November 14, 2022

FDA Comments on the California Environmental Protection Agency Office of Environmental Health Hazard Assessment Announcement of the Carcinogen Identification Committee Meeting Scheduled for December 14, 2022; Notice of Availability of Hazard Identification Materials for Bisphenol A (BPA) ("Evidence on the Carcinogenicity of Bisphenol A (BPA)")

FDA provides these comments in response to the document entitled "Evidence on the Carcinogenicity of Bisphenol A (BPA)," published in September 2022 by the Reproductive and Cancer Hazard Assessment Branch, Office of Environmental Health Hazard Assessment (OEHHA), California Environmental Protection Agency (Ref. 1).[1] FDA's multiple evaluations examining carcinogenicity and the results of the CLARITY-BPA study conducted at FDA do not support classifying BPA as a carcinogen. Detailed below, we provide an overview of our concerns related to the OEHHA analysis of data from the CLARITY-BPA core study.

The OEHHA document does not provide a statistical section, explaining the rationale for several statistical methods identified only as a footnote with: 'conducted by OEHHA' in numerous reported data tables (e.g., Ref. 1, Tables 8, 9, 12, 13, 15, 17, 19, 21, 23, 24, 25, 28).

The statistical analysis of bioassay data, particularly those of 2-year rodent cancer bioassays, is a highly delicate, scientific endeavor and an integral part of the predetermined experimental study design to be conducted only by qualified experienced professionals. It's important to note, separating selective histopathology data from their original statistical context often introduces bias, leading to unreliable analyses and conclusions. We note that the OEHHA author list (Ref. 1, pg. i) does not identify a toxicologic pathologist or statistician qualified in the analysis of animal bioassays (including the analysis of particularly complex 2-year rodent bioassays), and we believe the analysis would have benefitted from this type of expertise.

The OEHHA includes an extensive section on the CLARITY-BPA core study, which will be the focus of our comments.

The results of the CLARITY-BPA core study, conducted at FDA's National Center for Toxicological Research (NCTR), underwent a publicly peer-reviewed, globally recognized, quality verification process by a carefully-selected independent scientific expert panel before they were reported in a National Toxicology Program (NTP) study report (Ref. 2) and also published in a peer-reviewed journal article (Ref. 3).

[1] FDA previously submitted information and comments in response to the OEHHA proposal to classify BPA as a reproductive toxicant for Proposition 65. (Luciana Borio, FDA Acting Chief Scientist to Monet Vela, OEHHA, April 6, 2015, RE: OEHHA Proposition 65, potential listing of BPA). In these comments FDA stated that the findings of our 2014 assessment reaffirm FDA's determination that BPA is safe provided it is used in accordance with our regulations. FDA also stated that the results from the extensive range of studies completed at FDA's National Center for Toxicological Research (NCTR) do not support BPA as a reproductive toxicant.

In this context, it should be noted that the peer review process for the NTP carcinogenesis bioassays has been refined over decades to inform regulatory decision making in a balanced, unbiased manner with the highest degree of scientific rigor. Today, the NTP pathology peer review process represents a globally-respected gold standard for pathology data quality and analysis (Refs. 4 and 5).

The CLARITY-BPA core study authors reported no evidence in support of BPA low dose or non-monotonic carcinogenic or other dose responses (Ref. 2, pgs, 1, 39-45).

The OEHHA document entails a considerable number of pages tabulating various historical control data comparisons and what are referred to in the OEHHA document as 'rare tumors' (Ref. 1, pgs. 64-88).

Inappropriate application of Historical Control Data (HCD):

On pg. 63 of the OEHHA document (Ref. 1), the study authors identified three historical control databases of historical control tumor incidences for Sprague-Dawley rats:

- NTP (2008, 2010) (dietary/feed administration, SD (NCTR) rats, 1999 to 2003),
- Charles River (2013) (oral routes, Crl:CD®(SD)BR rats, 2001 to 2009), and
- NTP (2021b) (all routes, Hsd SD rats, 2007 to 2012).

It should be noted that the FDA interpretation of the CLARITY-BPA core study findings relied to a very minimal extent on historical controls, but appropriately, on concurrent control groups (Refs. 6 and 7). Also, as indicated in the NTP CLARITY-BPA core study report (Ref. 2) and in the OEHHA document (Ref. 1, pg. 63), there were no studies that fit the requirements for ideal historical controls as far as genetic background, husbandry, and time of study were concerned. As identified after careful consideration in the CLARITY-BPA core study report, the NTP studies of genistein and ethinyl estradiol were considered to be the only relevant historical controls**,** not only because of the genetic drift since the 1970's when the colony was established from Charles River SD rats, but also because of the animals' maintenance from that time on a relatively low isoflavone diet (NIH-31) and the use of a soy-free diet in the CLARITY-BPA core study itself. The study diet was listed in Table 10 of the OEHHA document (Ref. 1, pg. 64) but was not explained further in the text. Therefore, it should be noted that any use of the Charles River and NTP databases in the OEHHA document is scientifically inappropriate based on both genetic differences and diet differences.

Invalid analysis of rare tumor data:

Consistent with any 2-year rodent cancer bioassay, the CLARITY-BPA core study pathologist identified spontaneous, incidental neoplasia lacking dose responsiveness in several organ tissues with low incidences compared to concurrent controls. These findings do not inform the

carcinogenic discussion and are, therefore, not carried forward into the main part of the study report.

The OEHHA document states that "Historical control data enables the identification of rare spontaneous tumor types in an animal strain" (Ref. 1, pg. 63). However, and particularly in this case, historical control data do not enable the identification of rare spontaneous tumors. The identification of spontaneous tumors is performed via the histopathological microscopic evaluation of tissue slides and their careful weighing and comparison with concurrent control and treated animal tissues by highly trained toxicologic pathologists. Historical control data should only be used as a tool to help further characterize this analysis (Refs. 6 and 7).

In addition to the invalid use of historical control data, the OEHHA document attempts to re-define the diagnoses and professional categorizations by numerous qualified toxicologic pathologists (study and peer review pathologists) of spontaneous, incidental neoplasms into what the document calls 'rare tumor types' by applying undefined and nonstandard statistical methods to NTP reported data.

The OEHHA study authors chose to include numerous tabulations of these artificially-created 'RARE' tumor Tables (Ref. 1, Tables 10, 11, 12, 14, 16, 18, 20, 22) in comparison to the three historical control databases identified above. These tabulations are not specifically interpreted in the OEHHA document and, therefore, could mislead readers to interpret them as related to BPA exposure, selectively biassing the reported evidence.

Findings, whether rare or common, that are not statistically significantly different from the concurrent control in a pairwise comparison would not be considered treatment-related effects and, therefore, would not be reported in a document entitled "Evidence on the Carcinogenicity" for any regulated compound. The OEHHA document should instead have referred back to published literature cited (Ref. 8), pointing to quality assessments of animal cancer studies (Ref. 1, specifically Part E, pgs. 56-68 outlining animal cancer hazard identification). NTP utilizes at least five factors in the decision-making process:[9]

> 1) the statistical significance of a given tumor effect (single most important consideration in the decision-making process),
> 2) the historical rate of the tumor in question (is it a rare tumor or a common tumor),
> 3) survival histories (how do differences in survival affect the interpretation of the data),
> 4) the pattern of tumor incidence (is the effect dose related; did it occur in more than one sex-species group),
> 5) the biological meaning of the effect,

as well as many ancillary factors (listed in every technical report under "Explanation of Levels of Evidence of Carcinogenic Activity" (see, e.g., NTP TR 601, pgs. xii-xiv, available at https://ntp.niehs.nih.gov/go/tr601)).

The CLARITY-BPA core report (Ref. 2, pgs. 39-45) emphasized the essential nature of applying the interpretation of statistically significant findings in the context of biological plausibility, including consideration of dose-response, consistency across biologically related endpoints, as well as study dosing arms and sacrifice times. To be evaluated as treatment-related, factors such as dose-response and biological plausibility need to be considered, as well as comparisons to qualitative relevant historical controls.

Taken together, there is no scientifically sound basis to support any linkage of the OEHHA reported 'rare tumors' to BPA treatment. For example, tabulating the total number of animals with the non-statistically significant 'rare tumors' in all treatment groups combined (numbers in parenthesis) artificially inflates the positive findings (false-positives) of this assessment of carcinogenic evidence (Ref. 1, Table 25, pg. 88).

Nonstandard statistical analysis:

Trend test analysis

NTP (and NCTR) studies have a long history of applying a preplanned statistical methodology (Ref. 9) using the one-sided Fisher Exact test for pairwise comparison of each group to the concurrent control (with a poly-k or poly-3 test for age-adjusted mortality) and a Cochran-Armitage linear trend test to demonstrate any dose relationship (dose response). The CLARITY-BPA analysis used the preferred NTP poly-3 age-adjusted analysis that accounts for early animal removals.

By contrast, OEHHA, conducted their own statistical analyses of selected tumor data reported by NTP; OEHHA's use of their own chosen Exact trend test was not compared critically to the Cochran-Armitage test. In the footnotes of various tables, the document calls it an Exact trend test (e.g., Ref. 1, Table 8, pg. 54; Table 9, pg. 57). The OEHHA document fails to address why this specific test was performed and how the test is superior to other tests. Without clear justification for the specific test, the statistical method appears to inappropriately provide a lower p-value, biasing the outcome toward carcinogenic evidence only.

It should furthermore be noted, as was commented by one of the reviewers during the peer review of the NTP CLARITY-BPA core study report, that the group size used in chronic studies such as the CLARITY-BPA core study (50 per sex per group), while large relative to most animal studies reported in the literature, was still small for the detection of rare events. Therefore, the CLARITY-BPA study design was certainly not intended to detect rare tumors and should not be artificially construed to do so post hoc.

Incorrect denominator: n (tissues examined) – too few

In numerous tables (Ref. 1, Table 13, 15, 17, 19, 21), the OEHHA document reports the denominator as less than 50 depending on the tumor type, and reported tumor incidences were identified in the table footnotes "as the number of tumor-bearing animals over the number of

animals alive at the time of occurrence of the tumor." Thus, it appears that only animals that survived until a tumor was observed were included in the analysis, resulting in the elimination of numerous non-tumor-bearing animals for the OEHHA reported reanalysis of spontaneous tumors.

The correct denominator figures are critical for any independent statistical evaluation of the data (Ref. 10). Total examined tissue denominators are important for a 2-year rat study to identify 'true' carcinogenic treatment-related effects. Not including all study animals that were in fact examined histologically in the denominator (selecting only onset date tumor animals and those euthanized after that date) is not only unconventional but scientifically flawed. The conventional approach is using age-adjustment tests (poly-k or poly-3 test) for all group animals. NTP routinely conducts statistical evaluations on the overall rate, the adjusted rate for intercurrent mortality, and the terminal rate, and provides the p-value for the poly-3 test on the adjusted rate (e.g., NTP TR 517, Table 8, pg. 38). The inclusion of all animals with tumors and exclusion only of animals that died with no tumor in the OEHHA document did bias the result and is contrary to a scientifically harmonized approach to cancer bioassay analysis.

Incorrect denominator: n (tissue examined) - too many (e.g., Zymbal's gland)

On the other hand, the OEHHA document showed too many animals in the denominator of some tables (Ref. 1, Table 20, pg. 76). Here, the footnote reads (emphasis added):

> In the case of the historical control data for tumors of the Zymbal's gland and ear, tissues were not examined microscopically unless gross lesions were detected. The **denominator** represents the number of animals examined, either macroscopically **or** microscopically.

The study authors included in the analysis tumors of animals reported in tissues that were examined microscopically only when a gross lesion was observed and also included in the denominator of the incidence calculation animals that were only examined grossly (Ref. 1, Table 20, pg. 76, e.g., Zymbal's gland tumors).

Consequently, for several of the lesions re-evaluated statistically by OEHHA, organs of some animals were not evaluated microscopically if gross lesions were not detected but inappropriately included in the OEHHA statistical analysis.

Finally, since exact tests were conducted, the exact p-value should be reported in the tables rather than $p< 0.05$ or 0.01. This was present in some tables of the OEHHA document but not in all.

<u>Rebuttal to OEHHA critiques of the CLARITY-BPA core study:</u>

The OEHHA document repeated various critiques of the CLARITY-BPA core study design that have been made previously; for a balanced reporting of the evidence, the OEHHA document

would have included the numerous responses addressing these critiques in the published literature.

On page 89 (Ref. 1), the OEHHA document states without elaboration that "These issues are considered to be significant and may have limited the sensitivity of these studies, and thereby affect the ability of these studies to detect carcinogenic effects." However, the OEHHA document does not reference that many of these issues have been previously addressed in various forums (including publications related to the NTP-sponsored BPA and EE_2 studies conducted at NCTR (Refs. 11 and 12)) by the NCTR investigators involved in those studies.

On page 91 (Ref. 1), the OEHHA document states that "Additional concerns about the design of the CLARITY-BPA studies were raised by Vandenberg et al. (2020) and Uchtmann et al. (2020), such as the lack of an unhandled, non-gavaged control group and lack of EE_2-treated positive controls in the stop-dose arms of the core studies."

However, as stated in the NTP CLARITY-BPA core study report: "Resource limitations did not allow for the inclusion of stop-dose EE_2 groups. Likewise, although the NCTR BPA subchronic study had included a naïve control group that was not dosed by gavage, this group could not be included in the chronic study. The responses of the naïve and vehicle control groups in the NCTR BPA subchronic study were similar" (Ref. 2). The lack of these groups does not impair interpretation of the results.

The further comments here will be confined to a few major points.

There are several criticisms in the OEHHA document (e.g., Ref. 1, pgs. 59 and 90) of the rat strain used in the CLARITY-BPA study, either general comments on the Charles River Sprague-Dawley rat and its descendants or specific comments on the NCTR Sprague-Dawley rat. It is universally accepted that different rat strains will respond differently for different endpoints to test agents for a variety of reasons, but the long-standing generalization about the relative insensitivity of Sprague-Dawley rats derived from Charles River stock to BPA is challenged by a body of published evidence. Earlier studies suggesting that there is no intrinsic insensitivity of these rats to BPA include the following: Zsarnovszky *et al*., Endocrinology 146: 5388 – 5396, 2005; Moral *et al*, J. Endocrinol., 196: 101 – 112, 2008; and Jenkins *et al*., Environ. Health Perspect. 117: 910 – 915, 2009. The NTP CLARITY-BPA core study report (Ref. 2) discusses the background and previous use of the NCTR SD rat in multigenerational studies of estrogenic agents, as well as the extensive BPA pharmacokinetic data obtained in this rat strain. BPA dose-related effects have been reported in the NCTR SD rat (Refs. 2, 3, 11, 12, and 13).

The CLARITY-BPA core study report specifically stated (Ref. 2, pgs. xxi, 45) that the CLARITY-BPA study utilized low stringency statistical tests, which included multiple tests and higher p-value cutoffs than those scientifically justified and routinely applied by either the NTP or the FDA for histopathology endpoints, and that this increased the potential false-positive discovery rate.

On page 90 (Ref. 1), the OEHHA document states: "Vandenberg et al. (2019) also stated that the SD (NCTR) rat strain used in the CLARITY-BPA studies was insensitive to known estrogens, such as EE_2. For example, in the CLARITY-BPA core studies, several established estrogen-sensitive outcomes were not observed in the EE_2 positive control groups, such as any effects on the timing of vaginal opening in female SD (NCTR) rats undergoing puberty, or on testes weight, or chronic inflammation in the prostate in male SD (NCTR) rats." The lack of effects on the mentioned endpoints at the low doses (0.05 and 0.5 µg EE_2/kg bw/day) that were used in the CLARITY-BPA study is not surprising. The differences in pharmacokinetics of EE_2 in rats and humans and the circulating levels of EE_2 under the conditions of this study were discussed in the reports and manuscripts on the NTP-funded EE_2 and BPA studies conducted at NCTR (Refs. 2, 3, 11, and 12). Other studies using developmental gavage exposure to EE_2 in Long Evans rats did not report effects on the endpoints noted at EE_2 doses less than 5 ug/kg/day (Howdeshell et al., 2009; Ryan et al., 2010) and, as noted in the NTP CLARITY-BPA core study report (Ref. 2), effects on the estrous cycle, as observed in the CLARITY-BPA core study, may be a more sensitive endpoint.

The selection of the EE_2 doses was extensively discussed at planning meetings for CLARITY-BPA and is described in the NTP CLARITY-BPA study report (Ref. 2). As also indicated in the report, there was a clear dose-related and consistent pattern of EE_2 effects not evident for BPA, demonstrating that the NCTR SD rat was sensitive to this endocrine challenge.

The OEHHA document also suggests that background BPA contamination decreases the utility of the study. It states (Ref. 1, pg. 89): "Thus, it seems possible that contamination of animals with BPA was not adequately controlled for in the CLARITY-BPA core studies and this may have reduced the ability to detect differences in adverse outcomes (*e.g.,* cancer, hyperplasia) between control and BPA-treated animals." It is suggested that, despite the expressed concerns about the sensitivity of the animal model to BPA, this supposed contamination might be responsible for various rare tumors observed in controls.

The careful selection of feed and housing materials to minimize BPA background exposure of the study animals and the extent of analysis and reporting of potential background levels of test agent in the CLARITY-BPA studies and the earlier NCTR 90-day BPA toxicity study (Ref. 9) were far more extensive than the vast majority of BPA studies reported in the literature and were an important feature of the study designs. The results of these background assessments serve as a cautionary note for the conduct and interpretation of "low dose" studies and raise considerable questions on the value of the evidence brought forward by "low dose" studies that neglect to include these elements of design. The implications of such background in the CLARITY-BPA studies are discussed in a transparent manner and considered in the interpretation of the study data. For example, in the case of the potential background contamination mentioned above that would have been possible in approximately 20% of the animals, a sensitivity analysis was conducted in which all animals that potentially had exposure to BPA above that present in the

diet were excluded from analysis. The sensitivity analysis indicated minimal impact on the conclusions derived from the statistical tests (Ref. 2).

In summary, numerous errors and incorrect or inappropriate analyses of CLARITY-BPA core study results have been identified. We recommend OEHHA consider these issues for re-analyses, as the current methods applied by OEHHA lead to an unsupported conclusion of potential positive carcinogenicity of BPA. As stated above, FDA's multiple evaluations examining carcinogenicity and the results of the CLARITY-BPA study do not support classifying BPA as a carcinogen.

Sincerely,

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