence confirming the sensitivity of the system. The FEEDAP Panel notes that, albeit the PCE/NCE ratio was unchanged, the use of the intraperitoneal route and the dose-related induction of systemic toxicity support the systemic exposure to the test item. The FEEDAP Panel considers that the slight, albeit significant, increase of micronuclei observed only at 400 mg/kg bw in the 72 h group was accompanied by overt toxicity including

[30] Technical Dossier/Section III/References Sect. III/Ref 3.2.10.
[31] Technical Dossier/Section III/References Sect. III/Ref 3.2.11.
[32] Technical Dossier/Section III/References Sect. III/Ref 3.2.12.

treatment-related mortality. The OECD guideline 474 (Mammalian erythrocyte micronucleus test, 2014) indicates that relevant effects should be detected at exposure levels not exceeding the maximum tolerated dose, i.e. in the presence of some toxicity, but with no mortality or overt distress. Thus, the slight increase of micronuclei observed at the top dose level in the 72 h group is likely related to general toxicity. Accordingly, the test item was considered to be non-genotoxic *in vivo* under the conditions of the test.

Information on nitrilotriacetic acid trisodium salt genotoxicity

NTA-Na_3 is an impurity that may be present in GLDA-Na_4 containing products and was detected in the analytical data provided for five batches of Kelforce® with a mean value of 0.48 g/L additive (Section 3.1.2). NTA-Na_3 is classified by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to humans based on the consistent induction of renal tumours in rodent carcinogenicity studies (class 2B). According to the evaluation of the IARC, NTA acid and its disodium and trisodium salts were not genotoxic in mammalian cells *in vitro*, were not mutagenic to bacteria and were negative in experimental systems *in vivo*, except for the induction of aneuploidy in mouse germ cells (IARC, 1999). Aneuploidy, in the absence of DNA reactivity (that should result also in gene mutation and/or chromosome breakage), is assumed to origin from interference with the protein of the mitotic spindle and as such to occur through a thresholded mechanism only at rather high concentrations. The study of Nesslany et al. (2008) showed also *in vivo* and *in vitro* clastogenic activity in mammalian cells but attributed these effects to a consequence of the chelating properties of the substance that induce calcium deprivation, cytotoxicity and eventually clastogenicity as a secondary effect. This mechanism of genotoxicity is considered to have a threshold.

Conclusions on genotoxicity

GLDA-Na_4 did not induce any increase of gene mutations in the *in vitro* bacterial (*Salmonella* Typhimurium) reverse and mammalian cell gen (HPRT locus) tests. An *in vitro* chromosome aberration assay on the CHL cell line yielded equivocal results, but the outcome of the micronucleus assay in bone marrow of mice indicated that GLDA-Na_4 does not induce chromosome damage *in vivo*. Therefore, based on the weight of evidence, the FEEDAP Panel concludes that GLDA-Na_4 is non-genotoxic.

High doses of NTA-Na_3 induce renal tumours in rodents. The available evidence indicates that this compound is not a gene mutagen or a clastogen and that the tumorigenic effect occurs through a thresholded mechanism at high concentrations.

3.2.2.2.2. Subchronic repeated-dose oral toxicity study

The applicant provided a subchronic repeated-dose 90-day oral toxicity study with GLDA-Na_4 (95 ± 1%) given by daily gavage (OECD Guideline 408) to Wistar rats.[33] One control group and three treated groups (100, 300 and 1,000 mg GLDA-Na_4/kg bw per day) were tested, each consisting of 10 males and 10 females. An extra 10 animals per sex in the control and high-dose group were allowed to additional 14 days of observation, after the removal of test item on day 90.

Doses up to 1,000 mg GLDA-Na_4/kg bw per day caused no treatment-related changes in clinical appearance, functional observations, body weight and food consumption.

A number of significant changes (from the pairwise comparison with the control values) were observed at the highest dose of GLDA-Na_4 (1,000 mg/kg bw per day) in some of the haematological parameters: increased red blood cell counts in males, reduced mean corpuscular volume and mean corpuscular haemoglobin in both sexes, increased red cell distribution width and platelet counts in females. The only effect observed at 300 mg/kg bw per day was a reduced mean corpuscular haemoglobin in both sexes. Changes in clinical biochemistry parameters at 1,000 mg/kg bw per day at the end of the 90-day treatment included increased albumin and cholesterol levels, and reduced creatinine, inorganic phosphate and chloride levels in males and/or females. These changes were reversible as they were absent at the end of the 14-day observation period.

A number of changes were detected in the urinalysis values at 1,000 mg/kg bw per day, which included an increased sodium concentration/excretion in both sexes, and a reduced urinary volume with increased specific gravity, protein level and potassium concentration in females. In males, the increase in sodium concentration/excretion in urine was also observed at 300 mg/kg bw per day.

[33] Technical Dossier/Section III/References Sect. III/Ref 3.2.14.

These changes were absent at the end of the 14-day observation period, indicating that these were reversible in nature.

At the end of the 90-day treatment phase, there was an increase in absolute and relative kidney weight in males exposed to GLDA-Na_4 at 1,000 mg/kg bw per day. However, there was no histopathological evidence of an effect on kidneys. At the end of the 14-day observation period, kidney weights were similar in treated and control groups. In females treated with 1,000 mg GLDA-Na_4/kg per day, there was no effect on kidney size, but absolute and relative kidney weight was increased at the end of the observation period. No alterations were observed in organs and tissues other than kidney.

In summary, at 300 mg/kg bw per day, GLDA-Na_4 reduced mean corpuscular haemoglobin level and increased urinary sodium concentration/excretion. At 1,000 mg/kg bw per day, a range of more pronounced changes in blood and urine were noted, with increased kidney weight. Although no histopathological alterations were observed and most effects were reversible, the FEEDAP Panel considers that the observed changes are dose related and have a potential toxicological relevance; therefore, the FEEDAP Panel identifies a no observed adverse effect level (NOAEL) for GLDA-Na_4 of 100 mg/kg bw per day.

3.2.2.2.3. Reproduction toxicity studies (including prenatal developmental toxicity)

Study 1

A two-generation toxicity study of GLDA-Na_4 was conducted in rats exposed through the diet following OECD Guideline 416.[34]

The F_0-generation included five groups of 24 male and 24 female Wistar Han rats exposed to GLDA-Na_4 (91.0 ± 0.5%) at dose levels of 0 (group 1), 1,500 (group 2), 5,000 (group 3) or 15,000 mg/kg feed (groups 4 and 5). Groups 2–4 were exposed to GLDA-Na_4 included into a pelleted diet (total content of zinc: 88.6–93.5 mg per kg diet). Group 5 animals received a pelleted diet with GLDA-Na_4 at the high-dose level, supplemented with 1,000 mg zinc carbonate/kg (total concentration of zinc: 605–609 mg per kg diet). The additional dietary zinc in Group 5 was included in the experiment to compensate for possible (reproduction) toxic effects, if any, due to the chelating properties of GLDA-Na_4. Rats from the control group (Group 1) received the same pelleted diet as those from groups 2–4 but without GLDA-Na_4. F_0-males and F_0-females were exposed to GLDA-Na_4 from 10 weeks prior to mating and exposure was continued until euthanasia (males) or one day before euthanasia (females). Males were killed as soon as possible after the delivery of the litters. F_0-females were allowed to produce and rear a litter until day 21 of lactation and then killed (on day 21 post-partum or shortly thereafter). On day 4 of lactation, litters were reduced in size to eight pups (four of each sex when possible) by random culling. After weaning, one F_1-male and one F_1-female of each litter of groups 1–4 were selected for mating with offspring of another litter of the same dose group to produce a F_2-generation.

The F_1-generation included four groups of 24 males and 24 females Wistar Han rats exposed to GLDA-Na_4 at dose levels of 0, 1,500, 5,000 or 15,000 mg/kg feed. GLDA-Na_4 was included into a pelleted diet (total content of zinc: 83.8 mg per kg diet). No additional zinc-supplemented group was added in this phase of the study. Rats from the control group received the same pelleted diet but without GLDA-Na_4. After weaning, animals were treated for a minimum of 70 days prior to mating and continued until euthanasia (males) or 1 day before euthanasia (females). Males were killed as soon as possible after the delivery of the litters. F_1-females were allowed to produce and rear a litter until day 21 of lactation and then killed (on day 21 post-partum or shortly thereafter). On day 4 of lactation, litters were reduced in size to eight pups by random culling.

All animals were subjected to daily clinical observation. Body weight and food consumption were measured over the treatment period. Blood samples were collected for analysis from F_0-females with live offspring. At necropsy, urine samples were collected and macroscopic observations and organ weights were recorded. Reproduction parameters and breeding data were assessed. In addition, one male and one female per litter of the F1-pups selected for culling were examined for possible skeletal variations or malformations (excluding ossification). No other developmental toxicity parameters were recorded.

No unscheduled deaths occurred during the trial. No treatment-related clinical signs were observed and no adverse effects on body weight, body weight gain, food consumption, haematology, clinical biochemistry and urinalysis parameters, macroscopy or organ weights were noted following treatment with GLDA-Na_4 up to 15,000 mg/kg with or without zinc added. At 15,000 mg/kg without or with zinc

[34] Technical Dossier/Section III/References Sect. III/Ref 3.2.15.

(groups 4 and 5, respectively), increased mean kidney weight (absolute and relative to body weight) was observed in F_0- and F_1-adults. Histopathological renal changes were observed in F_1-adults, consisting in increased cortical tubular dilation in females and increased corticomedullary tubular basophilia in males. No adverse effects were identified in dose levels 1,500 and 5,000 mg/kg in all generations.

It was concluded that the parental NOAEL was 5,000 mg/kg feed, which is equal to 287–389 mg and 380–894 mg GLDA-Na_4 per kg bw per day for males and females, respectively.

Reproduction parameters (mating performance, number of implantation sites, duration of gestation and fertility parameters), breeding parameters (dead and living pups at first litter check, postnatal loss, living pups on day 4 post-partum and viability index) and skeletal variations or malformations were not affected up to a dose level of 15,000 mg/kg GLDA-Na_4 with or without zinc added. Based on the tested reproductive parameters, the dosage level of 15,000 mg/kg GLDA-Na_4 was considered as the NOAEL for reproductive toxicity, corresponding to at least 908 (males) and 1230 (females) mg GLDA-Na_4 per kg bw per day. However, based on renal findings in F_0 and F_1 adults, the overall NOAEL was 5,000 mg/kg feed, corresponding to at least 287 (males) and 380 (females) mg GLDA-Na_4 per kg bw per day.

Study 2

A developmental toxicity study with rabbits following OECD Guideline 414 was performed.[35] Eighty-eight mated female New Zealand white (NZW) rabbits were assigned to four dose groups each containing 22 animals. GLDA-Na_4 (87.3 ± 0.5%) was administered once daily by gavage from days 7–28 *post-coitum* at doses of 20 (Group 2), 75 (Group 3) and 300 mg/kg bw (Group 4). Rabbits of the control group (Group 1) were given water without GLDA-Na_4.

No treatment-related mortality occurred during the study. Maternal toxicity was noted at 300 mg/kg bw per day, with clinical signs such as an increased incidence of dark faeces, diarrhoea and reduced faeces output. Food and water consumption were reduced. In addition, body weight gain was decreased, with several animals showing a transient body weight loss. However, there were no statistically significant changes in (absolute) body weight or body weight gain corrected for uterus in any of the groups. In animals receiving 75 mg/kg bw per day, dark faeces, diarrhoea, reduced faeces production and slightly reduced food and water intake were also observed.

No significant differences were observed between control and treated groups regarding number of corpora lutea, implantation sites, viable or dead foetuses, early or late resorptions, or pre- and post-implantation loss. No developmental toxicity (viscera and skeleton) was observed.

The maternal NOAEL was identified as 20 mg/kg bw per day and the developmental NOAEL was 300 mg/kg bw per day.

3.2.2.3. Derivation of health-based guidance value

The FEEDAP Panel concludes that Kelforce® is non-genotoxic.

The lowest NOAEL found in animal studies with GLDA-Na_4 was 20 mg/kg bw per day, which corresponded to dose-related maternal gastrointestinal complications in rabbits. An overall uncertainty factor of 100 is applied to derive an acceptable daily intake (ADI) of 0.20 mg (200 μg) GLDA-Na_4/kg bw.

3.2.2.4. Assessment of consumer safety

The exposure to GLDA-Na_4 of consumers of products derived from animals receiving the additive was derived using the theoretical daily human consumption of tissues from birds of the standard food basket indicated in Regulation (EC) No 429/2008 (300 g of breast meat, 100 g of liver and 10 g of kidney) and the residues of GLDA-Na_4 (mean value plus 2 × standard deviation, reflecting the 95th percentile) detected in the study in chickens for fattening (Section 3.2.2.1.2). No data on the residues at the highest recommended dose (1,000 mg GLDA-Na_4/kg complete feed was made available); therefore, the data from animals receiving 10 times the highest inclusion level were considered. The calculation gave a theoretical intake of GLDA-Na_4 from food of animal origin of 7 μg/kg bw per day in a 60-kg adult which is only 3.5% of the ADI (200 μg GLDA-Na_4/kg bw). The consumption of food from chickens fed Kelforce® at 10 times the highest inclusion level of 1,000 mg GLDA-Na_4/kg complete feed would result in an exposure to GLDA-Na_4 28-fold lower than the ADI.

NTA-Na_3 is present in low concentrations in the additive but could not be quantified in tissues of chickens for fattening (LOQ 0.06 mg/kg) after exposure to the 10-fold of the highest applied feed concentration of the additive. Exposure of the consumers by ingesting 500 g animal products from

[35] Technical Dossier/Section III/References Sect. III/Ref 3.2.17.

treated birds would therefore not exceed 0.03 mg/person per day, assuming that all animal products contained NTA-Na_3 at LOQ concentration. Thus, the FEEDAP Panel considers that this impurity is not likely to pose a safety concern for the consumer.

3.2.2.5. Conclusions on safety for the consumer

No concern for consumer safety is envisaged. No other hazards in the additive represent a risk to the consumers.

3.2.3. Safety for the user

3.2.3.1. Effects on respiratory system

Because of the percentage of inhalable and respirable particles (19.5% of < 50 μm diameter and 2.8% of < 10 μm diameter)[36] and the substantial dusting potential of 30.6 g/m^3 of the additive,[37] the applicant submitted an inhalation study with GLDA-Na_4.

An acute inhalation study[38] was performed in accordance with OECD Guideline 403. Five male and five female Wistar rats were exposed nose-only to an intended concentration of 5 g GLDA-Na_4/m^3 for 4 h (aerosol from GLDA-Na_4 90 $\pm$ 1%) with a measured exposure concentration of 4.2 g GLDA-Na_4/m^3. Observed clinical signs (i.e. slight bradypnea during exposure and a soiled fur of the head, neck, back and/or abdomen shortly after exposure) were no longer evident on day 2. Sniffing was noticed in four animals shortly after exposure, in seven animals on day 1 and in one animal on day 2. A discharge from the eyes was seen in one animal on day 1. No other abnormalities were noted during the 14-day observation period. The limited and transient non-lethal effects observed in this study show that GLDA-Na_4 has low inhalation toxicity potential.

The dry form of the additive contains silicic acid as carrier. Measurements performed by X-ray diffraction on three batches of the additive, each analysed in triplicate, confirmed that crystalline silica (quartz) was not detected in the respirable dust.[39,40]

Due to its low inhalation toxicity, the exposure to GLDA-Na_4 in the additive is unlikely to pose a risk by inhalation. However, the Panel notes that the solid formulation as described has an exceptionally high dusting potential (30.6 g/m^3) and a risk from such a high level of dust, even if toxicologically inert, cannot be excluded. The occupational exposure limits for inert dust in the European Union (EU) Member States are 10 mg/m^3 for inhalable dust and 3–4 mg/m^3 for respirable dust.[41]

3.2.3.2. Effects on eyes and skin

3.2.3.2.1. Acute dermal toxicity

The acute dermal toxicity of GLDA-Na_4 (91.0 $\pm$ 0.5%) was evaluated in a study following OECD Guideline 402.[42] No mortality occurred and body weight gain during the observation period was within the range expected for rats of this strain and age. The macroscopic examination during necropsy showed no significant gross changes. Flat and/or hunched posture, piloerection and/or chromodacryorrhoea were observed in all animals from day 1 up to and including day 4. Slight scales and/or scabs were seen in the treated skin area of four females from day 3 up to and including day 9. Based on the results, the additive is considered to have a low dermal toxicity.

3.2.3.2.2. Skin irritation/corrosion

Irritancy potential of GLDA-Na_4 was assessed following one OECD Guideline 404 study.[43] A very slight erythema in the treated skin of two animals after 1 h was observed. All skin sites appeared normal after 24 h. The observations of the study showed that GLDA-Na_4 was not irritant to rabbit skin.[44]

[36] Technical Dossier/Section III/Annexes Sect. III/Annex III_3.
[37] Technical Dossier/Section III/Annexes Sect. III/Annex III_4.
[38] Technical Dossier/Section III/References Sect. III/Ref 3.3.01.
[39] Technical Dossier/Supplementary Information July 2017/Annex S-1.
[40] Technical Dossier/Supplementary Information July 2017/Annex S-2.
[41] https://www.eurosil.eu/sites/eurosil.eu/files/files/OEL-FULL-TABLE-Oct07-Europe.pdf; http://limitvalue.ifa.dguv.de/WebForm_gw2.aspx
[42] Technical Dossier/Section III/References Sect. III/Ref 3.3.03.
[43] Technical Dossier/Section III/References Sect. III/Ref 3.3.04.
[44] Technical Dossier/Section III/References Sect. III/Ref 3.3.05.

3.2.3.2.3. Eye irritation

The irritancy potential of GLDA-Na_4 was assessed following one OECD Guideline 405 study.[45,44] No corneal or iridial effects were observed. Minimal conjunctival irritation was noted in all treated eyes 1 h after treatment and persisted in one treated eye at the 24-h observation. All treated eyes appeared normal 48 h after treatment. Based on the results of the study, GLDA-Na_4 is not considered as an eye irritant.

3.2.3.2.4. Skin sensitisation

The sensitisation potential of GLDA-Na_4 (74.33%) was assessed in a Magnusson & Kligman maximisation test, following OECD Guideline 406.[46] None of the guinea pigs exposed to GLDA-Na_4 showed positive skin responses. GLDA-Na_4 is not considered as a skin sensitiser.

3.2.3.2.5. Silicic acid

Silica is not regarded as a substance of concern regarding dermal toxicity, skin and eye irritation and skin sensitisation (EFSA, 2004).

3.2.3.3. Conclusions on safety for the user

Due to its low inhalation toxicity, the exposure to GLDA-Na_4 in the additive is unlikely to pose a risk by inhalation. However, the Panel notes that the solid formulation as described has an exceptionally high-dusting potential and a risk from such a high level of dust, even if toxicologically inert, cannot be excluded.

Kelforce® has low dermal toxicity. It is not a skin or eye irritant or a skin sensitiser.

3.2.4. Safety for the environment

The active substance is not a physiological/natural substance of established safety for the environment. Consequently, according to Regulation (EC) No 429/2008, the Phase I assessment is required to determine the predicted environmental concentration (PEC).

Initially, a total residues approach is employed in Phase I and II of the assessment, meaning that the PEC is calculated based on the assumption that all the additives are excreted and that 100% remains as the parent compound.

3.2.4.1. Phase I Assessment

3.2.4.1.1. Physico-chemical properties of the additive

The physico-chemical properties of GLDA-Na_4 are summarised in Table 3.

Table 3: Physico-chemical properties of GLDA-Na_4

Property	Value	Unit
Molecular mass	351.13	Da
Dissociation constant pKa[(a)] (Values determined at 25°C with an ionic strength of 0.1 molar)	2.56/3.49/5.03/9.26[(b)]	–
Water solubility(21°C)[(c)]	At least 65,000	mg/L
Vapour pressure (20°C)[(d)]	80	Pa
K_{oc}: organic carbon–water partition coefficient[(e)]	< 32	L/kg
K_{ow}: octanol/water partition coefficient[(f)]	Log K_{ow} < 0	

(a): Technical Dossier/Section II/Annex II_22.
(b): Considering the four ionisation steps.
(c): Technical Dossier/Section II/Annex II_24.
(d): Technical Dossier/Section II/Annex II_25.
(e): Technical Dossier/Section II/Annex_II_27.
(f): Technical Dossier/Section III/Annex II_26.

[45] Technical Dossier/Section III/References Sect. III/Ref 3.3.06.
[46] Technical Dossier/Section III/References Sect. III/Ref 3.3.07.

3.2.4.1.2. Fate and behaviour

Fate in soil: Adsorption

The applicant provided information on the adsorption potential of the GLDA-Na_4. The provided K_{oc} value < 32 L/kg (value estimated by high-performance liquid chromatography (HPLC), following the OECD Guideline 121) represents the average measured K_{OC} value for GLDA-Na_4 in the ionised form (pH = 2) as well as in the non-ionised form (pH = 7).

Fate in soil/Biodegradation

The applicant did not provide data on degradation of the substance in soil. The applicant provided results on biodegradability from the closed bottle test (OECD Guideline 301D). In all experiments, the biodegradation percentage was between 72% and 83% at day 28, showing that GLDA-Na_4 is readily biodegradable in freshwater under aerobic conditions. A further prolonged closed bottle biodegradation test in seawater showed 7% biodegradation at day 14, 72% at day 28 and 83% at day 60.

Conclusion on fate and behaviour

The average measured K_{OC} value for GLDA-Na_4 is < 32 L/kg; therefore, the value used in the environmental assessment was 32 L/kg. The tested substance was found to be readily biodegradable in aquatic compartment. It is very unlikely that GLDA-Na_4 would persist and accumulate in soil. The value of DT_{50} of 28 days was used in the Phase I PEC calculation, as a surrogate for the DT_{50} in soil. The value of K_{oc} used in the assessment (32 L/kg) indicates that GLDA-Na_4 will be mobile in soil.

3.2.4.1.3. Predicted environmental concentrations (PECs)

The PECs in soil (PEC_{soil}), ground water (PEC_{gw}) and surface water (PEC_{sw}) were calculated according to EFSA FEEDAP Guidance (EFSA, 2008). At the proposed maximum use level of 1,000 mg/kg complete feed, the threshold values for soil and groundwater were exceeded (Table 4);[47] therefore, a Phase II environmental risk assessment is required.

Table 4: Initial PEC in soil, ground water and surface water at the inclusion level of 1,000 mg GLDA-Na_4 per kg feed of chickens for fattening

Inclusion level (mg/kg)	PEC in soil (µg/kg)	PEC in ground water (µg/L)[(a)]	PEC in surface water (µg/L)
1,000	5,194	7,612	2,537

(a): The surrogate for the PEC groundwater in the Phase I is pore water.

3.2.4.2. Phase II Assessment

3.2.4.2.1. Predicted environmental concentrations (PECs)

Refinement based on metabolism

GLDA-Na_4 is almost completely excreted from chickens for fattening as the parent compound; therefore, no refinement of PEC based on the metabolism of GLDA-Na_4 is possible.

Refinement based on degradation in manure

No data were submitted on the possible degradation of GLDA-Na_4 in manure. The applicant, however, provided information on degradation of GLDA-Na_4 in anaerobic conditions using digested sludge following OECD Guideline 311.[48] Results did not show any biodegradation (0% over 60 days), and therefore, GLDA-Na_4 can be regarded as not biodegradable under anaerobic conditions, such as those in manure.

3.2.4.2.2. PECs calculation in Phase II

The applicant did not provide data on soil degradability; thus, more sophisticated environmental exposure models cannot be used to determine PEC_{gw} and PEC_{sw}. Due to a lack of metabolism of GLDA-Na_4 in animals, the PEC values used in the exposure assessment are those indicated in Table 5.

[47] Technical Dossier/Supplementary Information November 2015.
[48] Technical Dossier/Section III/References Sect. III/Ref 3.4.08.

Table 5: Initial PEC in soil, ground water, surface water and sediment at the inclusion level of 1,000 mg GLDA-Na_4 per kg feed of chickens for fattening

Inclusion level (mg/kg)	PEC in soil (µg/kg)	PEC in ground water (µg/L)[(a)]	PEC in surface water (µg/L)	PEC sediment (mg/kg)
1,000	5,194	7,612	2,537	8,120

(a): The surrogate for the PEC groundwater in the Phase I is pore water.

3.2.4.2.3. Effect assessment

Ecotoxicity studies

Terrestrial compartment – Toxicity to microorganisms

In the 28-day soil microorganism test (OECD 216), GLDA-Na_4 (minimum 47%) was incorporated into the soil at geometric mean measured concentrations of 6.40, 10.7, 21.9, 43.1 and 90.3 mg GLDA-Na_4/kg soil dry weight (DW), respectively. A group with untreated soil was included as control. Concentrations of nitrate were measured in soil samples of each treatment at day 0 and following incubation for 7, 14 and 28 days. Nitrate formation rates were calculated from the difference in concentrations measured at each sampling occasion and those originally present on day 0 and compared to that of an untreated control.

No inhibition of the rate of nitrate formation was observed at any time point up to the highest GLDA-Na_4 concentration tested, 90.3 mg/kg soil DW. Indeed, GLDA-Na_4 addition to soil dose dependently increased the rate of nitrate formation (60% stimulation at 90.3 mg/kg soil DW). This concentration is 17-fold higher than the PEC_{soil} resulting from the maximum GLDA-Na_4 inclusion level and it is considered that GLDA-Na_4 in Kelforce® has no negative long-term impact on nitrogen transformation in soil (EFSA, 2008).

An activated sludge respiration inhibition test following OECD Guideline 209 was performed to evaluate the effects of GLDA-Na_4 in the environment and in biological wastewater treatment plants.[49] The toxicity to activated sludge was determined by measuring the respiration rate after a contact period of 30 minutes at various concentrations of GLDA-Na_4 (25.75 mg/L, 51.5 mg/L, 103 mg/L, 206 mg/L and 412 mg/L). Controls were measured at the beginning and at the end of the test. No inhibition of the respiration was measured at a concentration of 412 mg GLDA-Na_4/L, the highest concentration tested. Although activated sludge is not a compartment of relevance for the environmental fate of Kelforce® when used in chickens feed, the study does provide some further evidence of a high tolerance of microorganisms to GLDA-Na_4.

Terrestrial compartment – Terrestrial plants, growth test

A terrestrial plants, growth test (OECD 208) was carried out on GLDA-Na_4 (minimum 47 %). The test item was incorporated into soil at measured concentrations of 1.72, 3.33, 11, 43, 130 and 754 mg/kg DW, and seeds of one monocotyledon (oats, *Poaceae*), and two dicotyledons (rape, *Brassicaceae*; soybean, *Leguminosae*) were sown. Seeds sown in untreated soil served as control. Nitrate concentrations were measured in soil samples of each treatment at days 0, 7, 14 and 28. Nitrate formation rates were calculated from the difference in concentrations measured at each sampling occasion and those originally present on day 0 and compared to that of an untreated control.

No effects were observed in the terrestrial plants up to the highest concentration of GLDA-Na_4 tested, 754 mg/kg soil DW. Thus, the no observed effect concentration (NOEC) is $\geq$ 754 mg/kg soil DW.

Terrestrial compartment – Earthworm reproduction test

The OECD 222 Earthworm reproduction test was conducted using measured GLDA-Na_4 concentrations of 3.74, 9.39, 15.4, 38.0 and 248 mg/kg soil DW, administered to artificial soil containing 10% peat. No effects on adult earthworms were observed after exposure for 28 days. After another 28 days, the mean number of juveniles produced per replicate was 109 in the control and ranged from 92 to 162 in the test item treatments. There was no statistically significant reduction of reproduction compared to the control. Since no effects were observed at the highest concentration of GLDA-Na_4 tested, 248 mg/kg soil DW, this was considered the NOEC value.

[49] Technical Dossier/Section III/References Sect. III/Ref 3.4.09.

Freshwater compartment – Toxicity to aquatic algae

The effect of GLDA-Na_4 on the growth of the green algae *Scenedesmus subspicatus* was evaluated following OECD Guideline 201.[50] *S. subspicatus* was exposed to an aqueous solution of GLDA-Na_4 for 72 h and 24 ± 1°C, under constant illumination and agitation. The results of the initial range-finding study in the standard OECD growth medium showed that GLDA-Na_4 has no effect on growth at the test concentrations of 0.10 and 1.0 mg/L; however, significant inhibition of growth was observed at 10 and 100 mg/L. The applicant considered that the effect on growth of algae was due to chelating of nutrients caused by test substance. The range-finding test on modified micronutrients resulted in the NOEC of 10 mg/GLDA-Na_4, while the use of the modified macronutrient growth medium resulted in the NOEC of 100 mg/L. The range-finding test in a non-modified culture medium resulted in an ErC_{50} > 1 mg/L and a NOEC value of 1 mg GLDA-Na_4/L.

Freshwater compartment – Short-term toxicity to aquatic invertebrates

Acute toxicity of GLDA-Na_4 was tested in a 48-h acute immobilisation study[51] with *Daphnia magna* under static conditions and following OECD Guideline 202. Tested concentrations were 0 (control, 2 replicates) and 100 mg/L (4 replicates). No behavioural abnormalities and no immobility were observed during this limit test and the 48-h EC_{50} is therefore > 100 mg/L.

Freshwater compartment – Long-term toxicity to aquatic invertebrates

Chronic toxicity of GLDA-Na_4 on the reproductive efficacy of *Daphnia magna* was evaluated in a semistatic test following OECD Guideline 211.[52] *D. magna* was exposed to different GLDA-Na_4 concentrations (control, 0.5, 13.5, 36.5, 98.4 and 265.7 mg/L, 10 replicates each) for 21 days. Toxicity criterion was the reproductive capacity expressed as the number of neonates per daphnid per day.

Immobilisation of parent daphnids was tested during at least 6 days/week by gently shaking the test vessel. Living and dead neonates per brood or abortions were monitored and any abnormal observation recorded. No effects were observed on reproduction and parental length, and the NOEC is therefore ≥ 265.7 mg GLDA-Na_4/L, the highest concentration tested. There was likewise no effect at any concentration on immobilisation and the EC_{50} for parent survival was hence > 265.7 mg GLDA-Na_4/L.

Freshwater compartment – Short-term toxicity in fish

Acute toxicity of GLDA-Na_4 in rainbow trout (*Oncorhynchus mykiss*) was evaluated following a protocol in line with OECD Guideline 203.[53] Rainbow trout (three groups of 10 fish, 20 L tanks) were exposed to GLDA-Na_4 at a limit concentration of 100 mg/L (one control group and two replicates) for a period of 96 h. Mortalities were recorded at 3, 6, 24, 48, 72 and 96 h. No mortality was found in any of the replicates at any time point. A 96-h LC_{50} could not be established but was higher than the limit concentration of 100 mg GLDA-Na_4/L.

Short-term effects of GLDA-Na_4 to the embryo and sac fry stages of zebrafish (*Danio rerio*) were evaluated following OECD Guideline 212.[54] Eggs of zebrafish were exposed at different nominal concentrations of GLDA-Na_4 (control, 21.3, 47.0, 103, 227 and 500 mg/L) in a flow-through exposure design for 9 days. The highest concentration of GLDA-Na_4 tested (500 mg/L) increased the pH of the water to 9.93 (mean) compared with 7.65 (mean) in the control. Therefore, a second 500 mg/L exposure group was included in which the pH was adjusted to 7.67 (average). Measured concentrations of GLDA-Na_4 were 96–119% of the nominal in all groups throughout exposure. The following endpoints were evaluated: egg hatch, time to hatch, fry growth (expressed as length and weight), morphological and behavioural effects, post hatch survival, overall fry survival and mortality, respectively.

In the 500 mg/L group at pH 9.93, mortality was 100 %. However, mortality was likely aggravated by the high pH because in the pH-adjusted group at the same concentration, mortality was lower (30 %) and not statistically significantly elevated (one-way ANOVA with Dunnett's test, $p > 0.05$, $n = 3$). In the second highest concentration (227 mg/L), average mortality was 27% and this was also not statistically different from the overall average. Thus, the cumulative overall mortality was not increased in any of the groups where pH was maintained below 9 and the LC_{50} was > 500 mg/L. Hatching was the most sensitive endpoint with a NOEC of 103 mg/L.

[50] Technical Dossier/Section III/References Sect. III/Ref 3.4.10.
[51] Technical Dossier/Section III/References Sect. III/Ref 3.4.11.
[52] Technical Dossier/Section III/References Sect. III/Ref 3.4.12.
[53] Technical Dossier/Section III/References Sect. III/Ref 3.4.13.
[54] Technical Dossier/Section III/References Sect. III/Ref 3.4.14.

Conclusion on effect assessment for the soil, aquatic and sediment compartments

No inhibition of the rate of nitrate formation was observed at any time point up to the highest GLDA-Na_4 concentration tested, 90.3 mg/kg soil DW, leading to the conclusion that tested substance has no negative impact on nitrate formation in soil. No effects were observed in the terrestrial plants growth test up to the highest concentration of GLDA-Na_4 tested, 754 mg/kg soil DW. Thus, the NOEC is ≥ 754 mg/kg soil DW. In the earthworm reproduction test, no effects were observed at the highest tested concentration of 248 mg GLDA-Na_4 per kg of soil DW. This value was considered the NOEC for earthworm reproduction.

The NOEC for green algae in the modified macronutrient test estimated to be 100 mg GLDA-Na_4/L was selected for the assessment of risk in the aquatic compartment. However, the NOEC of 1 mg GLDA-Na_4/L from the range-finding test in a non-modified culture medium is also considered in the environmental risk assessment of GLDA-Na_4, since the reduction of nutrients may be considered a detrimental effect to the environment, in the absence of further information which may address the problem in a more realistic way. It was also noted that the temperature during the test (24 ± 1°C) was at the limit of acceptable temperature regimes for this test. The 48-h test of acute toxicity of GLDA-Na_4 led to the conclusion that EC_{50} for *D. magna* is > 100 mg/L. The reproductive effect of GLDA-Na_4 on *D. magna* was estimated as the highest tested concentrations resulting in no effect – NOEC of ≥ 265.7 mg GLDA-Na_4/L. Acute toxicity on fish was not observed at concentrations ≥ 100 mg GLDA-Na_4/L.

The applicant did not provide any data for the assessment of the risk for the sediment compartment.

3.2.4.2.4. Risk characterisation (PEC/PNEC ratio)

The risk characterisation ratios for terrestrial, freshwater and sediment compartments are reported in Tables 6, 7 and 8, respectively.

Table 6: Risk characterisation (PEC/PNEC ratio) for terrestrial compartment

Taxa	PEC_{soil} (µg/kg)	NOEC (mg/kg)	AF	PNEC (µg/kg)	PEC/PNEC
Earthworm	5,194	248	10	24,800	0.2
Plants		754	10	75,400	0.6

AF: assessment factor; PNEC: predicted no effect concentration.

Since the ratio PEC/PNEC is lower than 1, no risk is expected for terrestrial compartment.

Table 7: Risk characterisation (PEC/PNEC ratio) for freshwater compartment

Taxa	PEC_{sw} (µg/L)	$E(L,r)C_{50}$/NOEC (mg/L)	AF	PNEC (µg/L)	PEC/PNEC
Algae	2,537	> 100	50	2,000[a] 20[b]	1.27[a] 126.8[b]
Selenastrum subspicatus		100[a] 1[b]			
Aquatic invertebrates					
Daphnia magna acute		> 100			
Daphnia magna reproduction		265.7[c]			
Fish		103			
Brachydanio rerio					

AF: assessment factor; PNEC: predicted no effect concentration.
(a): NOEC for algae in the macronutrient-modified growth medium.
(b): NOEC for algae in the OECD standard non-modified culture medium.
(c): NOEC for daphnids.

Table 8: Risk characterisation (PEC/PNEC ratio) for sediment

Taxa	$PEC_{sediment}$ (µg/kg)	NOEC (mg/kg)	AF	PNEC (µg/kg)	PEC/PNEC
Sediment-dwelling invertebrates *Chironomus riparius*	8,120	/	/	/	/

AF: assessment factor; PNEC: predicted no effect concentration.

Risks for the aquatic compartment cannot be excluded, mainly due to the secondary effect of the additive on green algae (reduction of nutrients).

Risk of bioaccumulation and secondary poisoning

GLDA-Na_4 is a non-lipophilic compound. The Log of partitioning coefficient between octanol/water (Log K_{OW}) is below 0. Thus, the risk of bioaccumulation and secondary poisoning caused by this compound is considered very low.

Silicic acid

Silicic acid is ubiquitous in the environment, being a natural component of soil, sediments and living organisms. As such, its safety for the environment needs no further consideration.

3.2.4.3. Conclusions on safety for the environment

No risks for the terrestrial compartment were identified at the maximum use level of 1000 mg GLDA-Na_4/kg complete feed.

Risks for the aquatic compartment cannot be excluded based on the secondary effect of the additive on green algae (reduction of nutrients).

In the absence of data, the FEEDAP Panel cannot conclude on the safety for the sediment compartment nor on the possible contamination of the ground water.

The risk of bioaccumulation and secondary poisoning caused by the additive is considered very low.

3.3. Efficacy for chickens for fattening

In its scientific opinion on the potential reduction of the currently authorised maximum zinc content in complete feed, the FEEDAP Panel proposed a reduction of the total zinc content in feed for chickens for fattening to 100 mg Zn/kg feed (EFSA FEEDAP Panel, 2014). The Commission reacted to this Opinion by reducing the maximum total zinc contents in feed for chickens for fattening from 150 mg/kg diet (Regulation (EC) No 1334/2003)[55] to 120 mg/kg diet (Commission Implementing Regulation (EU) 2016/1095).[56]

The applicant proposes that Kelforce® (47% of GLDA-Na_4) enhances the nutritional availability of the zinc of the diets for chickens for fattening, by improving solubilisation or preventing precipitation in the gastrointestinal tract. Improving the absorption of zinc from the feed would allow reducing the supplementation of zinc and consequently decrease zinc emission through manure and would if efficacious, benefit the environment. To assess the increase of zinc bioavailability, bone zinc is the preferred marker. In addition, balance studies are suggested in order to assess total retention of zinc and the reduction of zinc excretion (EFSA FEEDAP Panel, 2011). According to the National Research Council (NRC), diets with 40 mg Zn/kg complete feed are recommended to meet zinc requirements of chicken for fattening (NRC, 1994; see also EFSA FEEDAP Panel, 2014).

3.3.1. *In vitro* studies

Six *in vitro* studies were submitted [redacted]

[55] 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.

[56] Commission Implementing Regulation (EU) 2016/1095 of 6 July 2016 concerning the authorisation of Zinc acetate dihydrate, Zinc chloride anhydrous, Zinc oxide, Zinc sulphate heptahydrate, Zinc sulphate monohydrate, Zinc chelate of amino acids hydrate, Zinc chelate of protein hydrolysates, Zinc chelate of glycine hydrate (solid) and Zinc chelate of glycine hydrate (liquid) as feed additives for all animal species and amending Regulations (EC) No 1334/2003, (EC) No 479/2006, (EU) No 335/2010 and Implementing Regulations (EU) No 991/2012 and (EU) No 636/2013. OJ L 182, 7.7.2016, p. 7.

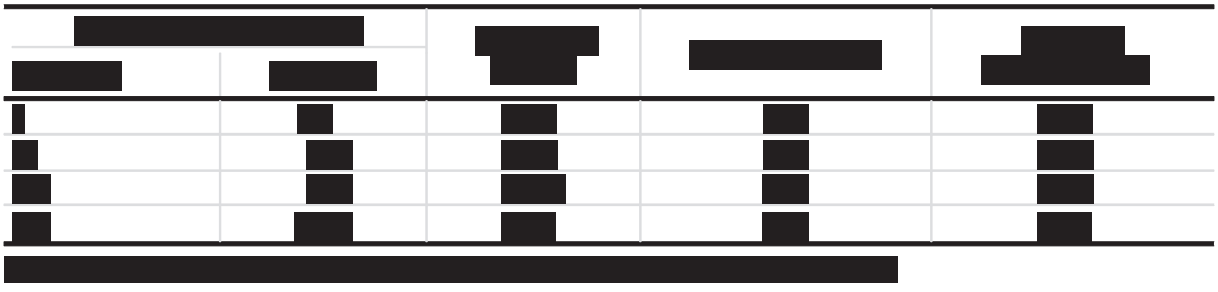
[redacted]

3.3.2. *In vivo* short-term studies

A total of five efficacy short-term studies were provided by the applicant to demonstrate that the additive may improve the nutritional availability of zinc in chickens for fattening. [redacted]

3.3.2.1. Study 1

[redacted]



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3.3.2.2. Study 2

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3.3.2.4. Study 4

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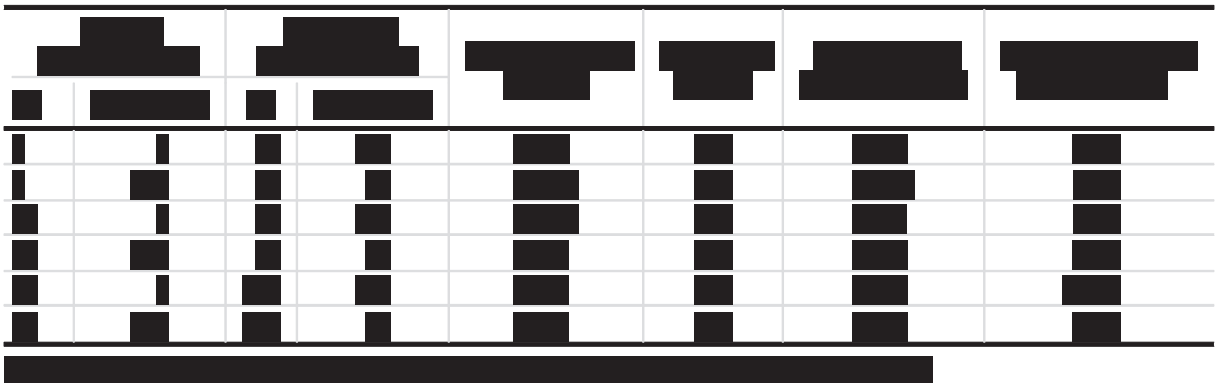

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3.3.2.5. Study 5

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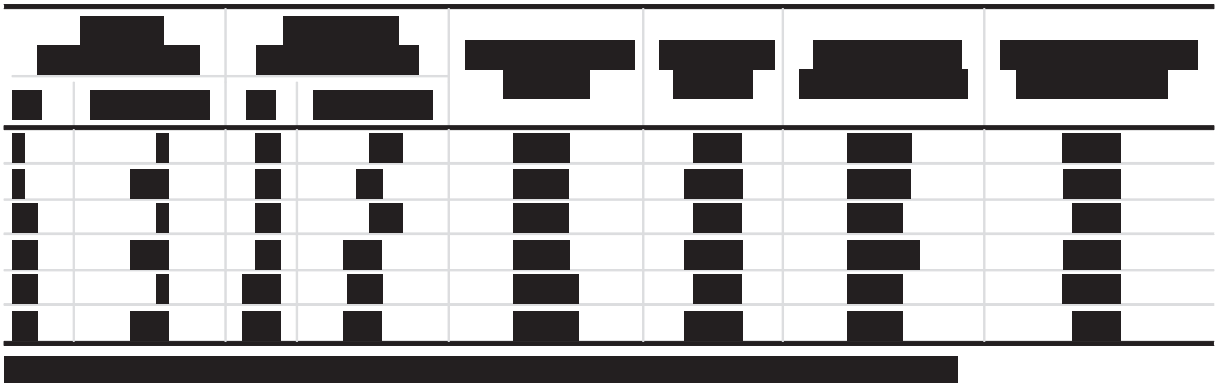

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3.3.2.6. Conclusions on efficacy

[redacted]

Therefore, the FEEDAP Panel cannot conclude on the efficacy of the additive either as an *Other zootechnical additive* or as a *Zootechnical additive favourably affecting the environment*.

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[64] and Good Manufacturing Practice.

4. Conclusions

The additive is safe for chickens for fattening when used at the maximum proposed level of 1,000 mg GLDA-Na_4/kg complete feed; this is equivalent to 2,110 mg liquid Kelforce®/kg feed or 3,333 mg solid Kelforce®/kg feed.

The FEEDAP Panel concludes that the use of Kelforce® as feed additive at the maximum proposed level of 1,000 mg GLDA-Na_4/kg complete feed of chickens for fattening is of no concern for consumer safety.

Due to its low inhalation toxicity, the exposure to GLDA-Na_4 in the additive is unlikely to pose a risk by inhalation. However, the Panel notes that the solid formulation as described has an exceptionally high-dusting potential and a risk from such a high level of dust, even if toxicologically inert, cannot be excluded. Kelforce® is not a skin and eye irritant or a skin sensitiser.

No risks for the terrestrial compartment were identified at the maximum use level of 1,000 mg GLDA-Na_4/kg complete feed. Risks for the aquatic compartment cannot be excluded based on the secondary effect of the additive on green algae (reduction of nutrients). In the absence of data, the FEEDAP Panel cannot conclude on the safety for the sediment compartment or the possible contamination of the ground water. The risk of bioaccumulation and secondary poisoning caused by the additive is considered very low.

Owing to the inconsistencies and conflicting results from the studies assessed, the FEEDAP Panel cannot conclude on the efficacy of the additive.

5. Recommendations

The specifications for the content of formaldehyde and cyanide in the active substance (aqueous solution of mg GLDA-Na_4) should be adjusted to reflect the analytical values; the FEEDAP Panel recommends to set both levels at $\leq$ 10 mg/L.

Documentation provided to EFSA

1) Kelforce®. L-glutamic acid, *N*,*N*-diacetic acid, tetrasodium salt (GLDA-Na_4) in the form of an aqueous solution of GLDA-Na_4 and GLDA-Na_4 [redacted]. May 2013. Submitted by Selko B.V.

[64] 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.

2) Kelforce®. L-glutamic acid, *N*,*N*-diacetic acid, tetrasodium salt (GLDA-Na_4) in the form of an aqueous solution of GLDA-Na_4 and GLDA-Na_4 [redacted]. Supplementary information June 2014. Submitted by Selko B.V.
3) Kelforce®. L-glutamic acid, *N*,*N*-diacetic acid, tetrasodium salt (GLDA-Na_4) in the form of an aqueous solution of GLDA-Na_4 and GLDA-Na_4 [redacted]. Supplementary information November 2015. Submitted by Selko B.V.
4) Kelforce®. L-glutamic acid, *N*,*N*-diacetic acid, tetrasodium salt (GLDA-Na_4) in the form of an aqueous solution of GLDA-Na_4 and GLDA-Na_4 [redacted]. Supplementary information July 2017. Submitted by Selko B.V.
5) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Kelforce®.
6) Comments from Member States.

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EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012c. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. https://doi.org/10.2903/j.efsa.2012.2539

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Nesslany F, Simar-Meintières S, Watzinger M, Talahari I and Marzin D, 2008. Characterisation of the genotoxicity of nitriloacetic acid. Environmental and Molecular Mutagenesis, 49, 439–452.

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Abbreviations

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
AF	assessment Factor
ANOVA	analysis of variance
bw	body weight
CAS	Chemical Abstracts Service
CHL	Chinese hamster lung
DW	dry weight
DT_{50}	half-life
EINECS	European Inventory of Existing Commercial Substances
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
GLDA	L-glutamic acid, *N*,*N*-diacetic acid
GLDA-Na_4	L-glutamic acid, *N*,*N*-diacetic acid, tetrasodium salt
HPLC	high-performance liquid chromatography

HPRT	hypoxanthine-guanine phosphoribosyltransferase
IARC	International Agency for Research on Cancer
IUPAC	International Union of Pure and Applied Chemistry
K_{oc}	organic carbon–water partition coefficient
K_{ow}	octanol/water partition coefficient
LC–MS	liquid chromatography–mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
MMC	mitomycin-C
NCE	normochromatic erythrocytes
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NRC	National Research Council
NTA	nitrilotriacetic acid
NTA-Na_3	nitrilotriacetic acid trisodium salt
NZW	New Zealand white
OECD	Organisation for Economic Cooperation and Development
PCBs	polychlorinated biphenyls
PCDDs	polychlorinated dibenzo-para-dioxins
PCE	polychromatic erythrocytes
PEC	predicted environmental concentration
PNEC	predicted no effect concentration
TEQ	toxic equivalent factor
TP	total protein
WHO	World Health Organization

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for feed additives on the method(s) of analysis for Kelforce®

In the current application authorisation is sought under article 4(1) for *Kelforce (GLDA-Na4)*, for the category/functional 4(c)(d) "zootechnical additives"/"substances which favourably affect the environment"/"other zootechnical additives" 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 *chicken for fattening*. *Kelforce* active substance is *L-glutamic acid N,N-diacetic acid tetrasodium salt (GLDA-Na4)*. The product is intended to be marketed in a liquid form, containing at least 47.4% of *GLDA-Na4* and less than 50% water (*GLDA-Na4 L)* and in a solid form containing minimum 30% of *GLDA-Na4* and maximum 36% silicic acid and 35% water (*GLDA-Na4 P*).

The *feed additive* is intended to be included into *premixtures* and/or *feedingstuffs* with a maximum *feed additive* content of 2110 or 3333 mg/kg *feedingstuffs* for the liquid or solid preparation, respectively.

For the determination of *GLDA-Na4* in the *feed additive* and *premixtures*, the Applicant submitted an in-house validated and further verified method based on Reversed Phase High Performance Liquid Chromatography coupled to spectrophotometric detection (RP-HPLC-UV), while for the determination of *GLDA-Na4* in the *feedingstuffs* the Applicant submitted an in-house validated and further verified method based on Reversed Phase High Performance Liquid Chromatography coupled to mass spectrometry detection (RP-HPLC-MS). The experimental evidence provided, allows the EURL to recommend for official control the RP-HPLC-UV method for the determination of *GLDA-Na4* in the *feed additive* and *premixtures* and the RP-HPLC-MS method for the determination of *GLDA-Na4* in *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.