Proposal to Streamline Several Sections of Cancer Hazard Identification Documents (HIDs) for Discussion with the Carcinogen Identification Committee

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Contents

1 Overview

Proposition 65, officially known as the Safe Drinking Water and Toxic Enforcement Act of 1986, was enacted as a ballot initiative in November 1986. The proposition protects the state's drinking water sources from being contaminated with chemicals known to cause cancer, birth defects or other reproductive harm, and requires businesses to inform Californians about exposures to such chemicals.

Proposition 65 requires the state to maintain and update a list of chemicals known to the state to cause cancer or reproductive toxicity. Cancer hazard identification documents (HIDs) are comprehensive scientific reviews of evidence on the carcinogenicity of chemicals prepared by OEHHA for the evaluation of cancer hazards by the Carcinogen Identification Committee (CIC), the “State’s Qualified Experts” for identifying carcinogens under Proposition 65. Hazard identification materials provided to the CIC to inform its decisions include the HID, other materials prepared by OEHHA, all papers cited, and public comments received on the materials.

This document describes the proposed content and organization of several parts of future HIDs, with an objective to promote discussion and solicit feedback from the CIC. Specifically, we propose to streamline the Introduction, Carcinogenicity Studies in Humans, and Carcinogenicity Studies in Animals sections by focusing on the more informative data and limiting the scope of discussion of less informative data, such as chemical uses/exposure, and various studies with major limitations in design or conduct.

The proposal will be discussed at the February 2024 CIC meeting and is an opportunity for the CIC to provide input on the most informative materials to include, and how best to present it, in the HID. The content and organization of the “Mechanistic Considerations and Other Relevant Data” section, such as pharmacokinetics and metabolism, structural activity comparisons, and data related to the Key Characteristics of Carcinogens, will be discussed at a future meeting.

The examples included in this proposal take excerpts from previous HIDs, and are meant to show how each section would look with the proposed changes. They are only for illustrative purposes.

As new tools and knowledge in epidemiology, toxicology and cancer hazard evaluations become available, the approaches proposed herein may be updated.

2 Changes to HID Introduction

The Introduction section of the HID includes chemical identity and properties, exposure-related information, and reviews by other health agencies.

Proposed changes

We propose to shorten the description of exposure-related information by providing a more concise summary of production, sources and uses, and occurrence and exposure.

The Introduction section currently includes four (sub)sections, which we propose to retain, but streamline as follows:

- Chemical identity: This section identifies the chemical or chemicals that are the subject of the document. Unique Chemical Abstract Service registry number(s) (CAS RN), when available, common synonyms, and key chemical properties that may affect exposure or metabolism (e.g., volatility or solubility) would be included in a table. No major changes are proposed for this section.
- Production, sources and uses: This section would be limited to one to two paragraphs. It would briefly summarize information on the production of the chemical, such as volume of production. It would broadly indicate sources of exposure, both natural and or anthropogenic. Common uses of the chemical that may lead to human exposure potential would be described (for example, uses in consumer products).
- Occurrence and exposure: This section would be limited to one to two paragraphs. It would briefly summarize the occurrence of the chemical in different environmental media (e.g., air or water) and human biomonitoring findings (e.g., in blood or urine samples) with a focus on California. Magnitude or temporal trend of exposure may be briefly discussed.
- Reviews by other health agencies: As is currently done, this section would briefly summarize the evaluation of carcinogenicity of the chemical by other health agencies, with a focus on bodies considered by the Carcinogen Identification Committee to be authoritative in the identification of chemicals as causing cancer (i.e., the International Agency for Research on Cancer [IARC], the National Institute for Occupational Safety and Health [NIOSH], the National Toxicology Program [NTP], the United States Environmental Protection Agency [US EPA], and the US Food and Drug Administration [US FDA]). No major changes are proposed for this section.

Example of proposed changes

The following example takes an excerpt from the bisphenol A (BPA) HID (OEHHA 2022) and shows how that section would look with the proposed changes. It is only for illustrative purposes. OEHHA is not using it to update the 2022 BPA HID.

Text on Occurrence and Exposure Section in the HID on BPA (OEHHA, 2022, pages 3-5):

1.3 Occurrence and Exposure

BPA has been measured in environmental media, biota, and humans. Though BPA is not considered to be a persistent chemical based on its physical properties (see Table 1), BPA can be considered ubiquitous as a result of high levels of production, use, and subsequent environmental introduction. Global environmental occurrence and environmental fate of BPA have been reviewed broadly (Corrales et al. 2015; Cousins et al. 2002) and by environmental medium, including a recent review of BPA in indoor and outdoor air (Vasiljevic and Harner 2021).

Briefly, BPA has been identified in sediments, soils, and biosolids, including those in California (Careghini et al. 2015; Maruya et al. 2022; United Nations Environment Program 2020). BPA has also been identified in drinking water, ground water, and surface waters, including various water bodies in California (Barnes et al. 2008; Maruya et al. 2022; United Nations Environment Program 2020).
BPA has been shown to be taken up by plants, including edible crops, and has also been measured in animals and in raw animal products (Flint et al. 2012; Repossi et al. 2016; Santonicola et al. 2019; Tao et al. 2021). BPA has been detected in foods; its presence is generally attributed to contact of the food with BPA-based processing materials or food packaging materials (Almeida et al. 2018; Vilarinho et al. 2019; Wang et al. 2022). BPA has been detected in indoor dust samples from businesses and homes, including in California (Caban and Stepnowski 2020; Mitro et al. 2016; Shin et al. 2020; Wang et al. 2015b).

Humans are exposed to BPA through ingestion of contaminated food and water, ingestion of dust, inhalation of indoor and outdoor air, and dermal contact with BPA-containing materials. Studies investigating the relative contribution of these exposure pathways have consistently identified ingestion of contaminated food and beverages as the predominant contributor to exposure for most individuals (Covaci et al. 2015; Huang et al. 2018b; Martínez et al. 2018; US FDA 2018). BPA has been detected in breast milk in the US and other countries, which contributes to dietary intake for infants and toddlers (Dualde et al. 2019; Nakao et al. 2015; Sayıcı et al. 2019; Zimmers et al. 2014). The relative importance of dietary intake to BPA exposure is further supported by intervention studies and randomized cross-over dietary studies that have demonstrated dietary modifications such as removal of canned or packaged foods or incorporation of more fresh foods result in decreases in urinary BPA levels by

50% or more (Carwile et al. 2011; Kim et al. 2020; Peng et al. 2019b; Rudel et al. 2011).

Other exposure pathways, e.g., dust ingestion, inhalation in indoor environments, or dermal contact can also contribute substantially to overall BPA exposure for certain individuals, such as some older infants and young children (Geens et al. 2011; Healy et al. 2015; Morgan et al. 2018; Wang et al. 2019d). Developing fetuses may also be exposed via in utero transfer; BPA has been detected in fetal cord blood, fetal liver, amniotic fluid, and the placenta (Dreshaj and Pasha 2021; Gerona et al. 2013; Lee et al. 2018b).

BPA has a short half-life in the body (approximately 6 hours) (see Section 5.1) and the general population is frequently exposed from multiple sources, resulting in BPA levels that vary widely within an individual, even within the span of a 24-hour period. Thus, biomonitoring approaches at best reflect an individual's short-term exposure to BPA. However, recent population biomonitoring studies of BPA report detection frequencies of over 90%, demonstrating that exposure in humans remains widespread (Centers for Disease Control and Prevention 2021; Colorado-Yohar et al. 2021; Huang et al. 2018b). Total BPA, which typically represents the sum of BPA and at least the two major conjugated metabolites (BPA glucuronide [BPA-G] and BPA sulfate [BPA-S]) following enzymatic hydrolysis, measured in urine using a liquid chromatography (LC) and tandem mass spectrometry (MS), is widely recognized as the standard biomonitoring measure. This is the approach currently used by major biomonitoring programs including Biomonitoring California and the US National Health and Nutrition Examination Survey (NHANES) (Calafat et al. 2015; Gavin et al. 2014; LaKind et al. 2019).

Several biomonitoring studies have reported total BPA levels in urine of California residents in recent years, including studies conducted by the Biomonitoring California program (https://biomonitoring.ca.gov) (Gerona et al. 2016; Harley et al. 2016; Kim et al. 2021; Lin et al. 2020; Smith et al. 2022; Waldman et al. 2016). Table A1 in Appendix A presents urinary BPA levels in Californians from select studies with urine samples collected between 2007 and 2020. Generally, both detection frequency and BPA levels in Californians have decreased in recent years, as some uses of BPA, such as its use in epoxy-resin linings of cans and bottles, have been reduced.

How the section would have looked under the proposal:

BPA is not a persistent chemical based on its physical properties (see Table 1). With its high production and uses, BPA is ubiquitous in the environment and has been detected in ground, surface, and drinking water, soil, air, and plants (Abraham and Chakraborty 2020). Humans are exposed to BPA predominantly through contaminated food and drinking water, with additional exposures from ingestion of dust, inhalation of indoor and outdoor air, and dermal contact with BPA-containing materials (Covaci et al. 2015; Huang et al. 2018b; Martínez et al. 2018; US FDA 2018). BPA is also

detected in breast milk, which contributes to dietary intake for infants and toddlers (Dualde et al. 2019; Nakao et al. 2015; Sayıcı et al. 2019; Zimmers et al. 2014).

BPA has a short half-life in the body (approximately 6 hours) (see Section 5.1) and the general population is frequently exposed from multiple sources, resulting in large temporal variation of BPA levels within an individual. Nevertheless, national and California biomonitoring studies report widespread exposure in humans (Centers for Disease Control and Prevention 2021; Hurley et al., 2016). BPA levels and detection frequency have decreased in recent years in California due to the reduction of uses (e.g., in epoxy-resin linings of cans) (Biomonitoring California, see https://www.biomonitoring.ca.gov/chemicals/bisphenol-bpa).

3 Changes to "Carcinogenicity Studies in Humans" Section

Evidence from studies of cancer in exposed humans, when available, can provide important information for cancer hazard identification. All pertinent studies of exposure to a chemical that report a cancer outcome are eligible for inclusion.

Generally, among observational epidemiologic studies, a greater focus is given to cohort and case-control studies (IARC 2019), although other designs may also play a role in cancer hazard identification. For example, ecologic studies and case-series, respectively, provided crucial evidence in the classifications of arsenic and aristolochic acid by IARC (IARC 2012a, b). Because cross-sectional studies measure exposure and outcome at the same time, they may be uninformative for assessing cancer outcomes with long latency periods, for which the potential for reverse causation and the potential for survivor bias in capturing only prevalent cases are key concerns (Savitz and Wellenius 2023).

Besides the types of limitations inherent to specific study designs (e.g., ecologic, cross-sectional), the "informativeness" of studies for use in cancer hazard identification also depends on their sensitivity and ability to detect a true association between the exposure and the outcome (Cooper et al. 2016). Specifically, study informativeness is determined by the presence or absence of biases (systematic errors) and other factors including (but not limited to) sample size, adequate exposure contrast, sufficient follow-up time to detect the presence of cancer. A non-exhaustive list of potential biases considered in such evaluations are selection and attrition bias, exposure measurement error and misclassification, outcome misclassification, potential for confounding, and analysis bias (Eick et al. 2020; IARC 2019; NTP 2015; Savitz et al. 2019).

Proposed changes

In presenting and summarizing the available evidence, we propose to focus on the most informative studies and limit the scope of discussion of less informative studies. This is a change from the two most recent HIDs, where all studies identified for inclusion (e.g., studies of the chemical and a cancer outcome, even without reported measures of association) have been presented in some detail in the text and in tables.

Specifically, the changes we propose regarding the less informative studies are to provide a discussion of the specific issues that limit study informativeness (e.g., clarity in study reporting, information bias from ascertainment of exposure or outcome, confounding, selection bias) followed by a brief summary of the studies, without detailed study descriptions or tables in the main report. For studies of very limited informativeness, discussion will be abbreviated, and a bibliography list of those studies

provided in an appendix. The most informative studies will continue to be summarized in the text and tables.

Proposed organization

Presentation of pertinent studies of cancer in exposed humans would be generally organized and similar to how it is currently, as follows.

1. Introduction: This section provides information on the scope of and search strategy for pertinent studies of cancer in exposed humans, the methods and approaches used to evaluate those studies, and key issues to be considered.
    a. Methods, including:
        i. Criteria for study inclusion and exclusion
        ii. General information on the scope of the studies identified for inclusion (e.g., number of publications, number of studies assessing a specific cancer site/type)
        iii. Approaches to the evaluation of study quality and informativeness for cancer hazard assessment
    b. Key issues in consideration of the available studies, e.g., exposure assessment limitations; study design limitations; confounding and other biases.
    c. Other information may be included, as appropriate, e.g., information on particular populations or cohorts studied.
2. Human Epidemiology Studies by Cancer Site: The presentation of the epidemiologic studies generally will be organized by cancer site/type, with greater detail provided for more informative studies, compared to less informative studies. The most informative studies for each cancer site/type will be summarized in the text and tables. For less informative studies, issues contributing to that determination will be discussed, and the studies briefly summarized. Studies of very limited informativeness will be mentioned in the text with issues contributing to that determination, and included as a bibliography list provided in an appendix. Individual cancer sites/types for which data are very limited will be mentioned in the text, and a bibliography list of those site/type-specific studies provided in an appendix.

Example of proposed changes

As the CIC discussed during the 2022 meeting on the carcinogenicity of BPA, none of the available epidemiologic studies were considered informative due to various study design issues. These issues were described in detail in section “3.1.2 Key issues in the consideration of the epidemiologic data on BPA and cancer” with the following organization:

- Exposure assessment limitations
    - Questionnaires and Job Exposure Matrices (JEMs)
    - Biological measurements
        - Number and timing of measurements
        - Biological matrix
        - BPA analytes
        - Detection method and limits of detection and quantification
        - Low detection frequency and handling of non-detects
- Study design limitations
- Confounding bias
    - Potential confounders in the association between BPA and cancer, by target organ

In the BPA HID, the key issues section was followed by "Human Epidemiology Studies by Cancer Site," where each available study was described in detail by cancer site. In future HIDs, we propose to keep the key issues section, and limit the scope of discussion of studies that are deemed less or not informative by providing only a citation with a brief justification. For example:

Text on human prostate cancer in the BPA HID (OEHHA, 2022, pages 39-42)

> ***3.2.2 Prostate cancer***
> *Both of the epidemiologic studies that assessed the association between BPA exposure and prostate cancer found evidence of an association. One cross-sectional study did not provide a risk estimate but is reported briefly below (Tarapore et al. 2014).*
>
> *In a case-cohort analysis, Salamanca-Fernández et al. (2021) included 575 prostate cancer cases and 3690 cancer free members of the Spanish EPIC cohort (1772 men) with available data on BPA exposure (Table 5). Total BPA was measured in serum samples collected prospectively at recruitment. There were no significant differences in serum BPA levels (p = 0.809) in prostate cancer cases (1.33 ng/ml) compared with the subcohort (1.29 ng/ml). BPA was not associated with prostate cancer in linear models (hazard ratio [HR] for 5 ng/ml increase in BPA: 0.989; 95% CI: 0.92–1.06). However, when BPA was analyzed as a log2 transformed continuous variable, there was a 3.5% increase in risk of prostate cancer with every ng/ml increase in BPA level (HR: 1.035; 95% CI: 0.99–1.08). The increases in risk were also observed when BPA was analyzed in tertiles compared to those below the limit of detection: HR tertile 1, 1.404 (95% CI: 1.05–1.88); HR tertile 2, 1.365 (95% CI: 1.02–1.82); HR tertile 3, 1.305 (95% CI: 0.98–1.74). [Exposure assessment limitations: single sample; biological matrix analyzed (serum); relatively high LOD; and imputation of exposure levels for 28.3% of participants below the LOD.]*

Tse et al. conducted a case-control study in Hong Kong among 431 incident prostate cancer cases and 402 age-matched controls (Tse et al. 2017; Tse et al. 2018). Cumulative BPA exposure through ingestion was assessed using a tool reconstructed through questionnaire data and a literature review of BPA levels, similar to construction of a JEM. Detailed data were collected on habitual use of specific types of food or beverage container including what the container is made of, the frequency of use (e.g., daily, weekly, etc.), the handling practice (e.g., for storing hot water, heating by microwave), and years of usage. The BPA assessment tool ranked specific items of food or beverage containers in terms of concentrations of BPA according to the literature review. Increasing cumulative BPA exposure was associated with prostate cancer (OR high exposure vs low exposure: 1.88; 95% CI: 1.24–2.86), with evidence of an exposure-response (p-value for trend = 0.014). The study adjusted for age, marital status, and unemployment status and therefore may have been over-adjusted. Hospital controls were recruited, which may differ in lifestyle habits from the general population. Misclassification of BPA exposure was possible as there were no considerations of exposure variations over time. Other routes of BPA exposure such as direct skin contact or inhalation were not measured by the index. The assessment tool was further validated by two experts in environmental hygiene and food safety who blindly rated the exposure intensity of BPA based on the same master list and same rating scale. High inter-rater and inter-method agreement was obtained with interclass correlation coefficients of 0.86 and 0.90, indicating a good replication of the tool for evaluating environmental BPA exposure via ingestion. [Exposure assessment limitations: this tool was limited to certain sources of dietary exposure and didn't capture other sources or routes.]

Tarapore et al. (2014) also assessed total BPA exposure and prostate cancer in a cross-sectional study, but did not present a risk estimate. Among the 60 urology patients included in this study, creatinine-adjusted urinary BPA levels in prostate cancer patients (5.74 mg/g [95% CI: 2.63, 12.51]) were significantly higher than in urology patients without prostate cancer (1.43 mg/g [95% CI: 0.70, 2.88]) (p = 0.012). This study is not included in Table 5. [Exposure assessment limitations: single sample; collection of spot urinary samples.]

Table 5 Prostate Cancer

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Exposed cases/ deaths	Co-variates controlled	Comments, strengths, and limitations
Salamanca-Fernández et al. (2021) Case-cohort Spain **Enrollment or follow-up:**	**Population:** Participants of subcohort from 4 EPIC centers in Spain: Gipuzkoa, Granada,	HR, Serum BPA levels (Categorized (ng/ml) and continuous)			Age, education level, BMI, physical activity, smoking status, alcohol consumption	**Exposure information**: LOD: 0.2 ng/ml total BPA levels: 1.33 ng/ml in prostate cancer cases and
		BPA levels (for 5 ng/ml increase)	0.989 (0.92–1.06)	NR		

EPIC cohort enrollment 1992–1996; 2011–2013 for case ascertainment	Murcia and Navarra Cases: 575; Controls: 1772 **Exposure assessment method:** serum; BPA measured prospectively in a single serum sample collected at recruitment (1992–1996) before case ascertainment (2011–2013); no measures to limit BPA contamination reported; exposure proxy: total BPA following enzymatic hydrolysis analyzed by UHPLC-MS/MS. Samples below LOD were imputed as the LOD/√2.	Log2(BPA)	1.035 (0.99–1.08)	NR		1.29 ng/ml in the subcohort **Strengths:** Prospective sample collection. Adequate follow-up time (median 16.9 years). Population is large. Used previously-validated questionnaires to characterize covariates. **Limitations:** High LOD. BPA detection in serum generally underestimates the levels of BPA excreted. Analysis of a single sample per person does not account for within-person variability over time and may limit ability to detect an effect. Exposure proxy (deconjugated BPA-G and BPA-S + BPA) may be affected by background contamination. Approximately 30% of samples were below the limit of detection, therefore decreasing capacity to characterize exposure.
		< LOD (0.2 ng/ml)	1	NR		
		Tertile 1 (0.2–1.8)	1.404 (1.05–1.88)	NR		
		Tertile 2 (1.8–5.1)	1.365 (1.02–1.82)	NR		
		Tertile 3 (5.1–68.9)	1.305 (0.98–1.74)	NR		
Tse et al. (2017; 2018) Case-Control Hong Kong **Enrollment or follow-up:**	**Population:** Cases: 431; Controls: 402 **Exposure assessment**	OR, Cumulative BPA Index (Categorical, main model)			Age, marital status, unemployment status	**Exposure information:** dietary exposure scores calculated from questionnaire
		Low	1	75		
		Middle	1.66 (1.15–2.4)	232		

2011–2016	**method:** questionnaire; Exposure was assessed using a tool that reconstructed BPA exposure through questionnaire data (use of specific types of food and beverage containers and handling conditions) and a literature review of BPA levels, similar to construction of a JEM. The BPA assessment tool rankings were validated against exposure intensity assessments by two experts.	High	1.88 (1.24–2.86)	124	Age at interview, marital status, unemployment status, family prostate cancer history, consumption of deep fried food, consumption of pickled vegetable, green tea drinking habits, nightshift work, cumulative BPA exposure index	responses using a literature review **Strengths:** Chronic BPA exposure via ingestion assessed using a validated tool with high interrater agreement. **Limitations:** Potential for selection bias: Use of hospital controls which may differ in lifestyle habits from the general population. Misclassification of BPA exposure possible: no considerations of exposure variations over time, exposure through sources other than specific types of food and beverage containers, or of exposure contributions through nondietary routes
		Trend-test p-value: 0.014				
		OR, Cumulative BPA Index (Categorical, full model)				
		Low	1	75		
		Middle	1.54 (1.05–2.26)	232		
		High	1.57 (1.01–2.44)	124		
		Trend-test p-value: 0.057				

How the section would have looked under the proposal:

3.2.2 Prostate cancer

Both of the epidemiologic studies that assessed the association between BPA exposure and prostate cancer found evidence of an association (Salamanca-Fernández et al. 2021; Tse et al. 2017; Tse et al. 2018). One cross-sectional study did not provide a risk estimate (Tarapore et al. 2014). These studies are not discussed further due to the limitations provided in the table below, and the very limited database with only one study that is considered somewhat informative (Tse et al. 2017; 2018).

Table 5 Prostate Cancer Studies: Study Design and Limitations

Reference (study design)	Limitations
Salamanca-Fernández et al. 2021 (case-cohort)	Exposure assessment limitations: single sample; biological matrix analyzed (serum); relatively high limit of detection (LOD); and imputation of exposure levels for 28.3% of participants below the LOD.
Tse et al. 2017; Tse et al. 2018 (case-control)	Exposure assessment limitations: although the tool to estimate BPA exposure attempts to capture chronic exposures via the (assumed) primary source of exposure (diet), it did not capture other routes and use of this tool has not been replicated in other studies.
Tarapore et al. 2014 (cross-sectional)	Cross-sectional study with no risk estimates. Exposure assessment limitations: single sample; collection of spot urinary samples.

4 Changes to "Carcinogenicity Studies in Animals" Section

Evidence from animal carcinogenicity studies is often a key component for characterizing potential carcinogenic hazards for humans.

Long-term carcinogenicity studies (also known as animal cancer bioassays) involving chronic exposure for most of the lifespan of an animal are generally accepted as scientifically valid testing methods for evaluation of chemical carcinogenicity.

Sub-chronic and short-term animal studies are sometimes used to screen for carcinogenic effects and to evaluate preneoplastic effects. These studies are often less informative due to the short exposure and study duration (US EPA 2005), but exceptionally may provide direct evidence when adequately designed and conducted. For example, under the conditions of 13-week sub-chronic toxicity studies conducted by the National Cancer Institute (NCI), direct blue 6, direct black 38, and direct brown 95 was each found to be carcinogenic in Fischer 344 rats,[1] inducing hepatocellular carcinomas and neoplastic nodules in the liver (NCI 1978).

Other, generally less informative, animal studies for purposes of cancer hazard identification include studies using genetically engineered animal models, other types of model systems (xenograft, syngeneic, and regenerated organs) using normal cells, and initiation-promotion studies.

Co-carcinogenicity studies and xenograft studies using established cancer cells are generally considered the least informative of studies that can provide information on observations of tumors in experimental animals because of the uncertainty in attributing the tumorigenic outcome to a specific chemical exposure. While these ("less" and "least" informative) studies may shed light on potential mechanisms of action, their contributions to the determination of carcinogenicity rest on the overall consistency of evidence.

Proposed changes

We propose some changes to the approach used to summarize the carcinogenicity evidence from whole animal studies in our HIDs. In this discussion we focus on the two most recent HIDs, the PFOS and BPA HIDs (OEHHA 2021, 2022). Specifically, we propose to focus on the most informative animal carcinogenicity studies, summarizing those studies in the text and tables. For studies of less informative design, including studies using genetically modified animal models, studies using normal human cells or tissues to construct xenograft, syngeneic, and regenerated organ models, and studies

[1] Direct blue 6 and direct black 38 dyes were carcinogenic in male and female Fischer 344 rats and direct brown 95 was carcinogenic in female Fischer 344 rats.

testing for tumor initiation or promotion activity, we propose providing a brief summary of the available studies and findings, without detailed study-by-study description and tables. The least informative animal studies, such as co-carcinogenicity studies, and studies using xenograft and other model systems with established cancer cell lines, may be provided in a bibliography list with abstracts.

Proposed organization

Reporting of available animal carcinogenicity studies will be generally organized as follows.

1. Animal cancer bioassays

The available animal cancer bioassays will be noted in the overview, together with general information on species/strain/sex and route of administration. In cases where several animal cancer bioassays are available, general information on study designs may be presented in tables, e.g., species, sex/group/size, study duration, route, doses, age at exposure and exposure duration.

Each study is then presented as follows:

- A summary of study protocol (animal stains/sex, number of animals per dose group, route, administered doses, age at exposure, experimental and exposure duration) will be presented. Lifetime average daily doses (mg/kg-day) will be reported when available. Summary of survival and body weight data will be described when available.
- Tumor findings will be summarized in text and data may be presented in tables when appropriate.
- Non-neoplastic or pre-neoplastic findings will be summarized as appropriate.

2. Sub-chronic and short-term animal toxicity studies and studies with small group sizes

Animal studies with lower power to detect a carcinogenic effect include sub-chronic and short-term animal studies and studies with small numbers of animals. These studies are typically not discussed in detail but may be included when judged to be adequately designed and conducted.

3. Other types of animal carcinogenicity studies

In cases where other types of animal carcinogenicity studies are available, e.g., of alternate design with regard to use of genetically or otherwise modified animal models or exposure to multiple chemicals or treatments (e.g., partial hepatectomy), such studies may be grouped and reported as follows.

- Studies using genetically modified animal models, studies using normal human cells or tissues to construct xenograft, syngeneic, and regenerated organ models, and tumor initiation-promotion studies – key findings from each study will be briefly summarized in the text, together with general information on study design (e.g., animal model, exposure).
- Co-carcinogenicity studies with information on control group and experimental group treated with the chemical of interest only – key findings will be briefly summarized.
- Co-carcinogenicity studies and xenograft studies using established cancer cells – these studies will be included as a bibliography list provided in an appendix.

Examples of proposed changes

Example #1: Data summary of Section 4.2.2 [from BPA HID (OEHHA, 2022)], and what the section may have looked like under the proposal.

Text for animal section 4.2.2 in the BPA HID (OEHHA, 2022, pages 92-94)

4.2 Studies Exposing Rodents Beginning In Utero or within the First Week of Life

4.2.2 Other Studies in Rats

Four other early life exposure studies (e.g., in utero with or without lactational exposures) were identified in rats (Table 1). One study was conducted in males and three studies were conducted in females (Acevedo et al. 2013; Ichihara et al. 2003; Murray et al. 2007).

These studies examined the effects of BPA on the development of neoplastic and/or preneoplastic lesions with broad ranges of doses and study durations, and via different early life exposures. In the male rat study (Ichihara et al. 2003), group sizes were small (12 animals per group) and only the prostate was examined. In the three female rat studies, the mammary gland was the only tissue examined (Acevedo et al. 2013; Murray et al. 2007). Other limitations of the studies in female rats included small group size (4–12 animals per group) and short study durations (PND50 to PND200).

Studies of short exposure duration and less than lifetime study duration may reduce the power to detect significant treatment-related effects. Such study designs render these studies inadequate for assessing the carcinogenic potential of BPA. Thus, studies of less than one year study duration are not included in Section 4.2.2, unless neoplasms were observed. Excluded studies include Ho et al. (2006), Durando et al. (2007), and Takashima et al. (2001).

Table 1 Overview of other studies exposing rats to BPA beginning in utero

Strain	F1 sex, group size	Study duration	Exposure route and design	Administered dose (µg/kg-day) to F0 dams	Exposure duration	Reference
F344	M, 12	65 weeks	In utero, and via lactation, F0 dams via gavage	0, 50, 7500, 120000	GD1 to PND21	Ichihara et al. (2003)
Sprague-Dawley	F, 5–12	PND50, 90, 140, 200	In utero, F0 dams via s.c. injections	0, 0.25, 2.5, 25, or 250	GD9 to birth	Acevedo et al. (2013)
Sprague-Dawley	F, 5–12	PND50, 90, 140, 200	In utero and via lactation, F0 dams via gavage	0, 0.25, 2.5, 25, or 250	GD9 to PND21	Acevedo et al. (2013)
Wistar-Furth	F, 4–6	PND50, 95	In utero, F0 dams via s.c. injections	0, 2.5, 25, 250, 1000	GD9 to PND1	Murray et al. (2007)

M, male; F, female; PND, postnatal day; GD, gestation day; s.c., subcutaneous

65-week study in male F1 F344 rats exposed in utero and via lactation (Ichihara et al. 2003)

Pregnant female F344 rats (F0 dams) were administered BPA at 0, 0.05, 7.5, or 120 mg/kg-day (0, 50, 7500, or 120000 µg/kg-day, as presented in Table 1) via gavage (in 0.5% carboxymethyl cellulose sodium salt) from gestation day (GD) 1 to PND21. F1 pups (12 animal per group) were weaned on PND21. F1 males were terminated for histological examination at 65 weeks of age.

No treatment-related tumors were observed in male rats. Seminal vesicles and ventral and anterior lobes of the prostate were examined for preneoplastic lesions, and none were observed in either the BPA-treated or control groups.

50- to 200-day study in female F1 Sprague-Dawley rats exposed in utero (mammary gland only) (Acevedo et al. 2013)

Pregnant female dams (F0) were administered BPA (0, 0.25, 2.5, 25, or 250 µg/kg-day in 50% DMSO) s.c. via osmotic pump implants from GD9 to birth. Mammary glands of F1 females (5–12 animals per group, each litter was represented only once) were removed on PND50, PND90, PND140, and PND200 for histological analysis. On PND50, no neoplastic lesions of the mammary gland were observed. Preneoplastic lesions were observed on PND50 only in BPA treatment groups, and included atypical ductal hyperplasia (control, 0/5; BPA0.25, 3/5; BPA2.5, 1/5; BPA25, 0/5; BPA250, 2/5). At later timepoints adenocarcinomas of the mammary gland were observed only in BPA treatment groups, on PND90 (one at BPA2.5), PND140 (one at BPA250) and PND200 (one at BPA0.25). In addition, one lobular alveolar hyperplasia was observed in the BPA250 group on PND90.

50- to 200-day study in female F1 Sprague-Dawley rats exposed in utero and via lactation (mammary gland only) (Acevedo et al. 2013)

Pregnant female dams (F0) were administered BPA (0, 0.25, 2.5, 25, or 250 µg/kg-day in 50% DMSO) s.c. via osmotic pump implants from GD9 to birth and through lactation (PND1 to PND21). Mammary glands of F1 females (5–12 animals per dose per exposure group, each litter was represented only once) were removed on PND50, PND90, PND140, and PND200 for histological analysis. On PND50, ductal carcinoma in situ was observed in one BPA-treated animal (control, 0/5; BPA25, 1/5). Atypical ductal hyperplasia was also observed on PND50 in BPA-exposed groups (control, 0/5; BPA2.5, 1/5; BPA25, 1/5; BPA250, 1/6). On PND90, lobular alveolar hyperplasia was observed in one BPA25 animal. On PND140, adenocarcinomas were observed in two BPA-exposed animals (one at BPA2.5, and one at BPA25) and one lobular alveolar hyperplasia was observed in the BPA0.25 group. On PND200, one fibroadenoma was observed in the BPA2.5 group.

50- and 95-day study in female F1 Wistar-Furth rats exposed in utero (mammary gland only) (Murray et al. 2007)

Pregnant female dams (F0) were administered BPA (0, 2.5, 25, 250, 1000 µg/kg-day) in 50% DMSO via osmotic pump implants from GD9 until PND1. F1 pups were weaned on PND21, and then fed normal diet. Female F1 animals were sacrificed on PND50 or PND95 and mammary glands were removed. Only one F1 from a given litter was assigned to each group for histopathological examinations. On PND50, significant (3–4-fold, $p < 0.05$) increases in the incidence of hyperplastic ducts of the mammary gland were observed in each of the treated groups compared to controls, and mammary gland carcinoma in situ was observed in one animal in each of the two highest dose groups (control, 0; BPA250, 1/4; BPA1000, 1/4). On PND95, a significant increase in mammary hyperplastic lesions was observed in the 2.5 µg/kg-day group compared to controls ($p = 0.032$), and mammary gland carcinoma in situ was observed in two animals in each of the two highest dose groups (control, 0; BPA250, 2/6; BPA1000, 2/6).

How the section would have looked under the proposal:

4.2 Studies Exposing Rodents Beginning *In Utero* or within the First Week of Life

4.2.2 Other Studies in Rats

Several studies starting exposure early in life are available, but they are typically of one year or less in duration and use small numbers of animals per dose group. Studies of duration substantially less than lifetime typically have low or no power to detect significant treatment-related neoplastic effects. The power is further reduced by the small numbers of animals used.

Three studies of less than one year study duration did not observe tumors in any dose groups: Ho et al. (2006), Durando et al. (2007), and Takashima et al. (2001).

Four other early life exposure studies (e.g., *in utero* with or without lactational exposures) were identified in rats with small group sizes (≤12 animals per group) (Table 1). One study was conducted in males and three studies were conducted in females (Acevedo et al. 2013; Ichihara et al. 2003; Murray et al. 2007).

These studies examined the effects of BPA on the development of neoplastic and preneoplastic lesions with broad ranges of doses and study durations, and via different early life exposures. In the male rat study (Ichihara et al. 2003), only the prostate was examined. In the three female rat studies, the mammary gland was the only tissue examined (Acevedo et al. 2013; Murray et al. 2007). Study designs and tumor findings are summarized in Table 26.

Table 2 Overview of other studies exposing rats to BPA beginning *in utero*

Strain	F1 sex, group size	Study duration	Exposure period	Exposure route and design	Administered dose (µg/kg-day) to F0 dams	Tissues examined	Results (Tumor findings)	Reference
F344	M, 12	65 weeks	GD1 to PND21	*In utero*, and via lactation, F0 dams via gavage	0, 50, 7500, 120000	All major organs	No treatment related increase of tumors	Ichihara et al. (2003)
Sprague-Dawley	F, 5–12	PND50, 90, 140, 200	GD9 to birth	*In utero,* F0 dams via *s.c.* injections	0, 0.25, 2.5, 25, or 250	Mammary gland only	Mammary gland adeno-carcinomas, only in BPA treatment groups, on PND90 (one at BPA2.5), PND140 (one at BPA250) and PND200 (one at BPA0.25).	Acevedo et al. (2013)
Sprague-Dawley	F, 5–12	PND50, 90, 140, 200	GD9 to PND21	*In utero* and via lactation, F0 dams via gavage	0, 0.25, 2.5, 25, or 250	Mammary gland only	On PND50, ductal carcinoma *in situ* (one at BPA25). On PND140, adenocarcinomas (one at BPA2.5; one at BPA25). On PND200, one fibroadenoma (BPA2.5).	Acevedo et al. (2013)
Wistar-Furth	F, 4–6	PND50, 95	GD9 to PND1	*In utero,* F0 dams via *s.c.* injections	0, 2.5, 25, 250, 1000	Mammary gland only	On PND50, mammary gland carcinomas *in situ* (one at BPA250, one at BPA1000). On PND95, mammary gland carcinomas *in situ* (two at BPA250, two at BPA1000).	Murray et al. (2007)

M, male; F, female; PND, postnatal day; GD, gestation day; *s.c.*, subcutaneous

Example #2: Data summary of tumor initiation-promotion studies.

This example shows how the summary of data for initiation promotion studies would change under the proposal.

Text for initiation-promotion studies in section 4.5.3 in the BPA HID (OEHHA, 2022, page 102)

Three publications investigated the effects of BPA administered after tumor initiation in female rats. BPA significantly increased tumors in the mammary gland after initiation with diethylnitrosamine, MNU, and N-bis (2-hydroxy propyl) nitrosamine (DHPN) (Zhang et al. 2021b). BPA either significantly reduced or had no effect on thyroid carcinomas or adenomas after initiation with DHPN (Takagi et al. 2001; Zhang et al. 2017a). For more information on the treatment plan and study findings of these studies, see Appendix Table D4.

How the section would have looked under the proposal:

The text summary would remain the same but the studies would not be summarized in an Appendix Table.

Three publications investigated the effects of BPA administered after tumor initiation in female rats. BPA significantly increased tumors in the mammary gland after initiation with diethylnitrosamine, MNU, and N-bis (2-hydroxy propyl) nitrosamine (DHPN) (Zhang et al. 2021b). BPA either significantly reduced or had no effect on thyroid carcinomas or adenomas after initiation with DHPN (Takagi et al. 2001; Zhang et al. 2017a).

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