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## Safety and efficacy of L-cysteine hydrochloride monohydrate produced by fermentation using *Escherichia coli* KCCM 80180 and *Escherichia coli* KCCM 80181 as a flavouring additive for all animal species

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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 L-cysteine monohydrochloride monohydrate produced by fermentation using two genetically modified strains of *Escherichia coli* K12 (*Escherichia coli* KCCM 80180 and *Escherichia coli* KCCM 80181) as a flavouring additive for all animal species. No safety concerns are derived from the use of these strains as production strains of the additive. The FEEDAP Panel concludes that L-cysteine hydrochloride monohydrate, produced by *E.coli* KCCM 80180 and KCCM 80181 at concentrations up to 25 mg/kg complete feed, is safe for the target species, for the consumer and for the environment. The product is proposed to be classified as respiratory irritant; however, exposure by inhalation is unlikely. L-Cysteine hydrochloride monohydrate produced by *E.coli* KCCM 80180 and *E.coli* KCCM 80181 was shown to be a skin and eye irritant but not a skin sensitiser. Since L-cysteine hydrochloride monohydrate is used in food as flavouring, it is to be expected that it can provide a similar function in feed and no further demonstration of efficacy is necessary when used at concentrations up to 25 mg/kg complete feed and the corresponding concentration in water.

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## 1. Introduction

### 1.1. Background and Terms of Reference

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

The European Commission received a request from CJ Europe GmbH<sup>2</sup> for authorisation of the product L-cysteine monohydrochloride monohydrate produced by fermentation with *Escherichia coli* KCCM 80180 and *Escherichia coli* KCCM 80181 when used as a feed additive for all animals (category: sensory additives; functional group: flavourings compounds).

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 19 October 2018.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product L-cysteine monohydrochloride monohydrate produced by fermentation with *E.coli* KCCM 80180 and *E.coli* KCCM 80181, when used under the proposed conditions of use (see Section 3.1.2).

### 1.2. Additional information

L-Cysteine hydrochloride monohydrate has been assessed by the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) as a flavouring substance in pets only (EFSA FEEDAP Panel, 2013). The FEEDAP Panel also evaluated L-cysteine hydrochloride monohydrate in an opinion on the safety and efficacy of the use of amino acids (chemical group 34) when used as flavourings for all animal species (EFSA FEEDAP Panel, 2014). In both opinions, the FEEDAP Panel did not conclude on L-cysteine hydrochloride monohydrate produced by fermentation because no information on the production strains was submitted.

L-Cysteine hydrochloride monohydrate has not been assessed for use as food flavour.

L-Cysteine hydrochloride monohydrate is listed in the European Union Register of Feed Additives as feed flavourings.

L-Cysteine hydrochloride monohydrate produced by chemical synthesis or protein hydrolysis instead of using production strains is currently authorised as a sensory additive for use in all animal species except cats and dogs in accordance with Regulation (EU) 2018/249<sup>3</sup> and L-cysteine hydrochloride monohydrate produced by hydrolysis of keratin from avian feathers is authorised in dogs and cats in accordance with Regulation (EU) 2015/2306.<sup>4</sup>

L-Cysteine hydrochloride [17.032], but not its monohydrate form, is listed in the European Union (EU) database of flavouring substances.<sup>5</sup>

Cysteine hydrochloride monohydrate is described in the European Pharmacopoeia as Monograph (MG) 0895 (PhEur, 2010).

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

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

<sup>3</sup> Commission Implementing Regulation (EU) 2018/249 of 15 February 2018 concerning the authorisation of taurine, beta-alanine, L-alanine, L-arginine, L-aspartic acid, L-histidine, D,L-isoleucine, L-leucine, L-phenylalanine, L-proline, D,L-serine, L-tyrosine, L-methionine, L-valine, L-cysteine, glycine, monosodium glutamate and L-glutamic acid as feed additives for all animal species and L-cysteine hydrochloride monohydrate for all species except cats and dogs. OJ L 53, 23.2.2018, p. 134.

<sup>4</sup> Commission Implementing Regulation (EU) 2015/2306 of 10 December 2015 concerning the authorisation of L-cysteine hydrochloride monohydrate as a feed additive for cats and dogs. OJ L 326, 11.12.2015, p. 46.

<sup>5</sup> Commission Implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1.

## 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>6</sup> in support of the authorisation request for the use of L-cysteine monohydrochloride monohydrate produced by fermentation with *E.coli* KCCM 80180 and *E.coli* KCCM 80181 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-cysteine monohydrochloride monohydrate produced by fermentation with *E.coli* KCCM 80180 and *E.coli* KCCM 80181 in animal feed. The Executive Summary of the EURL report can be found in Annex A.<sup>7</sup>

### 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of L-cysteine monohydrochloride monohydrate produced by fermentation with *E.coli* KCCM 80180 and *E.coli* KCCM 80181 is in line with the principles laid down in Regulation (EC) No 429/2008<sup>8</sup> and the relevant guidance documents: Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018), Guidance on the safety of feed additives for the environment (EFSA, 2008).

## 3. Assessment

The present opinion assesses the safety and efficacy of L-cysteine monohydrochloride monohydrate produced by fermentation using *E.coli* KCCM 80180 and *E.coli* KCCM 80181 as a sensory additive (functional group: flavouring compounds) for all animal species. The proposed inclusion level of the additive is 25 mg/kg feed for all animal species in accordance with Commission Implementing Regulation (EU) 2018/249.<sup>3</sup>

### 3.1. Characterisation

#### 3.1.1. Characterisation of the production organism

The additive is produced by two genetically modified strains of *E. coli* K12 which are deposited in the Korean Culture Collection of Microorganisms (KCCM) with accession numbers KCCM 80180 and KCCM 80181. The taxonomic identification of the production strains was confirmed by sequencing the 16S rRNA genes and comparing them to sequences from the GenBank database. The sequences showed 99% similarity with *E. coli*. The relationship of the production strains as *E. coli* K-12 derivatives was established [REDACTED]

The susceptibility of the production strains to the antibiotics recommended by the FEEDAP Panel (EFSA FEEDAP Panel, 2018) was tested [REDACTED]

[REDACTED]

<sup>6</sup> FEED dossier reference: FAD-2018-0042.

<sup>7</sup> The full report is available on the EURL website: <https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports/fad-2018-0042>.

<sup>8</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.

[REDACTED]

[Redacted]

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

#### *Characteristics of the recipient or parental microorganism*

[Redacted]

#### *Characteristics of the donor organism*

All the genes cloned in KCCM 80180

[Redacted]

#### *Description of the genetic modification process for *E.coli* KCCM80180*

*E.coli* KCCM 80180 has several genetic modifications

[Redacted]

- [Redacted]
- [Redacted]
- [Redacted]
- [Redacted]
- [Redacted]
- [Redacted]
- [Redacted]
- [Redacted]

All genetic modifications of *E. coli* KCCM 80180 have been verified by genome analysis of the production strain.

[Redacted]

### Description of the genetic modification process for *E. coli* KCCM 80181

*E. coli* KCCM 80181 was genetically modified [REDACTED]

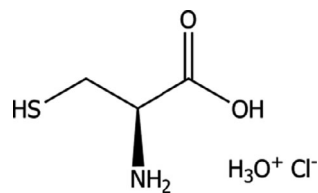
The absence of all antimicrobial resistance genes from the vectors transiently used during the genetic modification of the production strains *E. coli* KCCM 80180 and *E. coli* KCCM 80181, respectively, was demonstrated [REDACTED]

#### 3.1.2. Manufacturing process

The two production strains *E. coli* KCCM 80180 and *E. coli* KCCM 80181 [REDACTED]

#### 3.1.3. Characterisation of the additive

L-Cysteine hydrochloride monohydrate (International Union of Pure and Applied Chemistry (IUPAC) name: (R)-2-Amino-3-mercaptopropionic acid hydrochloride monohydrate; synonym  $\beta$ -Mercaptoalanine hydrochloride monohydrate) is a compound identified with the Chemical Abstracts Service (CAS) No 7048-04-6. It has a molecular mass of 175.6 g/mol. The molecular formula of L-cysteine hydrochloride monohydrate is  $C_3H_7NO_2S \text{ HCl} \cdot H_2O$ . The structural formula is given in Figure 1.



**Figure 1:** Molecular structure of L-cysteine hydrochloride monohydrate

The additive contains by specifications  $\geq 98.5\%$  L-cysteine hydrochloride monohydrate and  $\leq 12\%$  water.<sup>18</sup> The analysis of five batches showed an average value of L-cysteine hydrochloride monohydrate of 99.23% (range 99.20–99.31%).<sup>19</sup> Moisture average was 10.33% (range 10.28–10.38%).

Average cysteine content was 68.3% 'as is' (range 68.28–68.32), and average hydrochloride content was 20.5% (range 20.53–20.68). Other components analysed were sulphate 0.2% (in all batches), ammonium (detected in all five batches at 0.01%) and 0.13% free cysteine.<sup>19</sup> The sum of the identified material on 'as is' basis was 99.39%.

Analytical data on specific optical rotation of three batches of L-cysteine hydrochloride monohydrate showed an average value of  $+ 5.6^\circ$  (range  $+ 5.53^\circ$  to  $+ 5.59^\circ$ ), which is within the range described in the European Pharmacopoeia for this amino acid ( $5.5^\circ$ – $7.0^\circ$ ) and confirms the presence of the L-enantiomer.<sup>20</sup>

### 3.1.3.1. Impurities

Three batches of the additive were analysed for impurities.<sup>21</sup> Levels of heavy metals and arsenic were as follow: cadmium and lead were not quantifiable at the specific limit of detection (LOD),<sup>22,23</sup> mercury = 0.06 mg/kg and arsenic not quantifiable at the LOD.<sup>24</sup> Other metals were measured as impurities in the same three batches resulting in levels of chromium not quantifiable at the specific LOD,<sup>25</sup> copper between 0.2 and 2.58 mg/kg, nickel between 0.2 and 1.41 mg/kg and zinc between  $< 0.2$  and 1.16 mg/kg.

The same batches were analysed for dioxins (PCDD/F) and sum of PCDD/F and dioxin-like PCBs and the average values were 0.16 ng TEQ-WHO/kg and 0.30 ng TEQ-WHO/kg, respectively (in all batches). In reference to the microbial contamination, three batches were analysed. *Salmonella* spp. was negative (enriching process). Total bacterial counts, *E. coli*, yeasts and filamentous fungi were  $< 10^3$  colony-forming units (CFU)/g.

Analytical data from three batches showed levels of aflatoxins  $< 0.1 \mu\text{g}/\text{kg}$ ; the same batches were analysed for ochratoxin A, zearalenone and deoxynivalenol. In two out of three batches, these impurities were not detected; analytical data from the third batch showed levels of ochratoxin A  $< 1.0 \mu\text{g}/\text{kg}$ , zearalenone  $< 5.0 \mu\text{g}/\text{kg}$  and deoxynivalenol of  $< 100 \mu\text{g}/\text{kg}$ .<sup>26</sup>

The presence of viable cells of the production strains was investigated in three batches of the additive analysed in triplicate.

The presence of recombinant DNA from the production strains was analysed in three batches of the final product.

### 3.1.3.2. Physicochemical characteristics

The additive is an off-white crystalline powder, with a bulk density of 600–800 kg/m<sup>3</sup>,<sup>30</sup> a pH (10% water solution) ranging from 0.8 to 1.2, and a solubility in water (at 25 °C) of 50 g/L.

The particle size distribution (three batches of the additive) was analysed

<sup>18</sup> Technical dossier/Section II/Annex II\_1\_01.

<sup>19</sup> Technical dossier/Section II/Annex II\_1\_03.

<sup>20</sup> Technical dossier/Section II/Annex II\_1\_02.

<sup>21</sup> Technical dossier/Section II/Annex II\_1\_04.

<sup>22</sup> LOD for Cd,  $< 0.2 \text{ mg}/\text{kg}$ .

<sup>23</sup> LOD for Pb,  $< 1 \text{ mg}/\text{kg}$ .

<sup>24</sup> LOD for As,  $< 1 \text{ mg}/\text{kg}$ .

<sup>25</sup> LOD for Cr,  $< 0.2 \text{ mg}/\text{kg}$ .

<sup>26</sup> Technical dossier/Section II/Annex II\_1\_05.

<sup>27</sup> Technical dossier/Supplementary information May 2019/Annex\_SIN\_4.

<sup>30</sup> Technical dossier/Section III/ Annex\_III\_3\_01.





L-Cysteine is a physiological and natural component of proteins in animals and plants. It is not excreted as such but as urea or uric acid, sulfate and CO<sub>2</sub>. The use of L-cysteine in animal nutrition would not lead to any localised increase in its concentration in the environment. It is concluded that the use of this product as feed additive does not represent a risk to the environment.

The FEEDAP Panel concludes that the use of L-cysteine hydrochloride monohydrate, produced by *E.coli* KCCM 80180 and KCCM 80181 under the proposed conditions of use, is safe for the target species, for the consumer and for the environment.

### 3.2.3. Safety for the user

#### 3.2.3.1. Effects on the respiratory system

The pH of the additive (10% water solution) ranges from 0.8 to 1.2. The dusting potential of the additive, [REDACTED]

[REDACTED] exposure of users via inhalation is unlikely.

A valid acute inhalation test in laboratory animals, performed according to OECD guideline 403 showed an LC<sub>50</sub> greater than 5.14 mg/L in male and female rats.<sup>36</sup>

The applicant proposes to label the additive with the hazard statement H335 (may cause respiratory irritation)<sup>30</sup> according to the Regulation (EC) No 1272/2008.<sup>37</sup>

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

The skin irritation potential of the additive was tested in a valid study<sup>38</sup> performed according to OECD guideline 439. The results of the study indicate that the additive should be classified, according to the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (UN GHS) as an UN GHS Category 2: 'skin irritant'.<sup>30</sup>

The eye irritation potential of the additive was tested in a valid study<sup>39</sup> performed according to OECD guideline 437. The results of the study indicate that the additive should be classified, according to the UN GHS as an UN GHS Category 1: 'Serious eye damage'.<sup>30</sup>

In a valid dermal sensitisation study following OECD guideline 429<sup>40</sup> (local lymph-node assay), the additive did not show any skin sensitisation potential.

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

Although users' exposure via inhalation is unlikely, the product is proposed to be classified as respiratory irritant due to its low pH when in solution.

L-cysteine hydrochloride monohydrate produced by *E.coli* KCCM 80180 and *E.coli* KCCM 80181 was shown to be a skin and eye irritant but not a dermal sensitiser.

### 3.3. Efficacy

Since L-cysteine hydrochloride monohydrate is used in food as flavouring, it is to be expected that it can provide a similar function in feed and no further demonstration of efficacy is necessary when used in feed and the corresponding concentration in water.

### 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>41</sup> and Good Manufacturing Practice.

<sup>35</sup> Technical dossier/Section II/Annex\_III\_3\_03.

<sup>36</sup> Technical dossier/Section III/Annex\_III\_3\_04.

<sup>37</sup> Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006, OJ L 353, 31.12.2008, p. 1.

<sup>39</sup> Technical dossier/Section III/ Annex\_III\_3\_05.

<sup>40</sup> Technical dossier/Section III/ Annex\_III\_3\_07.

<sup>41</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.

## 4. Conclusions

The additive is produced by two genetically modified strains of *Escherichia coli* K12 (*E. coli* KCCM 80180 and *E. coli* KCCM 80181). None of the genetic modifications of the production strains raise a safety concern. The two production strains are free of all antibiotic resistance genes used during the genetic modification processes. The production strain and its DNA were not detected in the final additive. No safety concerns are derived from the use of these strains as production strains of the additive.

The FEEDAP Panel concludes that the use of L-cysteine hydrochloride monohydrate produced by *E. coli* KCCM 80180 and KCCM 80181 at concentrations up to 25 mg/kg complete feed, is safe for the target species, for the consumer and for the environment.

The product is proposed to be classified as respiratory irritant; however, exposure by inhalation is unlikely. L-Cysteine hydrochloride monohydrate produced by *E. coli* KCCM 80180 and *E. coli* KCCM 80181 was shown to be a skin and eye irritant but not a skin sensitiser.

Since L-cysteine hydrochloride monohydrate is used in food as flavourings, it is to be expected that it can provide a similar function in feed and no further demonstration of efficacy is necessary when used at concentrations up to 25 mg/kg complete feed and the corresponding concentration in water.

## Documentation provided to EFSA/Chronology

Date	Event
25/06/2018	Dossier received by EFSA
09/07/2018	Reception mandate from the European Commission
19/10/2018	Application validated by EFSA – Start of the scientific assessment
19/01/2019	Comments received from Member States
11/02/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation</i>
20/02/2019	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
22/03/2019	Request of supplementary information addendum in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – <i>Issues: characterisation</i>
28/06/2019	Reception of supplementary information from the applicant -Scientific assessment re-started
6/09/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation</i>
18/10/2019	Reception of supplementary information from the applicant
22/10/2019	Clarification received by e-mail – Scientific assessment re-started
10/01/2020	Opinion adopted by the FEEDAP Panel by written procedure. End of the Scientific assessment

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## **Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for L-cysteine monohydrochloride monohydrate produced by fermentation with *Escherichia coli* KCCM 80180 and *Escherichia coli* KCCM 80181**

In the current application, authorisation is sought under Article 4(1) for L-cysteine monohydrochloride monohydrate produced by fermentation with *Escherichia coli* KCCM 80180 and *Escherichia coli* KCCM 80181, under the category/functional groups 2(b) 'sensory additives/flavouring compounds' according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for all animal species. According to the Applicant L-cysteine monohydrochloride monohydrate has a minimum purity (mass fraction) of 98.5%. The feed additive is intended to be added directly into feedingstuffs or through premixtures and water for drinking. The Applicant proposed a maximum content of L-cysteine monohydrochloride monohydrate in feedingstuffs of 25 mg/kg.

For the quantification of L-cysteine monohydrochloride monohydrate in the feed additive the Applicant submitted an in-house validated analytical method based on reversed phase high-performance liquid chromatography coupled with ultraviolet detection (HPLC-UV). While in the frame of the validation study satisfactory performance characteristics were derived, the Applicant did not present a verification study or any additional test performed by a second independent laboratory applying the above-mentioned method.

However, in the frame of the stability studies, the Applicant presented experimental data obtained when analysing the feed additive with the VDLUFA method 4.11.6 designed for the analysis of lysine, methionine and threonine in feed additives and concentrated premixtures and based on ion exchange chromatography coupled with post-column derivatisation and optical detection (IEC-VIS/FD). The results presented are considered sufficient to demonstrate the suitability of the procedure for the determination of the amino acid in the feed additive. Furthermore, according to the experience of NRLs, the VDLUFA method and other ring trial validated methods designed for the analysis of amino acids and based on similar analytical procedure are fit-for-purpose. Hence, for official control, the EURL recommends this method (or equivalent) for the determination of L-cysteine monohydrochloride monohydrate in the feed additive.

For the quantification of the L-cysteine monohydrochloride monohydrate content in premixtures and feedingstuffs, the Applicant submitted the ring-trial validated Community method (Commission Regulation (EC) No 152/2009) based on 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. The Community method was further ring-trial validated by 23 laboratories for the determination of total cyst(e)ine (sum of cysteine and cystine, peptide bound and free) in feed and resulted in the equivalent standard method EN ISO 13903:2005. While the Applicant proposed a maximum content of L-cysteine HCl H<sub>2</sub>O in feedingstuffs of 25 mg/kg, a higher limit of quantification of 350 mg/kg feedingstuffs was derived for total cyst(e)ine. Therefore, the EURL is unable to recommend the Community method for the official control of this product in feedingstuffs when intended as flavouring feed additive. Nevertheless, based on the performance characteristics available, the EURL recommends for official control the ring-trial validated Community method based on IEC-VIS to quantify L-cysteine monohydrochloride monohydrate in premixtures. Moreover exclusively the procedure for the determination of free amino acid applies.

The Applicant did not submit any method for the official control of L-cysteine monohydrochloride monohydrate in water. However, in the frame of the stability studies, the Applicant presented experimental data obtained when analysing the amino acid with the above-mentioned ring-trial validated method VDLUFA – Method 4.11.6. The results presented are considered sufficient to demonstrate the suitability of the procedure for the analysis of L-cysteine monohydrochloride monohydrate in water. Hence, for official control, the EURL recommends this method (or equivalent) for the determination L-cysteine monohydrochloride monohydrate in water.

In the frame of the authorised 'Chemical Defined flavouring Group 34 – amino acids' (FAD-2010-0107), for the identification of L-cysteine monohydrochloride monohydrate in the feed additive, the EURL positively evaluated the European Pharmacopeia 2.2.56 (2009) method for amino acids based on IEC coupled with post column derivatisation and photometric detection (visible – VIS). Furthermore, the EURL recommendation has been included in the corresponding authorising regulation. In order to foster the use of the same method for identical substances, the EURL recommends this European

Pharmacopeia method for official control to identify L-cysteine monohydrochloride monohydrate in the feed additive.

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