The International Bromine Council

BSEF Comments on the Proposal for Harmonized Classification and Labelling of 2,2',6,6'-Tetrabromo-4,4'-Isopropylidenediphenol; Tetrabromobisphenol-A (TBBPA)

General Comments

BSEF Comment:

Herein we provide comments primarily regarding the carcinogenicity classification, along with some discussion on the evaluation of health hazards related to repeated-dose and reproductive toxicity.

Foremost are the comments related to the dossier not providing a convincing rationale to justify a Category 1B designation for carcinogenicity. As described in the individual comments, the CLP criteria have not been met for Category 1B; rather, evidence supports a Category 2 designation.

In BSEF's opinion the dossier does not adhere to GHS CLP guidance in determining the carcinogenicity classification. The text acknowledges that the data collectively demonstrate the involvement of secondary mechanisms with practical thresholds in uterine tumor formation, as well as other possible events associated with the mode(s) of action (MoA[s]). For this reason, these data support the criteria described in ECHA (2017) related to downgrading of a Category 1 to Category 2 classification based on a practical threshold.

The dossier does not address the potential for species differences related to sulfotransferases and glucuronide conjugate profiles and the role they may play in determining the strength of evidence (or lack thereof) related to CLP criteria. Further, multiple authoritative bodies have described human exposure in a way that would deny the plausibility of the biological pathway identified in the tested rat strain occurring in humans.

We appreciate the opportunity to provide comments related to clarifying the scientific discussion in the dossier to better reflect the body of evidence available for TBBPA relative to CLP criteria.

NOTE: *Please note that the attached document compiles all BSEF comments and contains an extra comment (the last comment of the document) regarding Section 5 (Identified Uses).*

The International Bromine Council

SECTION 10.9 CARCINOGENICITY

CLH Dossier – 10.9.3: "We propose TBBPA to be classified as a Category 1B carcinogen based on conclusive data (carcinogenic in animal studies and relevant mode of action for humans)."

BSEF Comment:

It does not appear that the CLP criteria for Category 1B have been met for TBBPA. The findings of the single animal study are aligned with the CLP criteria for "limited" in animals, as opposed to "sufficient," and thus support a Category 2 designation rather than 1B.

ECHA (2017) GHS CLP guidance Table 3.6.1 states on page 377 that (emphasis added):

> "Category 1B, presumed to have carcinogenic potential for humans, **CLASSIFICATION IS LARGELY BASED ON ANIMAL EVIDENCE.**
>
> The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived from:
> – [H]uman studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or
> – [A]nimal experiments for which there is sufficient [1] evidence to demonstrate animal carcinogenicity (presumed human carcinogen)."

As the dossier indicates, no human data are available, and thus, the category assignment is based on animal experiments. To be categorized as a 1B, the CLP criteria require that animal experiments provide "sufficient" evidence. [Note that a separate comment addresses footnote (1), which describes other considerations; this comment focuses specifically on the sufficiency of animal data relative to CLP definitions.] The evidence cited in the dossier to determine that TBBPA is "carcinogenic in animal studies" does not meet criteria for "sufficient" evidence, as outlined by the ECHA (2017) CLP guidance Annex I: 3.6.2.2.3.

Specifically, pages 378–379 of ECHA (2017) describe "sufficient" evidence of carcinogenicity as *"a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in"*:

(a) Two or more species of animals
Not fulfilled: Clear evidence of carcinogenicity was observed in only one species and sex, and was limited to a single tumor type—uterine lesions in female Wistar Han rats. The National Toxicology Program (NTP) concluded that lesions observed in male Wistar Han rats and or in

B6C3F1 mice (both sexes) were judged by the National Toxicology Program not to provide clear evidence of carcinogenicity.

No uterine tumors were observed in female mice, nor other tumors, leading to a conclusion of "no evidence of carcinogenicity."

Evidence was judged as equivocal (defined as a marginal increase of neoplasms that may be chemically related) in male rats for testicular adenomas by NTP, because the highest incidence (in high-dose rats, 3/50) was only one greater than in the historical control studies, and the incidence in the vehicle control was at the low end of the historical range (0/50).

There was some evidence in male mice for hepatoblastomas. However, these findings would not be considered to provide strong evidence, given: (a) the lack of a clear dose response, (b) the very high spontaneous control rate in control mice, and (c) the approach for assessing incidence of the lesion. It is well recognized in the literature that hepatoblastomas should not be considered a separate tumor type or incidence, because they represent a morphologically altered area of hepatocellular adenomas or carcinomas, rather than an independently derived tumor (e.g., Turusov et al, 2002; Thoolen et al, 2010; Cattley et al, 2013). This scientific understanding was observed in the study with TBBPA; all mice with hepatoblastomas also had adenomas and/or carcinomas (i.e., the combined rate of 78%, 84%, or 86% at 0, 250, or 500 mg/kg-day, respectively, is the same, whether for adenoma or carcinoma combined, or for adenoma, carcinoma, or hepatoblastoma combined).

For these reasons, clear evidence of carcinogenicity was not observed in two or more species, and thus, the CLP criteria to reach "sufficient" strength of evidence for this criterion have not been met.

(b) Two or more independent studies in one species carried out at different times or in different laboratories or under different protocols.
Not fulfilled: Only one study is available, so there are no data to support this criterion requiring two or more independent studies.

(c) An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence.
Not fulfilled: Clear evidence was limited to uterine tumors in female rats, and findings in male rats were equivocal; thus, there are no data supporting the criterion of an increased incidence of tumors in both sexes of a single species. Further, no evidence of carcinogenicity (including lack of uterine tumors) was reported in female mice.

(d) A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites."
Not fulfilled: The dossier indicates that a multi-site response was "not so evident."

The dossier does not describe how the evidence demonstrates that malignant neoplasms occur to an unusual degree. Malignancy of uterine tumor metastases was not described by the NTP as occurring to an unusual degree with regard to incidence, site, type of tumor, or age at onset. The type of tumor (MMRT) is rare, although a limited number of bioassays and historical control data preclude comprehensive assessment and overall confidence. .

In summary, the criteria to meet "sufficient" strength of evidence in animals have not been met. In contrast, the findings from the NTP study satisfy three of four ECHA (2017) criteria related to a limited strength of evidence . ECHA (2017) guidance defines limited evidence of carcinogenicity in experimental animals as "the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs."

For TBBPA, the data support the ECHA (2017) Guidance Criteria for "Limited Strength of Evidence" (2):
(a) The evidence of carcinogenicity is restricted to a single experiment
Fulfilled: Clear evidence of carcinogenicity is limited to a single experiment.

(b) There are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies
Fulfilled: The NTP (2014) bioassay used Wistar Han rats instead of the commonly used F344 strain. Wistar rats have been shown to have elevated estrogen levels and a higher estrogen/progesterone ratio, which would cause this strain to be more susceptible to these effects than other rat strains (Lai et al., 2015).
The NTP (2014) bioassay employed a novel histopathology technique (i.e., longitudinal sectioning, in contrast to a standard transverse section), for which there were very limited historical control data [see separate comment on use of historical control data].

(c) The agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential
Not fulfilled.

(d) The evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs
Fulfilled: It has been proposed that TBBPA interferes with estrogen sulfate conjugation, causing artificially elevated estrogen levels (Sanders et al., 2016) by competing for glucuronosyltransferases and/or sulfotransferases, thus indirectly resulting in higher serum estrogen and subsequent promotion of pre-existing Tp53 mutations in the uterus through increased DNA synthesis and cell proliferation, leading to uterine tumors.
When the available data are assessed in the context of the CLP criteria, the body of evidence does not meet the criteria for "sufficient." Rather, the animal data support a "limited" strength of evidence, which in turn, would support a Category 2 designation, even when the "additional considerations" are factored in [see separate comments on additional considerations].

CLH Dossier – Table 14: Scientific studies and reviews on the possible mode of action for TBBPA-induced uterine carcinogenesis in rats

BSEF Comment:

The dossier Table 14 presents studies and reviews on the possible mode of action (MoA), presenting five possible MoAs and associated references. However, only the first, "disruption of estrogen homeostasis," is supported by references as an MoA; the remaining possibilities do not provide MoA information, and thus, provide only the perception of additional evidence characterizing a mode of action.

The information provided for "disruption of thyroid hormone pathway" does not describe a mode of action but, rather, describes a lack of effect on thyroid hormones. Please consider clarifying the intent and interpretation of this information. Further, in the second row of Table 14, the reported findings in the second column clearly demonstrate that the substance is **NOT** disrupting the thyroid pathway per the definition of endocrine disruptors, because no adverse effects related to the observation of thyroid hormone levels were observed in all the studies. Therefore, BSEF respectfully requests to change the title of this column. The third, fourth, and fifth MoAs—oxidative stress, inflammation and immunosuppression, and genetic and related effects—are not themselves modes of action, nor does the information in the table for these entries present a mode of action. The hand-selected data points (not representative of the body of evidence) provided in the table for these entries report on activity in diverse assays (including

in vitro assays) that could be considered, at best, as key events in a mode of action. Further, several of these events can be associated with non-carcinogenic effects (see Bus et al., 2017, for example). No pathway-based analysis related to uterine tumors associated with TBBPA exposure was presented in the CLH dossier or in the references cited. As these are not actually MoAs, we request to consider omission of their description as such.

CLH Dossier – Section 10.9.1.1 According to IARC (2018), based on key characteristics of human carcinogens, there is strong evidence that TBBPA modulates receptor-mediated effects, induces oxidative stress and is immunosuppressive; there is moderate evidence that TBBPA induces chronic inflammation; and there is weak evidence that TBBPA is electrophilic, genotoxic or alters cell proliferation, cell death or nutrient supply.

BSEF Comment:

Section 10.9.1.1, Mode of Action (MoA) for uterine carcinogenesis in female rats and relevance to humans, begins with a statement from IARC related to the key characteristics and evidence of receptor-mediated effects, induction of oxidative stress, immunosuppression, and other characteristics. However, the key characteristics are not representative of a mode of action, and thus, the information should be omitted from the MoA section (or, if retained, should be evaluated further in the context of MoA).

As defined by the original authors of the KCC approach employed by IARC, each of the KCCs is not, in and of itself, a mechanism of carcinogenesis (Smith et al., 2016). Rather, the KCC "can provide a basis for systematically identifying, organizing, and summarizing mechanistic information as part of the carcinogen evaluation process" (Smith et al., 2016). It is widely recognized that the use of the KCCs has limitations in the way that is has been applied in practice, and specifically, for the misperception that the KCCs are equivalent to a mode of action (Becker et al., 2017; Fielden et al., 2018; Goodman and Lynch, 2017; Wikoff et al., 2019). The KCC approach applied by IARC does not assess the biological significance of mechanistic endpoints in context of specific carcinogenic responses in animals or humans (i.e., MoA or pathway-based assessment).

The ECHA CLP guidance specifically states the following regarding mode of action: "To establish a mode of action will usually require specific investigative studies over and above the standard carcinogenicity study. All available data must be considered carefully to judge if it can be concluded with confidence that the tumours are being induced through that specific mechanism. The IPCS Framework for Analyzing the Relevance of a Cancer Mode of Action for

Humans (2007) can be a useful way to construct and present a robust and transparent assessment of such data."

With respect to referencing IARC's evaluation of KCC, the dossier would be improved by acknowledging that the IARC did not conduct a systematic evaluation of KCC data for TBBPA and thus the summary statements from IARC do not represent the body of evidence. Further, the dossier could clarify that activity cited for some of the key characteristics was not assessed in the context of any specific cancer types—that is, data cited were not described as key events in a biological pathway that would lead to uterine tumors in a manner described by the ECHA CLP guidance.

Because the organization of data by key characteristics is much different from the scheme used in the IPCS framework referenced in the ECHA guidance, we suggest to omit the paragraph that refers to the KCC evaluation from IARC, because it does not represent an MoA, nor does it support one. Alternatively, it could be considered to conduct an MoA evaluation similar to that described in the IPCS framework, to assess events associated with the key characteristics relative to the specific tumor types observed in the NTP cancer bioassay in an MoA framework.

CLH Dossier – Section 10.9.3 We propose TBBPA to be classified as a Category 1B carcinogen based on conclusive data (carcinogenic in animal studies and <u>relevant mode of action for humans</u>)."

p. 31 — According to Lai et al. (2015) uterine tumours induced by TBBPA in rats are qualitatively applicable to humans by the described MoA, but that it is unlikely that thus MoA is quantitatively plausible for humans, especially taking into account the ADME and kinetic factors. In the DS´s view, this argument is relevant for risk assessment, and not for classification.

BSEF Comment:

The evidence base in support of demonstrating relevance of the mode of action for TBBPA carcinogenicity to humans appears less than comprehensive. For example, the discussion of TBBPA affecting estrogen homeostasis by competing with estrogen for estrogen sulfotransferases does not present a full account of the MoA that has been discussed and presented in the literature. Not presented is the context of these events, which require chronic, high-dose exposure for the threshold-based MoA to be operational. Also left unaddressed are the potential species and strain sensitivities of the MoA as it relates to the observations reported in the NTP study.

Although it is clear that the CLP evaluation is based on hazard potential and not risk (i.e., combined consideration of exposure), the mode of action has been demonstrated to be both threshold-based and dose-dependent. As such, the relevance of the MoA to humans must be determined by considering the plausibility of the biological pathway occurring in humans—which inherently involves consideration of the thresholds and dose-dependence, in addition to the relevance of the biological construct. That is, determining whether the pathway is "qualitatively applicable" to humans inherently involves assessing the plausibility of the initiating event and cannot be separated between hazard and risk as readily as the dossier suggests (p. 31). Multiple studies have demonstrated that the biological pathway associated with TBBPA-induced uterine tumors in Wistar Han rats would not be initiated in humans, based on observed kinetics (i.e., the threshold could not be plausibly reached under current and past exposure levels).

The dossier selectively cites a study by Borghoff et al. (2016) that provides strong evidence high-dose TBBPA administration changing the bioavailability of estrogens in rats, via—at least partly—influencing estrogen metabolism. Specifically, this influence involves the binding and inhibition of sulfotransferases. There are marked differences in the importance of the glucuronide versus the sulfate pathway in humans and rats. The conjugate profiles vary between species, because glucuronide conjugates are major metabolites in humans, whereas sulfate conjugates are major metabolites in rats [see separate comment on differing conjugate profiles between humans and rats]. Further, there are marked interspecies differences in internal dosimetry described as the competition of plasma TBBPA with estradiol for sulfation, which occurred in rats at >15,000-fold higher TBBPA serum concentrations compared than those measured in humans.

Of note, the dossier appears to recognize the importance of these species differences: "TBBPA-induced uterine tumours were seen in rats and not in mice, possibly because of differences between rats and mice as estrogen homeostasis is less affected in mice than in rats due to differences in capacity and/or capability of conjugating enzymes (Dunnick et al., 2015)."
The acknowledgement of differences between rodent species highlights that the discussion of species differences between rodents and humans, and the plausibility of the MoA being operable, is not comprehensive. We emphasize the importance of addressing these differences and acknowledging that there is no comparable ADME at the dose levels used in the cancer bioassay.

The "practical threshold" associated with the MoA is clearly relevant when considering that human exposure levels are estimated to range from 3.2×10^{-7} to 8.4×10^{-5} mg/kg-day, and lifetime average daily dose estimates are associated with a margin of exposure that is >32,000,000 for cancer (Wikoff et al., 2015).

Dose-dependency of other key events has also been demonstrated, including the incidence of atypical endometrial hyperplasia, as well as the occurrence of tumors (limited to the two highest dose groups based on combined incidence).

Further, multiple studies have indicated a potential species- and strain-specific sensitivity of the Wistar Han rats to the mode of action (e.g., Lai et al., 2015; Wikoff et al., 2019), as addressed in separate comments herein.

Given the evidence supporting a high-dose and threshold-based MoA, which has, to date, been observed in only a single, sensitive strain of rats (and not other rodents), data suggest that it is unlikely (if not implausible) that such an event would occur in humans. This finding is supported by the TBBPA exposure levels in humans being up to eight orders of magnitude lower (NTP, 2014; EU, 2006; Wikoff et al., 2015) than the doses associated with saturation of these key enzymes in animal studies.

CLH Dossier – Table 13: Compilation of factors to be taken into consideration in the hazard assessment

BSEF Comment:

The evidence summarized in Table 13 does not represent all of the additional considerations in CLH guidance. Specifically, Table 13 is missing two "additional considerations":

- Structural similarity (g)
- ADME between animals and humans (i).

Importantly, the evidence available for these considerations reduces the overall strength of the evidence [see separate comments on structural similarity and ADME between animals and humans]. The dossier would be improved by either including these criteria or presenting the rationale for omitting these criteria from Table 13.

CLH Dossier – p. 32 (g), Structural similarity TBBPA has little activity as an estrogen receptor agonist or antagonist compared to other bisphenols, e.g., bisphenol A.

BSEF Comment:

In the text provided for additional consideration (g), the “structural similarity” is not clear relative to the strength of evidence.

The single sentence indicates that TBBPA has little activity as an estrogen receptor agonist or antagonist compared to other bisphenols such as bisphenol A. The reference to “little” activity does not adequately describe the overall lack of estrogen receptor activity identified for TBBPA, based on the weight of evidence of a large data set on TBBPA activity in estrogen receptor binding and transactivation assays (Wikoff et al., 2016).

Further, not all ER agonists cause uterine tumors; it is requested that the dossier clarify this fact. This comment does not dispute what is drafted, but rather, requests that a clearer statement be provided with regard to the strength of evidence related to carcinogenicity. Specifically, we suggest clarifying that the lack of activity, despite the structural similarity of other chemicals that cause uterine tumors, decreases the strength of the evidence.

CLH Dossier – P. 32 Elimination half-life of TBBPA in experimental animals and humans do not differ considerably

BSEF Comment:

It is requested that rationale be provided to support the statement that elimination half-lives do not differ considerably between experimental animals and humans. The dossier is missing information which specifies which animals, including which strains, were used, as well as missing the information and dose levels that form the basis for this statement in each species.

It is important that the dossier address differences in ADME—including elimination—based on dose and species and acknowledge that there is no comparable ADME at the dose levels used in the cancer bioassay.

CLH Dossier – 10.9.2 Comparison with the CLP Criteria — Additional Considerations (p. 31 Table 13: Compilation of factors to be taken into consideration in the hazard assessment

BSEF Comment:

The information on the “additional considerations” is not presented clearly; available data do not provide a biologically coherent account of the evidence to support a determination of “sufficient” strength of evidence, consistent with a Category 1B designation.

In addition to the strength of evidence based on animal experiments, eleven “additional considerations” outlined by ECHA (2017) are considered. According to CLP guidance, a Category 2 is assigned when evidence is not sufficiently convincing for a 1A or 1B. This comment addresses the overall evidence for “additional considerations”; separate comments are provided on specific “additional considerations.”

For TBBPA, the characterization of the additional considerations relative to CLP begins on dossier p. 31, although the content relating to this topic is also summarized in Table 13 and the associated discussion. Table 13 presents a summary of the considerations based on species and sex; however, when compared to CLP, the information is combined in a misleading manner. The dossier would be improved by clarifying how the data for the individual additional considerations relate to CLP criteria, based on the separate data sets. This is quite important, given that each tumor type was observed in only one sex and species, and the additional consideration of information for one tumor type may not apply to others.

The evidence provided is not sufficiently robust to result in a Category 1B designation when focusing on the only tumor type with clear evidence of carcinogenicity (where applicable). The primary considerations that contributed to the strength of evidence are:

- Progression to malignancy (c)
- Reduced tumor latency (d)
- Route of exposure (h).

However, the considerations that reduced the strength of evidence include:

- Lack of a multi-site response (b)
- Lack of a multi-species response (f)
- Differences in ADME between animals and humans (i)
- Mode of action (k).

Other Considerations Impact on Strength of Evidence; Rationale Based on Summary of TBBPA Evidence for Uterine Tumors in Female Rats

a Tumor type and background incidence ↔ “Clear” evidence for uterine tumors, but in a particularly sensitive strain used infrequently by NTP, with no historical control data for the histopathology technique relied upon.

b Multi-site response ↓ Not observed. Multi-site responses limited to male mice in which evidence was only "some" or "equivocal."

c Progression of lesions to malignancy ↑ Endometrial atypical hyperplasia in all treated groups progressed to adenoma, adenocarcinoma, and/or malignant mixed Mullerian tumors (MMMT).

d Reduced tumor latency ↑ Some indication of reduced latency based on age of onset (668 d, 548 d, 321 or 442 d in control, 250, 500, or 1000 mg/kg-day groups, resp., for the combined rates).

e Single or both sexes ↔ Not applicable.

f Single or several species ↓ Uterine tumors not observed in female mice.

g Structural similarity ↔ TBBPA does not bind to ER, similar to other chemicals that cause uterine tumors.

h Route of exposure ↑ Gavage is considered a relevant physiological route.

i ADME between animals and humans ↓ TBBPA competes with estradiol for sulfation in rats; species differences in conjugate profiles. (Glucuronide conjugates are major metabolites in humans, whereas sulfate conjugates are major metabolites in rats.)

j Excessive toxicity confounds ↔ No appreciable signs of excess toxicity.

k Mode of action ↓ High-dose TBBPA interferes with estrogen sulfate conjugation, causing artificially elevated estradiol levels in a sensitive strain of rats.

Please clarify the discussion of "additional considerations" as they relate to the strength of evidence for uterine tumors. It would also be helpful to differentiate "additional considerations" for tumor types with lesser evidence.

CLH Dossier – 10.9.2 Comparison with the CLP Criteria – Additional Considerations (p. 31)
Table 13: Compilation of factors to be taken into consideration in the hazard assessment

BSEF Comment:

The table below summarizes the additional considerations for other tumor types observed, despite conclusions of equivocal or some evidence. In addition to none of these tumor types being classified as "clear" evidence, the additional considerations would not support a characterization of "sufficient" strength of evidence for Category 1B.

The dossier would benefit from clarifying how the additional considerations from other tumor types in other species were evaluated or used to support the proposed 1B classification. As drafted, these aspects are not described.

Limited "Strength of Evidence"
Other Considerations [left to right in the list below]:
(1) Liver (male mice) — "Some Evidence"
(2) Large Intestine (male mice) — "Equivocal Evidence"
(3) Hemangioma/ Sarcoma (male mice) — "Equivocal Evidence"
(4) Testes(male rats) — "Equivocal Evidence"

a	Tumor type and background incidence	(1)↓	(2)↔	(3)↔	(4)↔
b	Multi-site response	(1)↑	(2)↑	(3)↑	(4)↓
c	Progression of lesions to malignancy	(1)↑	(2)↓	(3)↑	(4)↓
d	Reduced tumor latency	(1)↓	(2)↓	(3)↓	(4)↓
e	Single or both sexes	(1)↓	(2)↓	(3)↓	(4)↔
f	Single or several species	(1)↓	(2)↓	(3)↓	(4)↓
g	Structural similarity to agents w/good evidence	(1)↓	(2)↔	(3)↔	(4)↔
h	Route of exposure (gavage)	(1)↑	(2)↑	(3)↑	(4)↑
I	ADME between animals and humans	(1)↓	(2)↓	(3)↓	(4)↓
J	Excessive toxicity confounds	(1)↔	(2)↔	(3)↔	(4)↔
K	Mode of action	(1)↔	(2)↔	(3)↔	(4)↔

CLH Dossier – P. 31 Uterine cancer in humans is of the same type as uterine tumours seen in rats.
10.9.1 Short summary and overall relevance of the provided information on carcinogenicity
10.9.2 Comparison with the CLP Criteria — Additional Considerations

BSEF Comment:

The rationale for the "additional consideration" related to tumor type is both unclear and unsubstantiated as drafted.

The CLH report states in multiple places, such as pages 23 and 32, that: “The predominant tumour type in rats was uterine adenocarcinoma, which is also the predominant uterine tumour type in humans.” First, the text as drafted is misleading statement and can easily be misconstrued that there is evidence of carcinogenicity of TBBPA in humans. Whereas there is no evidence that TBBPA causes any tumor type in humans. With respect to cancer in humans, IARC (2018) noted that “No data were available to the Working Group.”

Further, evidence is not directly provided to support the statement on p. 31: “Uterine cancer in humans is of the same type as uterine tumours seen in rats.” A subsequent sentence and citation of Dunnick et al. (2017) is provided, which addresses that uterine tumors are common in humans, but no connection to TBBPA is established in these conclusions.

In an earlier section, the Dossier briefly addresses tumor types in the context of the Tp53 mutations (p. 30). Of note, the dossier cites a study by Harvey et al. (2015) as providing a rationale for alterations of the Tp53 pathway to be relevant to humans. However, the scientific evidence presented on this matter in not complete, nor does the discussion include the study by Harvey et al. (2015), or any other study, to support a finding that the type of uterine tumors observed in rats exposed to TBBPA is the same as that observed in humans.

Available data do not fully support the findings of the dossier on tumor type. Harvey et al. (2015) discussed concordance between animal and human responses involving Tp53 mutations, stating that changes in gene expression and mutation spectra found in human endometrial carcinoma were similar to those identified in rat uterine carcinomas. These authors stated that changes in gene expression and mutation spectra found in human endometrial carcinoma were similar to those identified in rat uterine carcinomas, *regardless of TBBPA exposure*. The authors also noted that some morphological features of this tumor type were similar to human high-grade type I endometrial carcinoma, whereas other features (e.g., Her2 overexpression and increased Tp53 mutation frequency) were similar to human type II endometrial carcinoma.

The lack of concordance with Tp53 mutations and TBBPA exposure is notable, because the dossier apparently relies on these factors to support that the tumor types are the same. Harvey et al. (2015) evaluated the mutation frequency of Tp53 by non-randomly selecting tumors from 16 TBBPA-dosed Wistar Han rats and compared the frequency (combined across dose groups) to spontaneously induced tumors from control animals from other 2-year studies conducted in Wistar Han rats (which involved animals from both corn oil gavage studies and inhalation studies). Statistical analyses of the available data, despite the variable reporting, resulted in a lack of dose-response for the Tp53 mutations (Wikoff et al., 2016).

When considered collectively, although the TBBPA-induced uterine tumors in rats appear to be similar to human adenocarcinomas, classification as to the type of endometrial tumor (Type 1

vs. Type 2) is not clear at this time, most likely due to the lack of sufficient information to allow for meaningful comparisons of molecular genetic features.

For these reasons, the dossier would be improved with further elaboration on the science of this matter. A specific rationale, using available science, would help support the statement—as well as clarify uncertainties—that the uterine tumors observed in rats exposed to TBBPA are the same as those observed in humans.

CLH Dossier – 10.9 This makes the historical control database limited to 150 animals. According to Lai et al. (2015)[1] the Wistar Han strain resemble the SD strain, which are known to contain elevated levels of estrogens and a higher estrogen/progesterone ratio.

p. 32 The incidence in the 2-year rat study exceeds the incidence of these tumours in the historical control database which is of limited magnitude due to a change of rat strain by NTP.

BSEF Comment:

The complexities and limitations of uterine tumor comparisons to historical controls are not described adequately in the dossier.

In addition to the apparent high spontaneous incidence of uterine tumors in Wistar Han rats, there is also a general lack of historical control data from NTP for this strain, as well as a lack of comparison data for the histopathological approach used to assess the tissues. There is a lack of robust historical control data on the incidence of uterine tumors in the Wistar Han rat, because the NTP used this strain of rats only for a very short time.

The Wistar Han rats used in the NTP bioassays have a high background incidence of uterine adenocarcinomas (2%–18%) relative to that observed in F344 rats (0.29%) (NTP, 2013; Klaunig et al., 2015; Wikoff et al., 2016). Other studies in the literature also indicate that the Wistar Han strain may have a high background rate of uterine tumors (Deerberg et al., 1981; Harleman et al., 2012; Poteracki and Walsh, 1998), with incidence rates up to 39% (Deerberg et al., 1981).

Wistar rats have also been shown to have elevated estrogen levels and a higher estrogen/progesterone ratio, which would cause this strain to be more susceptible to these effects than other rat strains (Lai et al., 2015). Further, an inverse relationship between uterine and mammary tumors in the Wistar rat has been reported previously (Harleman et al., 2012). In the TBBPA bioassay conducted by NTP, no treatment-related mammary tumors were observed, which is consistent with the spontaneous pattern observed. Taken together, these data provide

evidence for potential strain sensitivity to this adverse outcome, and thus should be considered when evaluating the overall strength of the evidence.

Compounding these uncertainties, the NTP study with TBBPA was the first study that used longitudinal sectioning to evaluate the uterus. While this new approach provided useful information, there was also uncertainty in the historical control data available for the incidence of these lesions using the longitudinal approach, because data are limited to only 150 animals (50 of which were from the TBBPA study).

The dossier is not clear on these important aspects of background and historical control incidence. The footnotes to Table 13 do not sufficiently describe these differences and their potential impacts; thus, it is requested that the dossier better describe these aspects as they relate to uncertainty and unresolved issues according to CLP criteria.

CLH Dossier – h) [C]omparison of ADME between test animals and humans
Comparative studies in experimental animals and humans show that TBBPA was absorbed and metabolised rapidly in healthy volunteers as well as in experimental animals. No accumulation of TBBPA or metabolites found in uterus in female rats. TBBPA was metabolised by i.e., sulfate conjugation in humans and experimental animals and excreted predominantly via bile. Elimination half-life of TBBPA in experimental animals and humans do not differ considerably.

BSEF Comment:

The comparison of ADME between test animals and humans is very limited and does not address species differences specifically related to sulfotransferases—a toxicokinetic aspect recognized in the dossier to be important to TBBPA ADME, as well as the MoA for uterine tumors as it relates to alteration of estrogen homeostasis.

Because the MoA involves inhibition of estrogen sulfotransferase and a subsequent increase in the bioavailability of estrogens, it is important to compare the metabolism of estrogens in both humans and rats. As discussed in Borghoff et al. (2016), tissue distribution of sulfotransferases in humans has been relatively well characterized, including identification of major substrates. The endometrium is an estrogen-responsive tissue in both rats and humans and is known to express ES in human tissue. SULT1E1 is the isoform primarily responsible for estrogen metabolism in humans (Coughtrie et al., 2002; Falany et al., 1998; Xu et al., 2012).

However, much less information is available in other species, and the limited information that is available suggests potential species differences that are of critical importance to TBBPA. A tissue-specific evaluation of rat sulfotransferase messenger RNAs reported a lack of detection in the rat uterus (Dunn and Klassen, 1998). An *in vivo* study found that estradiol glucuronidation was more active in the uterus of Wistar Han rats than was estradiol sulfation, suggesting that the rat uterus is not a source of sulfotransferase enzymes (Blom et al., 2001).

Specific to TBBPA, there are differences in metabolism between humans and rats. In humans receiving an oral dose of TBBPA (0.1 mg/kg), the major metabolites are glucuronide conjugates. In contrast, after oral dosing of rats (300 mg/kg) sulfate conjugates predominated. Also, in rats, there are strain, gender, and dose differences in kinetics related to sulfation (Kuester et. Al., 2007; Knudsen et al., 2014; Schauer et al., 2006).

The dossier does not currently address the potential for species differences related to sulfotransferases and glucuronide conjugate profiles and the role they may play in extrapolating the high-dose uterine tumors observed in a sensitive rat strain to what would occur in high-dose-exposed humans.

CLH Dossier 10.9.3 Conclusion on classification and labelling for carcinogenicity A nongenotoxic mode of action is assumed (threshold carcinogen) relevant to humans.

Table 14 — Possible modes of action: Disruption of estrogen homeostasis
Section 10.9.1.1 — Mode of action (MoA) for uterine carcinogenesis in female rats and relevance to humans.

The DS agrees with Lai et al. (2015) that TBBPA is expected to exhibit a threshold for adverse effects….”

BSEF Comment:

The dossier does not acknowledge or consider ECHA CLP Guidance (2017) regarding application of CLP criteria, which indicates downgrades to Category 2 for chemicals with a practical threshold.

Specifically, p. 386 of the ECHA GLP Guidance states (bold added for emphasis):

> *[T]he existence of a **secondary mechanism** of action with the implication of a **practical threshold** above a certain dose level (e.g., **hormonal effects on target organs** or on*

mechanisms of physiological regulation, chronic stimulation of cell proliferation) may lead to a ***downgrading of a Category 1 to Category 2 classification****.*

The DS acknowledges and agrees with the existence of a threshold, as is stated throughout the dossier (selected examples are listed in the sections that follow). The DS also acknowledges that TBBPA is not genotoxic (also addressed in a separate comment).

All of the possible modes of action for uterine carcinogenesis in female rats discussed in the dossier, as well as those cited in IARC (2018), are associated with thresholds. Table 14 summarizes five types of events that have been evaluated [see separate comment differentiating these events from modes of action], all of which are recognized to be threshold based:

- Disruption of estrogen homeostasis
- Disruption of thyroid hormone pathway
- Oxidative stress
- Inflammation and immunosuppression
- Genetic and related effects: "direct or indirect via a secondary nongenotoxic event."

The threshold associated with the primary mode of action evaluated, involving modification of estrogen homeostasis, has been well characterized. That is, the evidence of a "practical threshold" has been investigated by multiple authors, including studies from NTP/NIEHS authors. These data repeatedly demonstrate that the threshold is due to the metabolic saturation of TBBPA sulfation in rats at high doses. [Note that human relevance and species specificity are addressed in a separate comment.]

As concluded by Knudsen et al. (2014), "Elimination pathways appeared to become saturated leading to delayed excretion after a single oral administration of the highest dose (1000 mg/kg); no such saturation or delay was detected at lower doses." The dossier references this study and cites two additional studies (Colnot et al., 2014, and Kuester et al., 2007) in stating, "At lower doses, over 95% of orally administered TBBPA is excreted, partially as parent compound and in the form of metabolites in feces within 72 hr after a single dose with associated little tissue retention or bioaccumulation."

The dossier also acknowledges, via the MoA, that the hormonal effects in the uterus are secondary to metabolic saturation. Cell proliferation, as acknowledged in dossier Table 11 and on page 26 (section 10.9.1.1 on Mode of Action), is also a secondary mechanism of action, but the dossier does not acknowledge it as such.

Data collectively demonstrate, as acknowledged in the dossier, that secondary mechanisms with practical thresholds are involved in uterine tumor formation, as well as other possible events associated with the MoA(s). Therefore, these data support the criteria described in ECHA (2017) related to downgrading of a Category 1 to Category 2 classification based on a practical threshold.

CLH Dossier: P. 33 TBBPA was tested by the IARC working group (IARC, 2018 pp. 63-64) across the full assay suite of ToxCast and Tox21[17] with data available for 836 assay end points[.]

BSEF Comment:

This sentence from the dossier would be more accurate if revised to reflect that the IARC working group did not test TBBPA. Rather, they evaluated publicly available high-throughput screening (HTS) data that were generated, processed, and analyzed by the USEPA and NTP (NIH), and by contract laboratories, as part of the ToxCast/Tox21 program.

Further, using the current version (invitrodb_v3.3, released September 4th, 2020), TBBPA was tested in 867 assays, not including background measurements. While the IARC assessment noted that the strong cytotoxic effect of TBBPA in the HTS assays may confound the results of other assay endpoints, they did not apply any formal criteria for contextualizing cytotoxicity for the various active assay endpoints referenced. The lower bound for cytotoxicity in the current version of the ToxCast/Tox21 database is 12.49 uM. For the majority (over 70%) of all active assays, not limited to KCC assays, activity occurred above the lower bound for cytotoxicity. Thus, an analysis that accounts for cytotoxic interference would not consider these assays to be active. We suggest addressing the discrepancy in accommodation for cytotoxicity in the HTS data if those data are to be used as supporting evidence.

CLH Dossier: The significant increased incidence of mutations in Tp53 gene (exons 5 to 8) in uterine adenocarcinomas from TBBPA dosed animals (10/16, 63%) compared to spontaneous uterine adenocarcinomas (1/9, 11%) may be a result of a direct genotoxic event from TBBPA or the result of a secondary nongenotoxic event.

BSEF Comment:

Several international regulatory authorities have concluded that TBBPA is not mutagenic or genotoxic (EU RAR, 2006; Health Canada, 2013; USEPA, 2015).

Page 32 in Section 10.9.3 of the dossier itself, titled "Conclusion on classification and labelling for carcinogenicity," recognizes "a nongenotoxic mode of action":

> "We propose TBBPA to be classified as a Category 1B carcinogen based on conclusive data (carcinogenic in animal studies and relevant mode of action for humans)… TBBPA administered orally by gavage for two years was clearly carcinogenic in female rats resulting in uterine tumours. TBBPA also resulted in liver tumours in male mice. **A NONGENOTOXIC MODE OF ACTION** is assumed (threshold carcinogen) relevant to humans" (emphasis added).

The statement in the dossier on page 30, indicating a "**DIRECT GENOTOXIC EVENT FROM TBBPA"** should be omitted, to be consistent with other sections (e.g., page 32, Section 10.9.3, which indicates that TBBPA is nongenotoxic) and other conclusions from authoritative bodies that TBBPA is not mutagenic or genotoxic. Furthermore, the expression of this mutation is generally secondary to the tumour formation and not at the origin of these tumours, and thus, is not related to the genotoxicity of a compound, but to the proliferation of the tumour once it is formed.

Section 3.6.2.5.3 of GHS emphasizes the importance of mutagenicity in the weight-of-evidence assessment. The absence of mutagenicity is an additional factor supporting a Category 2 classification.

CLH Dossier: TBBPA is an endocrine disruptor[.]

BSEF Comment:

TBBPA has not been identified as an endocrine disruptor; it is requested that the dossier be updated to reflect this.

CLH Dossier: ***Miscellaneous editorial comments throughout carcinogenicity and reproductive toxicity sections.***

BSEF Comments:

Some editorial changes are suggested:

- Page 18, "there was findings of uterine tumors" should be "there were findings of uterine tumors).
- Footnote 3 on page 20 misspells "tumour" as "tomour."
- On Page 23, a period is missing after the words (Poly-3 test).
- Page 23 notes that TBBPA resulted in a "dose-response increased incidence." This might be better phrased as a "dose-related increased incidence."
- Page 31 indicates, "No epidemiological data is available, so Category 1A is not warranted." The word "is" should be "are" (the word data is plural). Further, no human data of any type are available.
- Statistical significance is spelled out in most places, but abbreviated with a non-standard abbreviation in others (e.g., page 23, as "stat.sign.").
- On page 31, the word "is" should be "are" in this sentence: "…i) confounding effect of excessive toxicity at test doses: No signs of toxicity in female rats. Due to early mortality, tumour incidence data in the 1000 mg/kg bw male mice group <u>is</u> not presented."
- Page 38, first full paragraph, last sentence: "ant" is likely intended to be "and."
- Page 40: "No human data is available" should be, "No human data are available."

SECTION 10.10 REPRODUCTIVE TOXICITY

CLH Dossier: 10.10 Altered T4 levels are reported in Table 15 (pages 33, 35) and in Section 10.10.2 (page 36).

BSEF Comment:

The extensive discussion on T4 levels does not seem to be necessary, given the conclusion that no classification was proposed for reproductive toxicity and considering previous rigorous evaluations of this endpoint conducted by multiple authoritative bodies. Both Health Canada and the European Union concluded that reductions in T4 were not considered adverse in the absence of any other relevant thyroid-related effects (EU, 2006; Health Canada, 2013).

Further, as reviewed by Wikoff et al. (2015), Kim and Oh (2014) reported that TBBPA serum concentrations correlated weakly with thyroid hormones in humans, based on the observation of a positive relationship for free T4, although a negative relationship was observed for T3. When considered collectively, these data generally indicate that other effects commonly associated with thyroid hormone disruption (e.g., changes in T3, TSH, and thyroid weight and histopathology) do not consistently accompany the decreased levels of T4 (Schroeder, 2002a,b, 2003; van der Ven et al., 2008; NTP, 2013). Further, decreases in serum T4 levels have not been associated with adverse effects in reproductive and developmental toxicity studies that included neurobehavioral and neuropathology assessments (Schroeder, 2002b, 2003; Williams and Desesso, 2010). Taken together, these data indicate that decreased serum concentrations of T4 appear to have little adverse impact on parameters associated with a disruption in thyroid homeostasis in rat. This conclusion is similar to that reached by the EU (2006) and Health Canada (2013), as well as by Colnot et al. (2014).

Further, EFSA (2013) concluded that, due to “the limitations and uncertainties in the database,” it was inappropriate to use a $BMDL_{10}$ for decreased T4, to establish a health-based guidance value, concluding further that, based on large margins of safety, current dietary exposure to TBBPA does not raise a health concern, including for infants and young children.

CLH Dossier: Page 39, Anogenital distance (AGD) in females

BSEF Comment:

AGD is reported as being decreased in female pups on PND 7, which is a non-standard measurement day. OECD Test Guideline (TG) 415 (as well as other relevant TGs, such as OECD

416, 423, 453, etc.) recommends that AGD be measured on each pup on at least one time point between PND 0 and PND 4. Page 39 indicates that PND 4 AGD was not affected. Thus, the text should be revised to omit this sentence or acknowledge that there is no effect on AGD when measured according to OECD TG 415. If this sentence is to be retained in the final RAC opinion, we would suggest directly addressing the inconsistency of findings, and the impact of those inconsistencies, given that the dossier, as drafted, focuses on a timepoint that does not align with that recommended by OECD testing.

CLH Dossier: Page 39, Delayed time to vaginal opening

BSEF Comment:

Delayed vaginal opening is noted as occurring at a BMDL which was around the highest concentration. Because a BMDL is considered equivalent to a NOAEL, a BMDL around the highest concentration suggests that there is no effect on vaginal opening, consistent with the lack of effect on AGD or other reproductive parameters [BMDL — benchmark dose level; NOAEL — no-observed-adverse-effect level. The dossier would be improved by clarifying the lack of effect for this endpoint.

CLH Dossier: "[C]orrelations" are noted in several places, such as Table 15 and in the text.

BSEF Comment:

Information provided in the dossier is not sufficient to characterize "correlations." The word "correlation," in all instances, cannot be interpreted without additional statistical context, such as R^2 values. The dossier should be updated to clarify whether correlations are based on statistical significance reported by the authors, calculated by the DS, or otherwise.

CLH Dossier: All BMDL values

BSEF Comment:

All BMDL values should be reported with their corresponding benchmark response levels (e.g., BMR of 5%, 10%, 1SD, etc.) for context.

CLH Dossier: Table 15 lists multiple responses that were not statistically significant.

BSEF Comment:

All responses noted as lacking statistical significance should be omitted from Table 15, such as parietal thickness at 10 and 100 mg/kg/day, because this table is intended to summarize potentially adverse effects.

CLH Dossier: Organ weights (e.g., liver and testis)

BSEF Comment:

Table 15 and associated subsequent text (e.g., pages 39 and 40) report magnitudes for liver or testis weight increases without specifying whether absolute and/or relative weights were affected, which would provide context.

Table 17 (e.g., Cope et al., 2015) and associated subsequent text (e.g., page 52) reports magnitudes for liver-weight increases without providing context by specifying whether absolute and/or relative weights were affected.

CLH Dossier: Page 37-38 contexte on UDP-GT

BSEF Comment:

The dossier notes that "there are no data to support" the proposed MoA for decreases in T4 occurring secondary to the induction of UDP-GT. However, TBBPA data supporting this (and other) proposed MoAs have been reviewed by Lai et al. (2015), with the induction of UDP-GT considered the most plausible and supported MoA, based on decreases in T4 without concurrent compensatory increases in serum TSH or associated decreases in serum T3. Further, CD rats are more resistant to thyroid follicular changes, including carcinogenesis, because these changes are driven largely by excessive and sustained TSH stimulation in this strain (IARC, 2000, as cited by Lai et al., 2015), consistent with the lack of response on TSH in the

two-generation study with TBBPA in CD rats. Further, TBBPA did not produce thyroid follicular hyperplasia or tumors in the NTP (2014) study in Wistar Han rats or B6C3F1/N mice.

CLH Dossier: Table 15 "total spleen cell counts"

BSEF Comment:

"Total spleen cell counts" are noted as being increased, but it is unclear what cell type(s) were affected and whether the increases were statistically or toxicologically significant. The text regarding this endpoint should be clarified for these aspects.

CLH Dossier: Table 15 and page 38 parietal thickening

BSEF Comment:

Effects on parietal thickness are noted in high-dose groups on PND 11 but not PND 60. Neither of these measurement days complies with OECD 416, which indicates that measurements should be collected on PND 90 for neuro-morphometric analyses. This deviation suggests that parietal thickness is unlikely to have been affected if measured on PND 90. We recommend clarifying these discrepancies in the dossier and explaining the relevance of the early measurements to those directed by OECD testing guidance.

CLH Dossier: Table 16

BSEF Comment:

The study citation and route (i.e., gavage) should be included for reference.

CLH Dossier: Table 17 on page 46: "disturbance of liver function"

BSEF Comment:

The text in Table 17 notes that the only liver enzymes considered biologically significant were the increases in PROD (up to 23x), as indicative of "disturbance of liver function.". Increases in PROD are indicative of xenobiotic metabolism and detoxification (i.e., Cyp2b via CAR activation), rather than of a disturbance or adversity. The dossier would benefit from clarifying this distinction.

CLH Dossier: Table 15 on page 38-39

BSEF Comment:

Table 15 discusses brainstem auditory evoked potentials (BAEPs); however, the information provided could better reflect the body of evidence for these endpoints. On page 42 of the dossier, published letters are cited (Strain et al., 2009; Banasik et al., 2009) criticizing the BAEP that was performed. Additionally, EFSA (2013) noted that the ratios between BMDLs and their corresponding BMD values for BAEP responses were rather large, indicating a high uncertainty in these outcomes. The accuracy of the dossier would be improved by acknowledging this uncertainty, if the BMD values for BAEP are retained.

SECTION 5 – IDENTIFIED USES

CLH Dossier: Section 5, p. 8, section 5 "It is used in 90% of epoxy coated circuit boards (Cannon et al, 2019)

BSEF Comment:

Although the statement regarding the use in epoxy-coated circuit boards appears to be taken directly from the citation, it is not accurate (Cannon RE, Trexler AW, Knudsen GA, Evans RA, Birnbaum LS. 2019. Tetrabromobisphenol A (TBBPA) alters ABC transport at the blood-brain barrier. Toxicol. Sci 169(2):475–484).

To be accurate, the statement should read, "Tetrabromobisphenol A (TBBPA, CAS No. 79-94-7) is a brominated flame retardant used in 90% of **epoxy-based** circuit boards."

Further, Section 5 does not accurately describe that TBBPA is reacted with the epoxy and, therefore, is not present as TBBPA in its main use. That is, TBBA does not exist as a free

chemical in the final printed circuit boards. The DS may find the following information helpful in addressing the accuracy of Section 5: https://www.bsef.com/wp-content/uploads/2020/07/BSEF-TBBPA-Infographic-Digital.pdf

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Further information can be obtained from:
Dr Kevin Bradley
BSEF Secretary General

M: +34 67 23 98 118
E-mail: kbradley@bsef.org
www.bsef.com

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