

Human Biomonitoring of Butylated Hydroxytoluene (BHT) in Germany: Methods, Exposure Levels, and Health-Based Interpretation[‡]

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SUMMARY

Butylated hydroxytoluene (BHT) is an antioxidant which is used in a vast array of consumer products. The most sensitive toxicological endpoints of BHT are hepatic enzyme induction and reproductive effects. Because of its wide dispersive use and its potential relevance for human health, BHT was included in the human biomonitoring (HBM) cooperation between the German Federal Ministry for the Environment and the German Chemical Industry Association. An analytical method for the sensitive determination of 3,5-di-tert-butyl-4-hydroxybenzoic acid (BHT acid)—an oxidized metabolite of BHT which is excreted in urine—was developed. This method was then applied in several environmental and occupational HBM surveys, and BHT acid was detected in the vast majority of samples. Health-based guidance values as well as reference values for the interpretation of HBM results were derived for BHT acid. Thus, a fine-grained picture of the current state of BHT exposure in different populations in Germany is now available. Uncertainties arise from large variability in the fraction of dose excreted as BHT acid and incomplete understanding of human metabolism, which limits reverse dosimetry and risk assessment, particularly for children.

1. INTRODUCTION

Butylated hydroxytoluene (BHT, 2,6-di-tert-butyl-4-methylphenol, Figure 1) was first synthesized in the 1940s by American researchers [1, 2]. It was initially used as an antioxidant in the petrochemical and adhesives industries, but by the 1950s, it had found new applications in consumer goods, such as food and cosmetics [3]. Nowadays, BHT is added, *inter*

alia, to fuels, technical oils, rubbers, paints, cleaning products, animal feed, edible fats and oils (food additive E 321), chewing gum, plastics including food contact materials, cosmetics, and pharmaceuticals [4, 5]. With a long history of use in numerous areas, exposure of the general population to BHT is likely. Levels of exposure can be predicted from consumption data about relevant products and from permitted or reported use levels of BHT in these products [5].

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The empirical determination of actual exposures and intakes is best achieved through human biomonitoring (HBM), which involves measuring BHT or its metabolites in human biological samples such as blood and—preferably, since it is non-invasive—urine. Several studies conducted between the 1960s and 1980s examined the urinary excretion of BHT and its metabolites in humans following oral administration [6–9]. Because BHT metabolism is quite complex and varies significantly across different animal species—and because not all (human) metabolites could be elucidated with the analytical methods available at that time—our knowledge about specific urinary metabolites and their amounts remains incomplete. In 2006, Göen et al. were the first to perform HBM of a suitable BHT metabolite in the urine of individuals with no known exposure to BHT. Although they selected only a minor metabolite (3,5-di-*tert*-butyl-4-hydroxybenzoic acid, “BHT acid”, Figure 1) as a biomarker for BHT exposure, 14 out of 16 urine samples (88%) tested positive for this biomarker, with concentrations reaching up to $3.86 \mu\text{g l}^{-1}$ [10].

While the acute toxicity of BHT is low, the substance has attracted criticism in the past [3] and is currently being evaluated as a potential endocrine disruptor [4, 11]. In 2012, the European Food Safety Authority (EFSA) set an acceptable daily intake (ADI) of $0.25 \text{ mg kg}_{\text{bw}}^{-1} \text{ d}^{-1}$, based on a NOAEL of $25 \text{ mg kg}_{\text{bw}}^{-1} \text{ d}^{-1}$, reproductive effects and hepatic enzyme induction (with resulting thyroid hyperactivity) being the most sensitive endpoints [5].

Thus, at the beginning of the 2010s, the possible relevance of BHT for human health, as well as its potential for widespread exposure of the general population and workers, was obvious. In 2013, the

substance was therefore prioritized for method development and application in the German initiative to promote HBM—a 15-year cooperation project between the federal government and the chemical industry [12].

In this article, we summarize activities conducted since then (Figure 2) and their key outcomes. We discuss what we have learned about HBM of BHT and BHT exposure in Germany, the challenges we faced, and what remains unknown. We offer an outlook on ongoing studies and, finally, some suggestions for future research.

2. BIOMARKER AND SAMPLE MATRIX

Choosing adequate biomarkers for HBM of a new substance is not trivial — this step has even been described as “the key challenge in method development” [12]. In our case, despite the incomplete understanding of human BHT metabolism, a promising biomarker candidate already existed: BHT acid. Indeed, as mentioned above, BHT acid had been detected in the vast majority of samples in a small HBM study from Germany [10]. The structure of BHT acid is closely related to the parent substance BHT, hence adequate specificity could also be expected (Figure 1). The proven excretion of BHT acid in urine meant that method development could focus on this non-invasive and easily accessible sample matrix.

BHT acid was readily available from chemical suppliers as a reference standard, avoiding the costly and time-consuming process of custom synthesis, though this only applies to non-labeled BHT acid. A stable-isotope-labeled internal standard, essential for robust quantification by mass spectrometry, was not commercially available and had to be synthesized by a qualified lab [13]. BHT acid as a biomarker in HBM has limitations: it is a minor metabolite of BHT, meaning only a small portion of BHT intake is excreted as BHT acid in urine. Thus, a very sensitive analytical method is needed to detect low exposures in the general population. Göen et al. demonstrated such sensitivity with 16 samples, providing convincing proof of concept [10].

The main issue with BHT acid is that its quantitative significance is unclear, as the exact percentage

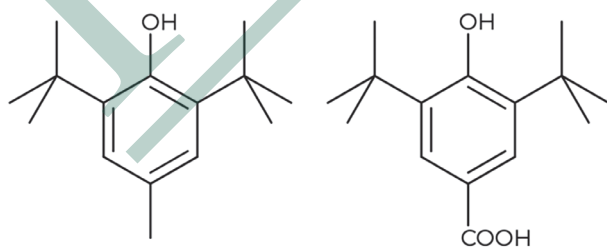


Figure 1. Structures of BHT (left) and its metabolite BHT acid (right).

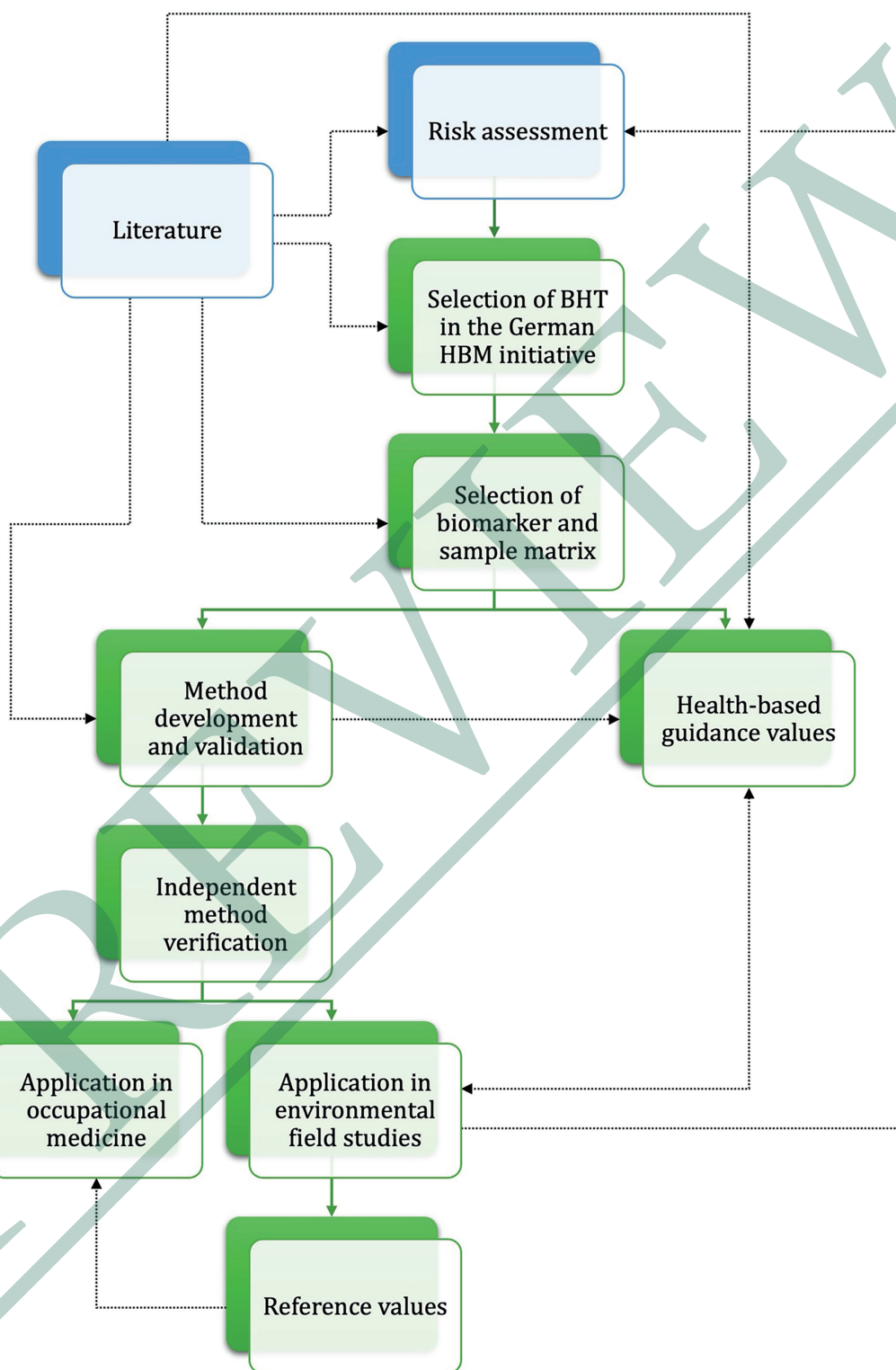


Figure 2. Flow chart of activities connected to HBM of BHT in Germany, between 2013 and 2025 (based on and modified from [12]).

of a BHT dose excreted in urine as BHT acid remains unknown. Molar urinary excretion factors (F_{ue} , equation 1) found in studies vary between less than 0.3% and 5.5% [6–9].

$$F_{ue} = \frac{\text{Cumulative urinary excretion BHT acid [mol]}}{\text{BHT exposure dose [mol]}} \quad (1)$$

Although we should always expect considerable inter-individual differences in metabolizing xenobiotics like BHT, the nearly 20-fold difference between the lowest and highest reported values for BHT acid is much greater than what is seen with many other substances, such as metabolites of 7-hydroxycitronellal [14], di(2-ethylhexyl)terephthalate [15], bronopol [16], ethylhexyl salicylate [17], or chloromethylisothiazolinone and methylisothiazolinone [18]. This isn't a problem for analytical measurements themselves, but it creates significant uncertainty when trying to estimate overall BHT intake from measured BHT acid levels in urine (reverse dosimetry). This uncertainty in exposure assessment will consequently lead to uncertainty in risk assessment.

However, no credible alternative to BHT acid as a biomarker was identified in the literature. While major metabolites that account for more than 20% of an oral BHT dose have been described, there has been considerable controversy over their relevance, and even their precise chemical structures remain uncertain [6–8]. Additionally, the reported structures are complex, involving oxidations at all three alkyl groups (methyl and both *tert*-butyl groups), which likely would have made the synthesis of authentic reference substances more difficult.

Despite these constraints, urinary BHT acid was considered a suitable exposure biomarker, allowing for the development of an analytical method targeting it. However, from the outset, it was clear that while it might be the *best available* biomarker, it would not be an *ideal* one.

3. METHOD DEVELOPMENT

The focus for the analytical method to be developed was on sensitivity, robustness and operational

simplicity, as well as speed. In fact, quantification of environmental exposure levels, transferability of methods between laboratories, and high sample throughput for large population studies are key requirements of the German HBM initiative [12].

The method was first published in 2017 in a condensed format [19] and later detailed in a full report [13]. Ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC–MS/MS) was the preferred technique. After thorough optimization of the calibration and reagents to reduce external contamination and blanks, an excellent limit of quantification (LOQ) of $0.2 \mu\text{g l}^{-1}$ was achieved. Thanks to a column-switching setup that enables automated online purification and enrichment, manual sample preparation was minimized to just dilution and enzymatic hydrolysis (to break down potential glucuronide conjugates) of urine samples. A UHPLC–MS/MS run time of only 10 minutes per sample was achieved, including system equilibration before each injection and online purification. Consequently, up to 144 urine samples can be analyzed daily; however, practical limits will be lower due to the inclusion of blank, calibration, and quality control (QC) samples.

The method was thoroughly validated for its precision, accuracy, and robustness across different urine matrices. The enzymatic hydrolysis was optimized and validated using native urine samples from occupationally exposed individuals. Additionally, the method was implemented and independently re-validated at a second laboratory (ABF – Analytisch-biologisches Forschungslabor GmbH, Munich, Germany), confirming its practicality and performance data [13]. This unique approach of independent and experimental method verification, as required by the Biomonitoring Working Group of the Deutsche Forschungsgemeinschaft (DFG), supports the reliability and reproducibility of the published procedures [20].

The first use of the final method on a group of people ($n = 80$) with no occupational exposure to BHT showed a BHT acid detection rate of 90% and a maximum concentration of $7.55 \mu\text{g l}^{-1}$ [19], confirming earlier findings by Göen et al. [10], and supporting the overall suitability of BHT acid as a biomarker at environmental exposure levels.

4. LARGE ENVIRONMENTAL FIELD STUDIES

The method was then ready to be applied in large-scale field studies. The main HBM instruments in Germany are the German Environmental Survey (GerES) and the German Environmental Specimen Bank (ESB). Both are operated by the German Environment Agency (Umweltbundesamt, UBA). While GerES is designed to be representative of the population at the national level, the ESB is a sample archive spanning several decades, but it is not population-representative [21].

4.1. GerES V – A Population-Representative Study of Children and Adolescents

Urinary BHT acid was first measured during GerES V, the fifth cycle of GerES [22, 23]. Samples of first morning void urine were collected from children and adolescents (aged 3–17 years) between 2015 and 2017. BHT acid was analyzed in a total of 2091 urine samples.

Almost all samples (99.7%) contained quantifiable levels of BHT acid, i.e., above $0.2 \mu\text{g l}^{-1}$. Ubiquitous exposure was thus confirmed again, now with nationwide representative data for Germany [22]. The median level was $2.18 \mu\text{g l}^{-1}$. BHT acid concentrations did not differ significantly between sexes. A highly significant ($p \leq 0.001$) age gradient was observed across all age groups, with the youngest children (3–5 years old) having the highest geometric mean BHT acid levels. The maximum value in the entire study population was $248 \mu\text{g l}^{-1}$, also measured in this age group. Possibly, higher concentrations of BHT acid in young children might result from increased ingestion of house dust, which is known to contain BHT, but was not analyzed for this substance in GerES V [22]. Therefore, young children are a subpopulation with relatively high exposure to BHT. Other than that, specific sources of exposure could not be identified, despite separate evaluation of data for different sociodemographic subgroups and for subgroups with certain dietary preferences or with varying usage of some personal care products [22].

4.2. ESB – A Time Trend Study of Young Adults

The ESB collects blood and urine samples from students (aged 20–29 years) at four universities in Germany. Sampling is performed at each site annually, and the samples are archived for retrospective measurement [21]. A particular feature of ESB samples is that 24-h urine is collected, which is ideal for determining daily excretion values. Thus, when we measured BHT acid in 329 urine samples from the ESB site Halle/Saale in central Germany, collected over six different years between 2000 and 2018, the original hypothesis was to identify possible time trends in exposure [24].

In fact, almost ubiquitous exposure was observed—once again (97.9% of samples above $0.2 \mu\text{g l}^{-1}$). However, temporal trends in urinary BHT acid were weak at most and not significant ($p > 0.05$). The fact that exposure levels remained approximately constant over nearly two decades was most evident in daily excretion data, after normalization to the body weight of the study participants [24]. As in GerES V (children and adolescents), sex-specific differences were not observed. BHT acid concentrations in ESB samples (median: $1.06 \mu\text{g l}^{-1}$, maximum: $18.1 \mu\text{g l}^{-1}$) were clearly lower than in GerES V. Still, the comparability of urinary concentrations between ESB and GerES V is somewhat limited due to the differences in sampling regimes and age groups [22, 24]. Interestingly, BHT levels in seabird eggs from the Canadian high Arctic were largely steady after the year 2000 [25]—similar to what we see for BHT acid in the human ESB samples from the same timeframe in Germany. Whether this similarity of temporal patterns in different species and regions is purely coincidental or not remains speculative.

4.3. GerES VI – A Population-Representative Study of Adults

The fieldwork and laboratory phase of the sixth cycle of GerES, GerES VI, was conducted and completed during 2023 and 2024 [26]. This time, BHT acid was analyzed in 1462 morning urine samples from adults across Germany (age range: 18–79 years). Widespread exposure to BHT was

also detected in this age group. Reporting and statistical analysis of laboratory data are currently in progress. Detailed results of GerES VI will be published elsewhere soon.

5. OCCUPATIONAL MEDICINE

BHT is a substance that is important in both environmental and occupational medicine. Reflecting its wide range of applications, BHT is used in many workplaces [4]. Inhalation is the primary route of exposure in occupational settings, while skin absorption plays a minor role [27]. Since 2018, under routine medical checkups and in accordance with German labor law, Currenta's Institute of Biomonitoring has been conducting HBM of urinary BHT acid for workers handling BHT at their workplaces. These workers come from different companies and sites, representing various jobs in the chemical industry. Due to confidentiality policies at both individual and company levels, detailed data from these activities cannot be published. However, anonymized and combined results have been reported in several cases (Table 1).

Some caution should be exercised when comparing concentrations across different groups, as various sampling methods were used (spot versus 24-hour urine). Nevertheless, it appears that protective measures implemented in industry can often reduce occupational BHT exposures to such low levels that they become difficult to distinguish

from the background levels in the general population (ESB). For example, the medians in the ESB group and the largest worker group are nearly identical (Table 1). However, in individual cases, quite high BHT acid concentrations were found in workers, possibly due to significant occupational BHT exposure. A definitive interpretation cannot be provided at this time, as it would require a detailed assessment of individual work situations.

6. REFERENCE VALUES AND HEALTH-BASED GUIDANCE VALUES

On their own, numerical results obtained from HBM measurements are insufficient for risk assessment. Once a chemical or its metabolite is identified and quantified in a person's urine, questions regarding interpretation and health implications naturally emerge. For example, is the measured concentration within a typical range observed in the population, or does it suggest heightened exposure? Furthermore, what are the potential health effects—are the levels harmless, or could they pose health risks? Is immediate action required, or can monitoring suffice? To evaluate whether exposure levels exceed relevant thresholds or pose health concerns, reference values and health-based guidance values are employed for comparison [28, 29].

Based on the studies conducted so far, reference values of urinary BHT acid have been established

Table 1. HBM results of urinary BHT acid in occupational exposure settings; ESB collective (university students without occupational exposure to BHT) shown for comparison.

	Worker collective 1	Worker collective 2	Worker collective 3	ESB (student) collective
Reference	[13]	[13]	[24, 27]	[24, 27]
Sample type	Spot urine	Spot urine	Spot urine	24-h urine
Number of samples	17	22	622	329
Maximum	32.5 $\mu\text{g l}^{-1}$	26.6 $\mu\text{g l}^{-1}$	142 $\mu\text{g l}^{-1}$	18.1 $\mu\text{g l}^{-1}$
95 th percentile	N/A	N/A	9.71 $\mu\text{g l}^{-1}$	5.44 $\mu\text{g l}^{-1}$
90 th percentile	N/A	N/A	4.93 $\mu\text{g l}^{-1}$	3.28 $\mu\text{g l}^{-1}$
Median	4.11 $\mu\text{g l}^{-1}$	4.55 $\mu\text{g l}^{-1}$	1.20 $\mu\text{g l}^{-1}$	1.06 $\mu\text{g l}^{-1}$
% $\geq$ LOQ (0.2 $\mu\text{g l}^{-1}$)	100.0%	100.0%	93.6%	97.9%

for different population groups in Germany. These reference values are purely statistical in nature—they do not indicate any potential health effects. Conversely, HBM-I health-based guidance values have also been established. They represent levels below which, according to current knowledge and in a single-substance assessment, no adverse health effects are expected, and therefore, no action is needed. However, results exceeding the HBM-I values do not necessarily mean that adverse effects will occur; they indicate that we are no longer within the “safe zone” [30]. Both types of values are summarized in Table 2.

It should be noted that the HBM-I values depend, among other variables, on the F_{uc} —with a wide range of published values (see above, “2. Biomarker and Sample Matrix”). Issues like this one led to the application of an additional uncertainty factor. Even so, uncertainties remain, preventing, for example, the calculation of an HBM-I value for children [31, 32].

In all general population studies so far, less than 0.2% of the samples exceeded the respective HBM-I values (Table 2), indicating safe exposure levels (based on single-substance assessment) for the vast majority of the population. However, this assessment should be considered provisional—first, due to the mentioned uncertainty regarding the F_{uc} of BHT acid and the inapplicability of the HBM-I values for children; second, because of potential endocrine-disrupting properties of BHT that are

still under investigation and may require considering mixture effects [22, 24].

7. OPEN QUESTIONS AND FUTURE RESEARCH DIRECTIONS

7.1. Laboratory Analysis

From the outset, a strong focus was placed on the quality of laboratory work [12, 13]. The results from external QC samples in GerES VI are just one example among many demonstrating the impressive performance of the analytical method in routine use. Along with the study samples, blinded native urine samples containing BHT acid (both free and conjugated) at an unknown concentration near the LOQ ($0.2 \mu\text{g l}^{-1}$) were shuffled into each batch of study samples sent to the contract laboratory (Currenta). Unblinding was carried out by UBA only after all results from the samples had been reported by Currenta. Over a total of 13 analytical cycles spanning more than a year, the overall relative standard deviation of the measured BHT acid levels in these QC samples was less than 20%, indicating the method's adequate precision [35], even under the most stringent conditions (blinded samples, low native analyte levels, long-term).

While the precision (repeatability) of a method can be reliably checked this way, the fact that the “true value” of an analyte, such as BHT acid, is generally unknown in a native QC material means that it is hard to determine the method's accuracy

Table 2. Reference and health-based guidance values for urinary BHT acid (after enzymatic hydrolysis), arranged by their $\mu\text{g l}^{-1}$ value in descending order. Reference values apply to Germany.

Name	Value type	Applicability	BHT acid	Comment	Literature
HBM-I	health-based	adult men, environmental medicine	$124 \mu\text{g l}^{-1}$	¹	[31–33]
HBM-I	health-based	adult women, environmental medicine	$106 \mu\text{g l}^{-1}$	¹	[31–33]
RV ₉₅	reference	boys (3–11 years), environmental medicine	$15 \mu\text{g l}^{-1}$	²	[34]
RV ₉₅	reference	girls (3–11 years), environmental medicine	$14 \mu\text{g l}^{-1}$	²	[34]
RV ₉₅	reference	girls (12–17 years), environmental medicine	$11 \mu\text{g l}^{-1}$	²	[34]
RV ₉₅	reference	boys (12–17 years), environmental medicine	$8.7 \mu\text{g l}^{-1}$	²	[34]
BAR	reference	working-age adults, occupational medicine	$7 \mu\text{g l}^{-1}$	³	[27]

¹Based on ADI, F_{uo} , daily urine volume, and an uncertainty factor.

²95th percentile in GerES V.

³Expert judgement based on 95th percentiles in several studies without occupational exposure, key study: ESB.

independently. Of course, the accuracy was verified during method validation; however, this has only been possible using samples that were spiked in-house with BHT acid. Certified reference materials (CRMs) or non-certified reference materials (RMs) can help establish metrological traceability, as they serve as external references rather than just in-house ones. However, in the field of HBM, such materials in biological matrices are rare, and certainly not available for emerging biomarkers like BHT acid [36]. Expanding the range of commercially available (C)RMs to include such biomarkers is desirable but very difficult.

In the absence of suitable (C)RMs, interlaboratory comparisons can also help increase confidence in the accuracy of analytical results. The German External Quality Assessment Scheme (G-EQUAS) is among the most comprehensive HBM inter-comparison programs worldwide, encompassing both routine and more specialized parameters, and has steadily expanded in scope over the years [37, 38]. Nonetheless, emerging parameters like BHT acid pose a challenge for such programs, as they are measured in very few laboratories.

Comparability of results between different laboratories can be challenging. However, experienced HBM laboratories have long been aware of these issues. Typically, they can produce consistent analytical results and accurately assess measurement uncertainty, even when external QC programs are not available.

7.2. Metabolism and Excretion Kinetics

We have discussed the gaps in the knowledge about metabolism, especially human excretion kinetics of BHT, and the implications for exposure and risk assessment (see “2. Biomarker and Sample Matrix” and “6. Reference Values and Health-based Guidance Values”). Conducting a small metabolism study, similar to other substances [14–18], might clarify remaining questions regarding F_{uc} or kinetic parameters like excretion half-lives, which are essential for sampling strategies, e.g., in occupational HBM. Nonetheless, the costs of such a project should not be overlooked, as they can easily reach five-figure euros: study planning is complex, ethical

approval must be obtained, volunteers need to be recruited, many urine samples must be collected and analyzed, data must be evaluated and interpreted, and a manuscript must be written and published.

An alternative method for evaluating reported F_{uc} values was attempted in the ESB study: Experimentally determined daily BHT intakes (based on various F_{uc} values from the literature and on BHT acid HBM results) were compared with predicted daily intakes (based on consumption statistics and BHT use levels in consumer products). Unfortunately, due to data limitations, no definitive conclusions could be made [24].

7.3. Specific Exposure Sources and Trends

The contribution of various exposure sources to overall BHT intake remains unresolved because HBM provides exposure estimates that encompass all possible sources and routes; combining HBM with other data has not yet enabled the identification of specific BHT sources [22, 24]. Several factors may compound this challenge. One such factor is the potential formation of BHT degradation products during food processing, such as heating or long-term storage of BHT-containing foods [39]. Natural sources of BHT, such as litchi and oak wood, or environmental sources of BHT and BHT acid, like water, may also warrant consideration [24, 39]. However, they are likely less relevant to human exposure than anthropogenic sources. The evaluation of GerES VI HBM data might provide insight into specific BHT sources, especially when combined with other data collected in GerES VI [26]. Population-representative data from GerES VI will likely enable further refinement of existing reference values, such as the BAR (Table 2, [27]). Since the most recent samples from the ESB time series date back to 2018 [24], the results from GerES VI (sampling in 2023–24) could reveal whether exposure levels have changed since then or if they remain stable.

Furthermore, it could be valuable to examine time trends of BHT exposure in other parts of the world. For example, while BHT was likely never a major additive in breakfast cereals in Europe, it was commonly used in cereals in North America at least

until 2015. Then, an activist blogger pressured cereal companies to remove BHT from their products sold in the area, and at least one large company voluntarily altered its cereal formulation or packaging. However, it claimed this change had been planned earlier [40]. If cereals were a significant source of BHT, this shift should be reflected in HBM results of historical urine samples in that region.

A few studies on HBM of urinary BHT acid in countries outside Germany have been published recently, using analytical methods different from those used in Germany [41, 42]. Since these study populations from the United States or various Asian countries are quite small and not representative ($n \leq 60$ per country), comparisons of concentration levels should be approached with caution. Nonetheless, two key points stand out. First, urinary BHT acid concentrations and detection frequencies are approximately similar across all countries, indicating widespread global exposure to BHT. Second, some country-specific differences in exposure appear to be present. It would be valuable to clarify this in larger studies.

8. CONCLUSION

Between 2013 and 2025, a significant amount of time, effort, and money was invested in HBM of BHT in Germany. Many key insights have been gained so far: widespread exposure of the general population to BHT—indeed, nearly universal. Children, especially very young children, are exposed to higher levels than adults. Exposure levels in adults remained quite stable over the first two decades of this century. Occupational exposure to BHT appears to be relevant for a relatively small percentage of workers handling BHT. A set of robust HBM reference values has been established to describe exposure levels among children, adolescents, and adults in Germany. Despite limited literature data on the toxicokinetics of BHT, it was possible to define health-based HBM guidance values, but only for adults. Most adults (> 99.8%) have BHT levels in their bodies that are considered safe based on current knowledge; however, clear assessments for children cannot yet be provided. Substantial uncertainties remain regarding the toxicological interpretation. The toxicology of BHT remains under investigation in the European Community

Rolling Action Plan (CoRAP). Some of the findings from HBM field studies in Germany [22, 24] have recently been considered in the CoRAP [11]. More results will be available soon from the GerES VI study, which is representative of the entire German adult population.

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