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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-2010-0132 CRL/100015
Feed Additive Name:	Butylated Hydroxy Anisole (BHA)
Active Substance(s):	Butylated Hydroxy Anisole (BHA)
Rapporteur Laboratory:	European Union Reference Laboratory for Feed Additives (EURL-FA) Geel, Belgium
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Report approved by: Date:	Christoph von Holst 13/01/2012

EXECUTIVE SUMMARY

In the current application authorisation is sought for *Butylated Hydroxy Anisole, E320 (BHA)* under Article 10, category/functional group 1(b) 'technological additives'/'antioxidants', according to the classification system of Annex I of Regulation (EC) No 1831/2003. *BHA* is already authorised as *feed additive* under Commission Directive 70/524/EEC.

According to the Applicant *BHA* is a white or slightly yellow waxy solid with a minimum purity of 98.5% *BHA*, containing at least 85% of 3-tert-butyl-4-hydroxyanisole (3-BHA) and 2-tert-butyl-4-hydroxyanisole (2-BHA) and a maximum of 0.2 % of hydroquinone. Specifically, authorisation is sought for the use of the *feed additive* for all animal species and categories. The *feed additive* is intended to be mixed in *premixtures* or added directly in complete *feedingstuffs.* Furthermore, the Applicant proposed a maximum level in the daily ration of 150 mg/kg for *BHA* alone or for the sum of BHA with *Butylhydroxytoluene* (*BHT*, E321) and/or *Ethoxyquin* (E324).

For the determination of *BHA* in the *feed additive*, the Applicant submitted the Food Chemical Codex 7 (FCC) method based on Gas Chromatography coupled to Flame Ionization Detection (GC-FID). Standard deviations for *repeatability* (RSD_r) of 2.0%, for *3-BHA*, and 6.0%, for *2-BHA*, were reported.

Based on the performance characteristics presented, the EURL recommends for official control the FCC 7 method based on GC-FID to determine *BHA* in the *feed additive.*

For the determination of *BHA* in *premixtures* and *feedingstuffs* the Applicant submitted a single laboratory validated and further verified multi-analyte method, based on Reversed Phase High Performance Liquid Chromatography coupled with UltraViolet-Diode-Array Detection (RP-HPLC-UV-DAD). The following correspondent performance characteristics were reported for concentrations in *premixtures* ranging from 5 to 120 g/kg and concentrations in *feedingstuffs* ranging from 35 to 226 mg/kg:

- a RSD_r ranging from 0.6 to 3.9%;
- a standard deviation for intermediate precision (RSD_{ip}) ranging from 2.6 to 6.4%;
- a recovery rate (R_{Rec}) ranging from 90.5 to 114%; and
- a limit of quantification (LOQ) below 35 mg/kg.

Based on the performance characteristics presented, the EURL recommends for official control, the single laboratory validated and further verified RP-HPLC-UV-DAD method, submitted by the Applicant, to determine *BHA* in *premixtures* and *feedingstuffs*.

According to the Applicant the above mentioned multi-analyte technique, submitted for the determination of *BHA* in *premixtures* and *feedingstuffs,* allows the quantification of other synthetic antioxidants such as *BHT* and *Ethoxyquin* (in *premixtures* only). Furthermore, the EURL identified the ring trial validated method by the Association of Official Analytical Chemists (AOAC 996.13 – "*Ethoxyquin* in feeds") based on isocratic RP-HPLC system coupled with fluorescence detection (RP-HPLC-FD). The following precisions (repeatability and reproducibility) were reported: ranging from 1.4 to 4.6 for *BHT* in *premixtures* and *feedingstuffs* and ranging from 2.1 to 5.4 and 4.5 to 29% for *Ethoxyquin* in premixtures and *feedingstuffs respectively.*

Based on the performance characteristics presented, the EURL considers suitable for the quantification of *BHT* and *Ethoxyquin*:

- the single laboratory validated and further verified RP-HPLC-UV-DAD method, submitted by the Applicant, for *BHT* in *premixtures* and *feedingstuffs* and *Ethoxyquin* in *premixture* only, and
- the ring trial validated RP-HPLC-FD method characterised by the "Association of Official Analytical Chemists" (AOAC 996.13) for *Ethoxyquin* 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.

KEYWORDS
Butylated Hydroxy Anisole (BHA), *E320*, technological additives, antioxidants, all animal species and categories, *Butylhydroxytoluene (BHT)*, E321, *Ethoxyquin,* E324.

1. BACKGROUND

In the current application authorisation is sought for *Butylated Hydroxy Anisole, E320 (BHA)* under Article 10, category/functional group 1(b) 'technological additives'/'antioxidants', according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1, 2]. *BHA* is already authorised as *feed additive* under Commission Directive 70/524/EEC [3].

BHA is a white or slightly yellow waxy solid with a minimum purity of 98.5% *BHA*, containing at least 85% of 3-tert-butyl-4-hydroxyanisole (3-BHA) and 2-tert-butyl-4-hydroxyanisole (2-BHA) and a maximum of 0.2 % of hydroquinone [4-6]. The *feed additive* is the product of the reaction among tertiary butyl hydroquinone with dimethyl sulphate in the presence of alkali and solvents under specific conditions [7]. According to the Applicant,

BHA is universally used as antioxidant and preservative in feed, food, food packaging, cosmetics, in rubber and petroleum products [8]. Specifically, authorisation is sought for the use of the *feed additive* for all animal species and categories. The *feed additive* is intended to be mixed in *premixtures* or added directly in complete *feedingstuffs.* Furthermore, the Applicant proposed a maximum level in the daily ration of 150 mg/kg for *BHA* alone or for the sum of BHA with *Butylhydroxytoluene* (*BHT*, E321) and/or *Ethoxyquin* (E324) [2, 9].

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 *BHA* 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 [10].

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

For the determination of *BHA* in the *feed additive*, the Applicant submitted the Food Chemical Codex 7 (FCC) method [5, 11], based on Gas Chromatography coupled to Flame Ionization Detection (GC-FID). The sample is dissolved and diluted to 10 ml with an "internal standard solution" (5 mg/ml of 4-tert-butylphenol in acetone). A "mixed standard solution" containing *3-BHA* and *2-BHA* (ratio 9:1 in the above mentioned "internal standard solution") is prepared in parallel. The "sample solution" and the "mixed standard solution" are separately injected into the GC-FID allowing the quantification of the two isomers in the *feed additive*. Standard deviations for *repeatability* (RSD_r) of 2.0%, for *3-BHA*, and 6.0%, for *2-BHA*, were reported [5].

Based on the performance characteristics presented, the EURL recommends for official control the FCC 7 method based on GC-FID to determine *BHA* in the *feed additive.*

For the determination of *BHA* in *premixtures* and *feedingstuffs* the Applicant submitted a single laboratory validated and further verified multi-analyte method, based on Reversed Phase High Performance Liquid Chromatography coupled with UltraViolet-Diode-Array Detection (RP-HPLC-UV-DAD) [11-15]. The method consists of an extraction of the *active substance* from the matrix using methanol (together with ascorbic acid for *premixtures*). The extract is further diluted with methanol and mixed thoroughly. The supernatant is filtered and injected into a gradient reversed-phase HPLC system. The *BHA* content is determined via UV-DAD adjusted at 285 nm. The concentration of *BHA* is determined using an external calibration curve with standards in the solvent. The following correspondent performance characteristics were reported for concentrations in *premixtures* ranging from 5 to 120 g/kg [12, 13] and concentrations in *feedingstuffs* ranging from 35 to 226 mg/kg [14, 15]:

- a RSD_r ranging from 0.6 to 3.9%;
- a standard deviation for intermediate precision (RSD_{ip}) ranging from 2.6 to 6.4%;
- a recovery rate (R_{Rec}) ranging from 90.5 to 114%; and
- a limit of quantification (LOQ) below 35 mg/kg [14].

Furthermore the NRLs recommended, based on their own previous experience, to include an additional purification step, using Solid Phase Extraction (SPE), to avoid any potential matrix interference.

Based on the performance characteristics presented, the EURL recommends for official control, the single laboratory validated and further verified RP-HPLC-UV-DAD method, submitted by the Applicant, to determine *BHA* in *premixtures* and *feedingstuffs*.

Moreover, the Applicant proposed a maximum level in the daily ration of 150 mg/kg *feedingstuffs* for BHA alone or for the sum of BHA and BHT (E321) and/or Ethoxyquin (E324) [2, 9]. According to the Applicant the above mentioned multi-analyte technique, submitted for the determination of *BHA* in *premixtures* and *feedingstuffs,* allows the quantification of other synthetic antioxidants such as *BHT* and *Ethoxyquin* (in *premixtures* only). Furthermore, the EURL identified the ring trial validated method by the Association of Official Analytical Chemists (AOAC 996.13 – "*Ethoxyquin* in feeds") [16]. The AOAC method consists of an extraction with acetonitrile followed by injection in an isocratic RP-HPLC system coupled with fluorescence detection (RP-HPLC-FD), adjusted at 360 nm for the excitation and 432 nm for the emission. The following precisions (repeatability and reproducibility) were reported: ranging from 1.4 to 4.6 for *BHT* in *premixtures* and

feedingstuffs and ranging from 2.1 to 5.4 and 4.5 to 29% for *Ethoxyquin* in premixtures and *feedingstuffs* respectively.

Based on the performance characteristics presented, the EURL considers suitable for the quantification of *BHT* and *Ethoxyquin*:

- the single laboratory validated and further verified RP-HPLC-UV-DAD method, submitted by the Applicant, for *BHT* in *premixtures* and *feedingstuffs* and *Ethoxyquin* in *premixture* only, and
- the ring trial validated RP-HPLC-FD method characterised by the "Association of Official Analytical Chemists" (AOAC 996.13) for *Ethoxyquin* 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 FCC 7 method based on GC-FID to determine *BHA* in the *feed additive*;
- a single laboratory validated and further verified method using RP-HPLC with UV-DAD detection to determine *BHA* in *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the quantification of *BHA* in *feed additive*:

- Gas Chromatography coupled to Flame Ionization Detection (GC-FID) (FCC7 method)

For the quantification of BHA in premixtures and feedingstuffs:

- Reversed Phase High Performance Liquid Chromatography coupled to UltraViolet-Diode-Array Detection (RP-HPLC-UV-DAD, 285 nm)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *BHA* 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. 1831/0022-2011

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

[3] Council Directive 70/524/EEC concerning additives in feedingstuffs - List of authorised additives in feedingstuffs (2004/C50/01)

[4] *Technical dossier, Section II: 2.1.3 Qualitative and quantitative composition

[5] *Technical dossier, Section II; Annex 2.2.1.1 BHA-FCC V1 MONOGRAPH_091218

[6] *Technical dossier, Section I; 2.2 Characterisation of the active substance(s)/agent(s)

[7] *Technical dossier, Section I: 2.3 Manufacturing process

[8] *Technical dossier, Section II: 2.1.2 Proposal for classification

[9] *Technical dossier, Section II: 2.5.1 Proposed mode of use in animal nutrition

[10] 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

[11] *Technical dossier, Section II: 2.6.1 Method of analysis for the active substance

[12] *Technical dossier, Section II; Annex: Antoxiac_Method of Analysis and Validation_Premix

[13] *Technical dossier, Section II; Annex: Antoxiac_Method verification_Premix-BHA

[14] *Technical dossier, Section II; Annex: Antoxiac_Method of Analysis and Validation_Feed

[15] *Technical dossier, Section II; Annex: Antoxiac_Method verification_Feed-BHA

[16] AOAC Official Method 996:13 – Etoxyquin in Feeds

* Refers to Dossier No. FAD-2010-0132

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

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- Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA) Speyer, Speyer (DE)
- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby (DK)
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)
- RIKILT-Instituut voor Voedselveiligheid, Wageningen (NL)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen. Jena (DE)
- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)