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Safety and efficacy of butylated hydroxyanisole (BHA) as a feed additive for all animal species

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP),
Guido Rychen, Gabriele Aquilina, Giovanna Azimonti, Vasileios Bampidis,
Maria de Lourdes Bastos, Georges Bories, Andrew Chesson, Pier Sandro Cocconcelli,
Gerhard Flachowsky, Boris Kolar, Maryline Kouba, Marta López-Alonso,
Secundino López Puente, Alberto Mantovani, Baltasar Mayo, Fernando Ramos, Maria Saarela,
Roberto Edoardo Villa, Robert John Wallace, Pieter Wester, Anne-Katrine Lundebye,
Carlo Nebbia, Derek Renshaw, Matteo Lorenzo Innocenti and Jürgen Gropp

Abstract

Butylated hydroxyanisole (BHA) is a waxy solid consisting for > 98.5% of the active substance, a mixture of 3-*tert*-butyl-4-hydroxyanisole and 2-*tert*-butyl-4-hydroxyanisole. It is intended to be used as an antioxidant in feedingstuffs for all animal species and categories. The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) considered BHA up to 150 mg/kg complete feed as safe for all animal species except for cats, for which a safe dose could not be established from the tolerance data. BHA is rapidly absorbed from the gastrointestinal tract; it is metabolised rapidly and excreted as such and as metabolites in the urine and faeces. The proportions of the different metabolites vary depending on species and dose. No accumulation of BHA or metabolites was observed in tissues. The Panel concluded that no concern for consumer safety would arise from the use of BHA as a feed additive at the maximum concentration of 150 mg/kg feed. The additive should be considered a skin, eye irritant and a potential skin sensitiser. Exposure of the user via inhalation was considered unlikely; therefore, a risk is not expected. The use of BHA at the maximum concentration proposed is unlikely to pose a risk to the environment. BHA is authorised as an antioxidant for food use at comparable use levels, therefore, no studies were required to demonstrate the efficacy of BHA as an antioxidant in feedingstuffs for all animal species.

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Correspondence: feedap@efsa.europa.eu

Panel members: Gabriele Aquilina, Giovanna Azimonti, Vasileios Bampidis, Maria de Lourdes Bastos, Georges Bories, Andrew Chesson, Pier Sandro Coconcelli, Gerhard Flachowsky, Jürgen Gropp, Boris Kolar, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Alberto Mantovani, Baltasar Mayo, Fernando Ramos, Guido Rychen, Maria Saarela, Roberto Edoardo Villa, Robert John Wallace and Pieter Wester.

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Summary

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 hydroxyanisole (BHA) as a feed additive for all animal species.

Butylated hydroxyanisole (BHA) is a waxy solid consisting for > 98.5% of the active substance, a mixture of 3-*tert*-butyl-4-hydroxyanisole and 2-*tert*-butyl-4-hydroxyanisole. It 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 feedingstuffs (alone or together with butylated hydroxytoluene (BHT) (E 321) and/or ethoxyquin (E 324)) and for dogs with a maximum content of 150 mg/kg complete feedingstuffs (alone or together with BHT (E 321))

Although (i) the classical tolerance study in chickens for fattening shows some weaknesses in experimental design and reduced sensitivity due to low body weight gain, (ii) the other studies, available only as publications and not as full report, were not designed as tolerance studies, and (iii) some uncertainties remain on the extrapolation of the data to all animal species, a weight of evidence of the limited data supports that 150 mg BHA/kg complete feed would be a safe dose for all animal species. However, a possible exception could be cat, with its known lower capacity for glucuronidation of phenolic compounds and for which no specific data were available.

BHA is rapidly absorbed from the gastrointestinal tract; it is metabolised rapidly and excreted as such and as metabolites in the urine and faeces. The proportions of the different metabolites vary depending on species and dose. No accumulation of BHA or metabolites was observed in tissues. The FEEDAP Panel retains the acceptable daily intake (ADI) of 1 mg/kg body weight (bw) proposed by the EFSA Scientific Panel on Additives and Nutrient Sources added to Food (ANS).

A highly conservative estimate of consumer exposure resulting from consumption of food from animals fed BHA at the highest feed concentration of 150 mg BHA/kg resulted in 5 mg BHA per person per day, corresponding to about 8% of the ADI. The FEEDAP Panel concludes that no concern for consumer safety would arise from the use of BHA as a feed additive at the maximum concentration of 150 mg/kg feed.

The additive should be considered a skin, eye irritant and a potential skin sensitiser. Exposure of the user via inhalation is considered unlikely; therefore, a risk is not expected.

The use of BHA at the maximum concentration proposed is unlikely to pose a risk to the environment.

Since BHA is authorised as an antioxidant for food use at comparable use levels, no studies are required to demonstrate the efficacy of BHA as an antioxidant in feedingstuffs for all animal species.

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003¹ 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 7 years after the entry into force of this Regulation for additives authorised without a time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from FEFANA asbl² for re-evaluation of the authorisation of the product Butylated Hydroxy Anisole (BHA), when used as a feed additive for all animal species (category: technological additive; 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 13 September 2011.

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 product Butylated Hydroxy Anisole (BHA), when used under the proposed conditions of use (see Section 3.1.2).

1.2. Additional information

The additive BHA is currently authorised as a technological additive in feedingstuffs for all animal species (in application of Article 9t (b) of Council Directive 70/524/EEC concerning additives in feedingstuffs (2004/C 50/01)) with a maximum content of 150 mg/kg complete feedingstuffs alone or together with butylated hydroxytoluene (BHT) (E 321) and/or ethoxyquin (E 324) (only for dogs: alone or in combination with E 321).

BHA is authorised according to Directive 95/2/EC¹ as a food additive (antioxidant), up to a maximum level of 400 mg/kg.

The Scientific Committee on Food (SCF), the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and EFSA ANS Panel have delivered several opinions on the use of BHA as a food additive (JECFA, 1974, 1976, 1982, 1986, 1987, 1989, 1999, 2006; SCF, 1989; EFSA ANS Panel, 2011, 2012).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier³ in support of the authorisation request for the use of BHA as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008⁴ and the applicable EFSA guidance documents.

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 BHA in animal feed. The Executive Summary of the EURL report can be found in Annex A.⁵

¹ European Parliament and Council Directive No 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners. OJ No L 61, 18.3.1995, p. 1.

² FEFANA asbl Avenue Louise 130A, 1050 Brussels, Belgium; Companies: Kemin NV, Lohmann Animal Health GmbH, Mitsui GmbH.

³ FEED dossier reference: FAD-2010-0132.

⁴ Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

⁵ The full report is available on the EURL website: <https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2010-0132.pdf>

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of BHA is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on technological additives (EFSA FEEDAP Panel, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011a), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008a), Guidance for the preparation of dossiers for the re-evaluation of certain additives already authorised under Directive 70/524/EEC (EFSA, 2008b), Guidance for the preparation of dossiers for additives already authorised for use in food (EFSA FEEDAP Panel, 2012b), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012c), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012d), Guidance on the assessment of additives intended to be used in pets and other non food-producing animals (EFSA FEEDAP Panel, 2011b) and Technical Guidance: Extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition (EFSA, 2008c).

3. Assessment

The additive under assessment is mainly composed of the active substance BHA. It is intended to be used as a feed additive (category: technological additives; functional group: (b) antioxidants) in feedingstuffs for all animal species.

3.1. Characterisation

The additive BHA is produced by the reaction of *tert*-butylhydroquinone with dimethyl sulfate and sodium hydroxide; it is then purified with *n*-hexane, filtered and crystallised in the form of flakes; it is a waxy solid at room temperature.

The additive is equivalent to the active substance, a mixture of 2-*tert*-butyl-4-hydroxyanisole and 3-*tert*-butyl-4-hydroxyanisole (Figures 1 and 2). It is specified to contain at least 98.5% of BHA (IUPAC name: 2-*tert*-butyl-4-hydroxyanisole and 3-*tert*-butyl-4-hydroxyanisole, Chemical Abstracts Service (CAS) number 25013-16-5, chemical formula $C_{11}H_{16}O_2$, and molecular weight 180.25) and not less than 85% of the 3-*tert*-butyl-4-hydroxyanisole isomer. The same specifications are set by the Commission Regulation (EU) No 231/2012⁶ for its use as a food additive.

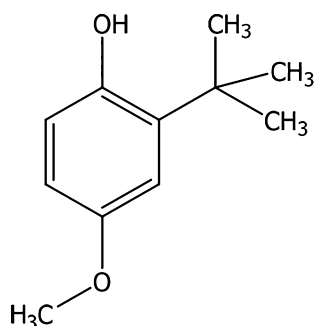


Figure 1: 3-*tert*-butyl-4-hydroxyanisole

⁶ 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 L 83, 22.3.2012, p. 1.

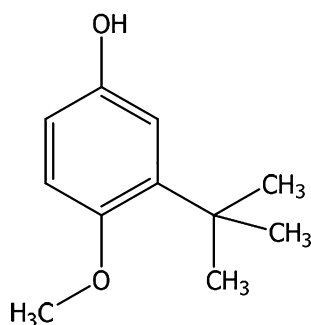


Figure 2: 2-*tert*-butyl-4-hydroxyanisole

The analysis of nine batches of the additive from two producers showed concentrations of BHA $\geq 99.3\%$ of which $\geq 90.0\%$ 3-*tert*-butyl-4-hydroxyanisole isomer,⁷ in compliance with the specifications. The impurities analysed (sulfated ash, phenolic impurities and arsenic in nine batches, and mercury in three batches)⁷ showed concentrations in compliance with the specifications for the food additive (sulfated ash $< 0.05\%$, phenolic impurities $< 0.5\%$, lead < 2 mg/kg, mercury < 1 mg/kg, arsenic < 3 mg/kg), while lead was found < 5 mg/kg in three batches and < 2 mg/kg in five batches.⁷ Toluene⁸ and hydroquinone⁹ were not detected in another eight batches of the additive.

The data provided on BHA indicate that the additive is a waxy solid with no dusting potential.¹⁰

3.1.1. Stability and homogeneity

The analysis of BHA in three batches of the additive stored in double high-density and high molecular high-density polyethylene bags inside fibre drums for 24 months at 25°C or for 6 months at 40°C showed a full recovery of the initial BHA concentration.¹¹

BHA 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 additive is soluble in fat, oils and emulsifiers (e.g. propylene glycol).¹² A homogeneous distribution of the additive in liquid antioxidant premixtures containing such substances is expected.

The analysis of 10 subsamples of a single batch of a vitamin–mineral premixture to which BHA was included through a liquid antioxidant premixture (containing 2% BHA) showed a coefficient of variation (CV) of 1.0%.¹³ Ten subsamples of one batch each of three feedingstuffs (one for poultry and two for lambs) with BHA at different inclusion levels (85, 50 and 95 mg/kg feed, respectively) were analysed for BHA concentration, showing CVs of 1.5%, 1.8% and 3.3%, respectively.¹³

3.1.2. Conditions of use

Butylated hydroxyanisole 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 feedingstuffs (alone or together with BHT (E 321) and/or ethoxyquin (E 324)) and for dogs with a maximum content of 150 mg/kg complete feedingstuffs (alone or together with BHT (E 321)).

3.2. Safety

3.2.1. Absorption, distribution, metabolism, excretion and residues

In its assessment of BHA as a food additive (EFSA ANS Panel, 2011), the ANS Panel of EFSA considered the available studies from earlier evaluations and new studies identified in the literature. The main issues concerning the metabolic fate of BHA, based on studies performed in rats, mice,

⁷ Technical dossier/Section II/Annex 2.1.3a, 2.1.3b.

⁸ Technical dossier/Section II/Annex 2.1.3b.

⁹ Technical dossier/Section II/Annex 2.1.3a.

¹⁰ Technical dossier/Section II/Physical state of each form of the product.

¹¹ Supplementary Information July 2012/Annex Qi_BHA_shelf-life_013-2005, Annex Qi_BHA_shelf-life_050-2006 and Qi_BHA_shelf-life_P4-685-2007.

¹² Technical dossier/Section II/Annex 2.1.5.

¹³ Technical dossier/Section II/Annex 2.4.2.

rabbits, dogs, monkeys and humans are that: (i) BHA is rapidly absorbed from the gastrointestinal tract, (ii) it is metabolised rapidly and excreted as metabolites in the urine and faeces, (iii) the major metabolites are glucuronides, sulfates and free phenols including *tert*-butylhydroquinone resulting from O-demethylation, (iv) the proportions of the different metabolites vary depending on species and dose, and (v) no accumulation of BHA or metabolites was observed in tissues.

The metabolism of BHA was examined in more detail in both rat and human microsomal fractions, purified isozymes of cytochrome P-450 and isolated rat hepatocytes. It was shown that *tert*-butylhydroquinone, *tert*-butylquinone and polar metabolites of 3-BHA including di-hydroxy-BHA, were formed in the presence of microsomal fractions or isolated cells, and that BHA metabolism appears to be mediated by cytochrome P-450. In addition, in the presence of reduced glutathione, a major metabolite was converted to a 3-BHA-glutathione conjugate (Cummings et al., 1985).

No data on the metabolic fate of BHA in birds or fish were found in the literature. Furthermore, there is very limited published data on BHA residues in tissues from target animal species fed BHA supplemented feeds.

In a study performed to investigate the stability of pig and chicken fat following administration of feeds supplemented with different antioxidants (François and Pihet, 1960), BHA residues in tissues were determined by spectrophotometry with a limit of quantification (LOQ) = 10 mg/kg wet tissue. Pigs (six animals per group, 50-kg body weight (bw)) and chickens (15 animals per group) were fed complete feeds supplemented with 0 or 1,000 mg BHA/kg, for 4 months and 8 weeks, respectively. At slaughter, three different fats, muscle, liver and kidneys were sampled from pigs, fat, muscle and liver from chickens. No BHA residues were found in any of the tissues.

In a study performed in Atlantic salmon (*Salmo salar*), groups of 50 fish (750–800 g) were fed fish meal and fish oil-based feeds supplemented with 0, 48.5, 92.5 and 225 mg BHA/kg feed for 12 weeks. Nine fishes per treatment were sampled at day 0, 28, 56, 84 of exposure and 98, the latter following to 2 weeks starvation of all groups, as in farming practice. Fillets and liver were taken for BHA analysis performed with an analytical method with a limit of detection (LOD) of 0.007 mg/kg. An increase in BHA concentration was observed in fish tissues during the period of exposure and according to the feed BHA concentration. A large variability was observed amongst animals that the authors attribute to suboptimal experimental conditions (it was necessary to give all groups medicated feed for anti-parasite treatment) and fish sampling performed 20–26 h postprandial, whereas BHA absorption peaks more rapidly. BHA concentrations in fillets and liver reached a plateau after 4 weeks, with maximum values of approximately (evaluation from a graph) 0.125 and 0.250 mg/kg, respectively (average values 0.085 and 0.125 mg/kg) for the high dose. After 2 weeks of starvation, BHA was not measurable in either fish fillets or liver (Petri et al., 2008).

Antioxidant residues, including BHA, were measured in various species of farmed fish (Atlantic salmon (*S. salar*), Atlantic halibut (*Hippoglossus hippoglossus*), Atlantic cod (*Gadus morhua*) and rainbow trout (*Oncorhynchus mykiss*)) from a wide geographical area and from different suppliers (Lundebye et al., 2010). The LOQ of the analytical method (high-performance liquid chromatography (HPLC) and fluorescence detection) was 0.005 mg BHA/kg wet tissue. BHA residues were detected only in salmon fillets (0.019 ± 0.024 mg/kg, range from < LOQ to 0.067 mg/kg).

In a survey study in which samples of milk were collected at farm level (organic and conventional dairy farms) and retail market, no residues (LOD = 0.001 mg/kg) of BHA were found in 53 samples of bovine milk (Pattono et al., 2009).

3.2.2. Toxicological studies

The toxicology of BHA has been previously evaluated by JECFA (JECFA, 1989) and by EFSA ANS Panel (EFSA, 2011) in relation to its use as a food additive. Both groups recognised that BHA gave positive results in some tests for genotoxicity, but considered that these effects were probably a consequence of the known pro-oxidant properties of high concentrations of BHA, which would not be evident at the lower concentrations in food to which consumers would be exposed. JECFA (JECFA, 1989) set an acceptable daily intake (ADI) of 0.5 mg/kg bw for BHA, by applying a 100-fold uncertainty factor to an no observed adverse effect level (NOAEL) of 0.1% diet (equivalent to 50 mg/kg bw per day) for forestomach hyperplasia (a precancerous lesion in the epigenetic production of squamous cell tumours of the forestomach) in rats.

The ANS Panel (EFSA, 2011) derived a lower confidence limit of the benchmark dose (BMDL₁₀) values of 83 and 115 mg/kg bw per day for forestomach hyperplasia seen in two carcinogenicity studies in rats. The ANS Panel did not base their ADI on the BMDL₁₀ values identified for forestomach

hyperplasia as it did not consider hyperplasia and tumorigenesis in the rat forestomach to be relevant to humans. The ANS Panel also set an ADI of 1 mg/kg bw for BHA, by applying an uncertainty factor of 100 to an NOAEL of 100 mg/kg bw per day for growth retardation, increased mortality and behavioural effects in pups born to female rats treated as part of a study of reproduction. It was noted that the BMDL₁₀ values were similar to the NOAEL that was used to derive their ADI.

The ANS Panel also noted that the potential endocrine effect of BHA has been investigated in a number of studies, but these were either performed at high dose levels or *in vitro* or not biologically relevant. In an *in vivo* study (Pop et al., 2013) on the anti-oestrogenic activity of BHA (and BHT), absolute and relative uterus weights were significantly decreased, in line with the finding observed in an older study (Kang et al., 2005). The available data do not allow conclusions on dose relationship of BHA and its anti-oestrogenic activity.

The FEEDAP Panel retains the ADI of 1 mg/kg bw proposed by the ANS Panel, and uses it in its evaluation of the consumer safety of BHA when used as a technological additive to animal feeds.

3.2.3. Safety for the target species

One tolerance study in chickens for fattening was provided by the applicant. The safety for the other target species was supported by published information: two publications based on a single pig study (Hansen et al., 1982; Würtzen and Olsen, 1986), one publication in fish and three publications with dogs.

3.2.3.1. Safety for chickens for fattening

A total of 504 one-day-old male chickens Ross 708 (initial bw 49.2 g) was fed diets supplemented with 0, 50 (0.3x maximum recommended dose), 150 (1x) or 500 (3.3x) mg BHA/kg (confirmed by analysis) for 35 days.¹⁴ Group size was 126 birds (nine replicates per treatment with 12-15 birds per pen). The basal diets (starter, grower, finisher) contained 20% barley and showed a low energy density (< 13 MJ ME/kg). Body weight and feed intake were measured after 10, 28 and 35 days since the beginning of the trial. On day 35, blood samples were taken from one bird per pen (selection criteria not described) and analysed for routine haematology¹⁵ and clinical biochemistry.¹⁶ The same animals were killed and necropsied. Statistical analysis was performed by analysis of variance (ANOVA) followed by t-test for group differences, for the zootechnical parameters based on the pen as experimental unit, for the other parameters on the individual bird.

Mortality was low (0–2.4%) and not treatment related. Final body weight was low (1,660–1,692 g), considering the duration of the study and the sex of the hybrid broiler strain used, thus reducing the sensitivity of the study. However, body weight and feed to gain ratio (1.79–1.82) did not show significant differences between the treatments. No relevant findings in necropsy analysis were observed in any group. The only significant differences seen in haematology were not dose related increased mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) in the treated groups compared to the control. No significant differences were observed in clinical biochemistry.

3.2.3.2. Safety for pigs

An embryotoxicity study was carried out on 43 gilts (Danish Landrace) for 110 days after mating. The results for gilts were described by Hansen et al. (1982), those for the fetuses by Wurtzen and Olsen (1986). Four groups of gilts (group size between 9 and 13) were given diets supplemented with 0, 5,000 (~ 33x the maximum recommended dose), 19,000 (~ 127x) and 37,000 (~ 247x) mg BHA/kg (corresponding to 0, 50, 200 and 400 mg/kg bw per day). Blood samples were taken at 2, 4 and 14 weeks after the start of the study and analysed for haemoglobin, packed cell value (PCV) and total erythrocyte, differential leucocyte and reticulocyte counts. After 110 days, all animals were killed and a thorough necropsy was performed. All fetuses were inspected, weighed, sexed and necropsied. A dorsal whole-body X-ray was made of each fetus after removal of the internal organs and the brain.

¹⁴ Supplementary Information July 2013/RF CZ13002BR_V4_16July13_MM final.

¹⁵ Red blood cells (RBC), haemoglobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC), lymphocytes, monocytes, neutrophils, eosinophil, basophils, platelets.

¹⁶ Glucose, calcium, inorganic phosphorus, cholesterol, triglycerides, phospholipids, uric acid, urea, creatinine, lactate dehydrogenase, alkaline phosphatase, glutamic oxaloacetic transaminase (GOT), glutamate pyruvate transaminase (GPT), total bilirubin.

No effects of BHA were observed on the clinical appearance of the dams and reproduction parameters, but a significant lower weight gain was observed in the high dose group. No differences in the haematological parameters were observed among the groups. The absolute and relative organ weights of the liver and the thyroid gland of dams were influenced by all the applied doses of BHA, showing a dose-related increase. The liver did not reveal any histopathological change. In the thyroid gland, large follicles with flattened epithelium were seen in some animals, especially in the highest dose group. Proliferative and parakeratotic proliferative changes of the stratified epithelium of the stomach were found in both control and treated pigs, so were not related to the dose of BHA. In addition, proliferative and parakeratotic changes of the oesophageal epithelium were observed in a few pigs in the two groups on the highest doses. No signs of a teratological effect on the fetuses were observed.

3.2.3.3. Safety for fish

Hendricks et al. (1994) fed for 8 months four groups of rainbow trout (*O. mykiss*) larvae (about 60 fish 2 weeks post swim-up) with diets containing 0 (two groups) or 6,000 (two groups) mg BHA/kg (~ 40x the maximum recommended dose). At the end of the study, all animals were killed, weighted and necropsied for tumour detection, with particular attention given to the stomach and liver. No differences in body weight were observed (final bw about 69 g). No stomach or liver tumours were found at the end of the study in either group.

3.2.3.4. Safety for dogs

A study was performed on 25 male and female beagle dogs (7–8 months old) (Tobe et al., 1986). Groups of three or four dogs were maintained for 6 months on diets containing BHA at concentrations of 0, 2,500 (~ 17x the maximum recommended dose), 5,000 (~ 33x) and 10,000 (~ 68x) mg/kg diet. The average daily intake of BHA was about 60, 110 and 220 mg/kg bw. General appearance and feed intake were recorded daily for each animal, body weight was measured weekly. Blood samples were collected 2 weeks before the start of treatment and 1, 3 and 6 months after for haematology¹⁷ and serum biochemistry¹⁸ analysis. At the end of the study, all animals were killed and tissues and organs¹⁹ were sampled and subject to histopathological examination. The stomach, oesophagus and duodenum were subjected to histometry and mitotic index analysis. All animals survived without showing signs of toxicity other than a dose-related retardation of growth, related to the significantly lower feed intake in the higher dose groups. Serum biochemical examinations conducted at 1, 3 and 6 months revealed a slight decrease in albumin concentration and an elevation of alkaline phosphatase and leucine aminopeptidase activity in the 5,000 and 10,000 groups. Histopathological and histometrical examination showed no evidence of mucosal alteration in the stomach, oesophagus or duodenum due to the administration of BHA. Relative liver weight was significantly increased in all BHA-treated groups in both sexes, absolute liver weight in male dogs only. However, no related histopathological changes were observed.

Beagle dogs (a total of 29 males (12 kg) and 30 females (10 kg)), were fed diets containing of 0, 10,000 (~ 68x the maximum recommended dose) and 13,000 (~ 87x) mg BHA/kg for 180 days (Ikeda et al., 1986). Feed intake were recorded daily for each animal, body weight was measured weekly. At the end of the study, all the dogs were killed and tissues and organs²⁰ were sampled and subject to histopathological examination. Both BHA doses resulted in a drastic reduction of feed consumption so that six animals had to be removed from the study. Body weight gain was reduced correspondingly, reaching significance for the highest dose. Liver weight was increased in both sexes at both BHA levels. The livers showed proliferation of smooth endoplasmic reticulum and hepatocytic cytoplasmic

¹⁷ Red blood cell counts, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelet count, white blood cell count and differential cell counts.

¹⁸ Total protein, albumin, albumin-globulin (A/G) ratio, blood-urea nitrogen, creatinine, glucose, phospholipid, triglyceride, total cholesterol, alkaline phosphatase, α -amylase, cholinesterase, glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase, γ -glutamyltransferase, leucine aminopeptidase, lactate dehydrogenase, calcium, inorganic phosphorus, sodium, potassium and chloride.

¹⁹ Brain, heart, lung, liver, kidney, spleen, adrenal, pituitary, thyroid, testis, ovary, prostate, epididymis, uterus, spinal cord, sciatic nerve, mesenteric lymph node, urinary bladder, sternum and femur.

²⁰ Adrenals, aorta, bone with marrow (lumbar vertebra), brain, diaphragm, oesophagus, external ears, eyes, epididymis, fallopian tubes, gall bladder, heart, kidneys, large intestine (caecum, colon, rectum), liver, lungs, lymph nodes (mesenteric), ovaries, pancreas, parathyroid, pituitary, prostate, salivary gland, sciatic nerve, skin with mammary gland and nipple, skeletal muscle (psoas), small intestine (duodenum, jejunum, ileum), spinal cord (lumbar), spleen, stomach, testis, thymus, thyroid, tonsils, trachea, urinary bladder and uterus.

myelinoid bodies. Light and electron microscopy revealed no proliferative or hyperplastic lesions of the stomach/gastric epithelium. Mixed function oxidases were increased in hepatic tissue.

Hodge et al. (1964) fed groups of three beagle dogs of either sex (about 10 kg bw) diets providing 0, 0.3, 3.0, 30 and 100 mg BHA/kg bw for 1 year (corresponding to approximately 0, 9 (0.06x the maximum recommended dose), 90 (0.6x), 900 (6x) and 3,000 (20x) mg BHA/kg diet). Body weight was measured individually; samples of urine were collected at the start and the end of the trial for the analysis of sugar and protein content. Blood samples were collected at the start of the study and at eight regular intervals during the year for analysis of haemoglobin concentration. At the end of the study, all dogs were killed and samples of tissues and organs²¹ were collected for macro- and microscopic examination and tissue deposition of BHA. No mortality occurred during the experiment. No effect of BHA on body weight, blood haemoglobin concentration, urine sugar and protein content, organs and tissues weight and histology was observed. BHA was not found in tissues up to the highest dose of ~ 3,000 mg BHA/kg diet (LOD 0.1 mg/kg).

3.2.3.5. Conclusions on safety for the target species

The 35-day tolerance study in chickens for fattening showed that 500 mg BHA/kg complete feed was tolerated. In a 110-day study on pregnant gilts, 5,000 mg BHA/kg complete feedingstuff, the lowest dose administered, is considered as a lowest observed adverse effect level (LOAEL). Rainbow trout from larval stage up to 8 months of age tolerated 6,000 mg BHA/kg feedingstuff without affecting body weight gain. Three studies with dogs with the duration between 6 and 12 months allow to select a LOAEL of 2,500 mg BHA/kg complete feedingstuff.

Although (i) the classical tolerance study in chickens for fattening shows some weaknesses in experimental design and reduced sensitivity due to low body weight gain, (ii) the other studies, available only as publications and not as full report, were not designed as tolerance studies, and (iii) some uncertainties remain on the extrapolation of the data to all animal species, a weight of evidence of the limited data supports that 150 mg BHA/kg complete feed would be a safe dose for all animal species. However, a possible exception could be cat, with its known lower capacity for glucuronidation of phenolic compounds and for which no specific data were available.

The FEEDAP Panel considers the use of BHA up to 150 mg/kg complete feed to be safe for all animal species except for cats, for which a safe dose could not be established.

3.2.4. Safety for the consumer

In its estimate of consumer exposure to BHA, the EFSA ANS Panel (2011) could not take into account the contribution of food of animal origin resulting from the use of BHA as a feed additive due to lack of data. Although tissue deposition of BHA is largely dependent upon species and dose, the limited data available on residue formation (Petri et al., 2008) and on pharmacokinetics (Conacher et al., 1986) suggest that BHA does not accumulate in mammalian and fish tissues (see Section 3.2.1). The limited data do not qualify for a conventional consumer exposure assessment for several reasons, mainly since (i) no studies with the currently authorised maximum feed concentration of BHA, and (ii) no studies of residues in eggs and milk were available. However, an estimate of the magnitude of exposure appears feasible, using the values of the LOQ for poultry and pig tissues and LOD for milk from a survey, and the highest concentration observed in fish. In an earlier study (François and Pihet, 1960) in which pigs and poultry were fed diets with 1,000 mg BHA/kg feed, no BHA residues were found in fat, muscle, liver and kidney (pigs only), however with an analytical method not satisfactory by current standards (high LOQ for BHA: 10 mg/kg). When applying the upper-bound procedure to that study in which dietary BHA exceeded the current maximum content by about seven times, 10 mg BHA/kg animal tissues/products would be considered as the maximum achievable BHA concentration. The data used for the calculation are described below, and the calculation of the estimates exposure is summarised in Table 1.

²¹ Fat, brain, liver and kidney.

Table 1: Estimate of consumer exposure calculated following the provisions of Regulation (EC) No 429/2008, considering a 60-kg adult

| Food product | Amount consumed (g) | BHA concentration (mg/kg fresh matter) | BHA daily intake (mg/person) | BHA daily intake (mg/kg bw) |
|--------------|---------------------|--|------------------------------|-----------------------------|
| Meat | 300 | 10 | 3 | 0.05000 |
| Liver | 100 | 10 | 1 | 0.01667 |
| Kidney | 50 | 10 | 0.5 | 0.00833 |
| Fat | 50 | 10 | 0.5 | 0.00833 |
| Milk | 1,500 | 0.001 | 0.0015 | 0.00003 |
| Fish flesh | 300 | 0.250 | 0.075 | 0.00125 |

BHA: butylated hydroxyanisole; bw: body weight.

The most conservative estimate of exposure resulting from consumption of food from animals²² fed BHA at the highest authorised feed concentration would then be 5 mg BHA/person per day (0.083 mg/kg bw for a 60-kg person). This figure corresponds to about 8% of the ADI proposed by the EFSA ANS Panel (1 mg/kg bw). Fillets from salmon (*S. salar*) fed diets with up to 225 mg BHA/kg for 3 months did not contain more than 0.250 mg BHA/kg (Petri et al., 2008). Consumption of 300 g of salmon fillet would result in a BHA exposure of ≤ 0.0013 mg BHA/kg bw per day (about 0.13% of the ADI). A conservative calculation of consumer exposure by milk (all values of a survey below LOD of 0.001 mg BHA/kg, (Pattono et al., 2009)) results in a value of 0.000025 mg BHA/kg bw and day. Both food items milk and fish do not substantially contribute to the exposure of consumer to BHA (Table 1). Although no residue data in eggs are available, the FEEDAP Panel considers it unlikely that the contribution to the consumer exposure from eggs would be of a higher order of magnitude.

In this context, it is noted that the exposure of adult consumers to BHA from its use as a food additive amounts to 0.1 (mean) to 0.14 mg/kg bw per day (97.5th percentile) (EFSA ANS Panel, 2011) and from its use as a food contact material to 0.4 mg/kg bw per day (EFSA ANS Panel, 2012).

3.2.4.1. Conclusions on safety for the consumer

No information on residue in eggs is available. A conservative estimate following Regulation (EC) No 429/2008 supports the conclusion that exposure of the consumer from BHA used in animal nutrition would not exceed 10% of the ADI. Therefore, the FEEDAP Panel concludes that no concern for consumer safety would arise from the use of BHA as a feed additive at the maximum concentration of 150 mg/kg feed.

3.2.5. Safety for the user

The exposure to BHA via skin has been associated with recurrent urticaria or exacerbations of urticaria in patients with chronic urticaria (Juhlin, 1981; Goodman et al., 1990) and a number of industrial cases in which skin contact has produced contact dermatitis have been described (Osmundsen, 1980). Therefore, the additive is considered a skin irritant and a potential skin sensitiser. No information was available on the eye irritation and sensitisation potential of undiluted BHA.

The data presented for the additive under application indicate the absence of dusting potential. Exposure of the user via inhalation is considered unlikely; therefore a risk is not expected.

3.2.6. Safety for the environment

BHA is not a physiological/natural substance of established safety for the environment. Consequently, according to the EFSA guidance (EFSA, 2008a) a Phase I assessment has to be conducted to determine the predicted environmental concentrations (PECs) of the feed additive in relevant environmental compartments.

3.2.6.1. Phase I assessment

All PECs for BHA were calculated according to the technical guidance for assessing the safety of feed additives for the environment (EFSA, 2008a), based on inclusion of 150 mg BHA/kg feed and 100% excretion. A Organic carbon normalised partition coefficient (K_{oc}) value of 871 L/kg and a solubility of 213 mg/L, as provided by the applicant, were used for the calculation. The highest PECs

²² 300 g meat, 100 g liver, 50 g kidney and 50 g fat, according to Regulation (EC) No 429/2008.

from the use of BHA as a feed additive for different animal categories were: PEC_{soil} 3,200 $\mu\text{g}/\text{kg}$; $PEC_{surface\ water}$ 71 $\mu\text{g}/\text{L}$; PEC_{sed} 32 mg/kg and PEC_{swaq} 0.4 $\mu\text{g}/\text{L}$.

The PECs for BHA exceeded the established limits of 10 $\mu\text{g}/\text{kg}$ in soil and sediment, and 0.1 $\mu\text{g}/\text{L}$ in surface water; therefore, a Phase II assessment is required.

3.2.6.2. Phase II assessment

Predicted no effect concentrations (PNECs) have been established in different animal species for BHA. They were summarised by REACH²³ as follow: (i) soil (terrestrial species): 13.38–13.62 mg/kg soil dry weight (dw); (ii) marine sediment (fish farmed in sea cages): 28.54–29.22 mg/kg sediment dw and (iii) freshwater (intermittent release): 15–19 $\mu\text{g}/\text{L}$. The highest PNECs and the PEC/PNEC ratios are summarised in Table 2.

Table 2: PNECs for BHA in experimental animals from different environmental compartments and ratios between PEC and PNEC, expected from the use of BHA as a feed additive

| Environmental compartment | PNEC | PEC/PNEC ratio |
|---|---|----------------|
| Soil | | |
| Terrestrial species | 13.38 mg/kg soil dw | 0.24 |
| Fresh water | | |
| Fish farmed in raceways, ponds, tanks, and recirculation systems | 15 $\mu\text{g}/\text{L}$ | 0.03 |
| Terrestrial animals | 15 $\mu\text{g}/\text{L}$ | 4.6 |
| Sediment | | |
| Fish farmed in sea cages | 28.54 mg/kg sediment dw | 1.1 |

PNEC: predicted no effect concentration; PEC: predicted environmental concentration; dw: dry weight.

The PEC/PNEC ratio is < 1 for soil and for fresh water, the latter considering fish farmed in raceways, ponds, tanks and recirculation systems. The ratio slightly exceeds the value of 1 (1.1) for sediment; however, considering that the PEC is calculated at first tier, without any refinement, it can be concluded that no specific concern is expected for the marine environment. The PEC/PNEC ratio for fresh water is up to 4.6, when considering terrestrial animals. The PEC values can be reduced taking in to consideration metabolism of BHA in the target animals and/or degradation in manure and/or using more sophisticated models.

Health Canada (Environment Canada HC, 2010) concluded that BHA did not meet the ecological categorisation criteria set out in the Persistence and Bioaccumulation Regulation. BHA is not persistent in water, soil or air, and is expected to have a low bioaccumulation potential and is not inherently toxic to aquatic organisms (Environment Canada HC, 2010). A similar conclusion was reached but the US EPA (2005), which concluded that half-life of BHA is short, some photolysis is likely but it is not hydrolysed in the environment. Primary degradation occurs in the order of days to weeks with ultimate degradation (mineralisation) in weeks to months.

The half-life was calculated using BioWin3 (Ultimate Survey Model), which gives a rating number. This rating number r (approximately 3 for BHA) was translated into a half-life using the formula by Arnot et al. (2005), resulting in a DT_{50} of about 10 days. Applying the first-tier leaching assessments of feed additives (see *Technical guidance for assessing the safety of feed additives for the environment*), the use of BHA as a feed additive for terrestrial animals at 150 mg/kg feed results in no concern for groundwater.

3.2.6.3. Conclusions on safety for the environment

The FEEDAP Panel concludes that is unlikely that the use of BHA up to 150 mg/kg feed in animal nutrition would pose a risk to the environment.

3.3. Efficacy

BHA is authorised to be added as an antioxidant to foods with a wide range of moisture content at concentrations of 40–400 mg/kg . Since the same effect can be reasonably assumed for feedingstuffs, no studies are required to demonstrate the efficacy of BHA as antioxidant in feedingstuffs for all animal species.

²³ Available at <https://echa.europa.eu/brief-profile/-/briefprofile/100.042.315>

4. Conclusions

The FEEDAP Panel considers the use of BHA up to 150 mg/kg complete feed to be safe for all animal species except for cats, for which a safe dose could not be established from the tolerance data.

BHA is rapidly absorbed from the gastrointestinal tract; it is metabolised rapidly and excreted as such and as metabolites in the urine and faeces. The proportions of the different metabolites vary depending on species and dose. No accumulation of BHA or metabolites was observed in tissues.

The FEEDAP Panel concludes that no concern for consumer safety would arise from the use of BHA as a feed additive at the maximum concentration of 150 mg/kg feed.

The additive should be considered a skin, eye irritant and a potential skin sensitiser. Exposure of the user via inhalation is considered unlikely; therefore, a risk is not expected.

The use of BHA at the maximum concentration proposed is unlikely to pose a risk to the environment.

Since BHA is authorised as an antioxidant for food use at comparable use levels, no studies are required to demonstrate the efficacy of BHA as an antioxidant in feedingstuffs for all animal species.

Documentation provided to EFSA

- 1) Butylated hydroxyanisole for all animal species. September 2010. Submitted by FEFANA asbl.
- 2) Butylated hydroxyanisole for all animal species. Supplementary information. July 2012. Submitted by FEFANA asbl.
- 3) Butylated hydroxyanisole for all animal species. Supplementary information. December 2012. Submitted by FEFANA asbl.
- 4) Butylated hydroxyanisole for all animal species. Supplementary information. July 2013. Submitted by FEFANA asbl.
- 5) Butylated hydroxyanisole for all animal species. Supplementary information. December 2016. Submitted by FEFANA asbl.
- 6) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Butylated hydroxyanisole.
- 7) Comments from Member States.

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Abbreviations

| | |
|--------------------|--|
| ADI | acceptable daily intake |
| ANOVA | analysis of variance |
| ANS | EFSA Scientific Panel on Additives and Nutrient Sources added to Food |
| BHA | butylated hydroxyanisole |
| BHT | butylated hydroxytoluene |
| BMDL ₁₀ | lower confidence limit of the benchmark dose |
| bw | body weight |
| CAS | Chemical Abstracts Service |
| CV | coefficient of variation |
| DT ₅₀ | period required for 50% dissipation |
| dw | dry weight |
| ECHA | European Chemicals Agency |
| EURL | European Union Reference Laboratory |
| FAO | Food Agricultural Organization |
| FCC | Food Chemical Codex |
| FEEDAP | EFSA Panel on Additives and Products or Substances used in Animal Feed |
| GC-FID | gas chromatography coupled to flame ionization detection |
| GOT | glutamic oxaloacetic transaminase |
| GPT | glutamate pyruvate transaminase |
| HPLC | high-performance liquid chromatography |
| JECFA | The Joint FAO/WHO Expert Committee on Food Additives |
| K _{oc} | Organic carbon normalised partition coefficient |
| LOAEL | lowest observed adverse effect level |
| LOD | limit of detection |
| LOQ | limit of quantification |
| MCH | mean corpuscular haemoglobin |
| MCHC | mean corpuscular haemoglobin concentration |
| MCV | mean corpuscular volume |
| NOAEL | no observed adverse effect level |
| PEC | predicted environmental concentration |
| PNEC | predicted no effect concentration |
| RBC | red blood cells |

| | |
|----------------|--|
| RP-HPLC-UV-DAD | reversed phase high performance liquid chromatography coupled with ultraviolet-diode-array detection |
| Rrec | recovery rate |
| RSDip | standard deviation for intermediate precision |
| RSDr | standard deviations for repeatability |
| SCF | Scientific Committee on Food |
| WBC | white blood cells |
| WHO | World Health Organization |

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Butylated Hydroxy Anisole (BHA)

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 (RSDr) 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 RSDr ranging from 0.6 to 3.9%;
- a standard deviation for intermediate precision (RSDip) ranging from 2.6 to 6.4%;
- a recovery rate (RRec) 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.