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**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**

Sunset Yellow FCF
(FAD-2010-0366; CRL/100338)



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in connection with the Application for Authorisation of a
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Dossier related to: **FAD-2010-0366 - CRL/100338**

Name of Product / Feed Additive: ***Sunset Yellow FCF***

Active Agent (s): **Sunset Yellow FCF**

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

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Date: **17/04/2020**

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Date: **20/04/2020**

EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 10(2) for *Sunset Yellow FCF* under the category/functional group 2(a) "sensory additives"/"colourants", 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 cats and dogs, ornamental fish, grain-eating ornamental birds and small rodents.

The *feed additive* is a synthetic orange-red powder or granules soluble in water, consisting of a minimum of 85 % (w/w) of "total colouring matters content" calculated as sodium salt of 6-hydroxy-5-[(sulfophenyl)azo]-2-naphthalene sulfonate (*Sunset Yellow FCF*). The Applicant states that the purity criteria for *Sunset Yellow FCF* as a food additive set in the Commission Directive 2008/128/EC (superseded by the Commission Regulation (EU) 2012/231) are applicable also for the current *feed additive*.

Sunset Yellow FCF is intended to be incorporated directly in *feedingstuffs* or as a solution in *water*. The Applicant proposed a maximum content of 150 mg/kg complete *feedingstuffs* only for grain-eating ornamental birds and small rodents.

For the determination of the "total colouring matters content" in the *feed additive*, the Applicant submitted the internationally recognised FAO JECFA monographs for food additives (recommended by Commission Regulation (EU) 2012/231), where the determination of the total colouring matters content of *Sunset Yellow FCF* is based on (i) spectrophotometry and (ii) titration with titanous chloride.

The EURL recommends for official control the above mentioned methods recommended by Commission Regulation (EU) 2012/231 and described in the FAO JECFA monographs for the determination of *Sunset Yellow FCF* in the *feed additive*.

For the determination of *Sunset Yellow FCF* in *feedingstuffs* the Applicant submitted a single-laboratory validated and further verified method based on high performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).

The following performance characteristics were obtained in the frame of the validation and verification studies for the determination of *Sunset Yellow FCF* in *feedingstuffs* with the mass fractions ranging from 1 to 100 mg/kg: a relative standard deviation for *repeatability* (RSD_r) ranging from 2.0 to 7.6 %, a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 2.8 to 16.4 %, a recovery rate (R_{Rec}) ranging from 83 to 101 % and a limit of quantification (LOQ) of 1 mg for *Sunset Yellow FCF* /kg *feedingstuffs*.

In addition, samples of a few commercial pet feed products (kibbles) have been analysed using the above mentioned method and acceptable precision was demonstrated. However, a

significantly lower mass fraction of *Sunset Yellow FCF* compared to the expected value was measured in one of the samples.

The Applicant has attributed the lower mass fraction observed in the sample to a lack of homogeneity of the samples and/or the adverse impact of specific production conditions of the kibbles. It is therefore recommended that additional measures are taken for checking the documentation related to the specific characteristics of the production process of the complete *feedingstuffs* in case when significantly lower mass fractions of the colourant in comparison to the ones indicated on the labels are obtained during the official control of pet feed samples.

The Applicant did not provide to the EURL a method for the determination of the above mentioned colourant in *premixtures*, as the *feed additives* (in the form of powder or as the solutions in water) are supposed to be added directly into *feedingstuffs*.

Based on the performance characteristics available the EURL recommends for official control a single laboratory validated and verified LC-MS/MS method for the determination of *Sunset Yellow FCF* in *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

Sunset Yellow FCF, sensory additives, colourants, cats and dogs, ornamental fish, grain-eating ornamental birds, small rodents

1. BACKGROUND

In the current application an authorisation is sought under Article 10(2) (re-evaluation of additives already authorised under the provisions of the Council Directive 70/524/EEC) for *Sunset Yellow FCF* under the category/functional group 2(a) "sensory additives"/"colourants", 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 cats and dogs, ornamental fish, grain-eating ornamental birds and small rodents [1,2].

The *feed additive* is a synthetic orange-red powder or granules soluble in water [3], consisting of a minimum of 85 % (w/w) of "total colouring matters content" calculated as sodium salt of 6-hydroxy-5-[(sulphophenyl)azo]-2-naphthalene sulfonate (*Sunset Yellow FCF*) [2,3]. The Applicant states that the purity criteria for *Sunset Yellow FCF* as a food additive set in the Commission Directive 2008/128/EC (superseded by the Commission Regulation (EU) 2012/231 [4]) are applicable also for the current *feed additive* [3]. *Sunset Yellow FCF* is

intended to be incorporated directly in *feedingstuffs* or as a solution in *water*. The Applicant proposed a maximum content of 150 mg/kg complete *feedingstuffs* only for grain-eating ornamental birds and small rodents [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 *Sunset Yellow FCF* 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 determination of the "total colouring matters content" in the *feed additive*, the Applicant submitted the internationally recognised FAO JECFA monographs for food additives [5,6] (recommended by Commission Regulation (EU) 2012/231), where the determination of total colouring matters content of *Sunset Yellow FCF* is based on (i) spectrophotometry at 485 nm in aqueous solution at pH 7, as specified and described in Commission Regulation (EU) 2012/231 [4] and Procedure 1 of the generic JECFA monograph [5]; and (ii) titration with titanous chloride as described in the specific JECFA monograph for *Sunset Yellow FCF* [6] and the Procedure 3 of the generic JECFA monograph [5].

The EURL recommends for official control the above mentioned methods recommended by Commission Regulation (EU) 2012/231 and described in the FAO JECFA monographs for the determination of *Sunset Yellow FCF* in the *feed additive*.

For the determination of *Sunset Yellow FCF* in *feedingstuffs* the Applicant submitted a single-laboratory validated and further verified method based on high performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) [7].

The sample (5 g) is sonicated with a mixture of methanol, aqueous bicarbonate solution and acetonitrile, shaken and centrifuged. The supernatant is separated and the extraction is repeated for the second time. The supernatants from the two extractions are combined, diluted

and an aliquot is evaporated after the dilution until dryness. The residue is dissolved in the mobile phase for further LC-MS/MS analysis. The analyte of interest is detected by mass spectrometry and the determination is performed in the multiple reaction monitoring (MRM) mode by using calibration with external standards [7]. The matrix-match calibration can be considered as well with the condition that it helps to compensate for the matrix effects when analysing samples in the frame of the official control.

The performance characteristics obtained in the frame of the validation [8] and the verification studies [9] for the determination of the above mentioned colourant in spiked samples of *feedingstuffs* are presented in Table 1. In addition, the Applicant reported a limit of quantification (LOQ) of 1 mg for *Sunset Yellow FCF* /kg *feedingstuffs* [8].

In addition, samples of a few commercial pet feed products (kibbles) have been analysed using the above mentioned method and acceptable precision was demonstrated. However, a significantly lower mass fraction of *Sunset Yellow FCF* compared to the expected value was measured in one of the sample [8]. The Applicant has attributed the lower mass fraction observed in the sample to a lack of homogeneity of the samples and/or the adverse impact of specific production conditions of the kibbles on these values [8]. If significantly lower mass fraction of the colourant than the ones indicated on the labels is obtained during the official control of pet feed samples, it is recommended that additional measures are taken for checking the documentation related to the specific characteristics of the production process of the complete *feedingstuffs*.

Table 1. The performance characteristics obtained in the frame of the validation and the verification studies for the determination of *Sunset Yellow FCF* in spiked samples of *feedingstuffs*.

	Validation	Verification
Mass fraction, mg/kg	1 – 100	10
RSD_r, %	2.0 – 7.6	2.8
RSD_{ip}, %	2.8 – 16.4	2.8
R_{rec}, %	83 – 97	101
Reference	[8]	[9]

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

The Applicant did not provide to the EURL a method for the determination of the above mentioned colourant in *premixtures* as the *feed additives* (in the form of powder or as the solutions in water) are supposed to be added directly into *feedingstuffs*.

Based on the performance characteristics available the EURL recommends for official control a single laboratory validated and verified LC-MS/MS method for the determination of *Sunset Yellow FCF* 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)

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 identification of *Sunset Yellow FCF*, the EURL considers as fit-for-purpose a spectrophotometric measurement at 485 nm in aqueous solution at pH 7.0 and a comparison of retention factors (R_f) determined by thin-layer chromatography (TLC) using several chromatographic conditions as specified and described in Commission Regulation (EU) 2012/231 [4] and the above mentioned JECFA monographs [5,6].

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 (i) the methods based on spectrophotometry at 485 nm and titration with titanous chloride as specified and described in Commission Regulation (EU) 2012/231 which refers to FAO JECFA Combined Compendium for Food Additive Specifications (Analytical methods Vol. 4) and the Monograph No. 11 (2011) "*Sunset Yellow FCF*" for the determination of the total colouring matters content of *Sunset Yellow FCF* in the *feed additive* and (ii) a single laboratory validated and verified method based on high performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) for the determination of *Sunset Yellow FCF* in *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the determination of the total colouring matters content of *Sunset Yellow FCF* in the *feed additive*:

- spectrophotometry at 485 nm and titration with titanin chloride as specified and described in Commission Regulation (EU) 2012/231 which refers to FAO JECFA Combined Compendium for Food Additive Specifications (Analytical methods Vol. 4) and the Monograph No. 11 (2011) "*Sunset Yellow FCF*".

For the determination of *Sunset Yellow FCF* in *feedingstuffs*:

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

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Sunset Yellow FCF* 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. 1831-0077-2019 & Annex I – submission number 1288638029069-1306
- [2] *Application, proposal for Register entry – Annex A
- [3] *Technical dossier, Section II: Identify, characterisation and conditions of use of the additive; methods of analysis
- [4] Commission Regulation (EU) No 231/2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council
- [5] FAO JECFA Combined Compendium for Food Additive Specifications - *Analytical methods, test procedures and laboratory solutions used by and referenced in the food additive specifications*, Monographs No. 1, Vol. 4
<http://www.fao.org/docrep/009/a0691e/a0691e00.htm> (last visited on 25/02/2020)
- [6] FAO JECFA Combined Compendium of Food Additive Specifications, *Sunset Yellow FCF*, Monograph No. 11 (2011)
http://www.fao.org/fileadmin/user_upload/jecfa_additives/docs/monograph11/additive-450-m11.pdf (last visited on 25/02/2020)
- [7] *Supplementary Information – RV77
- [8] *Supplementary Information – Synthetic dyes quantification in pet food (validation file)
- [9] *Supplementary Information – eurl-VF_E110

*Refers to Dossier no: FAD-2010-0366

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)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Wageningen Food Safety Research¹ (WFSR) (NL)
- Laboratoire de Rennes (SCL L35), Service Commun des Laboratoires DGCCRF et DGDDI, Rennes (FR)
- Instytut Zootechniki — Państwowy Instytut Badawczy, Krajowe Laboratorium Pasz, Lublin (PL)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)

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