DTU Updated assessment of BHA and BHT

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Updated assessment of BHA and BHT

Request from FVST

In English

The Danish Veterinary and Food Administration (FVST) has asked DTU National Food Institute to assess:

- Whether there are new studies (since the two EFSA risk assessments in 2011 and 2012) indicating a need for new hazard assessments of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT)? In addition, the relevance of setting a group-ADI should be addressed.
- Whether EFSA's exposure assessments of BHA and BHT are conservative enough to not alter the risk managing of BHA and BHT as food additives in foods and additives to food contact materials?
- Whether the current risk managing of BHA and BHT is sufficient in regard to exposure from other sources?

In Danish

DTU Fødevareinstituttet er af Fødevarestyrelsen, Kemi og Fødevarekvalitet, blevet bedt om at foretage en vurdering af:

- Om der er nye studier (siden 2012) der peger på et behov for at EFSA laver en ny farevurdering af BHA og BHA? Er der evt. behov for at fastsætte en gruppe-ADI for stofferne?
- Om EFSAs eksponeringsvurderinger af BHA og BHT er så konservative, at der fortsat ikke er behov for at ændre i håndteringen af BHA og BHT som tilsætningsstof til fødevarer og fødevarekontaktmaterialer?
- Om den nuværende håndtering af BHA og BHT tager tilstrækkelig højde for andre kilder?

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Conclusion

- On identification of new studies and the possibility of establishing a group-ADI:
  For BHA two articles were found to be possibly relevant (Hung *et al*., 2012; Sun *et al*., 2020). It was not possible to deduce from the study by Sun *et al*. whether the effects on the investigated endpoints (adipogenesis and lipid accumulation) could be considered adverse. The results of the study by Hung *et al*. point towards an adverse effect on the immune system, but there are concerns regarding the quality of the study, both in relation to the methods used and the statistical procedures undertaken. None of these studies is suitable for re-evaluation of the ADI. For BHT, none of the articles found in the open literature were considered relevant in relation to re-evaluation of the ADI. Regarding establishment of a group-ADI, this is considered relevant, as both substances show potential to disrupt the thyroid-hormone system. The ADI of 0.25 mg/kg bw per day set for BHT is proposed as a conservative group-ADI for BHA and BHT. Based on this DTU FOOD recommends to re-evaluate the hazard assessment of BHA and BHT.

- On the exposure assessment of BHT:
  The EFSA (2012a) assessment is considered highly conservative regarding oral exposure of BHT from its total use as food additive and food contact material (FCM) additive. The assessment does however, not take into account oral and dermal exposure from its use in personal care products (PCPs) or a potential oral exposure from carryover into food of BHT used in feed. Altogether, this makes the EFSA assessment uncertain with regard to how well the estimated exposure correspond to a realistic high intake exposure of BHT. When BHT is used up to the maximum permitted level (MPL) as food additive and as FCM additive, with a migration level equal to the specific migration limit (SML) into 1 kg food per day, the ADI is exceeded by children at the estimated mean and 95th percentile of exposure according to calculations made by EFSA (2012a). There is consequently no room for exposure from other sources within the given ADI. As reported by VKM (2019) however, also substantial exposure is estimated from PCPs through oral and dermal uptake (relative exposure of 37 and 17 %, respectively). Furthermore, carryover from feed to food is also a potential source of BHT exposure. To reduce the uncertainty related to the BHT exposure estimate, DTU FOOD recommends a refined exposure assessment (including more concentration data on actual use levels and migration levels) and inclusion of other sources such as PCPs and carryover from feed to food. Should the refined exposure estimate including all sources exceed the ADI, it may be suggested to consider allocation factors of the ADI for each application domain.

- On the exposure assessment of BHA:
  In EFSA (2012b), exposure estimates are based on MPLs of BHA as food additive and as additive to FCMs on a migration level equal to the SML in 1 kg of packed food per day. The EFSA estimate is thus highly conservative and it is assumed to substantially overestimate exposure of BHA. More data on BHA in FCMs and its potential migration onto food would be needed to have exact information on actual BHA levels in different kinds of packed food. No other sources (beside its use as food and FCM additive) contributing significantly to BHA exposure is reported in literature. The current risk management is thereby considered appropriate concerning inclusion of relevant sources of BHA. Due to the highly conservative BHA exposure estimate from its use as food additive and additive to FCMs it has been assumed that actual exposure levels probably lies below ADI, despite the EFSA estimate showing that most age groups exceeds the ADI on both average and high level consumption. DTU FOOD notes that a lower ADI for BHA would increase the need for a refined exposure assessment for BHA including concentration data on actual use levels and migration levels.

Background

BHA and BHT were re-evaluated as food additives by EFSA in 2011 and 2012, respectively (EFSA, 2011, 2012a). In addition, a statement on the safety assessment of the exposure to BHA applying a new exposure assessment methodology was issued in 2012 (EFSA, 2012b).

EFSA risk assessment of BHT

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the EU Scientific Committee for Food (SCF) established ADIs for BHT in 1996 and 1987, respectively. JECFA allocated an ADI of 0-0.3 mg/kg bw per day based on reproductive effects (including litter size, sex ratio and pup body weight gain during the lactation period) and hepatic enzyme induction seen in 2-generation studies in rats. The SCF established an ADI of 0-0.05 mg/kg bw per day based on thyroid, reproduction and haematological effects in the rat. In 2012, EFSA aligned with JECFA and established a new ADI of 0.25 mg/kg bw per day based on two 2-generation studies in rats using an assessment factor of 100 (EFSA, 2012a). It was concluded that exposure of adults to BHT used as food additive is unlikely to exceed the ADI, but that exposure of children for some European countries (Finland, The Netherlands) exceeds the ADI at the 95th percentile.

The EFSA (2012a) exposure estimate uses a very conservative scenario assuming BHT is used as food additive in all relevant foods (including chewing gum) at maximum reported use level from industry (the same level as the MPL). In addition, a worst-case exposure from food contact materials (FCMs) based on the assumption that every day throughout a lifetime an adult person weighing 60 kg consumes 1 kg of food packed in plastics containing BHT with a migration level equal to the SML is used.

EFSA risk assessment of BHA

An ADI for BHA of 0.5 mg/kg bw per day was previously established by JECFA and SCF, both in 1989. This ADI was based on proliferative changes in the rat forestomach. In 2011, EFSA established a new ADI of 1.0 mg/kg bw per day based on growth retardation, increased mortality and behavioural effects in rat pups and using an assessment factor of 100 (EFSA, 2011). It was considered that forestomach hyperplasia in rodents may no longer be relevant for human risk assessment. It was concluded that at the current levels of BHA used as food additive, refined intake estimates were generally below the ADI for all age groups. In 2012, EFSA issued a broader exposure assessment of BHA used as food additive in foods and as additive to FCMs (EFSA, 2012b). Actual use levels were not available so the exposure assessment could only be made on maximum permitted levels (MPLs).

The use as additive to FCMs was based on the conservative assumption that consumers from all the populations groups (toddlers, children, adolescents, adults and the elderly) consume 1 kg of food packed in plastics containing BHA at the migration level equal to the SML. It was concluded that combined exposure to BHA from its use as food additive in foods and additive to FCMs exceeds the ADI for most populations groups on average and on high level consumption.

VKM risk assessment of BHT

A more detailed exposure assessment of BHT was made by the Norwegian Scientific Committee for Food and Environment (VKM) in 2019. VKM (2019) evaluated whether the ADI established by EFSA (2012a) for BHT needed revision, by performing a literature search to retrieve articles reporting on adverse health effects related to BHT and published between 2012 and 2017. Three studies (Negritto *et al*., 2017; Pop *et al*., 2013 and Ma *et al*., 2013) on adverse health effects related to BHT were identified but were not considered relevant for the hazard identification and characterisation of BHT and did not lead to revision of the ADI established by EFSA (EFSA, 2012a).

The Norwegian exposure estimate on BHT is based on literature data on concentrations of BHT in different foods as well as consumption data from the Norwegian food survey (Totland *et al*., 2012) based on 1787 participants between 18 and 70 years and from a European study (Husøy *et al*., 2019). VKM (2019) presents a "realistic exposure scenario" including data on oral and dermal uptake of BHT (concentration data from Europe/USA). The relative contributions to exposure from different sources estimate that BHT exposure is mainly from oral intake from food (46 %), however, with substantial contributions from personal care products (PCPs) through oral (37 %) and dermal uptake (17 %). The "realistic exposure scenario" for BHT exposure is largely below the ADI of 0.25 mg/kg bw per day for both females and males.

VKM (2019) also presents a "high exposure scenario" from oral and dermal uptake based on concentration data from countries both inside and outside Europe/USA. The high total internal BHT exposure estimate exceed the ADI level up to three orders of magnitude for both females and males. It is concluded by VKM (2019) that the "high exposure scenario" has a rather high uncertainty due to a low number of concentration data on the high level food (butter, margarine and cheese) and due to no concentration data on these specific foods from Europe or USA.

The VKM (2019) exposure assessment of BHT is described in further detail under the *Evaluation of BHT exposure* on page 10 below and is used to evaluate the EFSA (2012a) opinion on BHT exposure and to answer the questions on BHT by FVST.

Literature search and inclusion criteria for re-evaluation of ADI

A literature search was conducted in PubMed covering articles published from 2011 to the search date (see Table 1). The search strings for BHA and BHT were identical, except for the chemical name and CAS registration number (RN). Toxicological relevance of the articles was screened in three steps based on 1) title, 2) abstract 3) full text article (Table 1). Each step included the following inclusion criteria:

- In the first step (Screen 1), the title was evaluated and the article included for further screening if it indicated a toxicological study of BHA or BHT in an *in vivo* or *in vitro* system. Studies where toxicological relevance was doubtful were included rather than excluded to avoid loss of important studies.
- In the second step (Screen 2), the abstracts of the chosen articles in Screen 1 were evaluated. Studies were included if they were conducted *in vivo*, if more than one dose of BHA or BHT was investigated and if it was a toxic rather than protective/treatment effect of BHA or

BHT that was investigated (many studies turned out to investigate protective or treatment effects of BHT in different disease scenarios).

- In the third step (Screen 3), full articles from Screen 2 were read and evaluated based on the method used and the endpoints investigated. To evaluate reliability the Klimisch score system (Klimisch *et al*., 1997) was used.

Table 1 shows the search string, date of search, number of hits and number of articles included in three different screening steps, which were used to narrow down the number of articles and identifying articles possibly relevant for ADI evaluation.

	Search string	Date of search	Number of hits in PubMed Screen 1 (title)	Screen 2 (abstract)	Screen 3 (full text)	Possibly relevant
BHA	((hydroxyanisole OR "25013-16-5") AND (Rats OR mice OR toxicity)) AND (("2011"[Date - Publication] : "2020"[Date - Publication]))	10/6 - 2020	134	39	7 *(National Toxicology Program, 2011; Hung et al., 2012; Chow and Mahalingaiah, 2016; Yuan and Marikawa, 2017; Yang et al., 2018; Liu and Mabury, 2019; Sun et al., 2020),*	2 *(Hung et al., 2012; Sun et al., 2020)*
BHT	((hydroxytoluene OR "128-37-0") AND (Rats OR mice OR toxicity)) AND (("2011"[Date - Publication] : "2020"[Date - Publication]))	12/6 - 2020	148	28	1 *(Shearn et al., 2011)*	0

For **BHA** two articles were found to be possibly relevant (Hung *et al*., 2012; Sun *et al*., 2020). Below a short summary of the studies is presented as also seen in Table 2.

Sun *et al*., 2020: This study cover endpoints related to obesity, more specifically adipogenesis and lipid accumulation. Male C57BL/6J mice (4 weeks old) were orally exposed to 3-BHA (3-*tert*.-butyl-4-hydroxyanisole, one of the two BHA isomers in E 320) for 18 weeks. Two doses were investigated, 1 and 10 mg/kg bw per day, and two cohorts of animals were included; normal diet or high fat diet (each with control and exposed groups, n = 8 per group). The specific endpoints included body weight gain, adipose tissue accumulation and distribution, adipocyte area, fasting blood glucose and insulin levels, oral glucose tolerance test, lipid content in blood as well as gene expression in fat. Effects were seen for several endpoints at both doses giving a lowest observed effect level (LOEL) of 1 mg/kg bw per

day. These are interesting results and shows that 3-BHA do seem to affect different aspects of adipogenesis, however, adversity of the effects, on the endpoints investigated, is difficult to deduce. As adversity is difficult to establish, this study is not suitable for re-evaluation of the ADI.

Hung *et al.*, 2012: This study investigated effects on the immune response. Male BALB7c mice (8 weeks old) were orally exposed to BHA for up to three weeks. The results point towards an adverse effect on the immune system, but there are concerns regarding the quality of the study, both in relation to the methods used and the statistical procedures undertaken. The study is therefore considered not reliable with a Klimisch score 3, and is not suitable for re-evaluation of the ADI.

For **BHT**, none of the articles found in the open literature were considered relevant in relation to re-evaluation of the ADI.

Table 2 Overview of method, effects, NOEL/LOEL as well as Klimisch score of the two relevant studies for BHA

Author	Method	Effects	NOEL/LOEL or NOAEL/LOAEL	Klimisch Score
Sun *et al.*, 2020	Male C57BL/6J mice 4 weeks old 3-BHA > 98 % pure Oral administration (intragastric) 2 doses: 1, 10 mg/kg bw per day) for 18 weeks Normal diet or high fat diet n = 8.	BW gain in the ND group not affected by exp. In the HFD group 1 mg/kg bw 3-BHA decreased BW and 10 mg/kg bw increased BS. Food intake was not affected. No haematological toxicity was seen. Adipose tissue accumulation and distribution was investigated (visceral fat (pWAT) and subcutaneous fat (iWAT). ND: 3-BHA slightly increased the relative weight of pWAT and iWAT compared to control. HFD: relative masses of iWAT and pWAT slightly reduced in 1 mg/kg bw. At 10 mg/kg bw iWAT was slightly increased and a significant weight elevation was seen in pWAT. Adipocytes area in 3-BHA exposed mice (ND) were significantly increased at 1 (40.3 %), 10 (31.7 %) mg/kg bw, whereas for HFD significant increase was seen in 10 (42.7 %) mg/kg bw only. Fasting blood glucose levels and insulin levels were not significantly affected in 3-BHA exposed groups. Oral glucose tolerance test at week 6, 12, and 18 (oral gavage of 2 mg/kg glucose): No significant affects seen in 3-BHA exposed groups. Triglyceride (TG), total cholesterol, high density lipoprotein cholesterol (HDL-C)	A NOEL was not identified in this study. The LOEL was 1 mg/kg bw, with effects on BW, adipocyte area, and blood lipids.	Reliability 2 - Acceptable, well-documented study, comparable to guideline standards.

		and low density lipoprotein cholesterol (LDL-C) was investigated in blood. 3-BHA significantly elevated TG levels in all exposed mice (ND, HFD, both 3-BHA doses). 3-BHA (both doses) increased total cholesterol in ND mice, no effect in HFD mice. HDL-C was significantly increased in 1 mg/kg bw ND mice, no effects seen on LDL-C. Gene expression was investigated in pWAT, showing dose dependent upregulation of mRNA levels of *PPARγ, Srebp1c, CD36, IL6* and *TNFα* in both ND and HFD groups. In HFD mice, a dose related upregulation was seen for *Acc* and *Hsl*.		
Author	**Method**	**Effects**	**NOEL/LOEL or NOAEL/LOAEL**	**Klimisch Score**
Hung *et al.*, 2012	Male BALB/c mice 8 weeks old. BHA (purity not reported). Oral gavage Dose: non-vehicle control, vehicle control (olive oil), 2 doses of BHA: 100, 200 mg/kg, for up to 3 weeks (they refer to another publication here) n =10 NOTE: Unclear if they use the vehicle control as control in statistical analysis. Also, they use students t-test. ANOVA would be appropriate.	No effects were seen on BW or weight of the spleen after BHA treatment (and no difference between non-vehicle control and vehicle control). Leukocytes were isolated from blood and surface markers investigated. CD3 and CD19 were significantly increased in both exposure groups. No effect was seen on Mac-3 and CD11b. Phagocytosis was investigated in leukocytes from peripheral blood mononuclear cells (PBMC) and peritoneal cavity. The results indicate increased phagocytosis after BHA exposure in PBMC, but not peritoneal cavity. Natural killer (NK) cell cytotoxicity was evaluated in splenocytes (*in vitro* culture). The results are rather confusing - the results heading and figure text says there are effects, but the graph shows no significance and reading the results text also reports no significance.	A NOEL was not identified in this study. The LOEL was 100 mg/kg bw, with effects on surface markers in leukocytes.	Reliability 3 - Uncertainties in statistical method as well as the statistical method chosen. ANOVA is the appropriate choice when more than the 2 groups are compared; however, in this study they used Student's t-test, which increases the risk of type 1 error. - The description of flow cytometry and data is not clear and does not follow the conventional way to evaluate flow cytometry. - It is unclear how many animals are used for the flow cytometry data. - There are uncertainties concerning

				the markers and cell types investigated in blood.

BW = body weight, ND = normal diet, HFD = high fat diet, pWAT = visceral fat, iWAT = subcutaneous fat, TG = triglyceride, HDL-C = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol, PBMC = peripheral blood mononuclear cells, NK cell = natural killer cell, NOEL = no observed effect level, NOAEL = no observed adverse effect level, LOEL = lowest observed effect level, LOAEL = lowest observed adverse effect level.

Relevance of a group-ADI

Both BHA and BHT show potential to disrupt the thyroid hormone system. In a mixture risk assessment report (Larsen *et al*., 2017), BHA and BHT were grouped with other thyroid hormone disrupting chemicals. For this purpose, we derived reference values specific to effects on the thyroid hormone system *in vivo*.

In brief, for BHA, a reference value of 1 mg/kg bw per day was derived from a reproductive toxicity study by Jeong *et al*., (2005), showing reduced serum T4 and altered thyroid gland histology in a 2-generation rat study using oral exposure. Three doses of BHA were administered to 12 rats of each sex per dose group from pre-gestation until 13 weeks of age of the offspring (F1). The thyroid disrupting effect of BHA was confirmed in a pig study showing increased absolute and relative thyroid weight (Hansen *et al*., 1982, cited in EFSA, 2011). For BHT, a reference value of 0.25 mg/kg bw per day was derived from two rat studies by Søndergaard and Olsen (1982) and Olsen *et al*., (1986). Both studies showed altered thyroid gland histology, but no changes in thyroid hormone levels after 13 and 4 weeks of exposure, respectively. The studies on BHT were used by EFSA (2012a) to set an ADI, whereas the study on BHA was not considered relevant for risk assessment by EFSA (2011). Specifically, the Panel noted deviations from OECD guidelines and that the decreases reported in the affected parameters were generally less than 10 % without a clear dose response, and that the ranges reported for these parameters overlap due to large standard deviations. Thus, the Panel concluded that the statistically significant (at $P < 0.05$) effects reported in this study were not biologically relevant and therefore cannot be used to derive a point of departure for the risk assessment on BHA. DTU FOOD considers the reduction of serum T4 relevant as an argument for grouping with BHT in relation to a group-ADI, as indications of thyroid effects were confirmed in the mentioned pig study.

The observed effects are considered relevant to a cumulative assessment group “Hypothyroidism”, which was defined by EFSA (2019) as “*an altered function of the thyroid gland resulting in follicular cell hypertrophy, hyperplasia and neoplasia*”, and corresponding to a cumulative assessment group: “Effects on follicular cells and/or the thyroid hormone system” in the Chemical Mixture Calculator (Boberg *et al*., *submitted*). This is based on the understanding that low thyroid hormone levels lead to increased thyroid-stimulating hormone (TSH) levels as a compensatory response of the hypothalamic–

pituitary–thyroid (HPT) axis, this condition is referred to as 'hypothyroidism'. If this stimulation is sustained over time, it increases the risk of morphological and/or histopathological changes in the thyroid (hypertrophy and/or hyperplasia). ECHA/EFSA Guidance for the identification of endocrine disruptors reflects specifically in its Appendix A on the human relevance of effects on the thyroid (ECHA and EFSA, 2018). Here, it is noted that: "*Substances inducing histopathological changes (i.e. follicular cell hypertrophy and/or hyperplasia and/or neoplasia) in the thyroid, with or without changes in the circulating levels of THs, would pose a hazard for human thyroid hormone insufficiency in adults as well as pre- and post-natal neurological development of offspring.*" For both substances, the observed effects mainly on thyroid gland histology is thus considered adverse and human relevant.
Overall, a group-ADI for BHA and BHT is considered relevant. FVST does not ask for considerations on the size of such a group-ADI. Although, as a conservative approach, a group-ADI could be set to the lowest of the two reference doses of the included substances. In this case, the ADI of 0.25 mg/kg bw per day set for BHT could be used, carrying in mind that the contribution of BHA to the overall risk may be overestimated. However, possible potency differences between BHA and BHT are not clear, given that thyroid hormone disrupting effects of these compounds have not been abundantly examined.

Evaluation of BHT exposure

This evaluation of BHT exposure is primarily based on the thorough exposure assessment made by VKM in 2019 (VKM, 2019) and on the exposure assessment made by EFSA in 2012 (EFSA, 2012a). Butylated hydroxytoluene (BHT) CAS RN 128-37-0, is authorised as food additive (E 321) in EU (EU Regulation 1333/2008[1] and amendments) for use in fats and oils (only for professional manufactures of heat treated food), in frying oil and frying fat (excluding olive oil), in lard, fish oil, beef, poultry and sheep fat and is permitted in concentrations up to 100 mg/kg fat (EFSA, 2012a). It is present in different foods such as dry breakfast cereals, potato flakes, enriched rice, and margarine (Nieva-Echevaria *et al.*, 2015). Moreover, BHT is authorised/permitted for the following uses:

- It is authorised for use up to 400 mg/kg in food supplements as given in Directive 2002/46/EC[2].
- It is authorised for use in feed for all species or categories of animals except dogs[3].

[1] 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)
[2] DIRECTIVE 2002/46/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements (OJ L 183, 12.7.2002, p. 51)
[3] REGULATION (EC) No 1831/2003 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 September 2003 on additives for use in animal nutrition (OJ L 268, 18.10.2003, p. 29)
[4] COMMISSION REGULATION (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food (OJ L 12, 15.1.2011) and amendments

- It is permitted in chewing gum alone or in combination with other antioxidants (e.g. BHA) at a maximum level of 400 mg/kg chewing gum[1].
- BHT is, as dual use additive, authorised for use as an antioxidant in plastic FCMs with a specific migration limit of 3 mg/kg food according to EU Regulation 10/2011[4].

Thus the total presence of BHT in food may be due to its use as food additive, by transfer from animal feed to food, by migration from FCMs into food (VKM, 2019) or from environmental contamination due to the presence of BHT in the wastewaters generated by these industries (Nieva-Echevarra *et al*., 2015). BHT is moreover permitted to use in PCPs according to EU Regulation 1223/2009[5] provided that the use is safe under normal or reasonable foreseeable conditions (VKM, 2019). No specific conditions for the use of BHT in PCPs is given. The presence of BHT in PCPs may be due to its use as a preservative in the product and/or due to migration from the packaging material (VKM, 2019). The multiple potential sources of BHT exposure need to be taken into account when performing exposure estimates of the substance.

BHT exposure assessment by VKM (2019)

Based on a thorough literature search, VKM (2019) lists levels of BHT reported in different kinds of food including cereals, vegetables, fruit, meat and meat products, fish (in particular farmed), egg, milk, various oils and in chewing gum. From these data (including Europe, USA and other countries) the highest contents of BHT was found in the given order: Chewing gum (135 mg/kg (n 10) – only data from Europe/USA), pepperoni (23 mg/kg (n 14)), and farmed fish (2.6 mg/kg (n 60)). For other food groups the BHT content was below 0.5 mg/kg including various oils from inside Europe/USA with a mean level of 0.006 mg/kg (n 14). In samples from countries outside Europe and USA the highest levels of BHT were reported in fish oil (144 mg/kg (n 5)) > margarine and butter (92 mg/kg (n 49)) > cheese (71 mg/kg (n 2)) > mayonnaise (48 mg/kg (n 6)) > vegetable cream (45 mg/kg (n 9)) > various oils (43 mg/kg (n 53)). The last group (various oils) was close to four orders of magnitude higher than oils from Europe/USA. No explanation was given for this. No data on BHT in margarine, butter and cheese in samples from Europe/USA was found according to VKM (2019). In addition, no studies reporting data on BHT in frying fat and frying oil in Europe/USA were identified. According to EFSA (2012a), the industry did not report any use of BHT in frying fat and oil in 2012. Based on the given data, exposure estimates were performed by VKM (2019). In the calculation of a “realistic exposure

[5] REGULATION (EC) No 1223/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 30 November 2009 on cosmetic products (OJ L 342, 22.12.2009,

scenario" of BHT, only data from Europe/USA were used. In the estimation of a "high exposure scenario", the above food groups of fish oil, margarine and butter, cheese, mayonnaise, vegetable cream and various oils were included by using the data of these foods from countries outside Europe/USA. Food consumption data used for "realistic" and "high" exposure estimates were obtained from EuroMix (Husoy *et al.*, 2019) and Norkost 3 (Totland *et al.*, 2012) based on 1787 participants between 18 and 70 years recording the food intake over two days. The result of the VKM (2019) report is presented in Table 3 and shows the following main contributions of BHT from the given food groups.

Table 3: Relative contribution of different food groups to BHT in a "realistic" and a "high" exposure scenario (VKM, 2019)

Dietary sources	Contribution of different food groups to estimated mean "realistic" BHT exposure*	Contribution of different food groups to estimated mean "high" BHT exposure**
Cheese	NA	46 %
Margarine and butter	NA	41 %
Fish oil	NA	5 %
Milk, cream, ice cream	43 %	3 %
Chewing gum	40 %	2 %
Fatty fish	17 %	1 %

*Only concentration data from Europe/USA. **All concentration data from both inside and outside Europe/USA. NA = not available.

In the "realistic exposure scenario" of BHT from food the two food groups "milk, cream, ice cream" and "chewing gum" are the main BHT sources. In the "high exposure scenario", the food groups "cheese" and "margarine and butter" are the main BHT sources. As seen in Table 3 no data from Europe/USA of BHT in the two food groups "cheese" and "margarine and butter" are available. These food groups were therefore not included in the VKM (2019) calculation of the "realistic" exposure scenario. The reason for the high level of BHT in fatty foods as butter/margarine (92 mg/kg) and cheese (71 mg/kg) from outside Europe/USA is not known. In Europe, BHT is not permitted as food additive in dairy products (butter and cheese) but whether it is due to another regulation outside Europe/USA permitting a use of BHT as food additive in such foods is not known. It seems unlikely to have such high levels as reported in Table 3 in European/Danish food of this kind. However, as BHT is authorised as additive in animal feed it may also add to BHT in food due to its carryover from feed to food in the food chain. With no data from Europe/USA on BHT in these food groups, this may potentially add to some underestimation of the calculated realistic exposure level from food. More quality data would therefore be needed to have sufficient information to evaluate if carryover of BHT from feed to food is a potential

significant source of the human BHT exposure. In margarine, BHT was not used according to European industry in 2012.

In the VKM (2019) report, some data on contamination of BHT from feed into food is included in the exposure assessment. According to NIFES[4] monitoring programme (NIFES *et al*., 2011) BHT was detected in farmed salmon fillets in a concentration range of > LOQ – 8.9 mg/kg with a mean concentration of 3.7 mg/kg fillet and in farmed trout fillet the concentration of BHT was 0.6 mg/kg wet weight. The results are due to BHT carryover from feed to fish fillets. The relative BHT contribution from fatty fish in the "realistic" and the "high" exposure scenario is 17 % and 1 %, respectively, as shown in Table 3.

In addition to food, other sources can contribute substantially to BHT exposure according to VKM (2019). This include oral exposure from PCPs (mainly from toothpaste and with a minor (15 %) contribution from lip gloss/stick/balm for females) as well as dermal BHT uptake from PCPs (VKM, 2019). In the PCP dermal category, body lotion and deodorant were estimated as major contributors to the estimated "realistic" exposure scenario (VKM, 2019). The combined exposure estimates (mean/median and high) including diet and cosmetics was obtained from the EuroMix study (Husøy *et al*., 2019). The EuroMix study allow for a combined exposure through diet and cosmetics, since consumption data for the same individuals for both routes are available according to VKM (2019). BHT is rapidly absorbed in the gastrointestinal tract after oral exposure and 100 % absorption can be assumed (EFSA, 2012a). Dermal absorption studies showed absorption values from 0.4 % to 14.4 % (Langian *et al*., 2002). The VKM panel has used an absorption factor of 4 % (as indicated by Lanigan *et al*.) when estimating internal dermal exposure and 100 % absorption for oral exposure. The relative contribution to overall internal exposure of BHT from the different oral and dermal sources is given in Table 4.

Table 4: Relative contributions to internal exposure of BHT from different sources (VKM, 2019)

Mean contribution	Diet/oral (%)	PCP/oral (%)	Dust/oral (%)	PCP/dermal (%)
All	46	37	0.02	17
Females	41	38	0.02	21
Males	59	34	0.02	7.1

[4] MONITORING PROGRAMME FOR RESIDUES OF THERAPEUTIC AGENTS, ILLEGAL SUBSTANCES AND OTHER UNDESIRABLE SUBSTANCES IN FARMED FISH (Conducted to fulfil Norwegian obligations as laid down in Council Directive 96/23/EC)

According to Table 4, 93 % and 79 % of total exposure was estimated to be due to oral intake (from food and other sources) for males and females, respectively. Overall, oral intake of BHT from the diet is the predominant source. However, for females the exposure from diet (41 %) and oral exposure from PCPs (38 %) (toothpaste and lip gloss/stick /balm) were estimated to be of similar magnitude.

The "realistic" total internal exposure (data from only Europe and USA used) for adults from all routes was estimated at these levels:

- 0.0014 – 0.0096 mg/kg bw per day for females
- 0.0008 – 0.0097 mg/kg bw per day for males

The estimated "realistic exposure level" is largely below the ADI of 0.25 mg/kg bw per day for both females and males. Regarding the given concentrations of BHT in food by VKM (2019), it is not stated if the data include packed food. It is therefore not possible to evaluate if (potential) migration from packaging is included in the given concentration data of BHT in food. This might add to underestimation of the "realistic" total concentration of BHT in food and to underestimation of the total internal exposure of BHT from food.

The "high" total internal exposure (all data included) from all routes was estimated at the given levels:

- 0.023 – 0.281 mg/kg bw per day for females
- 0.009 – 0.319 mg/kg bw per day for males

In the "high" scenario, the food groups "margarine and butter" and "cheese" were estimated as the main contributors to the exposure according to VKM (2019). This is because the level of BHT in these foods are significantly (up to two orders of magnitude) higher than BHT data from Europe/USA in other foods. VKM (2019) conclude that the uncertainty of these data is high due to a low number of concentration data on BHT (especially for cheese) and due to missing concentration data on these specific foods from Europe and USA. The range of the 95th percentile representing the potential "high" exposure is above ADI of 0.25 mg/kg bw per day for both females and males.

BHT exposure assessment by EFSA

EFSA (2012a) performed an estimate of BHT exposure of adults and children based on worst-case scenario of combining exposure from BHT as food additive from food categories where it is authorised and assuming a migration level equal to the SML of BHT from FCMs into 1 kg food. The estimated combined exposure from these sources by EFSA is given in Table 5.

Table 5: EFSA estimated worst-case combined exposure (mg/kg bw per day) of BHT used as food additive and used as FCM additive at maximum permitted levels (EFSA, 2012a)

	Adults Average	Adults P95	Adolescents Average	Adolescents P95	Children Average	Children P95
BHT exposure from use as food additive	0.01 – 0.02	0.02 – 0.16	0.01 – 0.04	0.03 – 0.13	0.01 – 0.09	0.04 – 0.30
Worst case BHT exposure from use as FCM additive*	0.05	0.05	–	–	0.2	0.2
Total exposure range	0.06 – 0.07	0.07 – 0.21	–	–	0.21 – 0.29	0.24 – 0.50

*Based on its use in FCMs with a SML of 3 mg/kg food and with the assumption that every day throughout a lifetime an adult person weighing 60 kg consumes 1 kg of food packed in plastics containing BHT at a maximum permitted level. Assuming that children weighing 15 kg also consume 1 kg of food packed in plastics containing BHT at the maximum permitted level.

As BHT is a rather lipophilic substance, a migration level equal to the SML in all packed food of 1 kg (including non-fatty food) is highly conservative and is not considered realistic. Detailed information on the use of BHT in food packing is not available. However, in literature it is mainly reported to be used in polypropylene (PP) and polyethylene (PE) plastics and suggested for use in polylactic acid (PLA) plastic.

Exposure to BHT as a food additive is estimated to exceed ADI for children for some countries (Holland and Finland) at the 95th percentile according to the EFSA (2012a) evaluation. If moreover also worst-case exposure from BHT at a migration level equal to the SML in FCMs is assumed, the ADI is exceeded by children at both the mean and at the 95th percentile total exposure level.

Oral exposure from PCPs is not part of the EFSA assessment. Dermal absorption of BHT from PCPs is not considered to produce substantial systemic exposure to BHT, as BHT appears to pass only slowly through the skin and the majority remaining on the surface.

Comparison of BHT exposure estimates by EFSA (2012a) and by VKM (2019)

To get a better overview of the difference between the VKM (2019) and the EFSA (2012a) exposure assessments of BHT the different sources of BHT are listed in Table 6.

Table 6: Summary of BHT exposure estimates from VKM (2019) and EFSA (2012a) and impact of used data on the exposure estimates (MR: add to a more realistic exposure estimate), (-: risk of underestimation of actual exposure estimate), (+: assume to overestimate actual exposure)

	Sources of BHT (included/excluded)	Impact on realistic exposure scenario MR or (+/-)	How to improve for a more realistic scenario
VKM (2019) exposure estimate:	The oral exposure estimate from food is based on concentration data from literature in different food groups.	More realistic estimates of oral exposure from food. MR	
	Includes oral exposure from PCPs.	MR	
	Includes exposure from chewing gum – assuming 100 % excretion (worst-case) of BHT from chewing gum*.	most likely +	
	Includes exposure from BHT contamination in farmed fish.	MR	
	Includes dermal exposure from PCPs.	MR	
	Includes exposure from indoor dust.	MR	
	Exposure estimate from BHT in FCMs is not included.	Risk of underestimation of realistic exposure. -	Information on actual use of BHT in FCMs – in which kind of plastic, in which packaging applications and what level of migration from packaging into food is needed to assess a realistic exposure estimate of BHT from FCMs.
	Exposure of BHT in cheese and “margarine and butter” and mayonnaise is not estimated due to lack of concentration data from Europe/USA in these foods.	Risk of underestimation of realistic exposure. -	Danish/European concentration data on especially BHT in cheese and “margarine and butter” and mayonnaise would be useful to reduce uncertainty of the total exposure assessment.

	Sources of BHT (included/excluded)	Impact on realistic exposure scenario MR or (+/-)	How to improve for a more realistic scenario
EFSA (2012a) exposure estimate	Includes food where BHT is authorised as food additive in the exposure assessment, and include BHT used as additive in FCMs at a maximum permitted level equal to the SML.	Oral exposure assessment is based on worst-case scenario regarding BHT used as additive in food and in FCMs when using maximum reported level of food additive and using the SML as migration level. This is supposed to overestimate actual exposure from these sources. +	Information on actual use of BHT in FCMs – in which kind of plastic, in which packaging applications and what level of migration from packaging into food is needed to assess a realistic exposure estimate of BHT from FCMs. As BHT is a lipophilic substance (K_{ow} of 5.1) it is assumed that migration into non-fatty or aqueous food will be low or absent depending also on time and temperature conditions. Assuming a migration level equal to the SML into all food is therefore considered highly conservative and to overestimate realistic migration of BHT from FCMs.
	Other sources (oral and dermal exposure from PCPs) of potential exposure are excluded as not considered to add substantially to systemic exposure	It is assessed to add to underestimation of total exposure when not including BHT from PCP sources (according to the VKM exposure assessment) -	

*Several studies have shown that the actual excretion from chewing gum is lower than 100 %, depending on chewing time. The estimated BHT exposure from chewing gum (as 100 % of BHT concentration) is therefore most likely an overestimation (VKM, 2019).

Exposure estimates by others

The estimated median daily intakes (EDIs) of BHT, calculated from urinary concentrations (urine samples from USA and Asian countries) in children and adults were 0.38–56.6 and 0.21–31.3 µg/kg bw

per day, respectively (Wang and Kannana, 2019). A main metabolite of BHT was found in 80 – 100 % of all samples. Elevated exposures to BHT (as high levels of BHT in urine) were noted in countries such as Japan, India, and the USA. The range of the estimated daily intake of BHT was 0.00021–0.372 mg/kg bw per day for adults and 0.00038–0.613 mg/kg bw per day for children, with some of the estimated exposure doses exceeding the ADI of 0.25 mg/kg bw/day.

In a recent German human biomonitoring study, the internal burden of BHT were analysed (as metabolite 3,5-di-tert-butyl-4-hydroxybenzoic acid) in 24-h urine samples from young non-specifically exposed adults of age 20-29 years (Schmidtkunz *et al*., 2020). In total, 329 samples were collected in the years 2000-2018. BHT acid was detected above the LOQ (0.2 µg/L) in 98 % of the samples. The median of the measured concentrations was 1.06 µg/L and the corresponding 90th percentiles was 3.28 µg/L. Daily intakes were estimated from excretion of BHT acid metabolite at approximately 0.1 mg/kg bw at the 95th percentile and estimated to be largely below ADI of 0.25 mg/kg bw per day. However, the authors also concluded that the intake assessments rely on very limited quantitative data regarding human metabolism of BHT and more knowledge is needed to validate the given estimates.

Leclercq *et al*. (2000) indicated that the exposure of the Italian population to BHT was up to 0.315 mg/kg bw per day based on theoretical maximum daily estimates assuming BHT concentrations at MPL in each food group. The main potential sources of BHT being “pastry, cake, and biscuits,” followed by “chewing gums,” and “vegetable oils and margarine”; overall, they contributed 74 % of the estimated theoretical maximum daily intake.

JECFA (2000) estimated that the population of the USA was exposed to 0.39 and 0.78 mg/kg bw per day for mean and high levels of consumption, respectively.

Occurrence of BHT in food

In a Danish study the level of BHT as food additive was tested in 122 food products of processed food of fruit and vegetables and different sauces (Danish Food Administration, 2006). The study concluded that none of the samples contained BHT above the limit of quantification (LOQ) at 20 mg/kg. As the LOQ of the used HPLC method with Photodiode Array (PDA) detector was at a very high level compared to reported levels of BHT in the VKM (2019) report it cannot be excluded if (some of) the samples contained BHT at a level below 20 mg/kg which would be of relevance for an exposure estimate of BHT.

BHT are together with other synthetic antioxidants authorised also for use as feed additives in the EU. This include feed for farmed fish such as Atlantic salmon, halibut and cod and rainbow trout. In a Norwegian study (Lundebye *et al*., 2010) the highest content of BHT was found in farmed Atlantic salmon fillets, at a level of 7.5 mg/kg and with a mean level of 3.9 mg/kg (n=24). The lowest concentration of the antioxidant was found in cod. According to Lundebye *et al*., a 300 g portion of farmed Atlantic salmon could potentially contribute up to 75 % of the ADI for BHT.

Migration of BHT from food packaging

According to literature, most plastics contain antioxidants to protect against degradation of the polymer and in particular PP, PE and polyvinyl chloride (PVC) need protection by addition of antioxidants. We do not have an overview on the given use of BHT in food packaging, including how much it is used in different packaging material and for which applications. However, addition of antioxidants such as BHT to polyolefins is mentioned to be a common practice to protect the plastic from degradation (Soto-Cantú *et al*., 2008). Packaging of PP and PE (which can have a content of BHT) is roughly assumed to account for about half of all packaging used in contact with food according to industry data on the internet and Econet AS (Econet, 2018). In a Chinese study, BHT (and BHA) was found in five different plastics for food contact (the kind of polymers is not given in the English abstract) in levels of 6 - 28 mg/kg polymer as well as in rubber FCMs (Xiong *et al.,* 2014). No data on migration from the materials was reported. BHT is a rather lipophilic substance (log Kow 5.1) and studies of migration of BHT from monolayer polyolefins show that the substance has a high diffusion coefficient and a high mobility into fatty foods (Soto-Cantú *et al*., 2008; Iberra *et al*., 2018; Mercea *et al*., 2020). Results show that the diffusion process is significantly influenced by temperature; however, the type of simulant also play an important role in the migration process. Accordingly, diffusion of BHT was significantly faster in isooctane (substitute for fatty food simulant) than with 50 % (v/v) and 95 % ethanol (v/v) (Ibarra *et al*., 2019). In another study BHT in PLA plastic shows no migration into the aqueous food simulant of 10 % ethanol (Jamshidan *et al*., 2012). In a former study by Wessling *et al*., (1998) the migration behaviour of BHT in low-density polyethylene (LDPE) film was tested into food simulants at different test temperatures. BHT can rapidly release from the material when in contact with fatty food simulants depending on temperature. After 1 week of contact at 4 °C the BHT level of the film was below limit of detection whereas at a test temperature of 20 °C the BHT content of the film was totally released after one day of contact with fatty food. BHT may also be used as antioxidant in active packaging films of e.g. PP with the intension to release BHT into the packed (fatty) food (Soto-Cantú *et al*., 2008; Fasihnia *et al*., 2020). Moreover, BHT is used as an antioxidant in PE water pipelines. The amount of BHT migrating into water from high density polyethylene (HDPE) pipes was low and

found to correspond to the background level (Skjevrak *et al*., 2003). It is thus regarded as insignificant to the migration of organic compounds from pipes into drinking water. In a Danish project, 28 samples of different food packagings (mainly plastic) were extracted with acetonitrile at room temperature at 6 hours of test time (FVST, 2014). The project did not find any migration of BHT above LOD (0.2 mg/kg food) at the given test conditions. In order to estimate potential worst-case migration of BHT and BHA in the samples it might have been appropriate to use worst-case test conditions by increasing the test temperature (and time) and/or by using the fatty food simulant substitute of isooctane to increase the diffusion rate of the lipophilic BHT.

Discussion and conclusion on human exposure to BHT

Different methodological approaches are used in estimating exposure of BHT as food additive from food. Whether estimates of exposure is based on BHT assumed to be present at MPL in all foods in which it is allowed (as by EFSA (2012a)) or by actual concentration data in selected foodstuffs (as by VKM (2019)) will greatly affect the estimated exposure level. Moreover, the age group, the eating habits (e.g. taken into account brand loyalty) and use of either medium or high consumers will significantly affect the estimated exposure levels. Food is the main contribution to human exposure of BHT according to VKM (2019). The exposure assessment by VKM is based on actual levels of BHT in different kinds of food, which gives a more realistic exposure assessment from food compared to the estimate by EFSA (2012a) using worst-case estimates assuming BHT at a MPL as food additive and worst-case migration level equal to the SML into 1 kg food. In addition, contamination of food due to carryover of BHT in feed for farmed fish was included in the calculations by the VKM. Moreover, VKM shows that also non-food sources may contribute significantly to human exposure of BHT. This include oral (37 % of total) and dermal exposure (17 % of total) from PCPs as the most important non-food sources, whereas dust was only estimated to be of minor importance to BHT exposure. Oral and dermal exposure from PCPs is not included in the EFSA opinion. All together, the EFSA opinion rely on a very rough estimate and is assumed to have a high uncertainty. According to the VKM “realistic” exposure assessment, the total internal BHT exposure is well below the ADI for both females and males. The VKM (2019) does, however, not include exposure estimates for children ≤ 17 years.

In the VKM (2019) exposure estimate, two conditions may add to uncertainty and potential underestimation of the “realistic” exposure assessment from food: 1) it is uncertain whether the given food include packed food and if the data on BHT content in food include any relevant migration from the packaging into the food, and 2) no data from Europe/USA on BHT in butter, margarine and cheese are available. These three foods were assessed to be the main contributors to the BHT exposure when

data from outside Europe/USA (with high levels of BHT) were included in the high (95th percentile) exposure estimate. In Europe, BHT is not allowed as food additive in butter and cheese, however, samples of butter and cheese may contain BHT due to 1) contamination of the food from animal feed through the food chain and/or 2) migration of BHT from packaging into butter and cheese (which are fatty foods) is not known. Data on this would be needed for a more exact estimate of both realistic and high exposures. In the opinion given by EFSA (EFSA, 2012a) a worst-case migration level equal to the SML into 1 kg food intake per day was assumed. If using the same estimate in addition to the given VMK (2019) estimate on "realistic" total internal exposure this will add 0.05 mg BHT/kg bw per day for an adult. This will bring the total maximum exposure to a level of 0.06 mg/kg bw per day for the "realistic" exposure scenario for females and males. This estimated total exposure level (including worst-case migration from FCMs) is well below the ADI of 0.25 mg/kg bw per day.

Evaluation of BHA exposure

Butylated hydroxyanisole (BHA) CAS RN 25013-16-5 is authorised as food additive (E 320) in EU for certain food products including cake mixes, cereal-based snack foods, bouillons, milk powder, dehydrated soups and dehydrated meat[5]. In plastic for food contact (FCM no. 635) it is authorized as plastic additive (antioxidant) with a SML of 30 mg/kg according to EU regulation 10/2011[6]. BHA is a mixture of two isomers (2-*tert*.-butyl-4-hydroxyanisole and 3-*tert*.-butyl-4-hydroxyanisole).

In Europe, the use of BHA is permitted in several foods like bouillons, gravies, dehydrated soups and dehydrated meat, individually or in combination with other antioxidants (VKM, 2019; Pop *et al*., 2013). The maximum limit is set to 200 mg/kg expressed on the fat content of the product.

According to ECHA, BHA is used in the following products: pharmaceuticals, polymers, cosmetics and PCPs, coating products and lubricants and greases. Release to the environment of this substance can occur from industrial use as processing aid and in the production of articles.

BHA exposure assessment by EFSA

The exposure assessment used by EFSA (2012b) to estimate exposure to BHA from its use as food additive is based on MPLs. By this method exposure at mean level is in the range of 0.04-0.23 mg/kg

[5] 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)

[6] COMMISSION REGULATION (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food (OJ L 12, 15.1.2011, p. 1)

bw per day for toddlers, 0.08-0.36 mg/kg bw per day for children, 0.06-0.18 mg/kg bw per day for adolescents, 0.03-0.12 mg/kg bw per day for adults and 0.02-0.11 mg/kg bw per day for the elderly. High level exposures at the 95th percentile was estimated in the range of 0.14-0.57 mg/kg bw per day for toddlers, 0.26-0.60 mg/kg bw per day for children, 0.12-0.38 mg/kg bw per day for adolescents, 0.08-1.12 mg/kg bw per day for adults, and 0.05-0.72 mg/kg bw per day for the elderly. MPLs were used for these estimations, as the amount of data on actual use levels were too limited. The few data made available to EFSA (2012b) indicate that BHA is either not used or found at levels below the limit of detection (10 mg/kg food).

An estimate for BHA exposure from FCMs was also performed by EFSA (2012b). Due to lack of data it was made on the assumption that consumers from all the population groups consume 1 kg of food packed in plastics containing BHA at the maximum permitted quantity, which would result in BHA exposure of 0.43, 0.6, 1.3 and 2.5 mg/kg bw per day for adults and the elderly, adolescents, children and toddlers, respectively.

Exposure estimates by others

Human exposure of plastic antioxidants, including BHA, was estimated by monitoring of the substance in urine samples from USA, China, India, Japan and Saudi Arabia sampled in 2010-2012 (Wei *et al.*, 2019). BHA was present in 39 % of the urine samples at concentrations < LOQ to 4.31 ng/ml. In comparison, BHT was detected in 88 % of the urine samples in levels from LOQ to 15 ng/ml. These data indicate a lower level of human exposure to BHA compared to exposure of BHT. However, as data from Europe is missing in the given study there is some uncertainty if the same apply for Europe.

Migration of BHA from food packaging

As for BHT, we do not have an overview of the use of BHA in FCMs and how much is used for different packaging applications, as the information from literature is very limited. In a Danish study from 2014, the level of BHA was analysed in 28 different samples of FCMs (mostly plastics) by extraction into acetonitrile for 6 hours at room temperature (FVST, 2014). BHA was not detected in any of the samples above LOD (0.2 mg/kg). Testing of BHA in PLA into 95 %, 50 % and 10 % ethanol showed an effective release of BHA into 95 % of ethanol (simulant for fatty food) however into 10 % ethanol (simulant for aqueous food) only a slight release of BHA was reported (Jamshidian *et al.*, 2012). These data indicate a low migration rate into aqueous/non fatty food.

Occurrence of BHA in food

BHA is also authorised in feed for all animal species with maximum level of 150 mg/kg feed alone or in combination with other authorised antioxidants. Maximum residue limits (MRLs) do not currently exist in EU for synthetic antioxidants in food products of animal origin (Lundebye *et al*., 2010). The use of BHA as additive in feed includes feed for farmed fish such as Atlantic salmon, halibut, cod and rainbow trout. In a Norwegian study (Lundebye *et al*., 2010) the highest content of BHA were found in farmed Atlantic salmon fillets, at a level of 0.07 mg/kg. The lowest concentrations of the antioxidant was found in cod. Lundebye *et al*. concluded that the consumption of farmed fish would not contribute measurably to the intake of BHA. This is also in good accordance with EFSA (2018) estimating a worst-case contribution of BHA from animal feed into food intake at a maximum of 8 % of ADI and concluding that BHA from feed is of no human health concern.

From 12 food categories, 133 samples of foods considered representative sources of BHA and BHT in the Korean diet were analysed. BHA was not detected in any of the food samples and the human intake was estimated to be low compared to the ADI (Suh *et al*., 2005).

Discussion and conclusion on human exposure to BHA

The exposure assessment made by EFSA (2012b) is highly conservative for both exposure from use as food additive and from additive to FCMs as it is based on MPLs and the assumption that 1 kg food per day is in contact with plastic that release BHA at the highest concentration permitted of 30 mg/kg (the SML). The few existing actual use levels of BHA as food additive indicate uses well below MPLs. The above studies on migration testing of BHA in PLA into 10 % ethanol indicating a low migration into aqueous food at both 20 °C and 40 °C. Fast migration rates is reported into the extraction solvent of 95 % ethanol (a substitute for fatty food simulant) but was significantly lower into 50 % at both 20 °C and 40 °C. Although we do not have exact migrations levels from different kinds of plastics the given results indicate that a maximum migration level equal to the SML from all packed food of 1 kg is highly unrealistic and exposure estimates based on this assumption is too conservative.

List of abbreviations

3-BHA	3-*tert*.-butyl-4-hydroxyanisole
ADI	acceptable daily intake
BHA	butylated hydroxyanisole
BHT	butylated hydroxytoluene
BW	body weight
CAS RN	Chemical Abstract Service Registration Number
DTU FOOD	Technical University of Denmark National Food Institute
ECHA	European Chemicals Agency
EDI	estimated median daily intake
EFSA	European Food Safety Authority
FCM	food contact material
FVST	Danish Veterinary and Food Administration
HDL-C	high density lipoprotein cholesterol
HDPE	high density polyethylene
HFD	high fat diet
HPLC	high performance liquid chromatography
HPT	hypothalamic–pituitary–thyroid
iWAT	subcutaneous fat
JECFA	the Joint FAO/WHO Expert Committee on Food Additives
Kow	n-octanol-water partition coefficient
LDL-C	low density lipoprotein cholesterol
LDPE	low-density polyethylene
LOAEL	lowest observed adverse effect level
LOD	limit of detection
LOEL	lowest observed effect level
LOQ	limit of quantification
MPL	maximum permitted level
MR	add to a more more realistic exposure estimate
MRL	maximum residue limit
NA	not available
ND	normal diet
NK cell	natural killer cell
NOAEL	no observed adverse effect level
NOEL	no observed effect level
OECD	Organisation for Economic Co-operation and Development
PBMC	peripheral blood mononuclear cells
PCP	personal care product
PDA	photodiode array
PE	polyethylene

PLA	polylactic acid
PP	polypropylene
PVC	polyvinyl chloride
pWAT	visceral fat
SCF	EU Scientific Committee for Food
SML	specific migration limit
TG	trigylceride
TSH	thyroid stimulating hormone
VKM	Norwegian Scientific Committee for Food and Environment

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