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## Safety and efficacy of a feed additive consisting of L-isoleucine produced by *Corynebacterium glutamicum* KCCM 80185 for all animal species (CJ Europe GmbH)

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### Abstract

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 a feed additive consisting of L-isoleucine produced by *Corynebacterium glutamicum* KCCM 80185 when used as a nutritional additive in feed and water for drinking for all animal species. The production strain is genetically modified, does not carry acquired antimicrobial resistance genes and no viable cells of the production strain were detected in the final product. The FEEDAP Panel could not exclude the presence of recombinant DNA from the production strain in the product. However, since no sequences of concern remain in the final production strain, the potential presence of recombinant DNA in the final product does not raise any safety concerns. The Panel concluded that the additive is safe for the target species, for the consumer and for the environment under the proposed conditions of use. Regarding the use in water, the FEEDAP Panel reiterated its concerns over the safety of L-isoleucine administered simultaneously via water for drinking and feed owing to the risk of nutritional imbalances and hygienic reasons. The FEEDAP Panel concluded that L-isoleucine produced by *C. glutamicum* KCCM 80185 is considered not toxic by inhalation, not irritant to skin or eyes and not a dermal sensitiser. However, due to the high dusting potential, exposure to dust *per se* might be a hazard for the user. L-Isoleucine produced by *C. glutamicum* KCCM 80185 is considered as an efficacious source of the essential amino acid L-isoleucine for non-ruminant animal species. For the supplemental L-isoleucine to be as efficacious in ruminants as in non-ruminant species, it would require protection against degradation in the rumen.

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## 1. Introduction

### 1.1. Background and Terms of Reference

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

The European Commission received a request from CJ Europe GmbH<sup>2</sup> for the authorisation of the additive consisting of L-isoleucine produced by fermentation with *Corynebacterium glutamicum* KCCM 80185, when used as a feed additive for all animal species (category: nutritional additives; functional group: amino acids, their salts and analogues).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). The particulars and documents in support of the application were considered valid by EFSA as of 2 April 2020.

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 feed additive consisting of L-isoleucine produced by fermentation with *C. glutamicum* KCCM 80185, when used under the proposed conditions of use (see Section 3.1.6).

### 1.2. Additional information

The subject of the assessment is the feed additive consisting of L-isoleucine produced by *C. glutamicum* KCCM 80185, intended for use as a nutritional additive (functional group: amino acids, their salts and analogues) for all animal species. This additive is not authorised in the European Union.

## 2. Data and methodologies

### 2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier<sup>3</sup> in support of the authorisation request for the use of L-isoleucine as a feed additive.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, 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 L-isoleucine in animal feed. The Executive Summary of the EURL report can be found in Annex A.<sup>4</sup>

### 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of L-isoleucine produced by *C. glutamicum* KCCM 80185 is in line with the principles laid down in Regulation (EC) No 429/2008<sup>5</sup> and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms

<sup>1</sup> Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

<sup>2</sup> CJ Europe GmbH, Ober der Roeth 4, 65824, Schwalbach/Taunus, Germany.

<sup>3</sup> FEED dossier reference: FAD-2019-0079.

<sup>4</sup> The full report is available on the EURL website: [https://ec.europa.eu/jrc/sites/default/files/finrep\\_fad-2019-0079-isoleucine.pdf](https://ec.europa.eu/jrc/sites/default/files/finrep_fad-2019-0079-isoleucine.pdf).

<sup>5</sup> Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

(EFSA FEEDAP Panel, 2018b) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

### 3. Assessment

The additive under assessment, L-isoleucine produced by fermentation using *C. glutamicum* KCCM 80185, is intended to be used as a nutritional additive (functional group: amino acids, their salts and analogues) in feed and water for drinking for all animal species.

#### 3.1. Characterisation

##### 3.1.1. Characterisation of the production organism

The additive L-isoleucine is produced by a genetically modified strain of *C. glutamicum* which is deposited at the Korean Culture Centre of Microorganisms (KCCM) with the accession number KCCM 80185.<sup>6</sup>

The taxonomic identification of the production strain, KCCM 80185, was confirmed [REDACTED]

The susceptibility of the production strain to relevant antibiotics was tested against the list of antimicrobials described for '*Corynebacterium* and other Gram-positive' in the Guidance on characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b).<sup>8</sup> All measured minimum inhibitory concentration (MIC) values were lower than the cut-off values specified in this guidance and, therefore, the strain is considered susceptible to the relevant antibiotics.

The WGS data of the production strain were searched for the presence of antimicrobial resistance (AMR) genes in [REDACTED]

[REDACTED].<sup>7</sup> No hits of concern were identified.

The WGS of the production strain was also interrogated for the presence of genes coding for toxins and virulence factors by [REDACTED]

[REDACTED].<sup>9</sup> No hits of concern were identified.

##### 3.1.1.1. Information related to the genetically modified microorganism

*Characterisation of the recipient or parental microorganism*

*Characterisation of the donor organisms*

*Description of the genetic modification*

<sup>6</sup> Technical dossier/Section II/Confidential Annex/Annex\_CONFID\_II\_2\_01.

<sup>7</sup> Technical dossier/Supplementary information June 2021/ANNEX\_SIN\_CONFID/Annex\_SIN\_CONF\_01.

<sup>8</sup> Technical dossier/Section II/Confidential Annex/Annex\_CONFID\_II\_2\_05.

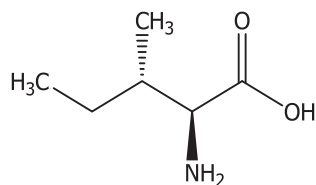
<sup>9</sup> Technical dossier/Section II/Confidential Annex/Annex\_CONFID\_II\_2\_04.

### 3.1.2. Manufacturing process

L-Isoleucine is produced by fermentation with *C. glutamicum* KCCM 80185.<sup>10</sup>

### 3.1.3. Characterisation of the additive

L-Isoleucine (International Union of Pure and Applied Chemistry (IUPAC) name: (2*S*,3*S*)-2-amino-3-methylpentanoic acid, a compound identified with the Chemical Abstracts Service (CAS) No 73-32-5, the European Inventory of Existing Commercial Chemical Substances (EINECS) No 200-798-2) is the active substance of the additive and has a molecular mass of 131.18 g/mol. The chemical formula of L-isoleucine is C<sub>6</sub>H<sub>13</sub>NO<sub>2</sub> and the structural formula is given in Figure 1.



**Figure 1:** Structural formula of L-isoleucine

The applicant declared that the product contains by specification  $\geq 90\%$  L-isoleucine (on a dry matter (DM) basis),  $\leq 2\%$  moisture and  $\leq 1\%$  ash. Batch-to-batch variation data were provided for five batches of the additive.<sup>13</sup> The content of the active substance for the additive was on average 91.9% (range: 91.8–91.9%) on a DM basis.<sup>14</sup> The moisture content was in the range of 0.21–0.27%.

The applicant analysed the same five batches for other components (on 'as is' basis) including free amino acids other than isoleucine (most of them were not detected, except 5.84%  $\alpha$ -amino-butyrac acid (range 5.81–5.86%), 0.13% alanine (range 0.12–0.13%), 0.44% valine (range 0.43–0.46%) and 1.02% phenylalanine (range 1.02–1.03%));<sup>15</sup> nitrogen containing components (ammonium

<sup>10</sup> Technical dossier/Section II/Confidential Annex/Annex\_CONFID\_II\_3\_01 and Supplementary information June 2021/ANNEX\_SIN\_CONFID/Annex\_SIN\_CONF\_04.

<sup>11</sup> Technical dossier/Section II/Confidential Annex/Annex\_CONFID\_II\_3\_12.

<sup>12</sup> Technical dossier/Section II/Annex/Annex\_II\_1\_01.

<sup>13</sup> Technical dossier/Section II/Annex/Annex\_II\_1\_03 and Spontaneous information August 2021/Annex\_SIN\_01.

<sup>14</sup> Isoleucine was analysed by HPLC amino acid analyser using fluorescence detector and phosphate hydroxide as mobile phase.

<sup>15</sup> Technical dossier/Supplementary information June 2021/ANNEX\_SIN/Annex\_SIN\_01. Limit of detection (LOD) in mg/kg were: 2 for phosphoserine; 1.8 for taurine; 1.6 for phosphoethanolamine; 0.6 for urea; 2.6 for aspartic acid; 1.8 for threonine; 1.6 for serine; 1.8 for glutamic acid; 2 for sarcosine; 1.6 for  $\alpha$ -amino adipic acid; 0.8 for glycine; 5 for tryptophan; 2 for alanine; 4 for citrulline; 1.8 for  $\alpha$ -amino-n-butyrac acid; 1.4 for cystine; 4 for cystathionine; 3.2 for lysine; 4 for leucine; 4.2 for tyrosine; 2.6 for phenylalanine; 3 for  $\beta$ -alanine; 2.2 for  $\beta$ -aminoisobutyric acid; 2.6 for  $\gamma$ -amino-n-butyrac acid; 4 for ethanol amine; 2.4 for valine; 4.2 for hydroxylysine; 2.2 for histidine; 2.6 for ornithine; 3.2 for 1-methylhistidine; 3.8 for anserine; 2.8 for carnosine; 4 for hydroxyproline; 3.6 for methionine; 2 for homoserine; 2.4 for arginine; 4.2 for proline.

0.21% (range 0.19–0.22%), nitrates, nitrites and betaine not detected);<sup>16</sup> organic acids (formic, acetic, citric, malic, succinic, lactic, not detected);<sup>17</sup> inorganic components (sodium 0.03% (range 0.03–0.04%), sulfate 0.13% (range 0.12–0.14%), potassium, magnesium, calcium, fluoride, bromide, chloride, phosphate not detected)<sup>18</sup>. Ash content was on average 0.08% (range 0.07–0.09%). The sum of identified material on DM basis was above 99%.

The specific optical rotation was measured in five batches of the final product and the average was +39.2° (range +39.0° to +39.4°),<sup>19</sup> which is according to the specifications set by the applicant (+38.5° to +41.5°). A deviation is noted with respect to the European Pharmacopoeia (PhEur, 2019) range of the specific optical rotation for this substance (+40° to +43°).

Three batches of the product were analysed for chemical impurities.<sup>20</sup> Heavy metals (lead, cadmium and mercury), arsenic and nickel were below their corresponding limit of quantification (LOQ), zinc was below its LOQ except one batch (0.65 mg/kg) and chromium ranged 0.21–0.39 mg/kg. Aflatoxins (B1, B2, G1 and G2), ochratoxin A, zearalenone and deoxynivalenol were also below their LOQs.

Polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar dioxin-like polychlorinated biphenyls (co-planar PCBs) were analysed in three batches and found below the corresponding LOQ.<sup>21</sup> The calculated (upper bound) levels of dioxins and the sum of dioxins and dioxin-like-PCBs were 0.07 ng WHO-PCDD/F-TEQ/kg DM and 0.14 ng WHO-PCDD/F-PCB-TEQ/kg DM, respectively (in all three batches). The calculated (upper bound) levels for non-dioxin-like PCBs (ICES-6) were 0.6 µg/kg (in all three batches).

The same three batches of the product were analysed for microbiological contamination: *Salmonella* spp. (not detected in 25 g), yeasts and moulds (< 100 colony forming units (CFU)/g), Enterobacteriaceae and *Escherichia coli* (< 10 CFU/g).<sup>22</sup>

The detected amounts of the above-described undesirable substances do not raise safety concerns.

The presence of viable cells of the production strain was investigated in three batches of the final product, each batch tested in triplicate.<sup>23</sup>

The presence of recombinant DNA of the production strain in the final product was analysed in three batches of L-isoleucine tested in triplicate.<sup>24</sup>

No DNA from the production strain was detected in the samples. However, the lysis buffers used to extract the DNA contain [REDACTED] which may not ensure the recovery of the DNA from non-viable cells of the production strain that could potentially remain in the product. Therefore, the FEEDAP Panel cannot exclude the presence of recombinant DNA from the production strain in the product.

### 3.1.4. Physical properties of the additive

The additive is a white to yellowish crystalline powder.<sup>12</sup> Its solubility in water is 3.0–3.5 g/100g water. The bulk density of the additive is 0.60–0.85 g/mL and its melting point of 270–290°C.

<sup>16</sup> Technical dossier/Supplementary information June 2021/ANNEX\_SIN/Annex\_SIN\_01. LOD in mg/kg were: 2 for ammonium; 4 for nitrates; 3 for nitrites; 3696.8 for betaine.

<sup>17</sup> Technical dossier/Supplementary information June 2021/ANNEX\_SIN/Annex\_SIN\_01. LOD in mg/kg were: 22 for formic acid; 40 for acetic acid; 16 for citric acid; 24 for malic acid; 30 for succinic acid; 6 for lactic acid.

<sup>18</sup> Technical dossier/Supplementary information June 2021/ANNEX\_SIN/Annex\_SIN\_01. LOD in mg/kg were: 2 for sodium; 2.5 for potassium; 5 for magnesium; 4 for calcium; 0.45 for fluoride; 0.5 for bromide; 0.2 for chloride; 14 for phosphate; 1.5 for sulfate.

<sup>19</sup> Technical dossier/Section II/Annex/Annex\_II\_1\_02.

<sup>20</sup> Technical dossier/Supplementary information June 2021/Annex\_SIN\_02. LOQ (in mg/kg) were 0.02 for lead, 0.01 for cadmium and mercury, 0.04 for arsenic, 0.20 for nickel and chromium, 0.50 for zinc. LOQ (in µg/kg) were 0.2 for aflatoxins (B1, B2, G1 and G2), 0.5 for ochratoxin A, 50 for deoxynivalenol, 10 for zearalenone.

<sup>21</sup> Technical dossier/Supplementary information June 2021/Annex\_SIN\_02. LOQs (in ng/kg) ranged between 0.01 to 1,000 for dioxins/furans and between 3 to 10 for mono-ortho PCBs. LOQ (in µg/kg) was 0.1 for non-dioxin like PCBs (ICES-6).

<sup>22</sup> Technical dossier/Section II/Annex/Annex\_II\_1\_04. The values reported represents LODs.

<sup>23</sup> Technical dossier/Section II/Confidential Annex/Annex\_CONFID\_II\_2\_07.

<sup>24</sup> Technical dossier/Supplementary information June 2021/ANNEX\_SIN\_CONFID/Annex\_SIN\_CONF\_03.

In three batches the dusting potential measured according to Stauber–Heubach ranged between 5.7 and 6.2 g/m<sup>3</sup>.<sup>25</sup>

Three batches of the additive were analysed for particle size distribution by sieving method.<sup>26</sup> The fraction of particles having a diameter < 105 µm ranged 63–70% and < 62 µm ranged 10–17%.

### 3.1.5. Stability and homogeneity

No information on the shelf-life, stability (in premixtures, feedingstuffs and water for drinking) and capacity of the additive under assessment to distribute homogeneously in feed was provided. The applicant provided information on the shelf life and stability (in a vitamin/mineral premixture, in a compound feed for chickens [mash and pelleted forms] and in water for drinking) with an L-isoleucine originating from a different strain (*C. glutamicum* KCCM 80189) of the same producer. Such studies were described in a previous opinion (EFSA FEEDAP Panel, 2020). As the production process is the same and the product characteristics are very similar, the FEEDAP Panel considers that the results of those studies can be applicable to the product under assessment.

### 3.1.6. Conditions of use

L-Isoleucine is intended to be used directly in feedingstuffs/complementary feedingstuffs or via premixture or water for drinking in all animal species. No proposed inclusion levels are provided, as the optimal daily allowance in quantitative terms depends on the species, the physiological state of the animal, the performance level and the environmental conditions, in particular on the amino acid composition of the unsupplemented diet.

## 3.2. Safety

### 3.2.1. Safety of the production organism

The production organism *C. glutamicum* KCCM 80185 was developed to increase the production of L-isoleucine. The production strain belongs to a species, *C. glutamicum*, that qualifies for the qualified presumption of safety (QPS) approach to safety assessment (EFSA, 2007) when used for production purposes (EFSA BIOHAZ Panel, 2020). The production strain was unambiguously identified as *C. glutamicum*. The genes inserted during the genetic modification do not raise safety concerns and the production strain does not carry acquired antimicrobial resistance genes. The production strain was not detected in the additive. Based on the data available, the FEEDAP Panel cannot exclude the presence of recombinant DNA from the production strain in the product. However, since no sequences of concern remain in the final production strain, the potential presence of recombinant DNA in the final product does not raise any safety concerns.

### 3.2.2. Safety for the target species, consumer and environment

Safety concerns from the additive may derive either from the amino acid or from the residues of the fermentation process/production strain remaining in the final product. L-Isoleucine under assessment is highly purified (less than 1% unidentified material). The production strain KCCM 80185 belongs to a species, *Corynebacterium glutamicum*, that qualifies for the QPS approach to safety assessment (EFSA, 2007) when used for production purposes (EFSA BIOHAZ Panel, 2020). Any potential presence of recombinant DNA in the final additive would not pose a safety concern. It can be concluded that no safety concerns for target animals, consumers and the environment would rise from the fermentation material present in the final additive.

L-Isoleucine is one of the three branched chain amino acid (BCAA) together with leucine and valine and one of the nine essential amino acids. The interaction of BCAAs fed at excessive levels has already been described in former FEEDAP opinions (EFSA, 2008; EFSA FEEDAP Panel, 2013, 2020).

The use of amino acids in water for drinking, in addition to complete diets with a well-balanced amino acid profile may represent a risk for the target species due to nutritional imbalances and hygienic reasons (EFSA FEEDAP Panel, 2010). Moreover, it may result in an increased nitrogen excretion via urine. Therefore, the FEEDAP Panel has concerns on the safety of the simultaneous oral administration of isoleucine-containing additives via feed and water for drinking.

<sup>25</sup> Technical dossier/Section II/Annex/Annex\_II\_1\_06.

<sup>26</sup> Technical dossier/Supplementary information June 2021/ANNEX\_SIN/Annex\_SIN\_03.



Regarding the safety of the amino acid for consumer, L-isoleucine supplemented to feed will be incorporated into proteins of tissues and/or products of animal origin but any of their potential excess will be metabolised and excreted as urea/uric acid and as carbon dioxide. Therefore, the composition of tissues and products of animal origin will not be modified by the use of L-isoleucine in animal nutrition.

The amino acid L-isoleucine is a physiological and natural component of the proteins of living organisms. When consumed, it will be absorbed, and the non-absorbed fraction will be incorporated into the intestinal microbial mass and excreted as such. Its use in animal nutrition would not lead to any localised increase of its concentration in the environment. Viable cells of the production strain *C. glutamicum* KCCM 80185 were not detected in the final product however, the FEEDAP Panel could not exclude the presence of recombinant DNA from the production strain in the product. Since no sequences of concern have been introduced in the final production strain, a risk for the environment resulting from the use of the additive under assessment in animal nutrition is not foreseen.

The FEEDAP Panel concludes that the use of L-isoleucine produced by *C. glutamicum* KCCM 80185 to cover nutritional needs of animals is safe for the target species, for the consumer and for the environment.

### 3.2.3. Safety for the user

The applicant did not submit studies performed with the additive under assessment to assess the safety for the user. Instead submitted an acute inhalation test performed according to OECD guideline 403,<sup>27</sup> a skin irritation test performed according to OECD guideline 439,<sup>28</sup> an eye irritation test performed according to OECD guideline 437,<sup>29</sup> and a skin sensitisation test following OECD guideline 429<sup>30</sup> performed with L-isoleucine produced by *C. glutamicum* KCCM 80189. Data from these tests were assessed in a previous opinion (EFSA FEEDAP Panel, 2020). As the composition, purity and physicochemical characteristics of the active substance under assessment is the same as of that assessed in the above-mentioned opinion and the production process is very similar, the FEEDAP Panel considers that the results of the studies performed with L-isoleucine produced by *C. glutamicum* KCCM 80189 can be used to support the safety for the user for the additive under assessment.

Based on the results with the other product, the FEEDAP Panel concludes that the additive should be considered not toxic by inhalation, not irritant to skin or eyes and not a dermal sensitiser. However, the Panel notes that this product has a high dusting potential (up to 6.2 g/m<sup>3</sup>) and that exposure to dust *per se* might be a hazard for the user.

### 3.3. Efficacy

Efficacy studies are not required for amino acids naturally occurring in proteins of plants and animals. The nutritional role of the amino acid L-isoleucine is well established in the scientific literature. The additive L-isoleucine is regarded as an effective source of isoleucine for non-ruminant animal species. For the supplemental L-isoleucine to be as efficacious in ruminants as in non-ruminant species, it would require protection against degradation in the rumen.

### 3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation<sup>31</sup> and Good Manufacturing Practice.

## 4. Conclusions

The additive is produced by a genetically modified strain of *Corynebacterium glutamicum* (*C. glutamicum* KCCM 80185). The production strain does not carry acquired antimicrobial resistance genes and no viable cells of the production strain were detected in the final product. The FEEDAP Panel could not exclude the presence of recombinant DNA from the production strain in the product.

<sup>27</sup> Technical dossier/Section III/Annex/Annex\_III\_3\_02.

<sup>28</sup> Technical dossier/Section III/Annex/Annex\_III\_3\_04.

<sup>29</sup> Technical dossier/Section III/Annex/Annex\_III\_3\_03.

<sup>30</sup> Technical dossier/Section III/Annex/Annex\_III\_3\_05.

<sup>31</sup> Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

However, since no sequences of concern remain in the final production strain, the potential presence of recombinant DNA in the final product does not raise any safety concerns.

L-Isoleucine produced by *C. glutamicum* KCCM 80185 is considered to be safe for the target species, for the consumer and for the environment. Regarding the use in water, the FEEDAP Panel reiterates its concerns over the safety for the target species of L-isoleucine administered simultaneously via water for drinking and feed owing to the risk of nutritional imbalances and hygienic reasons.

L-Isoleucine produced by *C. glutamicum* KCCM 80185 is considered not toxic by inhalation, not irritant to skin or eyes and not a dermal sensitiser. However, due to the high dusting potential, exposure to dust *per se* might be a hazard for the user.

L-Isoleucine produced by *C. glutamicum* KCCM 80185 is considered as an efficacious source of the essential amino acid L-isoleucine for non-ruminant animal species. For the supplemental L-isoleucine to be as efficacious in ruminants as in non-ruminant species, it would require protection against degradation in the rumen.

## 5. Documentation provided to EFSA/Chronology

Date	Event
05/12/2019	Dossier received by EFSA. L-isoleucine produced by fermentation with <i>Corynebacterium glutamicum</i> KCCM 80185 for all animal species. Submitted by CJ Europe GmbH.
23/12/2019	Reception mandate from the European Commission
02/04/2020	Application validated by EFSA – Start of the scientific assessment
25/05/2020	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation</i>
01/07/2020	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
07/10/2020	Comments received from Member States
13/10/2020	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation</i>
24/06/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
13/08/2021	Reception of spontaneous information from the applicant
10/11/2021	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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## Abbreviations

AMR	antimicrobial resistance
ANI	average nucleotide identity
BCAA	branched chain amino acid
CAS	Chemical Abstracts Service
CFU	Colony Forming Units
DM	dry matter
EINECS	European Inventory of Existing Commercial Chemical Substances
EURL	European Union Reference Laboratory
FCC	Food Chemical Codex
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
HPLC	high performance liquid chromatography
HPLC-FLD	high performance liquid chromatography coupled to fluorescence detection
IEC	ion-exchange chromatography
IEC-VIS	ion-exchange chromatography coupled to photometric detection

IUPAC	International Union of Pure and Applied Chemistry
KCCM	Korean Culture Centre of Microorganisms
LOD	limit of detection
LOQ	limit of quantification
MIC	minimum inhibitory concentration
OECD	Organisation for Economic Co-operation and Development
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzodioxin
PCDF	polychlorinated dibenzofuran
PCDD/F	polychlorinated dibenzo-p-dioxins and dibenzofurans
QPS	qualified presumption of safety
RSDr	relative standard deviation for repeatability
RSDR	relative standard deviation for reproducibility
TEQ	toxic equivalents
WGS	whole genome sequence
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 the Analysis for L-isoleucine produced by fermentation with *Corynebacterium glutamicum* KCCM80185

In the current application an authorisation is sought under Article 4(1) for *L-isoleucine produced by fermentation with Corynebacterium glutamicum KCCM80185*, under the category/functional group 3(c) 'nutritional additives'/amino acids, their salts and analogues', according to Annex I of Regulation (EC) No 1831/2003. The authorisation is sought for all animal species.

According to the Applicant *L-isoleucine* has a minimum purity (mass fraction) of 90%. The *feed additive* is intended to be added directly into *feedingstuffs* (or through *premixtures*) and *water* for drinking. However, the Applicant did not propose any minimum or maximum content of *L-isoleucine* in *feedingstuffs*.

For the quantification of *isoleucine* in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted an in-house validated analytical method based on high performance liquid chromatography coupled to fluorescence detection (HPLC-FLD). The Applicant did not present a verification study and therefore, the EURL cannot recommend the method for official control purposes.

For the quantification of *isoleucine* in feed a ring-trial validated European Union (EU) method (Commission Regulation (EC) No 152/2009) based on ion-exchange chromatography (IEC) coupled to photometric detection exists (IEC-VIS). This method, designed for the analysis of amino acids in *premixtures* and *feedingstuffs*, does not distinguish between the salts and the amino acid enantiomers. This method was further ring-trial validated resulting in the EN ISO 13903:2005 method. The following performance characteristics were reported for the quantification of total *isoleucine*: relative standard deviation for repeatability (RSD<sub>r</sub>) ranging from 2.0 to 5.4% and relative standard deviation for reproducibility (RSD<sub>R</sub>) ranging from 6.8 to 14.3%.

The Applicant did not provide experimental data to determine *isoleucine* in *water*. Nevertheless, as concluded in previous EURL reports, the EURL recommends the EU method for official control for the quantification of *isoleucine* in the *feed additive* and *water*. Furthermore, for the determination of *L-isoleucine* in the *feed additive* and *premixtures* the ring-trial validated method EN ISO 17180:2013 – "Animal feeding stuffs – Determination of lysine, methionine and threonine in commercial amino acid products and premixtures" is also applicable.

In addition, the EURL found the "L-isoleucine monograph" of the Food Chemical Codex (FCC) for the identification of *L-isoleucine* in the *feed additive*.

In the frame of the present request of authorisation the EURL recommends for official control (i) the "L-isoleucine monograph" of the Food Chemical Codex (FCC) for the identification of *L-isoleucine* in the *feed additive*; (ii) the ring-trial validated method EN ISO 17180:2013 based on ion-exchange chromatography coupled to visible or fluorescence detection (IECVIS/FLD) to quantify free *isoleucine* in the *feed additive* and *premixtures*; and (iii) the ring-trial validated European Union method based on IEC-VIS for the quantification of *isoleucine* in the *feed additive*, *premixtures*, *feedingstuffs* and *water*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005), as last amended by Regulation (EU) 2015/1761) is not considered necessary.