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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**

**Tertiary-Butylhydroquinone (TBHQ)**  
*(FAD-2012-0021; CRL/120008)*



**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-2012-0021 - CRL/120008**

Name of Product: ***Tertiary-Butylhydroquinone (TBHQ)***

Active Agent (s): **Tertiary-Butylhydroquinone**

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

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Date: **08/06/2015**

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Date: **08/06/2015**

## EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *Tertiary-Butylhydroquinone (TBHQ)* under the category/functional group 1(b) 'technological additives'/'antioxidants' according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for all categories and species.

According to the Applicant *TBHQ* is a white crystalline powder with a minimum purity of 99%. The Applicant states that the purity criteria/technical specifications set in Commission Regulation (EU) 231/2012 for the food additive apply also to the *feed additive*.

The *feed additive* is intended to be used into *feedingstuffs* through liquid or solid *premixtures*. The Applicant suggested a maximum *TBHQ* dose of 30 mg/kg complete *feedingstuffs*. When supplemented with other antioxidants (i.e. butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA)) a total maximum level of the antioxidants of 150 mg/kg *feedingstuffs* shall apply.

For the characterisation of the *feed additive* the Applicant submitted the internationally recognised FAO JECFA monographs for food additives cited by Commission Regulation (EU) 231/2012, where qualitative assays are based on: - solubility, - determination of melting point, and positive test for phenolic compounds; while quantitative assay of total amount of *TBHQ* and the impurities is based on titrimetry (the *TBHQ* content is derived by subtracting the amounts of impurities determined by gas chromatography). Even though no performance characteristics are provided the EURL recommends for official control the above mentioned methods described in the FAO JECFA monographs cited by Commission Regulation (EU) 231/2012 for the characterisation of the *feed additive*.

For the quantification of *TBHQ* in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted a single-laboratory validated and further verified method based on reversed phase high performance liquid chromatography with UV detection (HPLC-UV). The performance characteristics reported for the quantification of *TBHQ* in the *feed additive*, *premixtures* and *feedingstuffs* in the frame of the validation and verification studies are: *precisions* ranging from 0.2 to 10.4 %; a *recovery rate* ( $R_{rec}$ ) ranging from 87 to 105 %; and a limit of quantification (LOQ) of 9.8 mg *TBHQ*/kg *feedingstuffs*. In addition, on request from EURL the Applicant provided the experimental data allowing the proper chromatographic separation of *TBHQ*, *BHA* and *BHT*.

Based on the performance characteristics provided, the EURL recommends the above mentioned single-laboratory validated and further verified reversed phase HPLC-UV method for the quantification of *TBHQ* in the *feed additive*, *premixtures* and *feedingstuffs* within the validated and verified concentration range.

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**

*Tertiary-Butylhydroquinone (TBHQ)*, technological additives, antioxidants, all animal species and categories

## **1. BACKGROUND**

In the current application authorisation is sought under Article 4(1) (new *feed additive*) for *Tertiary-Butylhydroquinone (TBHQ)* under the category/functional group 1(b) 'technological additives'/'antioxidants' according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1,2]. Specifically, authorisation is sought for all categories and species [1,2].

According to the Applicant *TBHQ* is a white crystalline powder with a minimum purity of 99% [2,3]. The Applicant states that the purity criteria/technical specifications set in Commission Regulation (EU) 231/2012 for the food additive apply also to the *feed additive* [3].

The *feed additive* is intended to be used into *feedingstuffs* through liquid or solid *premixtures* [3]. The Applicant suggested a maximum *TBHQ* dose of 30 mg/kg complete *feedingstuffs* [2]. When supplemented with other antioxidants (i.e. butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA) a total maximum level of the antioxidants of 150 mg/kg *feedingstuffs* shall apply [3].

## **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 *Tertiary-Butylhydroquinone* 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 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 characterisation of the *feed additive* the Applicant submitted the internationally recognised FAO JECFA monographs for food additives [5,6] cited by Commission Regulation (EU) 231/2012, where qualitative assays are based on: - solubility; - determination of melting point; and - positive test for phenolic compounds; while quantitative assay of total amount of *TBHQ* and impurities (i.e. hydroquinone and 2,5-di-tertiary-butylhydroxyquinone) is based on titrimetry using ceric sulphate and p-diphenylaminesulfonic acid as an indicator - the *TBHQ* content is derived by subtracting the amounts of the impurities, determined by gas chromatography as described in the JECFA monograph [5].

Even though no performance characteristics are provided the EURL recommends for official control the above mentioned methods described in the FAO JECFA monographs as recommended by Commission Regulation (EU) 231/2012 for characterisation of the *feed additive*.

For the quantification of *TBHQ* in the *feed additive* and *premixtures* the Applicant submitted a single-laboratory validated and further verified method based on reversed phase high performance liquid chromatography with UV detection (HPLC-UV) [7]. Results from additional experiments demonstrated that this method is also applicable to the determination of *TBHQ* in *feedingstuffs* [8].

The sample (0.02 to 10 g) is treated with acetonitrile in ultrasonic bath. The solution is filtered or centrifuged and further analysed by reversed phase HPLC coupled to UV detection at 290 nm. Quantification of *TBHQ* is performed using calibration with external standards.

The performance characteristics reported for *TBHQ* quantification in the *feed additive* (containing of 75% and 99% of *TBHQ*), *premixtures* and *feedingstuffs* in the frame of the validation [8,9,10] and verification studies [10,11,12] are presented in Table 1. Furthermore, the limit of quantification (LOQ) of 9.8 mg *TBHQ*/kg *feedingstuffs* was reported by the Applicant [10].

**Table 1** The performance characteristics of the single-laboratory validated and verified reversed phase HPLC-UV method for the quantification of *TBHQ* in the *feed additive*, *premixtures* and *feedingstuffs*

	<i>Feed additive</i>		<i>Premixtures</i>		<i>Feedingstuffs</i>	
	Validation	Verification	Validation	Verification	Validation	Verification
Concentration, mg/kg	990000	762100	16300 - 40500	40000	25 - 1051	25 - 103
RSD <sub>r</sub> , %	2.4 - 2.6	0.7 - 0.9	0.7 - 1.9	2.0 - 2.5	0.7 - 10.4	0.2 - 0.9
RSD <sub>ip</sub> , %	2.6	1.2	2.0	2.7	10.4	0.9
R <sub>recr</sub> , %	100	102	101 - 102	100	87 - 105	100 - 103
Reference	[9]	[11]	[9]	[11]	[8,10]	[10,12]

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

In addition, on request from EURL the Applicant provided the experimental data demonstrating the selectivity of the method allowing the proper chromatographic separation of TBHQ, BHA and BHT [13].

Note: The analytical methods for determination of butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) in *premixtures* and *feedingstuffs* were evaluated and recommended by the EURL in the frame of FAD-2010-0132 (BHA) [14] and FAD-2010-0237 & FAD-2010-0300 (BHT) [15] reports.

Based on the performance characteristics provided, the EURL recommends the above mentioned single-laboratory validated and further verified reversed phase HPLC-UV method for the quantification of *TBHQ* in the *feed additive*, *premixtures* and *feedingstuffs* within the validated and verified concentration range.

The Applicant also submitted the ring-trial validated official AOAC method 983.15 [16], originally developed for fatty food matrices (such as oils and lard), for quantification of *TBHQ* in lipid based *feedingstuffs*. Considering the high HorRat values reported for butter oil and lard (ranging from 2.42 to 3.62) the EURL does not consider the AOAC method suitable for official control of *TBHQ* 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) is not considered necessary.

#### 4. CONCLUSIONS AND RECOMMENDATIONS

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

- the analytical methods described in the FAO JECFA monographs cited by Commission Regulation (EU) 231/2012 for characterisation of the *feed additive (Tertiary-Butylhydroquinone, TBHQ)*, and
- the single-laboratory validated and further verified method based on reversed phase high performance liquid chromatography with UV detection (HPLC-UV) for the quantification of *TBHQ* in the *feed additive, premixtures and feedingstuffs*.

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

For the characterisation of the *feed additive (Tertiary-Butylhydroquinone)*:

- FAO JECFA Monograph '*Tertiary-Butylhydroquinone*' as referred in Commission Regulation (EU) 231/2012

For the quantification of *Tertiary-Butylhydroquinone (TBHQ)* in the *feed additive, premixtures and feedingstuffs*:

- Reversed phase high performance liquid chromatography with UV detection (HPLC-UV)

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

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Tertiary-Butylhydroquinone* 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/G1: Forw. Appl. 1831/0066-2012
- [2] \*Application, Proposal for Register Entry
- [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 Combined Compendium of Food Additive Specifications, 'Tertiary butylhydroquinone', Monograph No. 1 (2006)  
<http://www.fao.org/ag/agn/jecfa-additives/specs/Monograph1/Additive-459.pdf>  
(last visited on 16/04/2015)
- [6] FAO JECFA Combined Compendium for Food Additive Specifications - Analytical methods, test procedures and laboratory solutions used by and referenced in the food additive specifications, Vol. 4  
<http://www.fao.org/docrep/009/a0691e/a0691e00.htm> (last visited on 16/04/2015)



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- [7] \*Technical dossier, Section II – Annex II\_20  
[8] \*Technical dossier, Section II – Annex II\_41  
[9] \*Technical dossier, Section II – Annex II\_35  
[10] \*Supplementary information – Verification report\_FAD-2012-0021\_Lohmann\_TBHQ\_signed  
[11] \*Technical dossier, Section II – Annex II\_36  
[12] \*Supplementary information – Annex\_1\_MoA\_TBHQ\_Feed\_2014  
[13] \*Supplementary information – FAD-2012-0021\_Lohmann\_TBHQ\_signed\_01-06-2015  
[14] #FAD-2010-0132 – JRC.D.5/CvH/SB/ag/ARES(2012)40826  
[15] #FAD-2010-0237 & FAD-2010-0300 – JRC.D.5/CvH/SB/mds/ARES(2012)490799  
[16] AOAC Official Method 983.15 Phenolic antioxidants in oils, fats and butter oil – liquid chromatographic method
- \*Refers to Dossier no: FAD-2012-0021  
# <https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports>

## 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:

- Fødevarestyrelsen, Ringsted (DK)<sup>1</sup>
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft. Geschäftsbereich 6 - Labore Landwirtschaft, Nossen (DE)<sup>2</sup>

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Name and addresses according to Regulation (EC) No 885/2009:

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