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

Subject: Addendum to the EURL evaluation report

References:

FAD-2019-0076 (chromium propionate) – JRC F.5/CvH/ZE/AS/Ares (2020)3710795

Background

In the evaluation report related to the present dossier [1] the EURL was not able to recommend an analytical method for the determination of *organic chromium* from *chromium propionate* in *feedingstuffs*. However, the enforcement of a corresponding limit of *organic chromium* would require a method for the determination of *organic chromium* or *chromium propionate* in *feedingstuffs*. Upon request of the Commission for providing such methods [2], the Applicant informed the EURL about limitations of potential methods in *feedingstuffs* [3], thus proposing the use of a microtracer added to the *feed additive* preparation at a specific ratio as an alternative means to monitor the correct use of *chromium propionate* in *feedingstuffs* [4].

The concept proposed by the Applicant foresees that the number of particles of the specific microtracer (Microtracer FS-green lake particles) present in the *feed additive* preparation are counted in *feedingstuffs* and the mass fraction of *organic chromium* is subsequently calculated by taking into account a conversion factor, which is the ratio of the content of *organic chromium* and of the microtracer in the *feed additive* preparation [4]. For establishing this conversion factor, the Applicant proposed the above-mentioned method for the determination of the number of particles of the microtracer in the *feed additive* preparation [4] and a method based on ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) for the determination of *chromium propionate/organic chromium* in the *feed additive* preparation [5]. Before establishing the abovementioned conversion factor [5], the mass fraction of *organic chromium* in the *feed additive* preparation is calculated stoichiometrically from the determined content of *chromium propionate*.

Evaluation

Determination of organic chromium in the feed additive preparation via the determination of chromium propionate

For the determination of *chromium propionate* in the *feed additive* preparation (KemTrace 0.4 % Cr) containing 1 % (w/w) of Microtracer FS-green lake particles, the Applicant submitted a single-laboratory validated and further verified method based on ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) [5].

According to the protocol of the method, the sample (0.2 g) is sonicated with a mixture of 20 ml of acetonitrile:water (1:1, v/v) for 20 min in an ultrasonic bath. An aliquot of the extract (1.5 ml) is centrifuged and the supernatant, after an appropriate dilution with the above-mentioned mixture, is analysed by UHPLC-MS/MS using a positive electrospray ionisation (+ESI) and multiple reaction monitoring (MRM). The following MRM transitions are acquired: m/z 610 > 356; m/z 610.0 > 209 and m/z 610 > 125. The transition m/z 610.0 > 209 is used for the quantification, while the other two ones are used for the identification. The quantification is performed by an external calibration curve using diluted aqueous solutions of *chromium propionate* as a standard substance [5]. The content of *organic chromium* in the *feed additive* preparation is subsequently calculated stoichiometrically from the determined content of *chromium propionate* (3.9 g of *chromium propionate* corresponds to 1 g of *organic chromium* [5]).

The performance characteristics obtained for the determination of *chromium propionate/organic chromium* content in the *feed additive* preparation in the frame of the validation [5] and verification [6] studies are summarised in Table 1.

Table 1: Performance characteristics obtained in the frame of the validation and verification studies of the UHPLC-MS/MS method for the determination of *chromium propionate/organic chromium* in the *feed additive* preparation formulated with 1 % (w/w) of Microtracer FS-green lake particles

	Validation	Verification
Method	UHPLC-MS/MS	
Mass fraction, expressed as Cr (%)	0.4(*)	
RSD_r (%)	5.3	5.7
RSD_{ip} (%)	-	14.4
R_{rec} (%)	85(**)	93(**)
Reference	[5]	[6]

RSD_r and RSD_{ip}: relative standard deviation for *repeatability and* for *intermediate precision;* R_{rec}*: recovery rate*;

(*)Expected Cr content; (**)based on expected Cr content.

Based on the acceptable performance characteristics obtained, the EURL recommends for official control the single-laboratory validated and further verified method based on UHPLC-MS/MS for the determination of the *chromium propionate/organic chromium* content in the *feed additive* preparation (KemTrace 0.4 % Cr) containing 1 % (w/w) of Microtracer FS-green lake particles.

Determination of number of particles of the microtracer in feedingstuffs, the feed additive preparation and premixtures

For the experiments with the microtracer the Applicant used the specific product "Microtracer FS Green lake", consisting of stainless steel particles, coated with a green colour and containing *ca.* 50000 particles per gram of the microtracer [4].

Following the experimental design of the validation studies, 5 g of the microtracer was mixed with 500 g of the *feed additive* preparation (KemTrace 0.4 % Cr). Samples of *feedingstuffs* were prepared by taking 10 g of the latter mixture (containing 9.9 g of KemTrace 0.4 % Cr and 0.1 g of Microtracer FS green lake) and mixing with 100 kg of *feedingstuffs*. This resulted in a theoretical content of 0.4 mg of chromium and *ca*. 50 particles / kg *feedingstuffs* [4].

According to the method, particles of the microtracer are separated from the sample (1000 g) of *feedingstuffs* by using a rotary detector. The particles collected on the rotary detector are further transferred and evenly distributed on a paper filter, which is activated with alkaline sodium carbonate solution. The filter is dried to develop green spots from the dye, which is present on each particle. For the enumeration, the coloured particles are counted visually or by using an automatic counting equipment [4].

In addition, for the enumeration of microtracer particles in the *feed additive* preparation (KemTrace 0.4 % Cr), containing 1 % (w/w) of Microtracer FS-green lake particles, the Applicant proposed the same method as for *feedingstuffs*. The only difference was that the amount of the sample used for the enumeration of the particles in the case of the preparation was 100 mg resulting in *ca*. 50 particles for this sample amount [4].

The content of *organic chromium* from *chromium propionate* in *feedingstuffs* (FS) can be calculated according to the formula by multiplying the conversion factor, which is the ratio of the *organic chromium* content and the number of specified (or measured) particles in the *feed additive* preparation with the number of measured of particles in the compound feed:

$$Cr_{organic}\left(\frac{mg}{kg}\right)_{FS} = conversion\ factor_{FA} * \left[\frac{Number_{Particles}}{kg}\right]_{FS} = \left[\frac{Cr_{organic}\left(\frac{mg}{g}\right)}{\frac{Number_{Particles}}{g}}\right]_{FA} * \left[\frac{Number_{Particles}}{kg}\right]_{FS}$$

The Applicant demonstrated acceptable performance characteristics of the enumeration method in the *feed additive* preparation and *feedingstuffs* in the frame of the validation [7-12] and verification [13-20] studies. In details, the validation and verification measurements were performed on 15 samples on two different days, separately for the *feed additive* preparation and *feedingstuffs*. The experiments also included measurements of the coloured particles in a visual manner and using an automatic counting equipment. In all cases, the relative standard deviation for counting of particles was ranging from 5.2 to 12.1 % and the recovery of the mictrotracer was ranging from 81 to 94 % [7-20].

For the determination of *chromium propionate/organic chromium* in *premixtures* (e.g. mineral/vitamin *premixtures* containing the above mentioned *feed additive* preparation and the microtracer) via the determination of the microtracer, the EURL recommends the use of the above mentioned methods. However, in case of *premixtures*, an appropriate sample amount has to be taken for the analysis in order to have *ca*. 50 particles for the counting.

Conclusions

In the frame of this authorisation the EURL recommends for official control:

- the single-laboratory validated and further verified method based on UHPLC-MS/MS for the determination of the *chromium propionate/organic chromium* content in the *feed additive* preparation (KemTrace 0.4 % Cr) containing 1 % (w/w) of Microtracer FS-green lake particles;
- the single-laboratory validated and further verified method, based on the enumeration of colour coated stainless steel particles (a microtracer), which are included in the *feed additive* preparation (KemTrace 0.4 % Cr) for the enumeration of the microtracer particles in the *feed additive* preparation (KemTrace 0.4 % Cr); and
- the single-laboratory validated and further verified method, based on the enumeration of colour coated stainless steel particles (a microtracer), which are included in the *feed additive* preparation (KemTrace 0.4 % Cr) at fixed mass ratio, for the determination of *chromium propionate/organic chromium* in *premixtures* and *feedingstuffs*.

It is important to underline, that the method based on the enumeration of the mictrotracer particles is applicable when the following criteria are fulfilled: (i) the microtracer has to be well characterised; (ii) the microtracer has to be added into the *feed additive* preparation before the mixing of the product with *premixtures* and *feedingstuffs*; and (iii) the inclusion rate of the microtracer, expressed as number of the particles per mass of the *feed additive*

preparation, has to be specified (e.g. 50 particles / 100 mg micro-tracered *feed additive* preparation in the case of 1 % (w/w) addition rate of the microtracer into the *feed additive* preparation). Moreover, it is recommended that these conditions are included in the Regulation authorising the *feed additive* preparation.

However, the official control for determination of the added content of *chromium propionate/organic chromium* in *premixtures* and *feedingstuffs* is not possible when this specific microtracer is used also for another feed additive(s) and both (all) are added to the same feed.

Recommended text for the registry entry (analytical method) (substituting the previous recommendations)

For the determination of *chromium propionate/organic chromium* in the *feed additive* preparation:

- Ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS)

For the determination of inclusion rate of the microtracer into the *feed additive* preparation:

- Enumeration of colour coated particles of the microtracer

For the determination of the added content of *chromium propionate/organic chromium* in *premixtures* and *feedingstuffs*:

- Enumeration of colour coated particles of the microtracer present at fixed mass ratio to *chromium propionate/organic chromium* content in the *feed additive* preparation

References

[1] EURL evaluation report: FAD-2019-0076 (chromium propionate) – JRC F.5/CvH/ZE/AS/Ares (2020)3710795

[2] *Supplementary information – Request Commission to Applicant.pdf

[3] *Supplementary information – Applicant response on request of methods.pdf

[4] *Supplementary information – MicroTracer FS validation.pdf

[5] *Supplementary information – 2021-SCT-R-2605 - Report_KEMIN_Method validation.pdf

[6] *Supplementary information – UHPLC-MS Verfication_SGS.pdf

[7] *Supplementary information – Annex_9_Kemin Premix Sabine Day 1 25.10.2021.xlsx

[8] *Supplementary information – Annex_10_Kemin Premix Claudia Day 2 26.10.2021.xlsx

[9] *Supplementary information – MTSE Premix-Statistics.xls

[10] *Supplementary information – Annex_11_Kemin Feed Sabine Day 1 25.10.2021.xlsx

[11] *Supplementary information – Annex_12_Kemin Feed Claudia Day 2 26.10.2021.xlsx

[12] *Supplementary information – Annex_13_Overview of Results_Validation.xlsx

[13] *Supplementary information – Verification Premixture.pdf

[14] *Supplementary information – Kemin Premix Mahendra Day1 11.11.2021.xlsx

[15] *Supplementary information – Kemin Premix Antonio Day2_12.11.2021.xlsx

[16] *Supplementary information – Kemin Premix-Statistics.xls

[17] *Supplementary information – Verification Feedingstuff.pdf

[18] *Supplementary information – Kemin Feed Mahendra Day1_11.11.2021.xlsx

[19] *Supplementary information – Kemin Feed AntonioDay2_12.11.2021.xlsx

[20] *Supplementary information – Kemin Feed-Statistics.xls

*Refers to Dossier no: FAD-2019-0076

Acknowledgments

The following National Reference Laboratories contributed to this addendum:

- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Centro di referenza nazionale per la sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Ústřední kontrolní a zkušební ústav zemědělský ÚKZÚZ), Praha (CZ)
- Wageningen Food Safety Research (WFSR)[1] (NL)

Addendum

- Prepared by Zigmas Ezerskis

- Reviewed and approved by Stefano Bellorini and Christoph von Holst (EURL-FA), respectively, Geel, 13/04/2022

[1] Name and address according to according COMMISSION IMPLEMENTING REGULATION (EU) 2015/1761: RIKILT Wageningen UR, Wageningen.

Ref. Ares(2020)3710795 - 14/07/2020

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate F - Health, Consumers & Reference Materials (Geel/Ispra)
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

Chromium propionate

(FAD-2019-0076; CRL/190040)

European Union Reference Laboratory
Feed Additives

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

Dossier related to:	**FAD-2019-0076 - CRL/190040**
Name of Product:	***Chromium propionate***
Active Agent (s):	**Chromium propionate**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
Report prepared by:	**Zigmas Ezerskis**
Report checked by: Date:	**Stefano Bellorini** **14/07/2020**
Report approved by: Date:	**Christoph von Holst** **14/07/2020**

EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 4(1) for *chromium propionate* under the category/functional group (4d) "zootechnical additives"/"other zootechnical additives", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, the authorisation is sought for the use of the *feed additive* for all growing poultry species.

According to the Applicant the active substance of the *feed additive* is *chromium propionate*. The *feed additive* is to be marketed as a liquid preparation with a content of *chromium propionate* ranging from 29 to 32 % (w/w), which corresponds to a *chromium* content ranging from 7 to 10 % (w/w). The *feed additive* is intended to be incorporated into *feedingstuffs* through *premixtures*. The Applicant proposed minimum and maximum levels of the *chromium* content added via the use of chromium propionate, which the Applicant defined as organic *chromium*, ranging from 0.2 to 0.4 mg/kg *feedingstuffs*.

For the quantification of the *chromium propionate* content in the *feed additive* the Applicant submitted two single-laboratory validated methods, namely a method based on liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) and a method based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The LC-MS/MS method was further verified and the following performance characteristics were obtained for the quantification of the *chromium propionate* content in the *feed additive* in the frame of the validation and verification studies: a relative standard deviation for *repeatability* (RSD_r) ranging from 2.0 to 7.2 %, a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 5.5 to 7.9 % and a *recovery* rate (R_{rec}) ranging from 91 to 103 %.

Based on the acceptable performance characteristics available, the EURL recommends for official control the single-laboratory validated and further verified method based on LC-MS/MS for the quantification of the *chromium propionate* content in the *feed additive*.

For the quantification of the *chromium propionate* content in *premixtures* and *feedingstuffs* the Applicant submitted the above mentioned methods based on LC-HRMS and LC-MS/MS after an appropriate sample preparation. However, the Applicant did not provide the EURL with proper validation and/or verification data when applying the LC-HRMS and/or LC-MS/MS methods for the quantification of *chromium propionate* in *premixtures* and *feedingstuffs*.

Based on the available performance information, the EURL is not able to recommend for official control the above mentioned methods based on LC-HRMS or LC-MS/MS for the quantification of the *chromium propionate* content in *premixtures* and *feedingstuffs*.

For the quantification of the total *chromium* content in the *feed additive* the Applicant submitted a single-laboratory validated and further verified method based on inductively coupled plasma-atomic emission spectrometry (ICP-AES). The following performance characteristics were obtained for the quantification of the total *chromium* content in the *feed additive* in the frame of the validation and verification studies: a RSD_r ranging from 0.3 to 0.9 %, a RSD_{ip} ranging from 0.9 to 1.1 % and a R_{rec} of 100 %.

Based on the acceptable performance characteristics available, the EURL recommends for official control the single-laboratory validated and further verified method based on ICP-AES for the quantification of the total *chromium* content in the *feed additive* (*chromium propionate*).

For the quantification of the organic *chromium* content in mineral-vitamin *premixtures* and *feedingstuffs* the Applicant proposed in-house methods based on ICP-AES and/or ICP-MS. Non-acceptable recoveries (lower than 60 %) were reported for an average organic *chromium* content in the analysed samples of *premixtures* and *feedingstuffs*.

Based on the available data, the EURL is not able to recommend for official control the proposed methods based on ICP-AES or ICP-MS, neither any other method for the quantification of the organic *chromium* content 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

Chromium propionate, *chromium*, zootechnical additives, all growing poultry species

1. BACKGROUND

In the current application an authorisation is sought under Article 4(1) (new *feed additive*) for *chromium propionate* under the category/ functional group (4d) "zootechnical additives"/"other zootechnical additives ", according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1]. Specifically, the authorisation is sought for the use of the *feed additive* for all growing poultry species [1,2].

According to the Applicant the active substance of the *feed additive* is *chromium propionate* [3]. The *feed additive* is to be marketed as a liquid preparation with a content of *chromium propionate* ranging from 29 to 32 % (w/w), which corresponds to a *chromium* content ranging from 7 to 10 % (w/w). The *feed additive* contains also propionic acid, sodium propionate, propylene glycol and water [2,3].

The *feed additive* is intended to be incorporated into *feedingstuffs* through *premixtures* [4].

The Applicant proposed minimum and maximum levels of the *chromium* content added via the use of chromium propionate, which the Applicant defined as organic *chromium*, ranging from 0.2 to 0.4 mg/kg *feedingstuffs* [2,3].

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 *chromium propionate* 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)

Chromium propionate

The Applicant applied two different methods for the determination of *chromium propionate* in the various matrices, based on liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).

Feed additive:

For the quantification of the *chromium propionate* content in the *feed additive* the Applicant proposed [5] and submitted a single-laboratory validated method based on LC-HRMS [6,7].

The following performance characteristics for the quantification of the *chromium propionate* content in three batches of the *feed additive* were obtained in the frame of the validation studies [7]: a relative standard deviation for *repeatability* (RSD_r) ranging from 2.3 to 5.1 %, a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 4.5 to 7.2 % and a *recovery* rate (R_{rec}) of 81 %. However, the Applicant submitted no verification studies for the quantification of *chromium propionate* in the *feed additive* when applying this LC-HRMS method.

In addition, for the quantification of the *chromium propionate* content in the *feed additive* the Applicant submitted a single-laboratory validated and further verified method based on LC-MS/MS [6,8].

The sample (0.2 g) of the *feed additive* is extracted with a mixture of 20 ml of acetonitrile:water (1:1, v/v) for 20 min in an ultrasonic bath. An aliquot of the extract (1.5 ml) is centrifuged and the supernatant, after an appropriate dilution with the above mentioned mixture, is analysed by LC-MS/MS using a positive electrospray ionisation (+ESI) and multiple reaction monitoring mode (MRM). The following m/z transitions were acquired: 610 > 356; 610.0 > 209 and 610 > 125. The transition 610.0 > 209 is used for the quantification, while the other two ones are used for the identification. The quantification is performed by an external calibration curve using diluted aqueous solutions of chromium propionate as a standard substance [6,8].

The performance characteristics obtained for the quantification of the *chromium propionate* content in the *feed additive* in the frame of the validation [8] and verification [9] studies are summarised in Table 1. Based on the acceptable performance characteristics available, the EURL recommends for official control the single-laboratory validated and further verified method based on LC-MS/MS for the quantification of the *chromium propionate* content in the *feed additive*.

Premixtures:

For the quantification of the *chromium propionate* content in *premixtures* the Applicant proposed [5] the above mentioned method based on LC-HRMS [6]. The Applicant analysed samples of commercial *premixtures* ("KemTRACE Cr 0.4 %") [6], where the *feed additive* is sprayed on limestone and of vitamin-mineral *premixtures* in the frame of a homogeneity study [10]. However, the Applicant submitted no verification studies for the quantification of *chromium propionate* in *premixtures* when applying the above mentioned LC-HRMS method.

Furthermore, for the quantification of the *chromium propionate* content in *premixtures* the Applicant applied the above mentioned LC-MS/MS method [6]. The Applicant analysed a sample of commercial *premixtures* ("KemTRACE Cr 0.4 %") and a RSD_r of 4.8 % and a R_{rec} of 102 % were obtained for an average content of *chromium propionate* of 17800 mg/kg *premixtures* [6].

Table 1: Performance characteristics of the LC-MS/MS method for the quantification of *chromium propionate* in the *feed additive* in the frame of the validation and verification studies

	Validation	Verification
Method	LC-MS/MS	
Mass fraction (%)	31.9	30.0
RSD_r (%)	7.2	2.0 – 5.5
RSD_{ip} (%)	7.9	5.5
R_{rec} (%)	91 – 98	103
Reference	[8]	[9]

RSD_r and RSD_{ip}: relative standard deviation for *repeatability* and for *intermediate precision;* R_{rec}: recovery rate.

The method performance studies performed with vitamin-mineral *premixtures* led to a RSD_r and a RSD_{ip} ranging from 6.6 to 10.4 % and a R_{rec} of 95 % for the content of 5943 mg *chromium propionate*/kg *premixtures* [11]. However, the report of this study contains also the statement that the use of the LC-MS/MS instrumentation/method gives unreliable results [11]. Moreover, the Applicant did not submit method performance studies obtained in another laboratory for the quantification of *chromium propionate* in *premixtures* when applying the above mentioned LC-MS/MS method.

Based on the available performance information, the EURL is not able to recommend for official control the above mentioned methods based on LC-HRMS or LC-MS/MS for the quantification of *chromium propionate* content in *premixtures*.

Feedingstuffs:

For the quantification of the *chromium propionate* content in *feedingstuffs* the Applicant applied the above mentioned LC-MS/MS method after an appropriate sample preparation [6]. Spiked samples containing 0.1 mg and 1 mg of *chromium propionate* /kg *feedingstuffs* have been analysed. The following performance characteristics were reported by the Applicant: a RSD_r ranging from 3.0 to 5.3 % and a R_{rec} ranging from 87 to 96 %. A limit of quantification (LOQ) of 0.05 mg of *chromium propionate*/kg *feedingstuffs* has been reported [6].

In addition, the Applicant used the above mentioned LC-HRMS method for the quantification of *chromium propionate* in five spiked feed samples demonstrating a RSD_r of 4.2 % and a R_{rec} of 85 % for an average content of *chromium propionate* of 740 mg/kg *feedingstuffs*. In addition, a limit of quantification (LOQ) of 0.04 mg of *chromium propionate*/kg *feedingstuffs* has been reported [6].

However, no verification studies for the quantification of *chromium propionate* in *feedingstuffs* were presented by the Applicant when applying the above mentioned LC-MS/MS or LC-HRMS methods.

Based on the available data, the EURL is not able recommend for official control the above mentioned methods based on LC-MS/MS or LC-HRMS for the quantification of the *chromium propionate* content in *feedingstuffs*.

Chromium

For the quantification of the total *chromium* content in the *feed additive* the Applicant submitted a single-laboratory validated and further verified method based on inductively coupled plasma-atomic emission spectrometry (ICP-AES) [12,13].

The sample (0.2 g) is dissolved in a mixture of acetonitrile:water (1:1, v/v). An aliquot of the solution is digested with hydrochloric and nitric acid at 105 °C for 2 h. The digestion solution is diluted with water for further ICP-AES analysis [12,13]. For the determination *chromium* the following emission wavelengths were used: 205.560 nm (for the quantification), 206.158 and 267.716 nm (for the identification). The quantification is performed by an external calibration using multi-elemental commercial mixtures containing *chromium* as a standard substance [13]. In addition, an internal standard (rhodium nitrate) is used for the correction of matrix effects [12,13].

The performance characteristics reported for the quantification of the total *chromium* content in the *feed additive* (*chromium propionate*) in the frame of the validation [7] and verification [14] studies are summarised in Table 2.

Based on the acceptable performance characteristics available, the EURL recommends for official control the single-laboratory validated and further verified method based on ICP-AES for the quantification of the total *chromium* content in the *feed additive* (*chromium propionate*).

Note: During the review process, some NRLs suggested using a pressure digestion as described in the EN 15621 and EN 17053 standard methods, when determining total *chromium* in the *feed additive*. The EURL agrees that the *feed additive* samples can be digested by pressure digestion with the condition that this type of the sample preparation does not affect negatively the overall performance characteristics of the ICP-AES method.

Table 2: Performance characteristics for the quantification of total *chromium* content in the *feed additive (chromium propionate)* in the frame of the validation and verification studies

	Validation	Verification
Method	ICP-AES	
Mass fraction (%)	8.54 – 8.70	8.6
RSD_r (%)	0.3 – 0.9	0.8
RSD_{ip} (%)	0.9*	1.1
R_{rec} (%)	100	100
Reference	[7]	[14]

RSD_r and RSD_{ip}: relative standard deviation for *repeatability* and for *intermediate precision;* R_{rec}: recovery rate; *derived from the largest value of RSD_r.

For the quantification of the organic *chromium* content in *premixtures* the Applicant proposed [5] an in-house method based on ICP-AES, which was used for the stability and homogeneity studies of the organic *chromium* content in *premixtures* [10]. According to the method, the sample is extracted with a mixture of acetonitrile and water (1:1, v/v). The extract is further digested after an evaporation with a mixture of nitric acid and hydrogen peroxide for further analysis by ICP-AES [10].

In the frame of the homogeneity study the Applicant has analysed ten samples of vitamin-mineral *premixtures* and reported a RSD_r of 8.2 % for an average content of organic *chromium* (expressed as *chromium*) of 46.2 mg/kg *premixures* [10]. In addition, the Applicant presented the results of the verification studies, where samples for organic *chromium* in vitamin-mineral *premixtures* have been analysed by using an inductively coupled plasma-mass spectrometry (ICP-MS) method [15] instead of the above mentioned ICP-AES method [10]. A precision (RSD_r and RSD_{ip}) ranging from 10 to 13 % and a R_{rec} lower than 60 % were reported for an average organic *chromium* content (expressed as *chromium*) of 1520 mg/kg *premixtures* [15].

Based on the available data, the EURL is not able to recommend for official control the proposed method based on ICP-AES or any other method for the quantification of the organic *chromium* content in *premixtures*.

For the quantification of the organic *chromium* content in *feedingstuffs* the Applicant proposed [5] in-house methods based on ICP-AES or ICP-MS, which were used for the stability and homogeneity studies of the organic *chromium* content in *feedingstuffs* [16]. In addition, the Applicant presented the results of the verification studies, where samples for organic *chromium* in *feedingstuffs* (mash and pelleted feed) have been analysed by using the

ICP-MS method [17,18]. The overall results demonstrated very low recoveries obtained when quantifying the organic *chromium* content of 0.4 mg/kg *feedingstuffs* [16-18].

Based on the available data, the EURL is not able to recommend for official control the proposed methods based on ICP-AES or ICP-MS, neither any other method for the quantification of the organic *chromium* content in *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)

For the quantification of *chromium* in *tissues* (i.e. muscle, skin, fat, kidney, spleen and liver) the Applicant proposed [5] the two standard methods, namely EN 17053 based on inductively coupled plasma-mass spectrometry [19] and EN 14083 based on graphite furnace atomic absorption spectrometry (GF-AAS) after pressure digestion [20]. However, the determination of *chromium* in animal *tissues* is not within the scope of the EN 17053 method [19].

The EN 14083 method was ring-trial validated for the quantification of *chromium* in beef liver and muscle. The RSD_r and RSD_R reported for the determination of a *chromium* content of 0.06 mg/kg beef muscle were 20.2 and 53.0 %, respectively; and the RSD_r and RSD_R reported for a *chromium* content of 190 mg/kg beef liver were 17.4 and 27.0 %, respectively. In addition, an average reported R_{rec} for the analysis of the chromium content in the certified reference material pig liver (ESB-SC-JUELICH) was 94 % [20].

Based on the performance information, the EURL is considering the EN 14083 method as fit-for-purpose for the quantification of *chromium* in the animal tissues within the concentration range investigated.

In addition, the Applicant submitted two single-laboratory validated methods based on isotope dilution using an inductively coupled plasma - sector field mass spectrometer (ID-ICP-SFMS) and graphite furnace-atomic absorption spectrometry (GF-AAS), respectively [21]. The Applicant performed some performance studies of both methods by analysing samples of animal muscle, fat, spleen, kidney and liver, without indicating which type of animal *tissues* have been analysed. Very large RSD_r values (up to 160 %) for the mass fraction of the *chromium* content in the *tissues* ranging from 10 to 500 µg/kg were reported when applying both methods [21]. Furthermore, no verification studies were submitted for the quantification of the *chromium* content in the *tissues*.

Based on the available data, the EURL is not able to conclude on the fitness-for-purpose of the above mentioned single-laboratory validated ID-ICP-SFMS and GF-AAS methods for the quantification of *chromium* in the analysed *tissues* at the concentration range investigated.

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

An evaluation of corresponding methods of analysis is not considered necessary by the EURL.

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 liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) for the quantification of *chromium propionate* in the *feed additive* and
- the single-laboratory validated and further verified method based on inductively coupled plasma - atomic emission spectrometry (ICP-AES) for the quantification of the total *chromium* content in the *feed additive*

Based on non-acceptable performance profiles of the methods proposed for the quantification of *chromium* propionate and the organic *chromium* content in *premixtures* and *feedingstuffs*, the EURL is not able to recommend any method for official control for the quantification of *chromium propionate* and the organic *chromium* content in *premixtures* and *feedingstuffs*.

Note: During the review process, some NRLs suggested using a pressure digestion as described in the EN 15621 and EN 17053 standard methods, when determining total *chromium* in the *feed additive*. The EURL agrees that the *feed additive* samples can be digested by pressure digestion with the condition that this type of the sample preparation does not affect negatively the overall performance characteristics of the ICP-AES method.

Recommended text for the register entry (analytical method)

For the quantification of *chromium propionate* in the *feed additive*:

- Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS)

For the quantification of total *chromium* in the *feed additive*:

- Inductively coupled plasma - atomic emission spectrometry (ICP-AES)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Chromium propionate* 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 SANTE_E5_FWD. APPL. 1831-0076-2020 & Application, Annex 1 – submission number 1574175406057-2474

[2] *Application, – Annex A

[3] *Technical dossier, Section II, 2.1. Identity of the additive

[4] *Technical dossier, Section II, 2.5. Conditions of use of the additive

[5] *Technical dossier, Section II, 2.6. Methods of analysis and reference samples

[6] *Technical dossier, Section II – Annex_II_28

[7] *Technical dossier, Section II – Annex_II_13

[8] *Supplementary information – KEMIN_Cr Prop_Validation study LC-MS

[9] *Technical dossier, Section II – Annex_II_29

[10] *Technical dossier, Section II – Annex_II_22

[11] *Technical dossier, Section II – Annex_II_30

[12] *Technical dossier, Section II – Annex_II_3

[13] *Supplementary information – KEMIN_Cr Prop base liquid_update 2018Dec_rev2019_addICP2020 (003)

[14] *Technical dossier, Section II – Annex_II_31

[15] *Technical dossier, Section II – Annex_II_32

[16] *Technical dossier, Section II – Annex_II_23

[17] *Technical dossier, Section II – Annex_II_33

[18] *Technical dossier, Section II – Annex_II_34

[19] EN 17053:2018 – *Animal feeding stuffs: Methods of sampling and analysis – Determination of trace elements, heavy metals and other elements in feed by ICP-MS (multi-method)*

[20] *EN 14083:2003 – Foodstuffs. Determination of trace elements. Determination of lead, cadmium, chromium and molybdenum by graphite furnace atomic absorption spectrometry (GFAAS) after pressure digestion*

[21] *Technical dossier, Section II – Annex_II_35

*Refers to Dossier no: FAD-2019-0076

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)
- [1]Wageningen Food Safety Research (WFSR) (NL)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, PESCA, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Centro di referenza nazionale per la sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- [2]Ruokavirasto Helsinki (FI)
- Univerza v Ljubljani. Veterinarska fakulteta. Nacionalni veterinarski inštitut. Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)

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