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Directorate F - Health, Consumers & Reference Materials (Geel)  
European Union Reference Laboratory for Feed Additives

JRC F.5/CvH/ZE/AS/Ares

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

**Ferric(III) ammonium hexacyanoferrate(II) (AFCF)**  
*(FAD-2010-0230; CRL/100278)*





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in connection with the Application for Authorisation of a  
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Dossier related to: **FAD-2010-0230 - CRL/100278**

Name of Feed Additive: ***Ferric(III) ammonium hexacyanoferrate(II)*  
*(AFCF)***

Active Agent (s): ***Ferric(III) ammonium hexacyanoferrate(II)***

Rapporteur Laboratory: **European Union Reference Laboratory for  
Feed Additives (EURL-FA)  
JRC Geel, Belgium**

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Date: **25/08/2020**

## EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 10(2) for *ferric(III) ammonium hexacyanoferrate(II) (AFCF)*, under the category/functional group 1(h) “technological additives”/“substances for control of radionuclide contamination”, according to Annex I of Regulation (EC) No 1831/2003. The authorisation is sought for ruminants (domestic and wild), pigs (domestic and wild), calves (prior to the state of rumination), lambs (prior to the state of rumination) and goat kids (prior to the state of rumination).

According to the Applicant, the *feed additive* is to be marketed as a crystalline powder preparation (Giese salt) containing 60 to 65 % (w/w) of *AFCF* as *active substance*.

According to the Applicant, the *feed additive* is intended to be used mostly in *salt licks* containing 2.5 to 5.0 % (w/w) of *AFCF*. The *salt licks* are given directly to the animals or the *feed additive* is added to *feedingstuffs* at levels of *AFCF* ranging from 50 to 500 mg/kg complete *feedingstuffs*.

For the quantification of *AFCF* in the *feed additive* (Giese salt) the Applicant submitted a single-laboratory validated and further verified method based on potentiometric titration with potassium permanganate.

The following performance characteristics were obtained in the frame of the validation and verification studies: a relative standard deviation for *repeatability* ( $RSD_r$ ) ranging from 0.1 to 0.6 %, a relative standard deviation for *intermediate* precision ( $RSD_{ip}$ ) ranging from 0.2 to 1.3 % and a *recovery* rate ( $R_{rec}$ ) ranging from 97 to 99 %.

Based on the acceptable performance characteristics, the EURL recommends for official control the single-laboratory validated and further verified method based on potentiometric titration with potassium permanganate for the quantification of *AFCF* in the *feed additive*.

For the quantification of *AFCF* in *salt licks*, the Applicant submitted another method based on potentiometric titration, which is almost identical to the method for the *feed additive*. However, no validation nor verification data were provided by the Applicant for the quantification of *AFCF* in the *salt licks*.

Based on the available information, the EURL is not able to recommend the proposed method for official control for the quantification of *AFCF* in the *salt licks*.

The Applicant did not provide any experimental method or data for the quantification of *AFCF* in *premixtures* and *feedingstuffs*. Therefore, the EURL is not able to evaluate or recommend any method for official control to quantify *AFCF* in *premixtures* and *feedingstuffs*.

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

## KEYWORDS

*Ferric(III) ammonium hexacyanoferrate(II) (AFCF)*, technological additives, substances for control of radionuclide contamination, ruminants (domestic and wild), pigs (domestic and wild), calves (prior to the state of rumination), lambs (prior to the state of rumination) and goat kids (prior to the state of rumination)

## 1. BACKGROUND

In the current application an authorisation is sought under Article 10(2) (re-evaluation of the already authorised additives under provisions of Council Directive 70/524/EEC) for *ferric(III) ammonium hexacyanoferrate(II) (AFCF)*, under the category/functional group 1(h) “technological additives”/“substances for control of radionuclide contamination”, according to Annex I of Regulation (EC) No 1831/2003 [1,2]. According to the Applicant, the *feed additive* is a non-absorbable binding agent for  $^{137}\text{Cs}$  and  $^{134}\text{Cs}$  present in contaminated feed [3] in order to reduce the absorption of these radionuclides in animals.

The authorisation is sought for ruminants (domestic and wild), pigs (domestic and wild), calves (prior to the state of rumination), lambs (prior to the state of rumination) and goat kids (prior to the state of rumination) [1,2,4].

The *feed additive* is to be marketed as a crystalline powder preparation (Giese salt) containing 60 to 65 % (w/w) of *AFCF* as *active substance* and 30 to 35 % (w/w) of ammonium chloride [3,4].

According to the Applicant, the *feed additive* is intended to be used mostly in *salt licks* containing 2.5 to 5.0 % (w/w) of *AFCF*, ammonium chloride and a rock salt (sodium chloride) [5,6]. The *salt licks* are given directly to the animals [5,6], however in exceptional (emergency) cases the *feed additive* is also to be used directly into *feedingstuffs* at the levels of *AFCF* ranging from 50 to 500 mg/kg complete *feedingstuffs* [6]. The use of the *feed additive* in slow release boli, feed concentrates and *premixtures* is not foreseen according to the updated conditions of use [5,6] contrary to the ones specified in the original dossier [7].

## 2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761, 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 the tasks of the European Union Reference Laboratory concerning applications for authorisations of feed additives, the EURL is requested to submit a full evaluation report to the European Food Safety Authority for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with *ferric(III) ammonium hexacyanoferrate(II) (AFCF)* and their suitability to be used for official controls in the frame of the authorisation were evaluated.

## 3. EVALUATION

***Description of the analytical methods for the determination of the active substance in the feed additive, premixtures, feedingstuffs and when appropriate water (section 2.6.1 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)***

For the quantification of *AFCF* in the *feed additive* (Giese salt) the Applicant submitted a single-laboratory validated and further verified method based on potentiometric titration with potassium permanganate [8].

The sample (0.7 g) is mixed with 100 ml of sodium hydroxide solution (1 M) and heated until the reaction is complete. The formed insoluble iron(III) hydroxide is removed by filtration when the solution is still hot, the filter is washed with water and the filtrate is acidified by addition of sulfuric and phosphoric acids. The hexacyanoferrate(II) anion present in the solution is further oxidised during potentiometric titration with a potassium permanganate solution (0.02 M). The amount of the analyte is calculated on the basis that 1 ml of the potassium permanganate solution (0.02 M) corresponds to 0.03039 g of *AFCF* monohydrate [8]. The performance characteristics obtained for the quantification of the *AFCF* in the *feed additive* (Giese salt) in the frame of the validation [9] and verification [10] studies are summarised in Table 1.

Based on the acceptable performance characteristics, the EURL recommends for official control the single-laboratory validated and further verified method based on potentiometric titration with potassium permanganate for the quantification of the *ferric(III) ammonium hexacyanoferrate(II) (AFCF)* in the *feed additive*.

**Table 1:** Performance characteristics of the method based on potentiometric titration for the quantification of *ferric(III) ammonium hexacyanoferrate(II) (AFCF)* in the *feed additive* (Giese salt)

	Validation	Verification
Method	Potentiometric titration	
Mass fraction (%)	58.1 – 64.3	62.7
RSD <sub>r</sub> (%)	0.1 – 0.2	0.6
RSD <sub>ip</sub> (%)	0.2	1.3
R <sub>rec</sub> (%)	98 – 99	97 – 98
Reference	[9]	[10]

RSD<sub>r</sub> and RSD<sub>ip</sub>: relative standard deviation for *repeatability* and for *intermediate precision*, respectively; R<sub>rec</sub>: *recovery rate*.

For the quantification of *ferric(III) ammonium hexacyanoferrate(II) (AFCF)* in *salt licks* containing *AFCF*, the Applicant submitted another method based on potentiometric titration, which is almost identical to the method for the *feed additive* [11].

Given the similar nature of the samples of the *feed additive* and of the *salt licks* and that both protocols are almost identical in terms of sample preparation and further quantification, a similar performance profile as the one demonstrated for the quantification of *AFCF* in the *feed additive* might be expected for the quantification of *AFCF* in the *salt licks*. However, no validation nor verification data were provided by the Applicant for the quantification of *AFCF* in the *salt licks*.

Based on the available information, the EURL is not able to recommend the proposed method based on potentiometric titration for official control for the quantification of *AFCF* in the *salt licks*.

The Applicant did not provide any experimental method or data for the quantification of *AFCF* in *premixtures* and *feedingstuffs*. Therefore, the EURL is not able to evaluate or recommend any method for official control to quantify *AFCF* in *premixtures* and *feedingstuffs*.

***Methods of analysis for the determination of the residues of the additive in food (section 2.6.2 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)***

An evaluation of corresponding methods of analysis is not relevant for the present application.

***Identification/Characterisation of the feed additive (section 2.6.3 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)***

For the additional characterisation of the *feed additive* (Giese salt) the Applicant submitted an in-house method to determine the ammonium chloride content. The method is based on

potentiometric titration with silver nitrate after an appropriate sample preparation [8]. The EURL considers this method fit-for-purpose for the additional characterisation of 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.

#### **4. CONCLUSIONS AND RECOMMENDATIONS**

In the frame of this authorisation the EURL recommends for official control the single-laboratory validated and further verified method based on potentiometric titration with potassium permanganate for the quantification of *AFCF* in the *feed additive*.

For the quantification of *AFCF* in the *salt licks* the Applicant provided no validation nor verification data. Furthermore, no experimental method or data were provided for the quantification of *AFCF* in *premixtures* and *feedingstuffs*. Therefore, the EURL is not able to evaluate or recommend any method for official control for the quantification of *AFCF* in the *salt licks*, *premixtures* and *feedingstuffs*.

##### ***Recommended text for the register entry (analytical method)***

For the quantification of *ferric(III) ammonium hexacyanoferrate(II) (AFCF)* in the *feed additive*:

- Potentiometric titration with potassium permanganate

#### **5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL**

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *ferric(III) ammonium hexacyanoferrate(II) (AFCF)* have been sent to the European Union Reference Laboratory for Feed Additives. The dossier has been made available to the EURL by EFSA.

#### **6. REFERENCES**

- [1] \*Application, Reference SANCO/G/1: Forw. Appl. 1831/0025-2013
- [2] \*Application, Annex I – submission number 1288012351695-1114
- [3] \*Technical dossier, Section II: II.1. Identity of the additive
- [4] \*Application, Proposal of Registry Entry – Annex A
- [5] \*Supplementary information – Annex\_to cover letter\_EFSA-Q-2013-00681\_FAD-2010-0230\_AFCF



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- [6] \*Supplementary information – Sect\_II\_Identity\_AFCF: II.5. Condition of use of the additives
- [7] \*Technical dossier, Section II: II.5. Condition of use of the additives
- [8] \*Technical dossier – Annex\_II\_6\_01\_MoA\_AFCF
- [9] \*Supplementary information – Annex\_II\_6\_03\_Validation report
- [10] \*Supplementary information – Annex\_II\_6\_04\_Verification report
- [11] \*Technical dossier – Annex\_II\_6\_02\_MoA\_AFCF in feed
- \*Refers to Dossier no: FAD-2010-0230

## **7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES**

The Rapporteur Laboratory for this evaluation is the European Union Reference Laboratory for Feed Additives, JRC, 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, as last amended by Regulation (EU) 2015/1761.

## **8. ACKNOWLEDGEMENTS**

The following National Reference Laboratories contributed to this report:

- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- <sup>1</sup>Wageningen Food Safety Research (WFSR) (NL)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, PESCA, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)
- <sup>2</sup>Ruokavirasto Helsinki (FI)

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<sup>2</sup> Name and address according to COMMISSION IMPLEMENTING REGULATION (EU) 2015/1761: Elintarviketurvallisuusvirasto/Livsmedelssäkerhetsverket (Evira), Helsinki/Helsingfors.