Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the Opinion proposing harmonised classification and labelling at EU level of

2,2',6,6'-tetrabromo-4,4'-isopropylidenediphenol; tetrabromobisphenol-A

EC Number: 201-236-9
CAS Number: 79-94-7

CLH-O-0000007043-83-01/F

Adopted

16 September 2021

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 2,2',6,6'-TETRABROMO-4,4'-ISOPROPYLIDENEDIPHENOL; TETRABROMOBISPHENOL-A

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties. Journal articles are not confidential; however they are not published on the website due to Intellectual Property Rights.

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Substance name: 2,2',6,6'-tetrabromo-4,4'-isopropylidenediphenol; tetrabromobisphenol-A
EC number: 201-236-9
CAS number: 79-94-7
Dossier submitter: Norway

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
29.01.2021	Belgium		MemberState	1
Comment received				
BECA would like to thank the Norwegian Environment Agency and the Danish Environmental Protection Agency for their work on this proposal for classification, and in particular, for their very clear assessment of TBBPA mode of action.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
Thank you for your comment.				

Date	Country	Organisation	Type of Organisation	Comment number
28.01.2021	Belgium	BSEF, the International Bromine Council	Industry or trade association	2
Comment received				
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).

ECHA note – An attachment was submitted with the comment above. Refer to public attachment BSEF-Comments on TBBPA CLH proposal-28Jan2021.pdf

Dossier Submitter's Response

DS` Response to BSEF attachment page 1:

Thank you for your comments. Our detailed response is provided below and also in a separate attachment.For references that are not included in the end of this document, please see the submitted CLH proposal.

In our view, the available data justify classification in category Carc. 1B. This is mainly based on the clear carcinogenic effect in the uterus of female rats with additional findings in the liver of male mice. We agree that the available mutagenicity and genotoxicity tests are negative and that there probably is a threshold involved in the mode of action (MoA) for carcinogenicity. There is strong evidence that TBBPA modulates receptor-mediated effects, induces oxidative stress and is immunosuppressive, while there is moderate evidence that TBBPA induces chronic inflammation Species differences between rodents and humans in the metabolism of TBBPA is not convincing to describe the MoA as not relevant to humans.

DS` Response to BSEF attachment page 26, paragraph 3, page 26, para 4 and page 27, para 1:

In the attached document from BSEF they have commented on section 5 – Identified uses:

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: https://www.bsef.com/wp-content/uploads/2020/07/BSEF-TBBPA-Infographic-Digital.pdf" Page 26, para 3: Thank you for the clarification in section 5 that TBBPA is used in epoxy-based circuit boards and not epoxy-coated circuit boards. Page 26, para 4 and page 27, para 1: Thank you for the clarification in section 5 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." DS notes that according to Birnbaum and Staskal, 2004, despite TBBPA's reactive properties, both additive- and reactive-treated products have been shown to release TBBPA and metabolites into the environment.
RAC's response
Thank you for your comments. RAC has evaluated the intrinsic hazard and considered the data in a weight-of-evidence assessment.

Date	Country	Organisation	Type of Organisation	Comment number
18.01.2021	Germany		MemberState	3
Comment received				
The DE CA agrees that TBBPA should be classified as Carc. 1B but not for the endpoints mutagenicity, reproductive toxicity and STOT RE.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
Thank you for your comment.				

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2021	Sweden	ChemSec	International NGO	4
Comment received				
We strongly support the proposed classification which should be implemented without delay. However in our opinion one part is missing in this suggested classification, the inclusion of persistence and endocrine disrupting properties. Such properties should not be set aside but complement this CLH proposal.				

Dossier Submitter's Response
Thank you for the support. We have included hazard classes referred to in CLP, art 36 (1) that shall normally be subject to harmonised classification and labelling. Other hazard classes may also be added on a case-by-case basis, if justification is provided demonstarting the need for such action at Community level. We have also included STOT-RE, which is closely related to the reproductive toxicity.
RAC's response
Thank you for your comments.

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2021	Netherlands		MemberState	5
Comment received				
NL-CA agrees with the dossier submitter's proposal for classification for carcinogenicity Category 1B. There was profound evidence of carcinogenic potential of TBBPA as demonstrated in a 2-year study by NTP (2014) in two species (rats and mice). Different types of tumors were found, including both benign tumors (e.g. interstitial cell adenoma of testes in male rats) and malignant tumors (e.g. uterine adenocarcinoma and malignant mixed Müllerian tumors in female rats, hepatocellular carcinoma or hepatoblastoma in male mice). Metastases were found throughout the body, demonstrating malignancy of tumors found in the uterus. In addition, carcinogenic mode-of-action (i.e. modulation of receptor-mediated effects, oxidative stress induction, immunosuppression) of TBBPA, relevant to human, have been described in in vitro and in vivo studies. Sufficient evidence is demonstrated supporting Category 1B, such as demonstration of malignant tumor formation relevant to human in two species, reduced latency, and relevant mode-of-action for humans.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
Thank you for your comments.				

Date	Country	Organisation	Type of Organisation	Comment number
29.01.2021	Sweden		MemberState	6
Comment received				
The Swedish CA agrees with the proposed classification of TBBPA as Carc. 1B, H350. The NTP study performed in rats shows clear evidence of carcinogenicity in females expressed as uterine tumours (adenomas, adenocarcinomas, malignant mixed Mullerian tumours), which are relevant for humans. The predominant tumour type in rats was uterine adenocarcinoma, which is also the main uterine tumour type seen in humans. The described carcinogenic mode of action (interference with the estrogen homeostasis by competing with estrogen for estrogen sulfotransferases) seems plausible and is of relevance for humans.				

Moreover, malignancy was observed in male mice as hepatocellular carcinomas, hepatoblastomas and hemangiosarcoma, the two latter being rare malignant tumour types.
Dossier Submitter's Response
Thank you for the support.
RAC's response
Thank you for your comments.

Date	Country	Organisation	Type of Organisation	Comment number
29.01.2021	Belgium		MemberState	7
Comment received				
BECA considers the available data as conclusive. Two 2-year studies on rats and mice are available (both NTP, 2014). Effects reported in rats were uterine tumours in females (statistically significant increase in adenoma, adenocarcinoma and/or malignant Müllerian tumours, starting at 500 mg/kg bw/d). these results were not confirmed in mice. However, liver tumours such as hepatoblastoma and/or hepatocellular carcinomas were reported in male mice, it was significantly increased at 250 mg/kg bw/d, but not at higher doses (500 mg/kg bw/d). Haemoangiosarcoma were also reported. We agree on the relevance of the tumours to humans and the relevance of TBBPA tumorigenic mode of action, as described by the dossier submitter. Strong supporting evidence can be found in IARC's assessment of TBBPA. Therefore, in conclusion, we support the proposal for classification as Carc. 1B for TBBPA.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
Thank you for your comments.				

Date	Country	Organisation	Type of Organisation	Comment number
28.01.2021	France		MemberState	8
Comment received				
There is clear evidence of an induction of uterine tumours by TBBPA in female rats based on an increased incidence of uterine adenocarcinoma (dose related and statistically significant from the mid-dose) as well as atypical rare malignant Müllerian tumours (incidence above HCD at low and high doses).These uterine tumours have a high metastatic rate and appear with a reduced latency. The carcinogenic potential of TBBPA is further supported by the induction a benign testicular tumours (above HCD at the high dose) in male rats as well as hepatoblastomas (no dose-response but > HCD from low dose) and haemangiosarcomas (dose-related, statistically significant at 500 mg/kg) in male mice. Because malignant tumours are observed in two species (uterine tumours in female rats and hepatoblastomas and haemangiosarcoma in male rats), the data fulfil the definition				

of a sufficient evidence of carcinogenicity in experimental animals.
The proposed classification Carc 1B is therefore supported.

The knowledge of a mode of action consistent with the effect would support the effects observed. However, the absence of a clear understanding does not alleviate the level of evidence seen from experimental data. ANSES agrees that some hypothesis for the mode of action have been proposed and can be involved in the effects observed but the mode of action remains largely uninvestigated. In particular, to our understanding there is no measurement of estrogen levels in any study. Could you clarify whether the hypothesis of competition with conjugation enzymes is expected to be specific of TBBPA metabolism towards estrogen metabolism (and why) or whether any substance that undergoes an active conjugation would be expected to similarly compete with estrogen metabolism (and whether it confirmed by other cases).

Finally, TBBPA is not genotoxic and shall not be considered as a non-threshold carcinogen but it should be emphasised that the dose-response and a potential threshold might be difficult to determine as non linear dose-responses are observed, e.g. for hepatoblastomas in male mice and malignant mixed Müllerian tumour in female rats.

Dossier Submitter's Response

Thank you for your support of the proposed classification for carcinogenicity. No measurement of estrogen levels was available in the 2y NTP study. We have not assessed whether or not this is a substance specific competition with conjugation enzymes or if it is relevant for other substances undegoing active conjugation. We agree that identification of the threshold may be difficult.

RAC's response

RAC took note of your comments. RAC has evaluated the intrinsic hazard and considered the data in a weight-of-evidence assessment.

Date	Country	Organisation	Type of Organisation	Comment number
28.01.2021	Belgium	BSEF, the International Bromine Council	Industry or trade association	9

Comment received

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 immunosuppres¬sion, 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 relevant mode of action for humans)."

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 x 10-7 to 8.4 x 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 Tox2117 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.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment BSEF-Comments on TBBPA CLH proposal-28Jan2021.pdf

Dossier Submitter's Response

DS` Response to BSEF attachment page 2, CLH dossier 10.9.3

Classification of a substance involves both evaluation of strength of evidence (enumeration of tumours and statistical significance) and consideration of all other relevant information. Concerning the strength of evidence, we consider the findings in female rats to constitute clear evidence of carcinogenicity of TBBPA (uterine tumours, with extensive metastases). The carcinogenic potential of TBBPA is further supported by statistically significant occurrence of some tumours in male mice (large intestine tumours

and hemangiosarcoma and Hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma), although not found in a dose-related manner.

DS` Response to BSEF attachment page 2, last para - page 4, 3rd para

a) Literature is inconsistent regarding the origin of hepatoblastomas.
Murine hepatoblastoma (HB) is a rare spontaneous tumor with controversial histogenesis. Older literature indicate that the origin is from hepatocellular adenoma and carcinoma precursor cells (Diwan, Hennemana, & Riceb, 1995) (Turusov, Diwan, Engelhardt, & Rice, 1997). The cell of origin for HB has not been established, although a pluripotential hepatic stem cell has been postulated (Cattley & Cullen, 2013). Others state that HB originate from liver blastema cells, neoplastic hepatocytes, oval cells, and biliary epithelial cells (Thoolen et al., 2010). We recognize that hepatocellular adenoma, hepatocellular carcinoma and hepatoblastoma are considered to represent a biological and morphological continuum. We consider the findings of hepatoblastoma to represent some evidence of carcinogenicity, but not clear since combined incidences of hepatocellular carcinomas and hepatoblastomas were significant only in the 250 mg/kg group and the trend test was not significant. Hepatoblastoma is a very rare and malignant tumour type. All in all, there was a causal relationship between the substance and an increased incidence of tumours in female rats and male mice.

b) We agree that only the NTP study (NTP, 2014) is available, but this study covers 2-years studies in two species - rats and mice. We consider the NTP studies to be robust and reliable with reliability score 1. We think this is sufficient and robust information to base the classification proposal for carcinogenicity on.

c) Findings in female reproductive organs likely triggered by increased levels of circulating estrogens can hardly be found in both sexes. We agree that no significant findings were made in female mice. In male rats there was only marginal increase of testicular adenoma that may have been related to TBBPA but attributed low weight of evidence in the overall assessment.

d) In addition to the adenocarcinomas of the uterus, uterine tumour metastases were found as carcinomas throughout the body in female rats, demonstrating malignancy. In addition, there were two findings of Malignant mixed Müllerian tumours in the top dose, but this was not statistically significant when assessed in isolation. The results are presented by IARC in an overview presented here for clarity (IARC, 2018):

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Purity Dose regimen No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, Wistar Han [Crl:WI(Han)] (F) 6–7 wks 105 wks NTP (2014)	Purity, > 99% 0, 250, 500, 1000 mg/kg bw 5 days/wk for 104 wks 50, 50, 50, 50 35, 34, 29, 33	*Uterus* (original transverse review)		GLP study Historical incidence of uterine adenocarcinoma (all routes): 7/150 (4.7%) in studies involving an original transverse examination; historical incidence of malignant mixed Müllerian tumour (all routes): 0/150 in studies involving an original transverse examination
		Adenoma: 0/50, 0/50, 3/50, 4/50	Trend: $P = 0.010$	
		Adenocarcinoma: 3/50, 3/50, 8/50, 9/50	Trend: $P = 0.016$	
		Malignant mixed Müllerian tumour: 0/50, 4/50, 0/50, 2/50	NS (rare tumour)	
		Adenoma, adenocarcinoma or malignant mixed Müllerian tumour (combined): 3/50, 7/50, 11/50*, 13/50**	Trend: $P = 0.003$; *$P = 0.013$; **$P = 0.005$	
		Uterus (residual longitudinal review)		
		Adenoma: 3/50, 2/50, 1/50, 3/50	NS	
		Adenocarcinoma: 4/50, 9/50, 15/50*, 15/50**	Trend: $P = 0.003$; *$P = 0.002$; **$P = 0.005$	
		Malignant mixed Müllerian tumour: 0/50, 0/50, 0/50, 1/50	NS (rare tumour)	
		Adenoma, adenocarcinoma or malignant mixed Müllerian tumour (combined): 6/50, 10/50, 16/50*, 16/50**	Trend: $P = 0.008$; *$P = 0.007$; **$P = 0.015$	
		Uterus (original transverse and residual longitudinal review combined)		
		Adenoma: 3/50, 2/50, 4/50, 6/50	NS	
		Adenocarcinoma: 4/50, 10/50, 15/50*, 16/50**	Trend: $P = 0.002$; *$P = 0.002$; **$P = 0.002$	
		Malignant mixed Müllerian tumour: 0/50, 4/50, 0/50, 2/50	NS	
		Adenoma, adenocarcinoma or malignant mixed Müllerian tumour: 6/50, 11/50, 16/50*, 19/50**	Trend: $P < 0.001$; *$P = 0.007$; **$P = 0.002$	

bw, body weight; F, female; GLP, good laboratory practice; M, male; NS, not significant; wk, week

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We consider TBBPA to have non-genotoxic properties, as all tests for genotoxicity are negative.

DS` Response to BSEF attachment page 4, 4th para - page 5, 2nd para

a) Please see our response in litra b above.

b) The NTP selected the Wistar Han rat as animal model to examine carcinogenesis since the frequently used F344 rat strain may have shown genetic drifts over time. According to Lai, Kacew and Dekant (in Lai et al., 2015[1]) " It is **conceivable** that Wistar Han strain rats **resemble** the SD strain, which are known to contain elevated levels of estrogens as well as a higher estrogen/progesterone ratio (Kacew et al., 1995) and this may account for the uterine carcinomas (the words conceivable and resemble are put in bold text by the DS). Thus, the wording is quite moderate in Lai et al. (SD = Sprague-Dawley in the quote).

Wistar is the original lab rat, outbred, with no genetic surveillance program until the last decades. SD rats originated from Wistars in 1925 and has been outbred since. Envigo, previously Harlan Sprague Dawley, writes on their web page that rats from different vendor colonies display divergent responses to experimental situations, stressing the need to identify colony-specific differences in endocrine and immune responses in Sprague Dawley® rats. (https://blog.envigo.com/sprague-dawley-the-workhorse-of-reproductive-endocrinology-research-part-1) The same could be said about Wistar rats.

[1] "Dr. Dekant and Dr Kacew both report personal fees from the American Chemical Council, during the conduct of the study; personal fees from Bromine Science and Environmental Forum (BSEF), outside the submitted work."

Wistar Han (WH) has been, and still is, bred in Hannover, Germany, and there is currently a genetic quality control program to lessen the impact of genetic drift which also occur in outbred colonies. The most prominent common feature in both stocks is albinism and, thus, easy to handle for humans. Albinism is not linked to endocrine susceptibility.

The statement in Lai et al. is misleading for two reasons - Reason 1: SDs were Wistars almost a hundred years ago, and both stocks differ considerably between vendors/labs. Reason 2: SDs were selected for endocrine experiments, while Wistar is a much more general stock. There is no reason to state that WHs are likely to be similar to SDs with respect to endocrine susceptibility.

Response to comment on histopathology technique: We refer to NTP summary minutes from the Technical Reports Peer Review Panel Meeting 22 May 2014, which states that residual longitudinal sectioning has now been incorporated as a standard protocol at NTP: "V. Residual Longitudinal Review for Identifying Uterine Proliferative Lesions. NTP Pathologist Dr. Susan Elmore briefed the panel on the uterine longitudinal sectioning protocol, which was used for Green Tea Extract, Indole-3-Carbinol, and CIMSTAR 3800. She discussed and illustrated the original transverse tissue review and the residual longitudinal tissue review. The residual longitudinal reviews revealed additional uterine tumors and nonneoplastic lesions in all groups and identified preneoplastic lesions in some groups. It also has helped to determine the primary site of invasive tumors and to avoid misinterpretation of gross lesion incidences. Dr. Elmore noted that residual longitudinal sectioning has now been incorporated as standard protocol for NTP subchronic and chronic studies."

For comments on historical control data, please see our response below.

c) No response necessary.

d) We agree that the studies demonstrate only promoting activity. Still we believe that when all data is taken into consideration, classification in Carc. cat. 1B is warranted. Please see litra d) above for findings of metastases following TBBPA dosing in female rats.

DS` Response to BSEF attachment page 5, 3rd para - page 7, 3rd para

CLH dossier - Table 14 - Possible mode of action (MoA)

We agree that only the "Disruption of estrogen homeostasis"-mode of action is supported by references as an MoA.

We agree that MoA is not the same as Key Characteristics of carcinogens, but we thought it was interesting and relevant to include the information from IARC (IARC, 2018). We see now that we could have turned the chapter around in a way that it was better to mention the proposed MoA first and then have reported the IARC analysis of the Key characteristics as additional information.

The evidence on the “key characteristics” of carcinogens (Smith et al., 2016) concerning whether TBBPA modulates receptor-mediated effects, induces oxidative stress, induces chronic inflammation, is immunosuppressive, alters cell proliferation, cell death, or nutrient supply, or is genotoxic, was summarized by IARC in Monograph 115 (IARC, 2018). It is reproduced here for clarity for RAC:

From IARC, 2018, p 280:
" 6.4 Rationale: In making its overall evaluation, a majority of the Working Group considered that the strong mechanistic evidence that tetrabromobisphenol A can operate through three key characteristics of carcinogens and that these can be operative in humans warranted an upgrade to Group 2A. Specifically, the evidence was strong for the modulation of receptor-mediated effects, for the induction of oxidative stress, and for the induction of immunosuppression: • Tetrabromobisphenol A interacts directly with several human nuclear receptors relevant to human cancers, including thyroid hormone and peroxisome proliferator-activated receptor-γ. Tetrabromobisphenol A modulates enzymes relevant for the endocrine system, inhibits aromatase, and is a potent inhibitor of sulfotransferase. • In multiple species in vivo and in human cells in vitro, tetrabromobisphenol A causes oxidative stress. • Immunosuppressive effects were observed in mice exposed in vivo. Multiple experiments in human natural killer cells exposed in vitro also showed effects consistent with immunosuppression. However, a minority of the Working Group judged that these data did not support a mechanistic upgrade."

As there is a strong link between CLP and the IARC classification criteria, as described in the CLP guidance (ECHA, 2017a), we think it is relevant to include this information here.

We agree that the human relevance framework presented by IPCS is relevant and useful, especially for risk assessment of carcinogens, as described by Boobis et al., 2008. According to the CLP guidance, such frameworks provide a basis for systematic assessments which may be performed in a consistent fashion internationally; however not intended to provide lists of criteria to be checked off.

DS` Response to BSEF attachment page 7, 4th para - page 9, 3rd para

CLH Dossier section 10.9.3

The database of longterm carcinogenicity studies of TBBPA in experimental animals is not expected to increase, so the one clearly positive study in female rats will be the main basis for the classification with additional findings in male mice taken also into consideration.

Conjugation reactions leading to e.g. glucuronides and sulphates differ according to species and sometimes result in species-specific toxicity. Both glucuronide and sulfate metabolites of TBBPA are formed both in humans and rats. However, TBBPA-sulfate was the major metabolite in rat plasma and urine, whereas TBBPA-glucuronide was present in much lower concentrations both in plasma and urine. In humans, TBBPA-glucuronide was detected in all blood samples in low levels, whereas TBBPA-sulfate was only detected in a few individuals at some timepoints (see Table 4 and the figures) (Schauer et al., 2006).

As mentioned in Boobis et al. (2006), the IPCS Workshop recognized that only infrequently is it likely that it will be possible to dismiss human relevance on the basis of quantitative differences in biological processes. The DS considers that information is not available to dismiss the effects of TBBPA in rats as non-relevant to humans as we do not have evidence for a substantial species-specific metabolism of this substance.

Also in the ECHA endpoint specific guidance R.7.12 (with reference to Ecetoc, 2006), it is discussed that only effects seen in the test animal that would under no circumstances be manifested in humans can be assessed without the regular conservatism (ECHA, 2017b). Even though this part of the guidance discusses risk assessment, we consider this as relevant here.

DS` Response to BSEF attachment page 9, 4th para - page 9, 6th para

CLH Dossier Table 13 - hazard assessment

You are correct that there is some information missing in the table. This information is available in other parts of the CLH report see e.g. 10.9.2 litra g) and h), but it could have been included in the table as well.

According to the text below the table in the CLH report template: "Some additional important factors to be taken into consideration may include whether responses are observed in single or several species; whether the substance of concern has similar structural similarity to a substance(s) for which there is good evidence of carcinogenicity; whether absorption, distribution, metabolism and excretion of the substance are similar between animals and humans; whether there is evidence of mutagenic activity in vivo."

DS` Response to BSEF attachment page 9, last para - page 10, 3rd para

CLH Dossier - p. 32 Structural similarity

TBBPA is a bisphenol and thus indeed it has structural similarity to other bisphenols. In addition, it is a brominated compound. TBBPA does not bind significantly to the estrogen receptor (ER) and we do not expect this to be the MoA for the carcinogenicity (see other parts of our response for the MoA discussion). The structural similarity to other bisphenols, e.g. bisphenol A, is probably not so relevant for the carcinogenicity. TBBPA probably affects estrogen homeostasis by competitive inhibition of estrogen conjugation, thereby disturbing the estrogen homeostasis.

DS` Response to BSEF attachment page 10, 4th para - page 10, 6th para

The statement that elimination half-lives do not differ considerably between experimental animals and humans is one of the conclusions in EFSA (2011).

Estimated half-lives are ~2 days and ~0.5 day in humans and rats (Sprague-Dawley), respectively. The observed half-life of approximately 2 days of TBBPA in humans was reported in several studies. In Schauer et al., 2006, the dose was given as a single gel capsule orally, 0.1 mg/kg bw. In Hagmar et al., 2000 and Sjodin et al., 2003, the half-life of TBBPA has been estimated in Swedish workers engaged in recycling processes.

The observed half-life of approximately 0.5 day of TBBPA in rats was reported in Schauer et al., 2006. After administration of a single oral dose of 300 mg/kg to rats (Sprague-Dawley), peak concentration of 103 lmol/l of TBBPA was achieved in plasma within 3 h after oral administration of TBBPA. TBBPA concentrations then declined following first-order kinetics with an elimination half-life of 13 h.

Please see our earlier response to the species differences in the metabolism of TBBPA (DS` Response to BSEF attachment page 7, 4th para - page 9, 3rd para).

DS` Response to BSEF attachment page 10, last para - page 13, second to last para

CLH Dossier 10.9.2

Thank you for your detailed analysis. Our analysis is given in the CLH report. When all information is taken into consideration, we still believe that classification in Carc.cat. 1B is justified mainly based on the clear findings in female rats, and some statistically significant findings in male mice, and a mode of action that is relevant to humans even if it contains a non-threshold MoA and we do not have evidence for a substantial species-specific metabolism of this TBBPA.

DS` Response to BSEF attachment page 13, last para - page 15, second para

CLH Dossier - P.31

We never intended to state that there is information available saying anything about the correlation between TBBPA and uterine tumours in women. We agree that such information is not available. Our point was merely to state that the type of uterine tumours seen in the rats (predominantly uterine adenocarcinoma) is similar to the type of uterine tumours frequently occurring in women, stemming from endometrial tissue. We note that Dunnick et al., 2017, states that "The TBBPA carcinogenic effect in the uterus is of concern because endometrial tumors are a common malignancy in women with an estimated 50,000 new cases per year in the U.S. (Siegel et al., 2013), and one million new cases per year worldwide (Webb, 2015)."

Harvey et al., 2015, is mentioned earlier in the dossier (p. 30) as you describe. We are sorry that this information was not also included in the discussion that follows in the dossier, together with the Wickoff et al., 2016, which is also mentioned earlier in the dossier.

DS` Response to BSEF attachment page 15, 3rd para - page 16, 3rd para

CLH Dossier 10.9 Historical Control Database

As previously described in our response, the NTP has changed to the Wistar Han rat as animal model to examine carcinogenesis. Unfortunately, this limits the historical database considerably.

As described in Greim et al., 2003, historical control data can only be used if several requirements can be fulfilled. This includes that 1) the historical control data and experimental data were obtained with the same species and strain of experimental animal, which were obtained from the one supplier who produced them in a continuous breeding programme, 2) The historical control data were produced in the same laboratory as the experimental data, 3) The study design, experimental methods and assessment criteria were the same (e.g. age at beginning, housing). So even if the number of historical control data is small (n=150) in the NTP report, they are more relevant than studies of Wistar Han rat uterine tumour background rates you list in your comments.

These background rates could have been mentioned in the dossier for completeness, though.

Concerning your comment on elevated estrogen levels in Wistar rats, please see our response to this above.

As you mention Harleman et al. (2012) observed an inverse relationship between uterine tumours and mammary tumours in Wistar rats in The RITA database (company sponsored database of peer-reviewed historical tumor data in control animals). According to Harleman et al., this association is likely to be related to prolactin. We agree that no mammary tumours were observed in rats in the NTP report of TBBPA (NTP, 2014). The level of prolactin is not analyzed/reported by NTP in NTP (2014). Harleman et al. states that 10-20% bodyweight gain reduction may induce a pattern of hormonal changes. In the NTP study, in female rats the body weights were similar to the controls throughout the study.

DS` Response to BSEF attachment page 16, 4th para - page 17, 3rd para

CLH Dossier h) ADME

Please see our earlier response to the species differences in the metabolism of TBBPA (DS` Response to BSEF attachment page 7, 4th para - page 9, 3rd para).

DS` Response to BSEF attachment page 17, 4th para - page 19, 1st para

A practical threshold *may* lead to a downgrading, it *must* not. Taking all available data into account and weighing the clear evidence of uterine cancer in female rats combined with some evidence in liver tumours in male mice as the most important evidence, we proposed to classify TBBPA in Carc.cat.1B.

DS` Response to BSEF attachment page 19, 2nd para - page 19, 4th para

We agree that the sentence about the IARC working group was inaccurate. They did not test TBBPA in the various high-throughput screening data tools, but only considered data generated by the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast™) research programmes. We agree that the lower-bound cytotoxicity limit given for the ToxCast/Tox21 data should be considered when evaluation the bioactivity profile of TBBPA. However, it should be regarded as a flag for greater scrutiny rather than an absolute limit for active substances.

As also mentioned by BSEF, ToxCast/Tox21 bioactivity data show that TBBPA is active in approximately 25% of the bioactivity assays at concentrations below the general lower-bound cytotoxicity limit.

DS` Response to BSEF attachment page 19, 5th para - page 20, 4th para

We agree that TBBPA is not mutagenic or genotoxic in the tests. The mechanism behind the increased incidence of mutations in the Tp53 gene in the tumours of the dosed animals vs. the incidence of mutations of Tp53 in spontaneous tumours in control animals is not evident to us. We have not found any references explaining your statement "expression of this mutation is generally secondary to the tumour formation...". NTP

(2014) states that: "In this study, the high rate of Tp53 mutations in uterine adenocarcinomas from tetrabromobisphenol A-dosed Wistar Han rats compared to spontaneous uterine adenocarcinomas suggests that the increased incidence of uterine adenocarcinomas in tetrabromobisphenol A-dosed animals may be driven at least in part through a Tp53-mediated mechanism."

DS` Response to BSEF attachment page 20, 5th para to end

Our intention in section 10.9.1.1 was to describe that TBBPA affects hormone homeostasis. We agree that TBBPA has not been identified as an endocrine disruptor and listed in the Candidate list according to REACH art. 57 f). However, it is under substance evaluation by DK based i.a. on a concern for endocrine disruption.

DS` Response to BSEF attachment page 21
Editorials
Thank you for your comments. As the CLH report is not to be amended by the DS, the editorials will be useful for later proposals. The word "data" is especially interesting and challenging as it is listed as both singular and plural in dictionaries. According to the dictionary we often use, "both constructions are standard. The plural construction is more common in print, evidently because the house style of several publishers mandates it". We suggest following ECHAs/RACs tradition on the singular or plural form of the word data and will try to do so in our next CLH proposal.

[1] "Dr. Dekant and Dr Kacew both report personal fees from the American Chemical Council, during the conduct of the study; personal fees from Bromine Science and Environmental Forum (BSEF), outside the submitted work."

References (if not already cited in CLH report and annex):

Boobis et al. 2008: IPCS Framework for Analyzing the Relevance of a Cancer Mode of Action for Humans, In Critical reviews in Toxicology, 2006, Vol 36, Issue 10, Pages 781-792 | Published online: 10 Oct 2008 link
Cattley & Cullen, 2013: Liver and Gall Bladder. Haschek and Rousseaux's Handbook of Toxicologic Pathology, Third Edition. pp 1509-1566.
Diwan, Henneman, & Rice, 1995: Further evidence for promoter-dependent development of hepatoblastoma in the mouse, In Cancer Lett 1995 Feb 10;89(1):29-35
ECETOC (2006) Toxicological Modes of Action: Relevance for Human Risk Assessment. 99. Brussels, ECETOC. Technical Report.
ECHA, 2017a: Guidance on the application of the CLP criteria
ECHA, 2017b: Guidance R.7.c Endpoint specific guidance R.7.12.2 TK in practice – derivation and generation of information
Greim et al. 2003: Evaluation of historical control data in carcinogenicity studies. In Human & Experimental Toxicology (2003) 22: 541/549
Harleman et al., 2012: A review of the incidence and coincidence of uterine and mammary tumors in Wistar and Sprague-Dawley rats based on the RITA database and the role of prolactin. in Toxicol. Pathol. 2012 Aug;40(6):926-30
Smith et al., 2016: Key Characteristics of Carcinogens as a Basis for Organizing Data on Mechanisms of Carcinogenesis. Environmental Health Perspectives • volume 124 | number 6 | June 2016
Thoolen et al., 2010: Proliferative and Nonproliferative Lesions of the Rat and Mouse Hepatobiliary System. Toxicologic Pathology, 38: 5S-81S, 2010.
Turusov, Diwan, Engelhardt, & Rice, 1997

RAC's response
RAC took note of your comments. RAC has evaluated the intrinsic hazard and considered the data in a weight-of-evidence assessment.

Date	Country	Organisation	Type of Organisation	Comment number
18.01.2021	Germany		MemberState	10
Comment received				
The evaluation of the carcinogenicity of TBBPA was based on two GLP-compliant NTP studies equivalent to OECD TG 451(/453) in rats and mice (NTP, 2014). In rats, TBBPA induced a dose dependent increase of the incidence of uterine tumors, which was significant in the mid and high dose groups (500 and 1000 mg/kg bw/day).The predomi-nant tumor type was uterine adenocarcinoma in female rats. In male rats there was a signif-icant trend in the incidence of testicular interstitial cell adenoma (exceeds historical control incidence in the highest dose group). In mice, TBBPA induced a dose dependent increase of the incidence of liver tumors in male mice that was significant in all dose groups (250, 500 mg/kg bw/day, due to early mortality, tumor incidence data in the 1000 mg/kg bw group was not presented). The incidence of hepatocellular adenomas was significantly increased the medium dose group (500 mg/kg bw). For the incidence of hepatoblastomas there was a treatment related increase that was significant in the low dose group (250 mg/kg bw). The incidence of hemangiosarcomas in all organs and large intestine tumors increased with increasing dose (significant in 500 mg/kg bw, significant trend). The incidence of hepatoblastoma in male mice exceeded the histori-cal control ranges (0-12%). TBBPA-induced uterine tumors were not seen in mice because estrogen homeostasis is probably less affected in mice than in rats due to differences in the capacity and/or capabil-ity of conjugating enzymes. Possible modes of action for uterine tumors are suspected to be disruption of estrogen homeostasis, oxidative stess, inflammation and immunosuppression, genetic and related effects (increased incidence of mutations in Tp53 gene, relevant for hu-mans). Tumors in uteri of female rats are relevant for humans since they are of the same type as uterine tumors seen in rats. Because of the clear dose dependent carcinogenicity of TBBPA and the human rele-vance (tumor type, mode of action), the DE CA agrees that classification of TBBPA as Category 1B carcinogen is appropriate.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
Thank you for your comments.				

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
29.01.2021	Belgium		MemberState	11
Comment received				
BECA considers the available data as conclusive. BECA supports the dossier submitter's assessment and conclusions. No classification is warranted for this endpoint.				

Dossier Submitter's Response
Thank you for the support.
RAC's response
Thank you for your comments.

Date	Country	Organisation	Type of Organisation	Comment number
28.01.2021	Belgium	BSEF, the International Bromine Council	Industry or trade association	12
Comment received				
Mutagenicity is included in the comments on carcinogenity. ECHA note – An attachment was submitted with the comment above. Refer to public attachment BSEF-Comments on TBBPA CLH proposal-28Jan2021.pdf				
Dossier Submitter's Response				
DS` Response to BSEF attachment page X, paragraph (para) Y - CLH dossier Z (hvis gitt)				
RAC's response				
Thank you for your comments.				

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2021	Netherlands		MemberState	13
Comment received				
NL-CA agrees with the dossier submitter's proposal for no classification for germ cell mutagenicity. In vitro tests, with or without metabolic activation (bacterial reverse mutation assays, mammalian chromosome aberration test or cell gene mutation test), and in vivo tests (mouse peripheral blood micronucleus assay) clearly indicate a non-mutagenic potential of TBBPA. Therefore, classification for germ cell mutagenicity is not warranted.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
Thank you for your comments.				

Date	Country	Organisation	Type of Organisation	Comment number
18.01.2021	Germany		MemberState	14
Comment received				
TBBPA was tested negative in 7 bacteria reverse mutation tests (OECD TG 471) using Salmonella typhimurium strains TA92, TA98, TA100, TA1535, or TA1537, TA1538 or E. coli strain WP2 uvrA/pKM101, with or without exogenous metabolic activation (NTP, 2014; Mortelmans et al., 1986; DOW Chemical Company, 1985; Velsicol Chemical Company,				

1977; Israel Institute for Biological Research, 1978; Litton Bionetics Inc., 1976; Ethyl Corporation, 1981). TBBPA was also not mutagenic in the mammalian erythrocyte micronucleus test (OECD TG 474) in mice and did not cause bone marrow toxicity (NTP, 2014). The in vitro mammalian chromosome aberration test (OECD TG 473) in human peripheral blood lym-phocytes (BioReliance, 2001) showed that TBBPA did not induce structural or numerical chromosomal aberrations. An in vitro mammalian cell gene mutation test from Helleday et al. (1999) using the hprt and xprt genes that was assumingly performed according to OECD TG 476 was negative as well. As none of the available studies indicate that TBBPA is mutagenic or genotoxic, the DE CA agrees that classification as mutagenic is not indicated.
Dossier Submitter's Response
Thank you for the support.
RAC's response
Thank you for your comments.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
28.01.2021	France		MemberState	15
Comment received				
In the two-generation study, changes in motor activity are observed in the F2 offsprings that were exposed only during development. The decrease is observed at several doses and points in time over different segments in both males and females. It is described that no effect on motor activity was found on PND13 in animals. A reduced activity was however observed for PND17 females exposed to 10 or 100 mg/kg/d. This was confirmed on PND21 for females exposed to 100 mg/kg/d. Males exposed to 100 or 1000 mg/kg showed also a reduced activity on PND60. The conclusion that the significant changes were not taken into account and considered as unrelated to the exposure is very surprising. The effects observed in males and females at different ages converge to a reduced activity of exposed animals. These should therefore not be considered as incidental findings. Besides, these decreases are statistically significant with $p < 0.05$, meaning that there is only a 5% probability that the effect observed is not related to treatment and therefore a strong evidence that it is not a chance finding. Similarly, in the one-generation study, effects on hearing capacities are reported in F1 (exposed during development + direct exposure) with several measured parameters that are affected. In these conditions, an effect of treatment was found in particular females. Interestingly, dosage of thyroid hormone levels showed a reduced level of thyroxin. The auditory system is also influenced by circulating levels of thyroid hormones during early stages of development. A significant decrease in the parietal cortex thickness at PND 11 is observed in both males and females (-17 and -24%, respectively). Although it is a transient effect that is not detected at PND60, this effect during the early phase of the offspring development cannot be discarded as irrelevant. Similar effects are not reported in the neurodevelopmental study (Hass, 2003). The lower maximum dose used in this study (250 mg/kg) may explain in part but not entirely the differences in the results because effects were observed from 10 or 100 mg/kg in the two-generation study. However, it is unclear if similar parameters have been investigated				

in all studies. Besides, in the neurodevelopmental study, exposure started at GD7 while multi-generation studies include a pre-and early-pregnancy exposure period, which may have consequences on the levels or periods of exposure.

In support to these effects, a decrease in T4 levels has been consistently observed in many studies (2-generation study, 1-generation study, NTP 90-day rat study, 90-day study by Osimitz 2016, 28-day study by Van der Ven 2008). TSH was not affected and T3 only occasionally increased.

Thyroid hormones play an important role in foetal and postnatal development and in particular in the development of the central nervous system. In humans, the major form of thyroid hormone in the blood is T4, which has a longer half-life than T3 (T4:T3 ratio of approx. 14:1, Mortoglou 2004). T3 is the main form activating thyroid hormone receptors and T4 is converted to the active T3 in the liver and in some tissues including brain by 5'-deiodinases (Peeters & Visser, 2017). Therefore, as highlighted by Dosiou & Medici (2017), the main TH needed for fetal neurodevelopment is T4. T4 enters the fetal brain, where it is converted by local deiodinases to T3, which then acts on local thyroid receptors to affect different aspects of neurodevelopment.

Dosiou & Medici (2017) have reviewed the consequences of isolated maternal hypothyroxinemia (IMH), which is the presence of low maternal T4 in the absence of TSH elevation. Based on animal models, it has been shown that even transient early IMH can cause structural and functional changes in developing brains in particular in the somatosensory cortex. In humans, a number of studies strongly suggest that IMH should be seen as a risk factor not only for impaired mental and motor neurodevelopment but also for neuropsychiatric diseases of the offspring (Dosiou & Medici, 2017). Similarly, US EPA (2019) has recently performed a comprehensive review of epidemiological studies that evaluated maternal thyroid hormone levels in early pregnancy and neurodevelopmental outcomes. The US EPA concluded that "maternal FT4, especially in the hypothyroxinemic range, is critical to proper offspring's neurodevelopment. Across different age ranges and neurodevelopmental indices, the impact of altered FT4 is seen even with small incremental changes in FT4."
All these elements support that a decrease in T4 should be considered in the assessment of the neurodevelopmental potential of TBBPA and support the significant changes observed in the two- and one-generation studies.

This is also in line with the ECHA/EFSA guidance on identification of ED in Biocides and Plant Protection Products (2018) stating that "Using the current understanding of thyroid physiology and toxicology (European Commission, 2017), it is proposed that the following be applied when interpreting data from experimental animals:
[…].
2) Substances that alter the circulating levels of T3 and/or T4 without histopathological findings would still present a potential concern for neurodevelopment."

Therefore, the association between the reduced locomotor activity and auditory effects with reduced T4 levels suggests that TBBPA may exert some effects on the nervous system probably in relation with the impairment of the thyroid axis. These neural effects cannot be excluded or considered as just obtained by chance.
The effects of changes in motor and hearing capacities with the support of a decrease in T4 shall be considered for a classification for development, at least in category 2.

As thyroid hormones regulate also cognitive processes, the evaluation of spatial memory assessed by the Water M-maze test is unfortunately not enough detailed to have a precise idea on the latency to reach the platform and the travelled distance during the trials for each exposed group per sex.

Editorial comments and clarification questions:
• Could you clarify the sentence on page 38: "There were a correlation between female uterine weight, endometrium thickness and CYP19 activity in the ovary to the increased male gonad weight at PND21 or necropsy." The relation between male gonadal weight and parameters in the female reproductive organs is unclear. Could you confirm that there was no effect on uterine weight and endometrium thickness?
• In the description of Saegusa et al, (2009), it would be less confusing to refer to PND 20 instead of "GD 20 after delivery"
• RDT: based on similar thyroid hormone values, it looks like the 90-day studies referenced as Osimitz 2016 and Unnamed 2002 is a single study.
• In the two-generation study, could you clarify when thyroid hormones were measures in F1 (age of the animals) and confirm that it was not measured in F2? This information can be important for the interpretation of neurobehavioural effects observed in F2.

Bibliographic references:
Dosiou C, Medici M. (2017). Management of endocrine disease: Isolated maternal hypothyroxinemia during pregnancy: Knowns and unknowns. Eur J Endocrinol 176:R21-R38.10.1530/EJE-16-0354
Mortoglou A, Candiloros H. The serum triiodothyronine to thyroxine (T3/T4) ratio in various thyroid disorders and after Levothyroxine replacement therapy. Hormones (Athens). 2004 Apr-Jun;3(2):120-6
Peeters RP & Visser TJ. Metabolism of Thyroid Hormone. [Updated 2017 Jan 1]. In: Feingold KR, Anawalt B, Boyce A, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK285545/
US EPA. United States Environmental Protection Agency (2019). Proposed Approaches to Inform the Derivation of a Maximum Contaminant Level Goal for Perchlorate in Drinking Water. Volume I. May 2019

Dossier Submitter's Response

Thank you for your comments. We agree with FR that there are some indications of effects on development, but we considered the effects as not sufficiently adverse to propose a classification as stated in section 10.10.7 in the CLH report.

The two-generation reproductive toxicity reported effects on motor activity (Unnamed 2002; EU RAR TBBPA, 2008; Cope et al., 2015). Motor activity was assessed at PND 13, 17, 21 and 60. The effects were reported in female rats exposed to 10 mg/kg/day in the 15-20 min segment and at 20 min period at 100 mg/kg/day PND 17. At PND 21 it was reported effects in female rats exposed to 100 mg/kg/day in the 5-10 min segment and over the 20 min test as a whole. At PND 60 effects were reported in males exposed to 100 and 1000 mg/kg/day in the 0-5 min segment and males exposed to 1000 mg/kg/day in the 5-10 min segment. EU RAR TBBPA, 2008 assessed these data. For PND 17 the data on horizontal activity were not accompanied by decrease in distance travelled and it was a lack of any response in males. Similar, at PND 21 there were no dose-response relationship and an absence of response in males. At PND 60 there were no effects in females and no consistent pattern of changes across animals of various ages (PND 13,

17, 21 and 60) and between sexes. The lower activity level seen in high dose TBBPA exposed males at PND 60 during the beginning of the test could be a treatment related effect and could indicate that exploratory behaviour was altered by TBBPA exposure in these males. These data were considered in the WoE assessment for reproductive toxicity. The two-generation reproductive toxicity also reported a decrease in the parietal cortex thickness at PND 11 (both sexes), but not at PND 60.

The one generation reproductive toxicity study (Van der Ven et al., 2008; Lilienthal et al., 2008) reported effects on Brainstem auditory evoked potentials (BAEPs). Effects were reported for females at the low frequency range up to 4kHz. It was further reported for both sexes slight exposure-related effects on latency of wave II, but wave II latency was not significantly altered after click stimulation. Prolonged wave IV latencies in the low frequency range was observed in exposed animals and was somewhat more pronounced in males. There were significant latency increases of wave IV after stimulation with clicks of 60 dB in female rats. There were also effects on interpeak latencies II-IV. The authors found effects of TBBPA exposure on brainstem auditory evoked potential. Based on their results they concluded that the outcome pattern suggested a predominant cochlear effect of TBBPA in females while in males neural effects were more apparent. The data published by Lilienthal et al., 2008 have previously been assessed by EFSA in Scientific opinion on Tetrabroomobisphenol A (TBBPA) and its derivatives in food (EFSA Journal 2011;9(12):2477 Scientific Opinion on Tetrabromobisphenol A (TBBPA) and its derivatives in food (wiley.com)) which reported that the ratios between the BMDL and the corresponding BMD from the BEAP were large, indicating a high uncertainty in these outcomes. Banasik et al. (2009) expressed concerns about Van der Ven et al. (2008) regarding the use of modelling software, methodology and conduct of the study. Some of the methods and statistical analyses of the findings presented by Lilienthal et al. (2008) were also questioned by Strain et al. (2009). Van der Ven et al. (2009) argued against the concern raised by Banasik and Strain in two response letters (see also response to comment 16).

Hass et al performed a developmental neurotoxicity study, the study was reported in the EU RAR TBBPA, 2008. Overall, this study provides some evidence of changes in the habituation behaviour of female offspring and indication of changes in the learning and memory in male offspring in the 250 mg/kg/day group (dose groups; 0, 50 and 250 mg/kg/day). It was not possible to draw a conclusion from the work, however, we agree that the data are not necessarily directly comparable to the one- and two-generation reproductive toxicity studies. The study has only been reported in the EU RAR TBBPA, 2008 and presented in the poster session at 30th Conference of European Teratology Society, Hannover, Germany and not published in a peer review scientific journal.

Most of the studies that evaluated thyroid hormone levels found a decrease in T4 serum levels (Cope et al., 2002, Van der Ven et al., 2008, NTP 2014, Osimitz et al., 2016). Effects on T3 were less consistent and TSH levels were not affected. Thyroid hormone levels were investigated in P and F1 generation of the two-generation reproductive toxicity study.

In conclusion, most studies reported reduced serum levels of T4. We agree that changes in thyroid hormones play an important role in development. There are also some evidence pointing towards TBBPA being a developmental neurotoxicant. However, when assessing

this data in a WoE matter we considered that the effects on neurobehavioral parameters do not suffice for classification of TBBPA as reprotoxic. We note that the EU Chemicals Strategy for Sustainability includes a proposal to amend the CLP regulation to introduce new hazard classes on endocrine disruptors.

Editorial comments and clarification questions:
• Could you clarify the sentence on page 38: "There were a correlation between female uterine weight, endometrium thickness and CYP19 activity in the ovary to the increased male gonad weight at PND21 or necropsy." The relation between male gonadal weight and parameters in the female reproductive organs is unclear. Could you confirm that there was no effect on uterine weight and endometrium thickness?
Van der Ven et al., 2008 performed correlation analysis. Details on the analysis are included in the CLH report Annex 3.10.1.3 p 67 (also included in the response to BSEF on reproductive toxicity, comment nr 16). The analysis was performed for parameters from the study that showed sensitive effects (i.e. BMDLs in the low-to mid-dose range) and used for correlation testing against all other parameters. Van der Ven et al., 2008 reports no effect on weight of female reproductive organs. However, a correlation of uterine weight, endometrium thickness, and CYP19 activity in the ovary to increased male gonad weight at either PND21 or final necropsy. Data on uterine weight are reported in the Supplementary Table 6-I of the Van der Ven et al., 2008 study and there are no significant dose response effects. However, it should be noted that six uterus samples from low and mid dose groups were excluded because of hydrometra.

• In the description of Saegusa et al, (2009), it would be less confusing to refer to PND 20 instead of "GD 20 after delivery"
Thank you for the correction.

• RDT: based on similar thyroid hormone values, it looks like the 90-day studies referenced as Osimitz 2016 and Unnamed 2002 is a single study.
There was no reference to Osimitz et al., 2016 at ECHA's dissemination site. However, going through the data reported by Osimitz et al., 2016 and Unnamed, 2002 we agree that the data from the OECD TG 408 test seem to be the identical. We agree that the studies most probably have the same data as origin. We apologise for reporting this as two separate studies in the CLH-report Section 10.12.

• In the two-generation study, could you clarify when thyroid hormones were measures in F1 (age of the animals) and confirm that it was not measured in F2? This information can be important for the interpretation of neurobehavioural effects observed in F2.
Thyroid hormone levels were measured in the P and F1 generation. The data are reported in Table 16 p 37 in the CLH report. T4, T3 and TSH were measured in serum from blood collected before sacrifice of the P and F1 generation (as reported in the CLH report annex 58).

RAC's response

RAC took note of your comments. RAC has evaluated the intrinsic hazard and considered the data in a weight-of-evidence assessment.

Date	Country	Organisation	Type of Organisation	Comment number
28.01.2021	Belgium	BSEF, the International Bromine Council	Industry or trade association	16

Comment received

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 BMDL10 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 R2 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.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment BSEF-Comments on TBBPA CLH proposal-28Jan2021.pdf

Dossier Submitter's Response

DS` Response to BSEF attachment page 22, paragraph (para) 1-3 - CLH dossier 10.10

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

We assessed CMR and STOT-RE. Thyroid hormones play an important role in fetal and neonatal development and information on thyroid hormone levels are considered relevant when evaluating reproductive toxicity, including possible effect on neurodevelopment. In addition, several of the studies assessed in the CLH dossier measured significant changes in thyroid hormone levels after exposure to TBBPA. To ensure transparency we are of the opinion that data on T4, T3 and TSH should be presented.

DS` Response to BSEF attachment page 22, para 4 and page 23, para 1 - CLH dossier page 38-39

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

The study performed by Van der Ven et al., 2008 and Lilienthal et al., 2008 are similar to OECD TG 415, but have some deviations from the test guideline. We agree that in test guidelines such as the Extended One Generation Reproductive Toxicity study (OECD TG 443) anogenital distance should be measured at least once from PND 0 to PND 4. In the study by Van der Ven et al., 2008 anogenital distance was measured at several timepoints, it was measured at PND 4, 7 and 21. Significant dose-response was observed at PND 7, but not at PND 4 and PND 21. Even though the test guideline does not specify the need for measuring AGD at PND 7 and 21, these effects should also be presented and included in the dossier and assessed in a weight of evidence approach.

DS` Response to BSEF attachment page 23, para 2 - CLH dossier page 39

CLH Dossier: Page 39, Delayed time to vaginal opening

A dose-response analysis of effects was based on external dosing (mg/kg bw) by using a nested family of purely descriptive (exponential) models with the PROAST software. From the best fitted curve, indicated by significance at 5% level the Benchmark Dose was calculated, the Benchmark Dose Level (BMDL) was determined at the lower confidence limit at 95% confidence interval. The statistical analysis was reported by Van der Ven et al., 2008 and also described in the CLH report Annex 3.10.1.3 p 67.

A significant dose-response was reported for day of vaginal opening. The CLH report also specify that the BMDL was around the highest dose, however we agree that additional clarity could have been given. The sentence could have been formulated as follows to better reflect what is stated in the Van der Ven study (p38, last paragraph): Female pups showed decreased anogenital distance at PND 7, but not at PND 4 and PND 21, and a delayed time to vaginal opening. The effects on anogenital distance and delayed vaginal opening were with a limited size and the BMDLs were around the top dose for absolute values when normalised for body weights.

DS` Response to BSEF attachment page 23, para 3 - CLH dossier page 35, 39 and 40

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

Information on the correlation analysis was reported in Van der Ven et al., 2008 and included in the CLH report Annex 3.10.1.3 p 67 (text from Annex added below).

Parameters from the study which showed sensitive effects, i.e. at BMDLs in the low- to mid-dose range, were used for correlation testing against all other parameters. This collection of univariate responses was screened for classes of correlation coefficients as defined in table 11, and all responses that fitted to those classes were reported in that table in a clustered manner. The results from the neurophysiologic tests (described in Lilienthal et al, 2008, see 3.10.1.2, study 2) were also included in the analysis. The correlation coefficient was based on group averages rather than comparison by individual, to allow comparisons across age cohorts and across sexes; this method ignores variability within groups, and these correlations should therefore be considered as indicative for clustering.

DS` Response to BSEF attachment page 23, para 4 - CLH dossier All BMDL values

CLH Dossier: All BMDL values

The BMR was reported as Critical Effect Size (CES) by Van der Ven et al., 2008 and Lilienthal et al., 2008. A description of the CES was included in the CLH report Annex 3.10.1.3. The default CES reported in Van der Ven et al. was 10%. The exceptions were for testis weight and bone parameters were the CES used was 5%. For liver weight and immune parameters a CES of 20% was used. Lilienthal et al. reported the CES used as 5%. Unfortunately, the CES used for liver, immune parameters and by Lilienthal et al. was not included in the CLH report Annex.

DS` Response to BSEF attachment page 24, para 1 - CLH dossier Table 15

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

In Table 15 we included the data that was considered in the weight of evidence approach. We also included summary of findings where no toxicity was observed. The data on parietal cortex was included since there were effects on parietal cortex thinning at the highest concentration. We see that the heading of the table may be misleading.

DS` Response to BSEF attachment page 24, para 2 - CLH dossier Table 15 page page 35 and text page 39 and 40

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

We agree that the organ weight data could have been further specified in Table 15. Organ weights were reported as absolute values by Van der Ven et al. As described in the response to the BMDL levels (above) the CES used for liver weight was 20%, the increased liver weight showed a maximum of 11.4% and no BMDL was calculated. The absolute weights on liver, testis and body weight at necropsy was reported in Van der Ven et al., 2008 in supplementary Table 6-II. For testis the CES used was 5% allowing calculation of BMDL (as described in the CLH report).

DS` Response to BSEF attachment page 24, para 3 - CLH dossier Table 17 page page 41

The slight significant effects on liver weight in maternal animals treated with 100 mg/kg/day was reported in EU RAR TBBPA, 2008 (page 93, para 2). There was no specification whether absolute and/or relative weights were affected. EU RAR TBBPA considered the effects to be unrelated to treatment.

DS` Response to BSEF attachment page 24, para 4 - CLH dossier page 37-38

CLH Dossier: Page 37-38 context on UDP-GT BSEF Comment

The study by Cope et al., 2015 suggests that the reduction of T4 may be caused by induction of hepatic uridine diphosphate glucuronyltransferase (UDP-GT). The study did

not investigate whether TBBPA exposure affects induction or activity of UDP-GT which was the reasoning behind the specification in the report page 37. Hence the statement is correct. Lai et al., 2015 proposes different hypothesis on mechanisms for explaining an absence of compensatory increase in TSH in response to lower T4. One of these hypotheses was linked to inactivation of T4 by TBBPA stimulation and upregulation of uridine diphosphate resulting in increased metabolism of T4 by UDP-GT. TBBPA seems to belong to a group of chemicals that consistently causes decreases in circulating T4 without concomitant increases in TSH. The mechanism behind this lack of compensatory TSH response is still not known. So while it is very likely that increased TH clearance by the liver causes the observed serum T4 reductions, it is important to keep the following statement from the ECHA/EFSA guidance (appendix A) in mind; … *In the absence of substance-specific data which provide proof of the contrary, humans and rodents are considered to be equally sensitive to thyroid-disruption (including cases where liver enzyme induction is responsible for increased TH clearance).*

NTP 2014 also investigated UDP-GT activity in the 3 months study in both rats and mice. UDP-GT measured at day 23 and week 14 in the F344/NTac rats and was significantly decreased in male and female rats at day 23 and in female rats in week 14 (Table G1 in NTP 2014). Considerably less effects were observed in mice. There was a significant decrease of UDP-GT activity in female mice at 50 mg/kg in the end of the study, but not in male mice or at any of the other concentrations (Table G2 NTP 2014).

DS` Response to BSEF attachment page 25, para 1 - CLH dossier page 35

CLH Dossier: Table 15 "total spleen cell counts" BSEF Comment

Some more information on immunotoxic and hematologic effects in the F1 animals is reported in the CLH report Annex I. Van der Ven et al. reports that there was an increase of total spleen cell count, apparently attributable to increase of all major spleen cell populations. The data were reported in supplementary Table 12-II in the Van der Ven study. Significant dose-response effects were reported for the absolute cell number per spleen, CD3, CD4, CD8 and CD45ra.

DS` Response to BSEF attachment page 25, para 2 - CLH dossier page 38

CLH Dossier: Table 15 and page 38 parietal thickening BSEF Comment

In general, the measurements and endpoints outlined in any given test guideline are the mandatory endpoint that need to be assessed. This however does not mean that results from additional endpoint assessments or from measurements performed at additional time points, should be ignored in a weight of evidence analysis. In this specific case, this is however not the question, as the time point for the performed neurological assessment is described in the relevant guideline.

The study reported by Unnamed, 2002; EU RAR TBBPA, 2008; Cope et al., 2015, was an OECD TG 416 study (GLP compliant) with additional neurological investigations. According to the Developmental Neurotoxicity test guideline (OECD TG 426) neuropathological

assessment should be investigated at termination and at PND 22 or at an earlier time point between PND 11 and PND 22. In the study reported by Unnamed 2002; EU RAR TBBPA, 2008; Cope et al., 2015, parietal cortex was measured at PND 11 and PND 60 which is in line with the OECD TG 426.

DS` Response to BSEF attachment page 25, para 3 - CLH dossier page 37

CLH Dossier: Table 16 BSEF Comment

The mean Thyroid hormone values was reported in the Two generation reproductive study (Unnamed, 2002; EU RAR TBBPA, 2008; Cope et al., 2015). Exposure route was oral (gavage) as reported in Table 15. We see that the measured levels of TSH are reported twice for both sex from the P and F1 animals.

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

DS` Response to BSEF attachment page 25, para 4 and page 26 para 1 - CLH dossier page 46.
Pentoxyresorufin-O-dealkylase (PROD) was significantly increased (up to 23x) which was reported by NTP as indicative of disturbance of liver function. Increased levels of PROD are indicative of xenobiotic metabolism.

DS` Response to BSEF attachment page 26, para 2 - CLH dossier page 38-39

CLH Dossier: Table 15 on page 38-39 BSEF Comment:

Strain et al., 2009 question the results from Lilienthal et al., 2008 by raising several questions on the statistical analysis and interpretation of the results from the Brainstem auditory evoked potential (BAEPs) alterations after TBBPA exposure. Some of the issues raised by Strain et al., 2009 was:

1. the lack of reported between-frequency pair-wise comparison for the click- and tone-evoked peak II latency and hearing thresholds
2. questioning the significant effects from tone-evoked peak IV latency
3. gender differences in click-evoked peak IV latency were to small to be physiologically meaningful.

Van der Ven et al., 2009 responded to the letter from Strain et al., 2009 and disagrees with the issues raised by Strain et al., 2009 and gives several explanations on the issues raised:

1. Argues that their study addresses dose-dependence with benchmark analysis and statistical trends, but they've also calculated between-frequencies effects (not pair-wise) which were significant.
2. Cochlear and neural processes contribute to the BAEP. The absolute latencies of BAEP waves can be influenced by effects on the cochlea and/or the neural part of the auditory pathway. While the interpeak latencies (such as IPL II-IV) are influenced only by neural effects as the cochlear contribution is the same to all waves. By substraction of the latencies, the cochlear part is removed and only the

neural transmission time is measured by IPL. Significant IPL and non-significant absolute latencies are not unusual and substraction reflects an additive standardization.

3. Acknowledge an error in one of the tables, but disagree that the effects have no physiological relevance. IPV II-IV was prolonged in tone -evoked BAEPs in males, but not in click-evoked potentials. It has to be considered that clicks in contrast to tones are composed by the whole spectrum of frequencies. The effects after stimulation with clicks are therefore likely to differ from effects in tone-evoked potential.

We evaluated the data from Van der Ven et al., 2008 and Lilienthal et al., 2008 with a reliability score 2, taking into account the criticism raised towards the studies. In our opinion the criticism raised by the Strain et al., 2009 were answered by the responding letter by Van der Ven et al., 2009. It could have been added some additional information around the criticism raised, but this will in our opinion not change the evaluation of the results. The report also refers to the two letters from Strain et al., 2009 and Banasik et al., 2009 and the corresponding responses from Van der Ven et al., 2009. Also see response to comment nr 15.

RAC's response

RAC took note of your comments. RAC has evaluated the intrinsic hazard and considered the data in a weight-of-evidence assessment.

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2021	Netherlands		MemberState	17

Comment received

NL-CA agrees with the dossier submitter's proposal for no classification for reproductive toxicity, based on fertility, sexual function and developmental toxicity.

No adverse effects on reproductive function/performance were observed in one- and two-generation generation studies. In regard to fetal developmental toxicity, effects on thyroid hormone levels were observed in multiple studies, but no significant adverse effects on fetal development were noted.
It is noted that a separate discussion/conclusion regarding adverse effects on/via lactation is not included in section 10.10 of the CLH-report. The NL-CA considers that the available data do not point towards adverse effects on pup development via lactation and classification is not warranted.

Dossier Submitter's Response

Thank you for the support.

RAC's response

Thank you for your comments.

Date	Country	Organisation	Type of Organisation	Comment number
18.01.2021	Germany		MemberState	18
Comment received				
Fertility: The evaluation of the endpoint sexual function and fertility was performed using two studies. The first one is an oral two generation reproduction toxicity study with a developmental neu-rotoxicity component in the F2 generation (OECD TG 416; Unnamed, 2002) in rats. The sec-ond study is an oral one generation reproduction toxicity study for endocrine and immuno-logical endpoints and additional analysis for bone and neurophysiological parameters similar to OECD TG 415 in rats (Van der Ven et al., 2008). In the parental generation, no general toxicity was observed as well as no effect on reproductive performance. In the F1 generation similarly no effects were seen, except for lower body weights and lower body weight gain in males exposed to 1000 mg/kg bw/day (high dose). In the F2 pups, no changes in body weight, sex ratio, survival to weaning or macroscopic findings and organ weights were found. There were effects on thyroid hormones: T4 levels were decreased in both sexes of P and F1 generations (at 100 and 1000 mg/kg/day for P males and F1 animals and 1000 mg/kg/day in P females). T3 levels were increased in P males exposed to 1000 mg/kg bw/day. However, there were no effects on TSH levels. The study showed no relevant neurobehavioral effects. The one generation study is a modified OECD TG 415 designed to determine benchmark doses (8 exposure groups: 0, 3, 10, 30, 100, 300, 1000 and 3000 mg/kg bw/day, 10 ani-mals/per sex in each group). However, the study was assigned a Klimisch score of 3 (not reliable) by the registrant, as benchmark modelling was considered inappropriate and other methodological shortages. No effects on reproduction was seen apart from a decrease in the anogenital distance at PND 7, but not at PND 4 and 21, and a delayed time for vaginal opening with a BMDL around the highest concentration in female F1 pups. Effects on thyroid hormones were found, equivalent to those in other studies (see STOT RE). The overall evidence revealed no effects on reproduction relevant for classification. The DE CA agrees that classification as reproductive toxicant is not warranted. Development: Developmental toxicity was evaluated based on a prenatal developmental toxicity study in rats according to OECD TG 414 (Unnamed, 2001), a developmental neurotoxicity study in rats according to OECD TG 426 (deviation: only two doses; Hass et al., 2003) and a non-guideline developmental study in rats (Saegusa et al., 2009). None of the studies shows evidence of developmental toxicity that is sufficient for classification. In the prenatal developmental toxicity study (OECD TG 414) no toxic effects in maternal animals or fetuses were seen, except for a slight significant lower liver weight ob-served in maternal animals treated with 100 mg/kg/day (low dose), that was not seen in the mid and high dose groups. In the developmental neurotoxicity study, no exposure related toxic effects were seen on physiology. Neurobehavioral effects were observed as changes in habituation behavior of female offspring and learning and memory in male offspring in the 250 mg/kg bw/day group (high dose) occured. However, the DS evaluates the evidence of developmental neurotoxicity as weakened by only small changes without consistent pattern, absence of consistent changes in the two genders and the lack of histopathological investiga-tions that could provide corroborative findings				

so it is not possible to draw definitive conclu-sions from this study. In the non-guideline developmental study no effects were seen in dams, except for increased body weight from day 9-20 after delivery (but not thereafter) in the 10 000 ppm (high dose, ~800 mg/kg bw/day) exposure group. Similarly, no (developmen-tal) effects were seen in offspring. The three studies in this section as well as the OECD TG 416 with developmental neuro-toxicity component could demonstrate some effects on development; however, these effects were not sufficiently adverse and reliability was partly questionable. DE CA agrees that classification is not warranted. PS: It has to be stated that the information in the registration dossier of TBBPA are not com-pliant with the standard information requirements for developmental toxicity in Annex X (> 1000 tpa). A prenatal developmental toxicity study in a second species is missing (rabbit). This information could further clarify this endpoint.
Dossier Submitter's Response
Thank you for the support.
RAC's response
Thank you for your comments.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
29.01.2021	Belgium		MemberState	19
Comment received				
Effects on thyroid with decreased T4 levels are consistently reported in different studies, only in rats. However, TSH and T3 levels were not affected (except in Van der Ven et al., 2008). Thyroid function or morphology do not appear to be adversely affected by the test substance in the presented data. In that regard, we agree with the dossier submitter's conclusion. Editorial remark: table 18 (pg. 49): about nose discharge would you please correct "clesar discharge" to "clear discharge"?				
Dossier Submitter's Response				
Thank you for the support and the editorial remark.				
RAC's response				
Thank you for your comments.				

Date	Country	Organisation	Type of Organisation	Comment number
18.01.2021	Germany		MemberState	20
Comment received				
Several studies are available for the evaluation of STOT RE, three studies according to OECD TG 408 and one non-guideline 12-weeks study. Additionally two subacute studies according to OECD TG 407 and a 14-day inhalation study in rodents described to be				

similar to OECD TG 412. An OECD TG 408 study (NTP, 2014) was conducted in rats and mice with 6 doses (0, 10, 50, 100, 500, 1000 mg/kg bw/day). Increases in liver weights (9-14%) were seen in the two highest dose groups in male mice and female and male rats, and in the highest dose group in female mice. Increase in liver CYP2B activity was seen in the two highest dose groups in mice and rats, but this was not accompanied by treatment-related liver lesions except for significantly increased incidences of renal tubule cytoplasmic alterations in 500 and 1,000 mg/kg male mice. Consistent, progressive, and dose-related decreases in total T4 concentra-tions occurred in 500 and 1,000 mg/kg male and female rats, but was not accompanied by decreases in T3 concentrations or increases in TSH concentrations.
In another subchronic study (Unnamed, 2002) no effects were found except for treatment related effects on serum thyroid hormone T4 levels, but not T3 and TSH (male and female, 100, 300, 1000 mg/kg bw/day). Effects on alkaline phosphatase levels and bilirubin are not considered toxicologically relevant (not adverse enough).
In the OECD TG 408 study by Osimitz et al. 2016 a significant decrease in all dose groups in mean serum T4 concentrations on day 33 (males and
Females) and day 90 (males) was noted, that returned to control
Levels (or lower) after 6 weeks recovery. Again, T3 and TSH were not affected.
In an academic 13 week study in rats (Dunnick et al. 2017), TBBPA induced upregulation of liver enzymes also induced the interferon (IFN) pathway transcripts, that might be involved in hepatic cancer. No other effects were observed.
In an 14 day inhalation study in male and female Crj: CD(SD) rats from 1975, no systemic toxic effects were observed up to the highest dose (18 mg/L), except for local irritation. In a 3 week dermal toxicity study in male and female New Zealand White rabbits from 1979 no systemic toxicity or unusual behaviour was observed.
One of the subacute studies (Borghoff et al. 2016, OECD TG 407) found no effects at all up to the dose of 1000 mg/kg bw/day in female rats. The other subacute study (Van der Veen et al. 2008, OECD TG 407) found a significant decrease in T4 values with a BMDL of 48 mg/kg bw/day and an increase in T3 values (BMDL 123, 8 mg/kg bw/day).

DE CA agrees that classification as STOT RE is not warranted. There are some but not severe toxic effects at moderate exposure concentrations, i.e. T4 reduction in several studies. However, we agree, that classification criteria are not fulfilled.

Any other hazard classes or endpoints:
Toxicokinetics:
Basic toxicokinetic of TBBPA was investigated using in vivo studies in humans and rats for absorption, distribution, metabolism and elimination. Additionally an in vitro study on der-mal absorption was conducted using human skin. The in vivo studies indicate rapid absorp-tion from the gastrointestinal tract with rapid metabolism to conjugates. The primary route of elimination is in the feces. The in vitro
dermal absorption was <1%. Estimated half-lives are ~2 days and ~0.5 day in humans and rats, respectively.
Systemic bioavailability of TBBPA is low (F < 0.05) due to extensive hepatic first pass bio-transformation to glucuronides and sulfates, which are predominantly excreted with bile from the liver due to their high molecular weight. TBBPA has been detected in the serum and milk of humans.

Dossier Submitter's Response
Thank you for the support.

RAC's response
Thank you for your comments.

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2021	Netherlands		MemberState	21
Comment received				
NL-CA agrees with the dossier submitter's proposal for no classification for STOT RE. Liver and spleen effects were observed in one 14-week repeated dose toxicity study in rats (NTP 2014); relative/absolute liver (9-14%) and spleen weights were increased in two species (rats and mice) in both sexes at 500 and/or 1000 mg/kg bw/day. However, liver effects were not supported by histopathological data and other liver adverse effects (liver enzyme levels) and/or in other studies. In addition, liver effects found were at 500 mg/kg bw/day (lowest dose reported), too high for classification on STOT-RE Category 2 (10 < concentration ≤ 100 mg/kg bw/day in 90-day repeated dose toxicity study). Effects on thyroxine (T4) hormone levels were observed in rats at ≥100 mg/kg bw/day or at a BMDL of 48 mg/kg bw/day, but not in other species, and were not classified as severe. Effects observed upon exposure to TBBPA were not classified as significant or adverse.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
Thank you for your comments.				

PUBLIC ATTACHMENTS
1. BSEF-Comments on TBBPA CLH proposal-28Jan2021.pdf [Please refer to comment No. 2, 9, 12, 16]