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European Union Reference Laboratory for Feed Additives

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

Dossier related to:	FAD-2010-0345 CRL/100314
Feed additive:	Quinoline Yellow (E 104)
Active Substance(s):	Quinoline Yellow
Rapporteur Laboratory:	European Union Reference Laboratory for Feed Additives (EURL-FA) Geel, Belgium
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EXECUTIVE SUMMARY

In the current application authorisation is sought under articles 4(1) and 10(2) for *Quinoline Yellow* under the category/functional group group 2(a)i "sensory additives"/"colourants - substances that add or restore colour in feedingstuffs", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* for all animal species and categories.

Quinoline Yellow is a synthetic yellow powder or granules, soluble in water, consisting of a minimum of 70 % of total colouring matters content calculated as the sodium salts. The total colouring matters content of *Quinoline Yellow* contains at least 84 % of disodium 2-(2-quinolyl)indan-1,3-dione-disulfonates (principal component) and a maximum of 11 % of sodium 2-(2-quinolyl)indan-1,3-dione-monsulfonates and 7 % trisodium 2-(2-quinolyl)indan-1,3-dione-trisulfonates. Furthermore, the purity criteria set in the Commission Directive 2008/128/EC for the food additive apply to the requirement for the *feed additive*. *Quinoline Yellow* is intended to be incorporated directly in *feedingstuffs* as a solution in *water* (either added directly as a solid to the *feedingstuffs* in the presence of water or by addition of an aqueous solution), with a maximum content of 100 mg/kg *feedingstuffs*.

For the determination of total colouring matters content of *Quinoline Yellow* in the *feed additive*, the Applicant proposed the internationally recognised FAO JECFA monograph for food additives. Identification and quantification of total colouring matters content of *Quinoline Yellow* is based on spectrophotometry at 411 nm in aqueous acetic acid solution, as recommended by Commission Directive 2008/128/EC laying down specific purity criteria concerning colours for use in foodstuffs. The total colouring matters content is quantified using JECFA Procedure 1. The Applicant applied this JECFA method to quantify the total colouring matters content of *Quinoline Yellow* in the *feed additive* and reported acceptable performance characteristics.

The Applicant applied the JEFCA method mentioned above for the quantification of total colouring matters content of *Quinoline Yellow* in *feedingstuffs*. The following performance characteristics were reported for a *Quinoline Yellow* concentration in *feedingstuffs* ranging from 5 to 25 mg/kg: - RSD_r ranging from 2.9 to 5.7 %; - a *recovery* rate (R_{rec}) ranging from 79 to 96 %. The Applicant used the lowest concentration analysed to derive a limit of quantification (LOQ) of 5 mg/kg *feedingstuffs*.

Based on the experimental evidence and the performance characteristics provided by the Applicant, the EURL recommends for official control the JECFA monograph method - recommended by Commission Directive 2008/128/EC - based on spectrophotometry at 411 nm - for the quantification of total colouring matters content of *Quinoline Yellow* in the

feed additive and *feedingstuffs*. However, other colouring substances present in the *feedingstuffs* may absorb/interfer in the 400-500 nm range, thus influencing the determination of total colouring matters content of *Quinoline Yellow*. Whenever the spectrophotometric reported values are above the maximum content, an additional chromatographic separation may be required for confirmation.

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

Quinoline Yellow, sensory additives, colourants, all animal species and categories

1. BACKGROUND

In the current application authorisation is sought under articles 4(1) (new use) and 10(2) (re-evaluation of additives already authorised under the provisions of the Council Directive 70/524/EEC) for *Quinoline Yellow* under the category/functional group 2(a)i "sensory additives"/"colourants - substances that add or restore colour in feedingstuffs" [1], according to the classification system of Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* for all animal species and categories [2].

Quinoline Yellow is a synthetic yellow powder or granules, soluble in water (130 g/L at 20°C) [3], consisting of a minimum of 70 % of total colouring matters content calculated as the sodium salts [2]. The total colouring matters content of *Quinoline Yellow* contains at least 84 % of disodium 2-(2-quinolyl)indan-1,3-dione-disulfonates (principal component) and a maximum of 11 % of sodium 2-(2-quinolyl)indan-1,3-dione-monsulfonates and 7 % trisodium 2-(2-quinolyl)indan-1,3-dione-trisulfonates [3]. The Applicant states that the purity criteria set in the Commission Directive 2008/128/EC for the food additive apply to the requirement for the *feed additive* [3]. *Quinoline Yellow* is intended to be incorporated directly in *feedingstuffs* as a solution in *water* (either added directly as a solid to the feedingstuffs in the presence of water or by addition of an aqueous solution), with a maximum content of 100 mg/kg *feedingstuffs* [2].

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 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 *Quinoline Yellow*, and their suitability to be used for official controls in the frame of the authorisation, were evaluated.

3. EVALUATION

Identification /Characterisation 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, lead, mercury, mycotoxins, and dioxins) are available from the respective European Union Reference Laboratories [4].

Description of the analytical methods for the determination of the active substance in feed additive, premixtures and feedingstuffs

For the determination of total colouring matters content of *Quinoline Yellow* in the *feed additive*, the Applicant proposed the internationally recognised FAO JECFA monograph for food additives [5], where:

- Identification of *Quinoline Yellow* is based on (i) spectrophotometry at 411 nm in water at pH 5 and (ii) Thin Layer Chromatography (TLC) with Retention factors (R_f) determined using several chromatographic conditions for confirmation, while
- Quantification of total colouring matters content of *Quinoline Yellow* is based on spectrophotometry at 411 nm in aqueous acetic acid solution, as recommended by Commission Directive 2008/128/EC. Total colouring matters content is quantified using JECFA Procedure 1 [5].

The Applicant applied this JECFA method to quantify the total colouring matters content of *Quinoline Yellow* in the *feed additive* and reported a relative standard deviation for *repeatability* (RSD_r) of 0.7 % and a minimum total colouring matters content of 70 % [6].

Furthermore, the Applicant suggested High Performance Liquid Chromatography (HPLC) with UV/visible spectrophometry detection (HPLC-UV/Vis) for the determination of three main constituents of *Quinoline Yellow*:

- disodium 2-(2-quinolyl)indan-1,3-dione-disulfonates,
- sodium 2-(2-quinolyl)indan-1,3-dione-monosulfonates,
- trisodium 2-(2-quinolyl)indan-1,3-dione-trisulfonates,

and provided the reference chromatogram showing the characteristic profile of *Quinoline Yellow* [7], to confirm the technical specifications required by Commission Directive 2008/128/EC.

The Applicant applied the JEFCA method mentioned above for the quantification of total colouring matters content of *Quinoline Yellow* in *feedingstuffs* [5]. The sample is extracted with purified water. The aqueous solution is then filtered. The filtrate is adjusted to pH 5 with sodium hydroxide and acetic acid buffer solutions for further analysis. Total colouring matters content of *Quinoline Yellow* is determined by spectrophotometry at 411 nm using the reference absorption value ($E_{1cm}^{1\%}$ = 865) of total colouring matters content of *Quinoline Yellow* solution.

The following performance characteristics were reported for a *Quinoline Yellow* concentration in *feedingstuffs* ranging from 5 to 25 mg/kg [8]:

- RSD_r ranging from 2.9 to 5.7 %; and
- a *recovery* rate (R_{rec}) ranging from 79 to 96 %.

The Applicant used the lowest concentration analysed to derive a limit of quantification (LOQ) of 5 mg/kg *feedingstuffs*.

Based on the experimental evidence and the performance characteristics provided by the Applicant, the EURL recommends for official control the JECFA monograph method - recommended by Commission Directive 2008/128/EC - based on spectrophotometry at 411 nm - for the quantification of total colouring matters content of *Quinoline Yellow* in the *feed additive* and *feedingstuffs*.

However, other colouring substances present in the *feedingstuffs* may absorb/interfer in the 400-500 nm range, thus influencing the determination of total colouring matters content of *Quinoline Yellow*. Whenever the spectrophotometric reported values are above the maximum content, an additional chromatographic separation may be required for confirmation.

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

In the frame of this authorisation the EURL recommends for official control the identification tests and the spectrophotometric method (at 411 nm) recommended by Commission Directive 2008/128/EC and described in the JECFA monographs No. 1 (Vol. 4), Combined Compendium for Food Additive Specifications, for the determination of total colouring matters content of *Quinoline Yellow* in *feed additive* and *feedingstuffs*.

However, other colouring substances present in the *feedingstuffs* may absorb/interfer in the 400-500 nm range, thus influencing the determination of total colouring matters content of *Quinoline Yellow*. Whenever the spectrophotometric reported values are above the maximum content, an additional chromatographic separation may be required for confirmation.

Recommended text for the register entry (analytical method)

For the quantification of total colouring matters content of *Quinoline Yellow* in the *feed additive* and *feedingstuffs*:

- Spectrophotometry at 411 nm (Commission Directive 2008/128/EC referring to FAO JECFA monographs No. 1, Vol. 4)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Quinoline Yellow* 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/D/2 Forw. Appl. 1831/(00177) (10505)-2010

[2] *Application, Proposal for Register Entry – Annex A

[3] *Technical dossier, Section II: Identity, characterisation and conditions of use of the additive; Methods of analysis

[4] 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

[5] FAO JECFA monographs No. 1 (Vol. 4), Combined Compendium for Food Additive Specifications

[6] *Technical dossier, Section II - Annex II_5 – validation report_feed additive

[7] *Supplementary information – Chromatographic profile of Quinoline Yellow

[8] *Supplementary information – Validation report_feedingstuffs

*Refers to Dossier No. FAD-2010-0345

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was European Union 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, as last amended by Regulation (EC) No 885/2009.

8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- 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)
- Skúšobné laboratórium – Oddelenie analýzy krmív, Ústredný kontrolný a skúšobný ústav poľnohospodársky, Bratislava (SK)
- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)
- Univerza v Ljubljani, Veterinarska fakulteta. Nacionalni veterinarski inštitut, Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)