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Community Reference Laboratory for Feed Additives

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CRL Evaluation Report on the Analytical Methods submitted in connection with the Application for the Authorisation of a Feed Additive according to Regulation (EC) No 1831/2003

Dossier related to:	**FAD-2010-0024** **CRL/100009**
Name of additive:	**Choline Chloride**
Active Agent (s):	***Choline Chloride***
Rapporteur Laboratory:	**Community Reference Laboratory for Feed Additives (CRL-FA), Geel, Belgium**
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Report checked by: Date:	**Piotr Robouch (CRL-FA)** **20/09/2010**
Report approved by: Date:	**Christoph von Holst (CRL-FA)** **21/09/2010**

EXECUTIVE SUMMARY

In the current application authorisation is sought for *choline chloride* under the category nutritional additives, functional group '3a' vitamins, pro-vitamins and chemically well-defined substances having similar effect, according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of *choline chloride* for all animal species and categories. The active substance is a white crystalline powder containing at least 99% total (on anhydrous basis) expressed as *choline chloride*. It is intended to be used in *premixtures*, *feedingstuffs* as a formulated product and in *water* as pure substance. According to the applicant, the typical formulation is in the range of 70 to 80 wt % if liquid, of 50 to 70 wt. % if on vegetable carrier and around 50 wt. % on silica carrier. The applicant does not propose any minimum or maximum *choline chloride* concentration in *feedingstuffs* or *water*.

For the determination of *choline chloride* in the *feed additive*, *premixtures*, *feedingstuffs* and *water* the applicant proposed a single laboratory validated and further verified ion chromatographic method with conductivity detection (IC-CD). The method is based on the determination of the *choline chloride* content in an aqueous solution. The following performance characteristics derived from the validation and verification studies - performed at *choline chloride* concentrations ranging from 100 to 25000 mg/kg - were reported by the applicant:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 0.34 to 4.1 %,
- a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 0.31 to 3.3 %, and
- a recovery rate (R_{Rec}) ranging from 91.5 to 99.5 %.

Based on the above mentioned performance characteristics the CRL recommends for official control this single laboratory validated and further verified IC-CD method for the determination of *choline chloride* in *feed additive*, *premixtures*, *feedingstuffs* and *water* within the concentration range covered by the validation study.

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.

KEYWORDS

choline chloride, nutritional additive, vitamins, all species

1. BACKGROUND

In the current application authorisation is sought under articles 4(1) (new use in feed) and 10(2) (re-evaluation of the already authorized feed additive – Council directive 70/524/EEC) for *choline chloride* under the category nutritional additives, functional group '3a': vitamins, pro-vitamins and chemically well-defined substances having similar effect, according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1]. Specifically, authorisation is sought for the use of *choline chloride* for all animal species and categories [1]. *Choline chloride* belongs to the family of water soluble B-vitamins (also known as vitamin B4). The active substance is a white crystalline powder with a purity of at least 99 % total (on anhydrous basis) [2]. *Choline chloride* is a quaternary amine salt ($C_5H_{14}ClNO$) [3]. Depending on the feed application, it is placed on the market via various formulations. According to the applicant, typical mass fractions of *choline chloride* are in a range of 70 to 80% wt. for liquid (water), between 50 and 70 wt.% on vegetable carrier, and around 50 wt.% on silica carrier [3]. However the applicant suggested no minimum or maximum levels for the *feed additive* in *premixtures, feedingstuffs* and *water* [4],[5].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the duties and tasks of the Community Reference Laboratory concerning applications for authorizations of feed additives, the CRL is requested to submit a full evaluation report to the European Food Safety Authority (EFSA) for each application or for each group of application. For this particular dossier, the methods of analysis submitted in connection with *choline chloride* and their suitability to be used for official controls in the frame of the authorisation, were evaluated.

3. EVALUATION

Identification/Characterization of the feed additive

Qualitative and quantitative composition of impurities in the additive

When required by EU legislation, analytical methods for official control of undesirable substances in the additive (e.g. arsenic, cadmium, mercury, lead, dioxins and PCBs) are available at the respective Community Reference Laboratories [6].

Description of the method for the determination of the active substance in the feed additive, premixtures, feedingstuffs and water

For the determination of *choline chloride* in the *feed additive* and *water*, the applicant proposed a single laboratory validated and further verified ion chromatographic method. The method is applicable to aqueous solutions and to dry choline chloride. The substance is determined, after dilution with water, by an ion chromatograph equipped with a suppressed conductivity detector (IC-CD). The quantification is done by calibration with external standard [7]. The performance characteristics derived from the validation [7] and verification studies [8] are presented in Table 1.

For the determination of the active substance in *premixtures* and *feedingstuffs* the applicant proposed a single laboratory validated and further verified ion chromatographic method with conductivity detection (IC-CD) after suppression. C*holine chloride* in *premixtures* and *feedingstuffs* is dissolved in water. To obtain a clear solution, the proteins are precipitated with 1,1,1-trichloroethane and centrifuged. The supernatant is injected and analysed by ion chromatography. The quantification is done by calibration with external standard [9]. The performance characteristics derived from the validation [9] and verification studies [10] – performed at concentration levels ranging from 250 to 25000 mg/kg for *premixtures* and from 100 to 10000 mg/kg in *feedingstuffs* - are presented in Table 1.

Furthermore, the applicant reported the following limits of detection (LOD) and quantification (LOQ):

- LOD = 0.3 mg/kg and LOQ = 1 mg/kg for *choline chloride* in *water* [8],
- LOD = 30 mg/kg and LOQ = 100 mg/kg for *choline chloride* in *feedingstuffs* [9].

Table 1: Method performance characteristics for the determination of *choline chloride* in the *feed additive* (FA), *premixtures* (PM), *feedingstuffs* (FS) and *water* (W)

	RSD_r (%)		RSD_{ip} (%)		R_{Rec} (%)	
	Validation	Verification	Validation	Verification	Validation	Verification
FA & W	0.83 [7]	0.34 [8]	0.83 [7]	0.31 [8]	99.2 [7]	99.5 [8]
PM	3.3 - 4.1 [9]	1.2 - 1.9 [10]	3.6 [9]	2.2 [10]	-	94.2 [10]
FS	2.0 - 3.5 [9]	0.9 - 2.4 [10]	2.1 [9]	2.3 [10]	-	91.5 [10]

RSD_r and RSD_{ip}: relative standard deviation for *repeatability* and *intermediate precision*

R_{Rec}: *recovery* rate

Based on the above mentioned performance characteristics and the concentration range investigated, the CRL recommends for official control the single laboratory and further verified IC-CD method submitted by the applicant for the determination of *choline chloride* in *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) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

The CRL recommends for official control the single laboratory validated and further verified ion-chromatographic method submitted by the applicant, for the determination of *choline chloride* in *feed additive*, *premixtures, feedingstuffs* and *water*.

Recommended text for the register entry, fourth column (Composition, chemical formula, description, analytical method)

For the determination of *choline chloride* in the *feed additive, premixtures, feedingstuffs* and *water*:

- ion chromatography with conductivity detection (IC-CD)

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *choline chloride* have been sent to the Community Reference Laboratory for Feed Additives Authorisation. The dossier has been made available to the CRL by EFSA.

6. REFERENCES

[1] *Application/Ref:SANCO/D/2:Forw.Appl.1831/018-2010

[2] *Technical dossier, Section II: 2.2 Characterisation of the active substance

[3] *Technical dossier, Section II: 2.3 Manufacturing process, including any specific processing procedures

[4] *Application, Proposal for Register Entry, Annex A

[5] *Technical dossier, Section II, 2.5 Condition of use of the additives

[6] Commission Regulation (EC) No 776/2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards to Community Reference Laboratories

[7] *Technical dossier, Section II, Annex 2.6.1.a "Validation report - Determination of choline chloride in feed additive by ion chromatography with conductivity detection"

[8] *Technical dossier, Section II, Annex 2.6.1.b "Verification report - Determination of choline chloride in feed additive by ion chromatography with conductivity detection"

[9] *Technical dossier, Section II, Annex 2.6.1.c "Determination of choline chloride in premix and feed via ion chromatography – analytical method, validation and verification"

[10] *Technical dossier, Section II, Annex 2.6.1.d "Verification of analytical method – Determination of choline chloride in premix and feed via ion chromatography"

* Refers to Dossier No. FAD-2010-0024

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was Community Reference Laboratory for Feed Additives, IRMM, Geel, Belgium. This report is in accordance with the opinion of the consortium of National Reference Laboratories as referred to in Article 6(2) of Commission Regulation (EC) No 378/2005.

8. ACKNOWLEDGEMENTS

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