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Safety and efficacy of a feed additive consisting of butylated hydroxytoluene (BHT) for all animal species (Katyon Technologies Limited)

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Abstract

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of butylated hydroxytoluene (BHT) as a feed additive for all animal species. The additive BHT is considered safe for chickens for fattening and weaned piglets at the maximum proposed concentration of 150 mg/kg complete feed. This conclusion is extended to chickens reared for laying and extrapolated to pigs for fattening. In the absence of data, no conclusion on the safety for the other target species could be drawn. The exposure of the consumer to BHT from tissues and products of animals fed the additive ranged from 1% to 3% of the acceptable daily intake (ADI). The FEEDAP Panel concluded that the use of BHT as a feed additive at the proposed conditions of use is of no concern for the safety of the consumers. Exposure of the user to BHT via inhalation is likely; however, the Panel is not in the position to conclude on the potential inhalation toxicity of the additive. BHT is a skin and eye irritant, no conclusions can be drawn on the potential of the additive to be a skin sensitiser. In the absence of data, the FEEDAP Panel cannot conclude on the safety of BHT for the environment. The additive BHT is considered an efficacious antioxidant in feedingstuffs for all animal species.

Keywords: Butylated hydroxytoluene, BHT, technological additive, antioxidants, safety, efficacy, all animal species

Requestor: European Commission

Question number: EFSA-Q-2011-01149

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of this Regulation for additives authorised without a time limit or pursuant to Directive 82/471/EEC In particular, Article 10(2) of that Regulation specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, within a maximum of seven years after the entry into force of this Regulation.

The European Commission received a request from the company Katyon Technologies Limited[2] for re-evaluation of butylated hydroxytoluene (BHT), when used as a feed additive for all animal species (category: technological additives; functional group: antioxidants).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 28 March 2012.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the feed additive consisting of butylated hydroxytoluene (BHT), when used under the proposed conditions of use (see Section 3.1.2).

1.2. Additional information

Butylated hydroxytoluene (BHT) is included in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003.

BHT is authorised for use in food as antioxidant[3] up to a maximum level of 400 mg/kg.

The BHT was assessed in the past by the Scientific Committee for Food (SCF) in 1989 (European Commission, 1989), by the by FAO/WHO Expert Committee on Food Additives (JECFA) several times, the latest in 1996 (WHO, 1996), by EFSA in 2012 (EFSA ANS Panel, 2012) and more recently by the Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES) (ANSES, 2016), by the Norwegian Scientific Committee for Food and Environment (VKM) (VKM, 2019) and the Scientific Committee on Consumer Safety (SCCS) (SCCS, 2021).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[4] in support of the authorisation request for the use of BHT as a feed additive.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers and other scientific reports to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active substance in animal feed. The Executive Summary of the EURL report can be found in Annex A.[5]

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] Katyon Technologies Limited, Kyriakou Matsi 20, 1082, Nicosia, Cyprus.
[3] Regulation (EC) no 1333/2008 of the European Parliament and of the council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.
[4] FEED dossier reference: FAD-2010-0237.
[5] The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports_en

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of BHT is in line with the principles laid down in Regulation (EC) No 429/20087 and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012); Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a); Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEEDAP Panel, 2017b); Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c); Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018); and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

3. Assessment

Butylated hydroxytoluene (BHT) is intended to be used as a technological feed additive (functional group: antioxidant) in feed for all animal species.

3.1. Characterisation

3.1.1. Characterisation of the additive

Butylated hydroxytoluene (BHT) is a crystalline powder produced by chemical synthesis, following two similar different synthetic routes. In the first one, [redacted]

The additive is equivalent to the active substance and contains by specification at least 99.5% of BHT (IUPAC name 2,6-di-tert-butyl-4-methylphenol, Chemical Abstracts Service (CAS) number 128-37-0, chemical formula $C_{15}H_{24}O$ and molecular weight 220.35 g/mol). The additive is in form of crystals, flakes or powder. The structural formula of the additive is given in Figure 1.

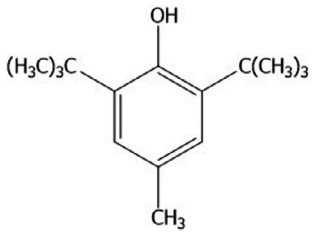

Figure 1: Structural formula of BHT

The analysis of five batches of each form of the additive showed concentrations of BHT ≥ 99.7%,[6] in compliance with the specifications and with the specifications set by the Commission Regulation (EU) No 231/2012[7] for its use as food additive (BHT ≥ 99%).

The impurities analysed in the same batches showed concentrations in compliance with the specifications for the food additive (phenolic impurities < 0.5%, lead < 2 mg/kg, mercury < 1 mg/kg, arsenic < 3 mg/kg); cadmium was measured to be < 0.1 mg/kg.[8] No information was available on concertation of sulfated ash. Polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) were analysed in one batch of each form of the additive and found below the corresponding limit of quantification (LOQ). The calculated (upper bound) levels of dioxins ranged 0.19–0.25 ng WHO-PCDD/F-TEQ/kg. The concentrations of these impurities are considered of no concern.

The particle size distribution of the additive was measured by laser diffraction on one batch of each form of the additive.[9] In the flake form, about 0.05% of particles were < 50 μm and no particles were < 10 μm; in the crystalline form, about 0.18% of particles were < 50 μm and no particles were < 10 μm; In the

[6] Technical dossier/Section II/Annex II_2 to Annex II_29 and Supplementary Information October 2012/Annex_2_1_to Annex 2_32.
[7] COMMISSION REGULATION (EU) No 231/2012 of 9 March 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. OJ No L 83/1, 22.3.2012, p. 1.
[8] Technical dossier/Section II/Annex II_31 and Supplementary Information October 2012/Annex_3_1_and Annex 3_2.
[9] Supplementary Information October 2012/Annex 4_1 to Annex 4_3.

powder form, about 44.6% of particles were < 50 μm, and about 18% of the particles were < 10 μm; the amount of particles with a diameter < 10 μm in the dust generated by the powder form was about 82%.

The dusting potential of the additive was measured (four replicate analysis) in the same batches, using the Stauber-Heubach method. The results indicated that the flake form has a dusting potential of 0.130 g/m^3, the crystalline form has a dusting potential of 0.075 g/m^3 and the powder form has a dusting potential of 14.45 g/m^3.

Stability and homogeneity

The analysis of four batches of each form of the additive when stored in polyethylene containers[10] for 36 months under room conditions showed a full recovery of the initial BHT concentration.

BHT is added to feedingstuffs and premixtures as an antioxidant. Antioxidants are not considered stable in feed materials and compound feed; therefore, there is no need to assess the stability in these matrices.

The flake and crystalline form of the additive are incorporated in fats and oils, in which they dissolve; therefore, a demonstration of a homogeneous distribution in these matrixes is not considered necessary. The homogenous distribution of the powder form of BHT was investigated in an in vitro study,[11] in which 10 subsamples of one batch each of a complete feedingstuffs for laying hens, for pigs and of ruminants, with BHT at inclusion level of 150 mg/kg feed complete feed, were analysed for BHT concentration, showing coefficients of variation (CV) of 7.9%, 7.9% and 4.4%, respectively. To support the homogenous mixing of the feedingstuffs, a microtracer was also added during the test. The microtracer recovery rate (97, 89 and 90%, respectively) confirms the appropriate mixing of the feedingstuffs.

3.1.2. Conditions of use

Butylated hydroxytoluene is intended to be used as an antioxidant in feedingstuffs for all animal species and categories except dogs with a maximum content of 150 mg/kg complete feed (alone or together with BHA (E320) and/or ethoxyquin (E324)) and for dogs with a maximum content of 150 mg/kg complete feed (alone or together with BHA (E320)).

3.2. Safety

3.2.1. Safety for the target species

To support the safety of the additive for the target species, the applicant provided two tolerance studies, one with chickens for fattening and one with weaned piglets, assessed below. In addition, the applicant provided studies published in the public literature (13 publications)[12] in which the dietary supplementation of BHT at various inclusion levels was studied on different physiological parameters of various animal species (chickens for fattening/rearing, laying hens, turkeys for fattening, pigs for fattening, quail, dogs, cats, rabbits, guinea pigs, rats, mice and Atlantic salmon). None of the 12 studies were further considered in the assessment as they contained many experimental drawbacks and reporting flaws (e.g. lack of control and multifold treatment groups, lack of replicates, insufficient number of animals used, short duration).

3.2.1.1. Safety for chickens for fattening

A total of 540 1-day-old male chickens for fattening (Ross 708) were distributed in 36 pens in groups of 15 animals and allocated to four dietary treatments (nine replicates per treatment).[13] Three basal diets (starter, from day 1 to day 10; grower, from day 11 to day 28; finisher, from day 29 to day 35), based on maize and soybean meal, were either not supplemented (Control) or supplemented with 150 (1× the maximum use level), 1,000 (6.7×) or 1,500 (10×) mg BHT/kg feed (confirmed by analysis). Diets were offered in mash form ad libitum for 35 days. Health status and mortality were monitored daily, and most probable cause of death determined by necropsy. Animals were weighed on days 1, 10, 28 and 35 of the experiment (pen basis), feed intake was registered per pen and daily body weight gain and feed to gain ratio calculated. At the end of the experiment (day 35), blood

[10] Supplementary Information October 2012/Annex 5_1 to Annex 5_15.
[11] Supplementary Information October 2012/Annex_6_1.
[12] Supplementary Information October 2012/Annex_1_1 to annex 1_13.
[13] Supplementary information March 2014/Question 1/Annex 1_0-9 Tolerance chickens.

samples were obtained from one chicken per pen (randomly selected) for routine blood haematology and biochemistry.[14] These birds were killed for carcass/organs evaluation.[15] An analysis of variance (ANOVA) was performed with the data considering the pen as the experimental unit and group means were compared. Significance level was set at $p < 0.05$.

Mortality and culling of birds were low in each treatment and not treatment related (< 0.4% total). Performance parameters were not affected by treatments and mean values were for daily feed intake 77.3 g/day, for final body weight 1.63 kg, for body weight gain 45.2 g/day and for feed to gain ratio 1.71. No effects were observed in the haematological and biochemical parameters, with the exception of monocytes percentage, which were increased significantly at 150 mg BHT/kg feed (1×) compared to the control and 10× groups and of urea and creatinine concentrations, which were decreased at 1,000 mg/kg feed (6.7×) compared to the control and 1× groups; however, these changes were not considered treatment related. No treatment effects were observed either in carcass/organs evaluation.

The BHT supplementation up to 10-fold the maximum recommended level of 150 mg/kg feed did not adversely affect the health and performance of the chickens. Therefore, the FEEDAP Panel concludes that the product is safe for chickens for fattening at the maximum recommended use level.

3.2.1.2. Safety for weaned piglets

A total of 80 castrated male and 64 female piglets (Italian Duroc × Goland, 32 days of age, body weight 7.7 kg) were distributed in 36 pens in groups of four animals and allocated to four dietary treatments (nine replicates per treatment; representing five pens with castrated males and four pens with females per treatment).[16] Two basal diets (starter, from day 1 to day 14; grower, from day 15 to day 42), based on maize, wheat and soybean meal, were either not supplemented (control) or supplemented with 150 mg BHT/kg feed (1× the maximum concentration), 1,000 mg BHT/kg feed (6.7×) or 1,500 mg BHT/kg feed (10×) (confirmed by analysis). Diets were offered in mash form ad libitum for 42 days. Health status and mortality were monitored daily. Animals were weighed on days 1, 14 and 42 of the experiment (pen basis), feed intake was registered per pen and daily weight gain and feed to gain ratio calculated. At the start of the experiment (day 1), blood samples were obtained from 10 castrated males and 10 female piglets, and at the end of the experiment (day 42), blood samples were obtained from six castrated males and six female piglets per treatment (randomly selected, in total 24 castrated males and 24 female piglets) for routine blood haematology and biochemistry.[14] No gross pathology was performed. An analysis of variance (ANOVA) was performed with the data considering the pen as the experimental unit and group means were compared. Significance level was set at $p < 0.05$.

No mortality occurred. Performance parameters were not affected by treatments and mean values were for daily feed intake 583.3 g/day, for final body weight 20.7 kg, for body weight gain 313.5 g/day and for feed to gain ratio 1.72. No effects were observed in the haematological and biochemical parameters, with the exception of haemoglobin and haematocrit; however, these changes were not considered treatment related, since they were increased only in the group supplemented with 150 mg BHT/kg feed (1×). Lactate dehydrogenase was increased at 1,500 mg BHT/kg feed (10×) compared to the other groups (334, 345, 335 and 371 U/L, for control, 1×, 6.7× and 10×, respectively); however, the change was considered small and not biologically relevant.

The BHT supplementation up to 10-fold the maximum inclusion level of 150 mg/kg feed did not adversely affect the health and performance of weaned piglets. Therefore, the FEEDAP Panel concludes that the product is safe for weaned piglets at the maximum inclusion level.

[14] Haematology: Red blood cell count (RBC), haemoglobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell count (WBC), lymphocytes, monocytes, neutrophils, eosinophils, basophils, platelets, reticulocyte count, reticulocyte production index (RPI), mean platelet volume (MPV), platelet crit (PCT), platelet distribution width (PDW). Biochemistry: Glucose, calcium, inorganic P, cholesterol, triglycerides, phospholipids, uric acid, urea, creatinine, lactate dehydrogenase, alkaline phosphatase, aspartate transaminase (GOT), alanine transaminase (GPT), total bilirubin, gamma-glutamyl transpeptidase (GGT), haptoglobin, serum albumin, serum total protein, blood clotting: quick/INR (International Normalised Ratio) value, prothrombin time (PT), thyroid hormones (TSH-thyroid stimulating hormone, T3-triiodothyronine, T4-thyroxine).

[15] Skin, eyes, feet, ears, head and tail, mouth and anus, gut – oral cavity, oesophagus, stomach, upper, mid and lower small intestine, caecum and colon, pancreas, spleen liver/gall bladder, kidneys, genitals, abdominal fat, omentum, heart and lungs, skeletal muscle and fat.

[16] Supplementary information March 2014/Question 1/Annex 2_0-9 Tolerance piglets.

3.2.1.3. Conclusions on safety for the target species

The FEEDAP Panel concludes that BHT is safe for chickens for fattening and weaned piglets at the maximum intended concentration of 150 mg/kg complete feed. This conclusion is extended to chickens reared for laying and to pigs for fattening and extrapolated to all growing avian species. In the absence of data, no conclusion on the safety for the other target species could be drawn.

3.2.2. Safety for the consumer

For the current assessment, the applicant made reference to the re-evaluation of butylated hydroxytoluene BHT (E 321) as a food additive (EFSA ANS Panel, 2012), the relevant studies at the basis of these evaluations, absorption, distribution, metabolism and excretion studies in laying hens and mini pigs and residue studies in milk, edible tissues of laying hens and mini pig.

The FEEDAP Panel noted that that other evaluations on the safety of BHT were performed by the Scientific Committee for Food (SCF) (European Commission, 1989) and FAO/WHO Expert Committee on Food Additives (JECFA) (latest, WHO, 1996)) before the submission of the dossier. More recently, the safety of BHT was evaluated by the Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail (ANSES) (ANSES, 2016), by the Norwegian Scientific Committee for Food and Environment (VKM) (VKM, 2019) and the Scientific Committee on Consumer Safety (SCCS) (SCCS, 2021).

The FEEDAP Panel re-evaluated the main results of the studies assessed in the previous evaluations and assessed the studies done by the applicant for the current application.

3.2.2.1. Absorption, distribution, metabolism and excretion

3.2.2.1.1. ADME in laboratory animals

In the opinions of the EFSA ANS Panel (2012) and more recently of the SCCS (2021), the ADME of BHT in laboratory animals (mice, rats, rabbits, dogs, monkeys) has been reviewed. It can be summarised as follows: (i) after oral exposure BHT is highly bioavailable, with 30–40% urinary excretion and significant enterohepatic recirculation; BHT is generally distributed to and metabolised by the liver and is distributed to body fat, (ii) more than 40 metabolites have been separated/identified; the main primary metabolic pathway leads to the production of BHT alcohol, BHT aldehyde and BHT acid by stepwise oxidation of the p-methyl group in the rat and rabbit; these metabolites are conjugated (glucuronide, acetylcysteine and sulfate) and cleared; a BHT dimer is formed also, (iii) an unstable quinone methide metabolite is formed in all species via oxidation mechanisms.

3.2.2.1.2. ADME in target species

In the current application, two studies on the ADME of BHT in the target species were submitted.

A study performed in laying hen[17] comprised two trials. The first one was designed to establish the duration of the exposure to BHT required to reach BHT residue steady state in the eggs. Ten hens were fed with BHT at 150 mg/kg complete feed for 28 days. Eggs (one per hen) were collected after 6–8, 13–16, 20–22 and 27–28 days of dosing; the mixture yolk plus egg white was prepared on the day of collection and kept at −20°C until analysis. BHT was determined using an HPLC/UV detection method internally validated. The results indicated that a steady state was reached after 14 days of exposure.

In the second trial, six hens were fed for 20 consecutive days a complete feed supplemented with 150 mg BHT ([phenyl-U-14C]-BHT plus unlabelled BHT)/kg feed. Eggs were collected between days 15 and 20 and yolk plus egg white combined. Animals were slaughtered 22 h after the last administration of the test item and liver, kidney, muscle and skin/fat of all birds were sampled. Metabolic profiles were established using a radio-HPLC analysis of the total radioactive residue (TRR) extracted from pooled tissues and eggs, with the exception of the muscle due to very low TRR. The average extraction yield of TRR was 98%, 73%, 74% and 82% for the liver, kidney, skin/fat and egg, respectively. The chromatographic runs allowed the complete recovery of the TRR extracts injected. BHT (2.9%) and a great number of metabolites (22 individual or groups of), all amounting to less than 10% of the TRR (with the major one (compound B) to 4.5%), were separated in the liver. In the kidney, the same situation was observed, with BHT (4.6%) and two major compounds, A and C, amounting to 2.4% and 5.4% of the TRR. In the skin/fat, BHT amounted to 41.4% and many metabolites (17 individual or groups of) were separated representing each less than 10% of the TRR, except for one metabolite

[17] Supplementary Information June 2019/6.FR000889.

(component B) amounting to 10.4%. In the egg, BHT amounted to 38% and one metabolite (compound A) to 10% of the TRR. Co-injection of liver, skin/fat and egg extracts with standards of four potential BHT metabolites (i.e. 2,6-di-tert-butyl-1,4-benzoquinone, BHT-alcohol, BHT-acid and BHT-aldehyde) confirmed that none of them corresponded to components A, B or C.

Tentative identification of metabolites from the pooled TRR extracts from skin/fat and eggs was carried out. BHT and metabolites were separated on a liquid chromatographic column which flow was split 80% into a radiodetector and 20% into a high-resolution mass spectrometer (positive and negative ionisation runs). The two known BHT dimers were not identified. Four potential BHT metabolites were detected; two exhibited molecular ions corresponding to acetylated BHT whereas the other two molecular ions corresponded to hydroxylated BHT; however, their full chemical structure was not established further. Two other compounds were detected with molecular ions corresponding: (i) one to multiple biotransformations such as the loss of two methyl groups, oxidation and hydroxylation; (ii) the second to hydration and di-hydroxylation; their full chemical structure was not established either. No correspondence could be established between these potential metabolites and the compound B not detected radiochemically in the skin/fat extract.

To study the metabolism in pig species, the applicant conducted a study using mini pig, which were chosen to allow a proper use of radiolabelled test substance, considering the lower bodyweight compared to pigs for fattening.[18] Considering the substantial metabolic similarity between the two breeds, the FEEDAP Panel considered this approach as acceptable.

In this study, four animals (Göttingen strain, two castrated males and two females) were fed a complete feed supplemented with 150 mg BHT/kg for 7 days, followed by 150 mg [phenyl-U-14C]-BHT plus unlabelled BHT/kg for another 7 days. The animals were killed 21 h after the last administration of the test item and liver, kidney, muscle and fat sampled. Metabolic profiles were established using a radio-HPLC analysis of the TRR extracted from tissues pooled by gender, with the exception of the muscle due to very low TRR. The average extraction yield was 60% for the liver, 82% for the kidney and 77% for the fat. The chromatographic runs allowed the complete recovery of the TRR extracts injected. In the liver, BHT and a great number of metabolites were separated, all these compounds amounting to less than 10% of the TRR. In the kidney, BHT and many metabolites were below 10% of the TRR; only one metabolite (compound A) represented 11.9% of the TRR in the male but only 3.8% in the female. In the fat, BHT and many metabolites were separated representing each less than 10% of the TRR, with the exception of one metabolite (component B) amounting to 10.4%. Co-injection of kidney and fat extracts with standards from four potential BHT metabolites (i.e. 2,6-di-tert-butyl-1,4-benzoquinone, BHT-alcohol, BHT-acid and BHT-aldehyde) confirmed that none of them corresponded to components A or B.

3.2.2.1.3. Conclusions on ADME

The commonality of the metabolic pathways of BHT in the laboratory animals and target species for which ADME studies are available is established. BHT is considered as the marker residue and liver is the target tissue.

3.2.2.2. Residue studies

Three studies analysing BHT residues' concentration in milk, in eggs and edible tissues of laying hens and in edible tissues of mini pigs were made available.[19]

3.2.2.2.1. BHT residues in milk

A residue study of BHT in milk was performed in eight Holstein cows (630 ± 43 bw, 1.88 ± 0.83 parity).[20] A residue study of BHT in milk was performed in eight Holstein cows (630 ± 43 bw, 1.88 ± 0.83 parity). The duration of the study was 24 days corresponding to 14 days acclimatisation plus 10 days experiment during which the animals were fed a total mixed ration (52.5% dry matter) supplemented with 88.3 mg BHT/kg (analytical values) equivalent to 150 mg BHT/kg complete feed (88% dry matter). Milk from morning and evening milkings were collected at days 0, 8, 9 and 10 of the experiment. BHT analysis in milk was carried out after n-hexane extraction, centrifugation and

[18] Supplementary Information June 2019/7.FR001102.

[19] The methods of analysis for BHT residues in milk, in eggs and in edible tissues of laying hens were evaluated for their quality by the EURL. The methods of analysis were considered fit for purpose for the analysis of BHT residues in the matrixes and at the concentrations considered in these studies.

[20] Supplementary Information January 2016/RF_CZ14010VL_Amendment_3_January_2016.pdf.

overnight defatting in freezing conditions. BHT determination was performed by gas chromatography/mass spectrometry using the two ions m/z + 205 and 220.[21] BHT residues in milk were all below the limit of detection (LOD) (< 0.005 mg/kg) in all the samples analysed. The FEEDAP Panel considered that the more appropriate value for residues of BHT in milk to be used for the assessment of the exposure of the consumer should be the LOQ value. Therefore, the residues of BHT in milk of cows exposed to BHT at the level of 150 mg/kg complete feed to be considered for consumer exposure assessment are < 0.012 mg/kg.

In a published survey study (Pattono et al., 2009), samples of cow milk were collected at farm level (organic and conventional dairy farms) and retail market; BHT residues were determined using a GC-MS method with an LOQ of 1 μg/L. Residues of BHT were found in 10 samples of conventional milk and in 11 samples of organic milk (sampled either at farm level or at retail level). Since no information was available on the BHT content of the cow diets related either to the contribution of the additive as antioxidant components of the diet or to contaminations, the results of this study cannot be used to estimate consumer exposure under the proposed conditions of use of the additive.

3.2.2.2.2. BHT residues in laying hen edible tissues and egg

The ADME study of BHT in the laying hen already described included (add ref to chapter) the analysis of residues in the tissues and eggs. Data on total radioactive residues in tissues and eggs are reported in Table 1. The ratios of BHT to total radioactive residue (TRR) were calculated from the metabolic profiles data and amounted to 2.9%, 4.6%, 41.4% and 38% for the liver, kidney, skin/fat and egg, respectively. In the absence of direct determination of the marker residue in tissues and eggs, a back calculation of the BHT concentration from TRR applying these ratios was retained.

Table 1: Total radioactive residues (TRR) (expressed as equivalent μg BHT/kg fresh tissue or whole egg) and back-calculated marker residue (MR) (μg BHT/kg) concentrations in eggs (steady state) and pooled tissues (22 h withdrawal) from laying hens administered 150 mg BHT ([^{14}C]-BHT plus unlabelled)/kg complete feed

	Liver	Kidney	Muscle	Skin/fat	Egg
TRR	1,886 ± 454	618 ± 206	58 ± 56	1,191 ± 661	544 ± 157
Ratio MR/TRR	2.9	4.6	nd[1]	41.4	38.0
MR	55 ± 13	28 ± 10	nd	493 ± 273	207 ± 60

nd[1] = not determined due to too low TRR.

3.2.2.2.3. BHT residues in mini pig edible tissues

The ADME study of BHT in the mini pig already described included the analysis of residues in the tissues. Data on total radioactive residues in tissues are reported on Table 2. Considering the low number of animals (4), the highest individual value has been retained. The ratios of BHT to TRR were calculated from the metabolic profiles data and amounted to 0.4%, 2.7% and 22.8% for the liver, kidney and fat, respectively. In the absence of direct determination of the marker residue in tissues, a back-calculation of the BHT concentration from TRR applying these ratios was retained.

Table 2: Total radioactive residues (TRR) (expressed as equivalent μg BHT/kg fresh tissue) and back-calculated marker residue (MR) (μg BHT/kg) in tissues of mini pigs administered a feed supplemented with 150 mg BHT ([14C]-BHT plus unlabelled)/kg complete feed, after 21-h withdrawal

	Liver	Kidney	Muscle	Skin/fat
TRR*	896	776	49	363
Ratio MR/TRR	0.4	2.7	nd[1]	22.8
MR	4	21	nd	83

nd[1] = not determined due to too low TRR.
*: highest individual value.

[21] The method was validated in milk with recovery tests at 0.012, 0.6, 1.6 and 12 mg BHT/kg spiking levels. A BHT reference standard was used for both calibration and spiking of the samples. The limit of detection (LOD), set at the lowest concentration having a signal-to-noise signal of 3 as minimum, amounted to 0.005 mg/kg. The limit of quantification (LOQ) was 0.012 mg/kg, based on the lowest spiking level at which both accuracy and precision were adequate (recovery 111 ± 3%).

3.2.2.3. Toxicological studies

BHT was evaluated by the SCF in 1987 (European Commission, 1989) and by JECFA in 1996 (WHO, 1996). The SCF established an acceptable daily intake (ADI) of 0–0.05 mg/kg bw per day based on thyroid, reproduction and haematological effects in the rat. At its last evaluation, JECFA allocated an ADI of 0–0.3 mg/kg bw for BHT, based on effects in the reproduction segments and hepatic enzyme induction seen in two separate two-generation studies in rats. Noting the discrepancy between the SCF and JECFA evaluations, the ANS Panel reviewed the toxicology of BHT (EFSA ANS Panel, 2012), considering the studies that had become available since the last review. In the ANS Panel opinion, studies on acute toxicity in mice rats, rabbits, guinea pigs and cats were assessed, in addition to repeated-dose toxicity studies in mice, rats and dogs, chronic toxicity/carcinogenicity studies in mice and rats, various mutagenicity studies, reproduction/developmental toxicity studies in mice, rats and monkeys, special studies of thyroid effects in rats, case reports and epidemiological studies in humans and numerous studies of the mechanism of action. The main outcomes of the ANS Panel assessment are summarised below.

The acute oral toxicity of BHT was low in all species tested. The results of repeated-dose studies showed that short-term or subchronic exposure to BHT can cause histopathological changes in the liver of mice and rats. In addition, BHT has been shown to increase the relative thyroid and adrenal weight in rats and thyroid hyperactivity in chronic studies. An no observed adverse effect level (NOAEL) of 25 mg/kg bw per day was identified for histopathological changes in the thyroid, based on electron microscopy analysis. Lung or liver tumours were seen in some studies in mice or rats exposed orally to BHT as the single test substance, and there was some evidence that BHT could promote lung cancers induced in mice by other agents. Benchmark dose (BMD) analyses of the data on the incidence of lung neoplasia in mice induced by BHT revealed a BMDL10 of 38 mg/kg bw per day, and a $BMDL_{10}$ of 247 mg/kg bw per day on the incidence of hepatocellular carcinomas in male rats induced by BHT.

The ANS Panel noted that the majority of the genotoxicity studies were negative for the induction of point mutations and chromosomal aberrations and for the interaction with DNA. The Panel recognised that positive results reported in some *in vitro* genotoxicity studies with BHT and BHT metabolites can be attributed 'to pro-oxidative chemistry giving rise to formation of quinones and reactive oxygen species and that such a mechanism of genotoxicity is generally considered to have a threshold'.

The ANS Panel also noted that BHT and, to a larger extent, its metabolite 6-t-butyl-2-(hydroxy-butyl) 4-methylphenol were found to inhibit gap junctional intercellular communication (GJIC) in mouse lung epithelial (C10) and rat liver epithelial (WB-F344) cell lines and concluded that BHT at high doses can exert tumour-promoting effects in some animal models. In conclusion, the tumorigenic activity of BHT observed in long-term studies is attributed to non-genotoxic thresholded mechanisms.

No overt effects on reproduction were reported in mice or rhesus monkeys. The lowest NOAEL identified in any of the reproduction studies was 25 mg/kg bw per day for effects on litter size, sex ratio and pup body weight gain during the lactation period of the reproduction segment of a rat study that also considered carcinogenicity. Human studies, including an epidemiological investigation of risk of stomach cancer, revealed no areas for concern over the safety of BHT.

The ANS Panel set an ADI of 0.25 mg/kg bw applying an uncertainty factor of 100 to the NOAEL of 25 mg BHT/kg bw per day, which was derived from the results from the reproduction segment of a study in rats (effects on litter size, sex ratio and pup body weight gain during the lactation period), and to the same NOAEL for thyroid effects seen in a study in rats where electron microscopy analysis of the thyroid glands of rats exposed to 500 mg BHT/kg bw per day for 28 days showed an increase in the number of follicle cells.

After the EFSA ANS opinion (2012), an *in vivo* study (Pop et al., 2013) investigated the possible endocrine-disrupting (ED) activity of BHT using the immature rat uterotrophic assay. Wistar female rats ageing 17–21 days were orally administered with BHT at 75 mg/kg bw (i.e. three times the calculated NOAEL) for three consecutive days. The absolute and relative decrease in uterus weight pointed to an anti-oestrogenic activity of BHT.

In a subsequent document concerning the analysis of the most appropriate Risk Management Option (RMOA), ANSES (2016) examined the full data set of toxicological effects of BHT. As regards the possible ED activity, ANSES highlighted the need to further evaluate the adrenal and thyroid effects and to get more insight into the interaction with androgenic and oestrogenic receptors reported *in vitro*. Based on the available data, ANSES was not in the position to conclude on the ED activity of BHT. Overall, also due to other uncertainties on the tumorigenic effects and the reported negative effects on reproduction, ANSES proposed BHT as a candidate for the REACH evaluation process.

An assessment was performed in 2019 by the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food, and Cosmetics of the Norwegian Scientific Committee for Food and Environment (VKM). In this evaluation, as regards the Pop et al. (2013) study, the endpoint «uterus weight in rats» was considered to be relevant for an assessment of potential risks in humans. According to the evaluation of the weight of evidence, however, an association between BHT and the reported anti-oestrogenic effect was considered unlikely, and the study was not used for the hazard identification and characterisation. No further original studies since the EFSA ANS Panel (2012) evaluation were retrieved. Consequently, the VKM did not see any reason to modify the ADI of 0.25 mg/kg bw per day established by the EFSA ANS Panel in 2012.

The Scientific Committee on Consumer Safety (SCCS) has recently issued an opinion on BHT (2021) as to its use as a cosmetic ingredient. No activity towards oestrogen, androgen or thyroid receptors alpha or beta could be demonstrated *in silico* through the VEGA QSAR programme. The SCCS opinion also reported on the results of the *in vitro* US EPA ToxCast Endocrine Screening Program. The test assesses the agonism/antagonism towards androgen or oestrogen receptors, or endpoints related to thyroid function or oestrogen synthesis (aromatase). In all tests, BHT was either inactive or active only at cytotoxic concentrations. Based on the evaluation of four *in vitro* studies (Wada et al., 2004; Pop et al., 2016, 2018; Yang et al., 2018) not covered by previous risk assessments, the SCCS concluded that BHT may behave as either weak oestrogen or anti-oestrogen according to the cell system used. Finally, SCCS attributed the observed histological signs of thyroid hyperactivity reported in previous evaluations to the increase in the activity of liver enzymes participating in thyroid hormone catabolism and agreed on the ADI 0.25 mg/kg bw established by the EFSA ANS Panel in 2012.

The FEEDAP Panel, having reviewed the studies and evaluations above, supports the conclusions reached by the EFSA ANS Panel in 2012 and considered the ADI of 0.25 mg/kg bw as adequate and used this ADI as the basis of its assessment of the consumer safety of the use of BHT in animal feeds.

3.2.2.4. Assessment of Consumer exposure and Consumer safety assessment

In its estimate of consumer exposure to BHT, the ANS Panel (2012) could not take into account the contribution of food of animal origin resulting from the use of BHT as a feed additive due to lack of data. The ANS Panel noted that 'exposure of adults to BHT from its use as food additive is unlikely to exceed the newly derived ADI of 0.25 mg/kg bw per day at the mean and for the high consumers (95th percentile). Exposure of children to BHT from its use as food additive is also unlikely to exceed this ADI at the mean, but is exceeded for some European countries (Finland, The Netherlands) at the 95th percentile. If exposure to BHT from its use as food contact material is also taken into account, the new ADI would be exceeded by children at the mean and at the 95th percentile'.

In the current assessment, the FEEDAP Panel performed an exposure assessment following the methodology described in the Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a) (Appendix A), using the residue data in milk from dairy cows, eggs and edible tissues from laying hens and edible tissues from mini pigs described in the residue section (see Section 3.2.2.2). The FEEDAP Panel notes that, in the absence of residue data in fish, the exposure calculated refers only to the consumption of tissues and products from terrestrial animals.

Comparing mini pigs with standard pigs for fattening, mini pigs can be characterised by a lower body weight gain (if at all under study conditions) and by a higher body fat proportion. The intake of feed per body weight gain unit is higher than in pigs for fattening. The residues measured in mini pigs could be therefore taken as a conservative estimate of residues expected in pigs for fattening after oral exposure to 150 mg BHT/kg complete feed.

The BHT exposure was calculated on the basis of the highest reliable percentile (HRP) of food consumption (raw agricultural food commodities), expressed in mg/kg bw per day for the different population categories and compared with the ADI established by the EFSA ANS Panel in 2012 (EFSA ANS Panel, 2012). The input data of BHT TRR content used to estimate exposure are reported in Table 3.

Table 3: Input data on BHT content in food of animal origin used for the consumer exposure assessment

Animal product	BHT (mg/kg wet tissue/product)
Birds' fat tissue	2.513
Birds' liver	2.794
Birds' meat(1)	0.404
Birds' offals and slaughtering products (other than liver)(2)	1.030
Mammals' fat tissue	0.363
Mammals' liver	0.896
Mammals' meat(3)	0.112
Mammals' offals and slaughtering products (other than liver)(2)	0.776
Milk	0.012
Whole eggs	0.868

(1): Calculated by default as 90% muscles and 10% fat tissue.
(2): Kidney values taken by default.
(3): Calculated by default as 80% muscles and 20% fat tissue.

The results of the dietary exposure to BHT for the different population categories are reported in Table 4.

Table 4: Chronic human dietary exposure to BHT. Maximum highest reliable percentile expressed in mg/kg bw per day

Population class	Number of surveys	Maximum highest reliable percentile	% ADI*
Infants	6	0.0061	2
Toddlers	10	0.0068	3
Other children	18	0.0060	2
Adolescents	17	0.0037	1
Adults	17	0.0028	1
Elderly	14	0.0026	1
Very elderly	12	0.0023	1

*: ADI: Acceptable daily intake: 0.25 mg/kg body weight and day.

The exposure of the consumer to BHT from products of terrestrial animals fed the additive ranged from 1% to 3.0% of the ADI. The population class with the highest exposure was toddlers with an intake amounting to about 3% of the ADI, exposure of the population classes adults, elderly and very elderly ranged around 1% of the ADI.

In this context, it is noted that the exposure of adult consumers to BHT from its use as a food additive amounts to 0.01–0.03 mg/kg bw per day (mean) and 0.03–0.17 mg/kg bw per day (95th percentile) and from its use as a food contact material to 0.05 mg/kg bw per day (EFSA ANS Panel, 2012). Exposure of children to BHT from its use as a food additive amounts to 0.01–0.09 mg/kg bw per day (mean) and 0.05–0.30 mg/kg bw per day (95th percentile) and from its use as a food contact material to 0.2 mg/kg bw per day.

3.2.2.5. Conclusions on safety for the consumer

The FEEDAP Panel concluded that the exposure of the consumer to BHT from its use as a feed additive in terrestrial animals is of no concern for the safety of consumers.

3.2.3. Safety for the user

3.2.3.1. Effects on the respiratory system

No data were provided on the effects of the additive on the respiratory system, including inhalation toxicity. Considering the particle size distribution of the additive and its dusting potential, exposure of the user via inhalation is likely.

3.2.3.2. Effects on the eyes and skin

The effects of BHT on skin and eyes were summarised in the Organisation for Economic Co-operation and Development (OECD) Screening Information Dataset (SIDS) (OECD, 2002). BHT was reported to be slightly irritating to the skin of rabbits. The irritating effects observed were mild and reversible within 72 h. BHT was also slightly irritating to the eye of rabbits. The irritating effects observed were mild and completely reversible within 72 h. No information was provided on skin sensitising potential.

3.2.3.3. Conclusions on the safety for the user

The FEEDAP Panel concludes that exposure of the user to BHT via inhalation is likely; however, the Panel is not in the position to conclude on the potential inhalation toxicity of the additive. BHT is a skin and eye irritant, no conclusions are possible on the potential of the additive to be a skin sensitiser.

3.2.4. Safety for the environment

The active substance BHT is not a physiological/natural substance of established safety for the environment. Consequently, the Phase I assessment has to be continued to determine the predicted environmental concentration (PEC).

In Phase I, a total residue approach was used meaning that the predicted environmental concentrations were calculated based on the assumption that the additive is excreted 100% as parent compound.

3.2.4.1. Phase I

The values used to perform an estimation of BHT in the environment, assuming 150 mg/kg concentration of the additive in feed, are described in Table 5.

Table 5: BHT characteristics

Parameters	Value
Vapour pressure	1.1 Pa*
Molar Mass	220.36 g/mol
Water solubility	0.6 mg/L*
Organic carbon normalised partition coefficient (K_{oc})	8183 dm^3/kg**

*: From OECD 2002.
**: EPI Suite calculation.

The Phase I assessment results in a predicted environmental concentration in soil (PEC_{soil}) ranging from 700 to 3,200 μg/kg depending on the species considered; and a predicted environmental concentration in groundwater (PEC_{GW}) in the range of 3–5 μg/L.

The Phase I PEC trigger values are exceeded both for soil and for groundwater. Therefore, a Phase II assessment is considered necessary.

3.2.4.2. Phase II

No experimental data were provided by the applicant neither for the exposure assessment nor for ecotoxicological assessment.

In the absence of any experimental data, the FEEDAP Panel cannot conclude on the safety of BHT for the environment.

3.3. Efficacy

BHT is authorised to be added to food matrixes on a fat basis with the function of antioxidant at concentrations of 40–400 mg/kg. The food categories in which its use is authorised cover a wide range of moisture content. Since the same effect can be reasonably assumed for feedingstuffs, no studies are required to demonstrate the efficacy of BHT as antioxidant in feedingstuffs for all animal species.

The effects of the additive on egg quality were studied by Hayat et al. (2009). Groups of ISA Brown Leghorn laying hens (24-week-old at start), six replicates of two hens each, received a diet containing 10% flaxseeds either without BHT or supplemented with 50, 100 and 150 mg BHT/kg complete feed for 56 days. Twelve eggs per treatment (two per replicate cage) were selected every 2 weeks to determine egg quality characteristics in yolk, albumen and eggshell (yolk weight, shell

weight, albumen weight and height, Haugh units, yolk colour and shell thickness) and lipid composition of eggs. Since none of the measured parameters was negatively affected, it can be concluded that dietary BHT supplementation at levels up to 150 mg/kg feed had no adverse effects on egg quality.

4. Conclusions

The additive BHT is considered safe for chickens for fattening and weaned piglets at the maximum proposed concentration of 150 mg/kg complete feed. This conclusion is extended to chickens reared for laying and extrapolated to pigs for fattening. In the absence of data, no conclusion on the safety for the other target species could be drawn.

The exposure of the consumer to BHT from tissues and products of animals fed the additive ranged from 1% to 3% of the ADI. The FEEDAP Panel concluded that the use of BHT as a feed additive at the proposed conditions of use is of no concern for the safety of the consumers.

Exposure of the user to BHT via inhalation is likely; however, the Panel is not in the position to conclude on the potential inhalation toxicity of the additive. BHT is a skin and eye irritant, no conclusions can be drawn on the potential of the additive to be a skin or respiratory sensitiser.

In the absence of data, the FEEDAP Panel cannot conclude on the safety of BHT for the environment.

The additive BHT is considered an efficacious antioxidant in feedingstuffs for all animal species.

5. Documentation provided to EFSA/Chronology

Date	Event
03/11/2010	Dossier received by EFSA. Butylated hydroxytoluene (BHT) for all animal species. Submitted by Angefero Limited LLC.
08/11/2011	Reception mandate from the European Commission
20/12/2011	Application validated by EFSA – Start of the scientific assessment
29/03/2012	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: safety for the target species, characterisation*
20/04/2012	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
19/03/2014	Reception of supplementary information from the applicant - Scientific assessment re-started
06/06/2014	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: safety for the consumer*
05/01/2015	Reception of supplementary information from the applicant - Scientific assessment re-started
17/02/2015	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: safety for the consumer*
07/04/2015	Reception of supplementary information from the applicant - Scientific assessment re-started
03/06/2015	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: safety for the consumer*
01/09/2015	Reception of supplementary information from the applicant - Scientific assessment re-started
10/11/2015	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: safety for the consumer*
09/02/2016	Reception of supplementary information from the applicant - Scientific assessment re-started
29/07/2016	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: safety for the consumer*
26/04/2019	Reception of supplementary information from the applicant - Scientific assessment on hold pending evaluation method of analysis for residues in tissues and products
21/09/2021	Reception of the evaluation of the methods of analysis - Scientific assessment re-started
23/03/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ADI	acceptable daily intake
AFC	EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
ANS	EFSA Scientific Panel on Additives and Nutrient Sources added to Food
BW	body weight
CAS	Chemical Abstracts Service
CD	Commission Decision
CG	chemical group
CV	coefficient of variation
DM	dry matter
EINECS	European Inventory of Existing Chemical Substances
EURL	European Union Reference Laboratory
FAO	Food Agricultural Organization
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FLAVIS	The EU Flavour Information System
FL-no	FLAVIS number
GC-MS	gas chromatography-mass spectrometry
IUPAC	International Union of Pure and Applied Chemistry
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
LOQ	limit of quantification
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
SCF	Scientific Committee on Food
WHO	World Health Organization

Appendix A – Detailed results of chronic exposure calculation

Table A.1: Chronic dietary exposure of consumers to residues of BHT per population class, country and survey (mg/kg body weight per day) based on residue data

Population class	Survey's country	Number of subjects	Highest Reliable Percentile value	Highest Reliable Percentile description
Infants	Bulgaria	523	0.00612	95th
Infants	Germany	142	0.00204	95th
Infants	Denmark	799	0.00270	95th
Infants	Finland	427	0.00159	95th
Infants	Italy	9	0.00077	50th
Infants	United Kingdom	1,251	0.00307	95th
Toddlers	Belgium	36	0.00387	90th
Toddlers	Bulgaria	428	0.00683	95th
Toddlers	Germany	348	0.00399	95th
Toddlers	Denmark	917	0.00357	95th
Toddlers	Spain	17	0.00518	75th
Toddlers	Finland	500	0.00338	95th
Toddlers	Italy	36	0.00363	90th
Toddlers	Netherlands	322	0.00425	95th
Toddlers	United Kingdom	1,314	0.00431	95th
Toddlers	United Kingdom	185	0.00392	95th
Other children	Austria	128	0.00393	95th
Other children	Belgium	625	0.00419	95th
Other children	Bulgaria	433	0.00595	95th
Other children	Germany	293	0.00365	95th
Other children	Germany	835	0.00340	95th
Other children	Denmark	298	0.00312	95th
Other children	Spain	399	0.00444	95th
Other children	Spain	156	0.00598	95th
Other children	Finland	750	0.00383	95th
Other children	France	482	0.00419	95th
Other children	Greece	838	0.00453	95th
Other children	Italy	193	0.00406	95th
Other children	Latvia	187	0.00342	95th
Other children	Netherlands	957	0.00357	95th
Other children	Netherlands	447	0.00323	95th
Other children	Sweden	1,473	0.00327	95th
Other children	Czechia	389	0.00506	95th
Other children	United Kingdom	651	0.00336	95th
Adolescents	Austria	237	0.00263	95th
Adolescents	Belgium	576	0.00173	95th
Adolescents	Cyprus	303	0.00164	95th
Adolescents	Germany	393	0.00264	95th
Adolescents	Germany	1,011	0.00164	95th
Adolescents	Denmark	377	0.00168	95th
Adolescents	Spain	651	0.00282	95th
Adolescents	Spain	209	0.00369	95th
Adolescents	Spain	86	0.00227	95th
Adolescents	Finland	306	0.00172	95th
Adolescents	France	973	0.00245	95th

Population class	Survey's country	Number of subjects	Highest Reliable Percentile value	Highest Reliable Percentile description
Adolescents	Italy	247	0.00231	95th
Adolescents	Latvia	453	0.00260	95th
Adolescents	Netherlands	1,142	0.00231	95th
Adolescents	Sweden	1,018	0.00201	95th
Adolescents	Czechia	298	0.00336	95th
Adolescents	United Kingdom	666	0.00190	95th
Adults	Austria	308	0.00186	95th
Adults	Belgium	1,292	0.00156	95th
Adults	Germany	10,419	0.00160	95th
Adults	Denmark	1,739	0.00126	95th
Adults	Spain	981	0.00214	95th
Adults	Spain	410	0.00200	95th
Adults	Finland	1,295	0.00182	95th
Adults	France	2,276	0.00174	95th
Adults	Hungary	1,074	0.00254	95th
Adults	Ireland	1,274	0.00175	95th
Adults	Italy	2,313	0.00159	95th
Adults	Latvia	1,271	0.00208	95th
Adults	Netherlands	2,055	0.00176	95th
Adults	Romania	1,254	0.00278	95th
Adults	Sweden	1,430	0.00206	95th
Adults	Czechia	1,666	0.00218	95th
Adults	United Kingdom	1,265	0.00146	95th
Elderly	Austria	67	0.00186	95th
Elderly	Belgium	511	0.00140	95th
Elderly	Germany	2,006	0.00147	95th
Elderly	Denmark	274	0.00130	95th
Elderly	Finland	413	0.00146	95th
Elderly	France	264	0.00160	95th
Elderly	Hungary	206	0.00207	95th
Elderly	Ireland	149	0.00169	95th
Elderly	Italy	289	0.00135	95th
Elderly	Netherlands	173	0.00142	95th
Elderly	Netherlands	289	0.00143	95th
Elderly	Romania	83	0.00259	95th
Elderly	Sweden	295	0.00191	95th
Elderly	United Kingdom	166	0.00134	95th
Very elderly	Austria	25	0.00106	75th
Very elderly	Belgium	704	0.00150	95th
Very elderly	Germany	490	0.00145	95th
Very elderly	Denmark	12	0.00099	75th
Very elderly	France	84	0.00157	95th
Very elderly	Hungary	80	0.00209	95th
Very elderly	Ireland	77	0.00180	95th
Very elderly	Italy	228	0.00129	95th
Very elderly	Netherlands	450	0.00133	95th
Very elderly	Romania	45	0.00225	90th
Very elderly	Sweden	72	0.00205	95th
Very elderly	United Kingdom	139	0.00127	95th

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for butylated hydroxytoluene (BHT)

In the current applications, authorisation is sought for Butylated hydroxytoluene, E321 (BHT) 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. BHT is already authorised as feed additive under Commission Directive 70/524/EEC.

According to the two applicants (FAD 2010-0237 & FAD 2010-0300), BHT is a white or colourless solid powder with a minimum purity of 99.5%. 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 applicants proposed a maximum level of 150 mg/kg for BHT alone or for the sum of BHT with butylated hydroxy anisole (BHA, E321) and/or ethoxyquin (E324).

For the determination of BHT in the feed additive, applicant (FAD-2010-0237) submitted the internationally recognised FAO JECFA method based on gas chromatography coupled to flame ionisation detection (GC-FID). Even though no performance characteristics of this method are provided, the EURL recommends for official control the internationally recognised FAO JECFA method based on GC-FID to determine BHT in the feed additive.

For the determination of BHT in premixtures and feedingstuffs, applicant (FAD-2010-0300) submitted a single laboratory validated and further verified multi-analyte method, based on reversed phase high-performance liquid chromatography coupled with UltraViolet or Diode-Array Detection (RP-HPLC-UV or DAD). The following performance characteristics were reported for concentrations ranging from 5 to 120 g/kg and from 35 to 226 mg/kg, for premixtures and feedingstuffs, respectively:

- a standard deviation for repeatability (RSDr) ranging from 1.4 to 6.6%;
- a standard deviation for intermediate precision (RSDip) ranging from 3.3 to 11.4%;
- a recovery rate (RRec) ranging from 86.9 to 114%; and
- a limit of quantification (LOQ) below the lowest concentration investigated of 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 (or DAD) method, submitted by the applicant, to determine BHT in premixtures and feedingstuffs.

According to the applicant (FAD 2010-0300), the above-mentioned multi-analyte technique, submitted for the determination of BHT in premixtures and feedingstuffs, allows the quantification of other synthetic antioxidants such as BHA 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 relative precisions (repeatability and reproducibility) were reported: ranging from 0.6 to 6.4% for BHA 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 the following methods suitable for official control:

the single laboratory validated and further verified RP-HPLC-UV (or DAD) method, submitted by the applicant, for the determination of BHA in premixtures and feedingstuffs and for the determination of 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 the determination of 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.