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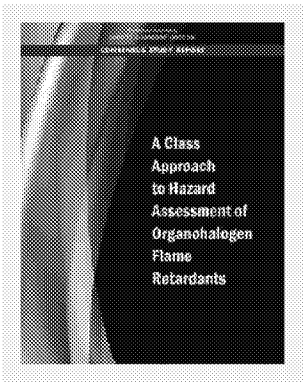

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A Class Approach to Hazard Assessment of Organohalogen Flame Retardants

Committee to Develop a Scoping Plan to Assess the Hazards of Organohalogen Flame Retardants

Board on Environmental Studies and Toxicology

Division on Earth and Life Studies

A Consensus Study Report of
The National Academies of
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Summary

One of the biggest challenges for the risk-assessment community is how to move from the traditional chemical-by-chemical approach to analyses that evaluate multiple chemicals together. The primary problems with the traditional approach are that chemicals on which data are insufficient are typically treated as not hazardous, that untested chemicals are often substituted for hazardous chemicals, and that cumulative exposure and risk are often ignored. One method of assessing multiple chemicals is a class approach in which chemicals that have similar chemical structures or physicochemical properties are evaluated together on the basis of an assumption that they have similar biologic activity. Over the last few decades, that approach has been used for a few chemical classes, including phthalates and cholinesterase-inhibiting pesticides. Although it is challenging to evaluate chemical groups, the number of chemicals in use today demands a new approach to risk assessment, and the class approach is a scientifically viable option.

In light of the momentum to regulate by chemical class, a coalition of organizations and individuals petitioned the Consumer Product Safety Commission (CPSC) to initiate regulatory action under the Federal Hazardous Substances Act (FHSA) to ban several products that contain nonpolymeric, additive organohalogen flame retardants (OFRs).[1] To decide whether a ban should be enacted, CPSC must first conduct a hazard assessment to determine whether the chemical is toxic as defined in the FHSA. The term *toxic* is applied to "any substance that has the capacity to produce personal injury or illness through ingestion, inhalation, or absorption through any body surface".[2] If the chemical is found to be toxic, CPSC conducts a quantitative risk assessment in which dose–response relationships, bioavailability, and exposure are considered to determine whether the chemical is a "hazardous substance" under the FHSA.

ORGANOHALOGEN FLAME RETARDANTS AND THE PETITION

In the 1970s, flame retardants began to be added to synthetic materials to meet strict flammability standards. Over the years, diverse flame retardants have been manufactured and used in various products. Some flame retardants have migrated out of the products, and this has led to widespread human exposure and environmental contamination. There also is mounting evidence that many flame retardants are associated with adverse human health effects. As a result, some flame retardants have been banned, restricted, or voluntarily phased out of production and use.

The petition submitted to CPSC in 2015 specified four product categories that contain OFRs: infant, toddler, or children's products; upholstered furniture; mattresses; and plastic electronic casings. The petitioners argued that the entire chemical class is toxic, that consumers are exposed to OFRs because they migrate from the products into the environment no matter how the products are used, and that their use therefore poses a risk to consumers. However, CPSC staff recommended that the commission deny the petition because OFRs constitute a broad chemical class that is defined primarily by function—to suppress combustion and increase the probability of escape from fire—rather than by any specific toxicity characteristic or chemical feature other than a halogen. Several other considerations also influenced the recommendation to deny the petition. The commission voted, however, to grant the petition, which required staff to proceed with the hazard assessment based on the chemical class. Because of the likely complexities of

[1]The abbreviation *OFR* in this report refers specifically to nonpolymeric, additive organohalogen flame retardants.

[2]CPSC (Consumer Product Safety Commission). 2017. Staff briefing package in response to petition HP15-1, requesting rulemaking on certain products containing organohalogen flame retardants. May 24, 2017. Available: https://www.cpsc.gov/content/ballot-vote-petition-hp-15-1-requesting-rulemaking-on-certain-products-containing [accessed July 18, 2018].

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an assessment of this chemical class, CPSC asked the National Academies of Sciences, Engineering, and Medicine (the National Academies) to develop a scoping plan to conduct the hazard assessment for OFRs as a chemical class.[3] As a result of the request, the National Academies convened the Committee to Develop a Scoping Plan to Assess the Hazards of Organohalogen Flame Retardants, which prepared this report.

HAZARD-ASSESSMENT SCOPING PLAN

The committee's recommended scoping plan is shown in Figure S-1 and described in detail in Chapter 2 of this report. The first step in the process is to determine whether a class approach to the chemicals of interest is viable for conducting a hazard assessment for CPSC. Answering that question might involve determining whether subclasses need to be formed if the chemicals in the class cannot all be assessed as a single class. Forming and evaluating broad subclasses is still a class approach. If a class approach is viable, the second step is to survey the literature to determine the availability of toxicity data (from human, animal, in vitro, and other relevant studies) and to identify relevant end points to investigate. If relevant data are available on any chemical for a given end point, the next steps are to extract, evaluate, and integrate the relevant data to reach a decision regarding potential hazard that can be applied to the entire class or subclass.

The committee conducted its own analysis to determine whether OFRs can be treated as a single class. It first created an inventory of 161 OFRs from several sources and then identified analogues on the basis of functional, structural, and predicted bioactivity information. To evaluate similarity, the committee compared the OFR inventory to the analogues and found that the OFRs cannot be treated as a single class for the purposes of a CPSC hazard assessment. The OFRs can, however, be divided into subclasses on the basis of chemical structure, physicochemical properties, and predicted biologic activity. The committee identified 14 subclasses that can be used to conduct a class-based hazard assessment and concluded that the best approach is to define subclasses as broadly as is feasible for the analysis; defining subclasses too narrowly could defeat the purpose of a class approach to hazard assessment.

The committee surveyed the literature and selected two subclasses—polyhalogenated organophosphates and polyhalogenated biphenol aliphatics—to illustrate various aspects of its proposed scoping plan. In conducting its case studies, the committee identified four scenarios that would likely arise in a class-based hazard assessment of OFRs. Scenario 1 involves a subclass that has many data-rich members on which data are concordant. For that scenario, the hazard determination for the subclass should be relatively straightforward.

In Scenario 2, there are no relevant data on any subclass member that can be used to conduct the hazard assessment. The lack of data should not imply that an OFR subclass is *not* hazardous. The committee identified the following options to move the assessment forward:

- Option 2-1: Generate toxicity data on the subclass. The committee recommends a tiered approach that initially relies on new approach methodologies (NAMs) that encompass computational modeling, in vitro assays in animal and human cells and tissues, and toxicity testing that uses alternative animal species, such as zebrafish. The results of such studies can help to identify potential end points of interest and one or more chemicals in the subclass for targeted animal toxicity studies.
- Option 2-2: Expand the analysis beyond the set of chemicals that were identified as OFRs and use toxicity data on structurally related chemicals.
- Option 2-3: Reclassify the subclass so that data-poor members are distributed in other data-rich subclasses. Many OFRs have multiple functional groups and could be placed in multiple subclasses; reclassification might help to minimize the number of data-poor categories. Confidence in the reclassification can be increased when concordant biologic responses are seen among the members of the newly expanded subclasses, for example, if additional data show a common mechanism or effect.

[3]The verbatim statement of task is provided in Chapter 1 of this report.

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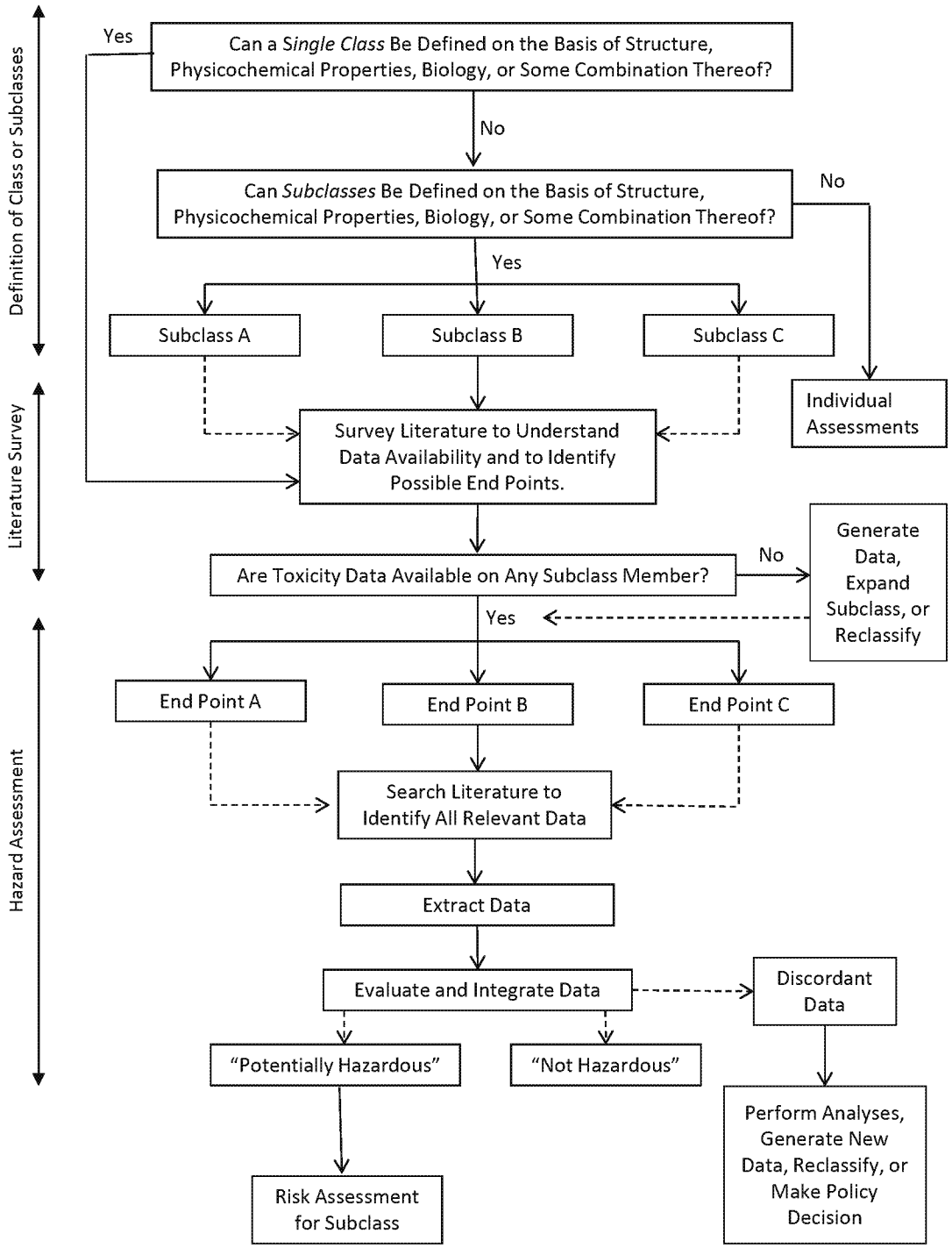

FIGURE S-1 Scoping plan to conduct a hazard assessment for the Consumer Product Safety Commission (CPSC) by using a class approach. *Toxicity data* is an inclusive term that refers to data from human, animal, in vitro, and other studies for end points relevant for CPSC. *End point* is used here to refer to toxic effects that CPSC considers relevant for hazard assessment under the FHSA. *Literature* is used broadly here to refer to scientific literature and databases.

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In Scenario 3, there are sufficient coherent data on one or two chemicals for assessment, but there are few or no data on the other class members. The few available data, however, might suggest that the subclass members have similar biologic activity. In this case, the committee identified the following possible options to move the assessment forward:

- Option 3-1: Make a science-based policy decision, for example, to classify the subclass as potentially hazardous on the basis of the data-rich chemicals in the subclass.
- Option 3-2: Use the data-rich chemicals to serve as an anchor as suggested above and extrapolate or interpolate to other chemicals in the subclass.
- Option 3-3: Generate toxicity data on data-poor subclass members to the extent that satisfactory confidence is gained; testing could involve NAM studies, targeted animal testing, or a combination thereof.

Scenario 4 is the most difficult to address. There are data on some chemicals in the subclass but few or no data on others, and the available data are so heterogeneous or inconsistent with respect to biologic activity that a discordant-data designation is reached. The committee identified the following possible options, which are discussed in further detail in Chapter 3:

- Option 4-1: Make a policy decision, for example, to extend the most conservative conclusion regarding hazard to the subclass.
- Option 4-2: Reclassify members in such a way that biologic similarity is improved; generate data to increase confidence that reclassification has resulted in biologically similar members.
- Option 4-3: Perform analyses that would help to explain the discordance and allow the assessment to move forward.
- Option 4-4: Generate new data that could increase clarity and the scientific basis of a decision.

CONCLUSIONS

In this report, the committee has provided a scoping plan for using a class approach to hazard assessment and illustrated aspects of the plan with case studies. Ultimately, the time and resources required to implement the plan will depend on several policy decisions that are beyond the committee's charge. For example, CPSC will need to decide whether it will accept NAM data to set testing priorities for chemicals or to conduct its hazard and risk assessments. If not, the cost and time implications are dramatic and a class-based hazard assessment for all relevant OFRs will be unlikely to be achieved. Specifically, relying solely on traditional (whole-animal) toxicology studies will require resources that are orders of magnitude greater than would be needed if NAM data or a combination of NAM data and targeted animal studies were used. CPSC will also have to determine the type and quantity of data necessary to achieve the confidence needed to draw conclusions about hazard and risk. If CPSC requires some data on each chemical in a subclass, the cost and time implications again are substantial. Moreover, as noted, the type of data required will have a strong bearing on the resources required. Ideally, the class approach provides a mechanism for extrapolating data on data-rich chemicals to data-poor chemicals and eliminates the need to collect data on all chemicals in a specific class.

The committee hopes that the scoping plan that it has described will give CPSC a means to use a class approach to assessing the hazards posed by OFRs. A class approach will likely result in increases in efficiency and decreases in cost compared with the traditional approach of evaluating individual chemicals. Although the challenges to a class approach might appear daunting, the alternative—individual assessments of hundreds of chemicals—is unrealistic. The only possible practical approach for a set of chemicals as large as the OFRs is a class approach.

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1

Introduction

In the 1970s, flame retardants began to be added to synthetic materials to meet strict flammability standards. Over the years, a diverse array of flame retardants have been produced and used in various products. Some flame retardants have migrated out of the products and resulted in widespread human exposure and environmental contamination (Iqbal et al. 2017), and there is mounting evidence that many flame retardants are associated with adverse human health effects (Linares et al. 2015; Hou et al. 2016). As a result, some flame retardants have been banned, restricted, or voluntarily phased out of production and use.

In 2015, a petition was submitted to the Consumer Product Safety Commission (CPSC) to initiate regulatory action under the Federal Hazardous Substances Act (FHSA) that would ban nonpolymeric, additive organohalogen flame retardants (OFRs)[1] in four product categories (Gartner and Weintraub 2015). To decide whether a ban should be instituted, CPSC first conducts a hazard assessment to determine whether the chemical in question is "toxic" as defined in the FHSA and, if so, to conduct a quantitative risk assessment that considers dose–response relationships, bioavailability, and exposure to determine whether the chemical is a "hazardous substance" under the FHSA.[2] The petition was unique in that it requested action on an entire chemical class rather than a single chemical. Because of the complexities of conducting a hazard assessment of a chemical class, CPSC asked the National Academies of Sciences, Engineering, and Medicine (the National Academies) to develop a scoping plan for the hazard assessment of OFRs as a chemical class. As a result of the request, the National Academies convened the Committee to Develop a Scoping Plan to Assess the Hazards of Organohalogen Flame Retardants, which prepared this report.

CONCEPTUAL ADVANTAGES OF A CLASS APPROACH

Regulatory evaluation of chemical hazards and risks has traditionally relied on a chemical-by-chemical approach wherein a regulator reviews the full suite of available hazard, dose–response, and exposure data on an individual chemical and determines whether the information is sufficient to support an assessment of hazard or risk. If the information is sufficient, the process proceeds in a manner similar to that described in *Risk Assessment in the Federal Government: Managing the Process* (NRC 1983). If the regulator considers the available information to be insufficient, the assessment of hazard or risk is not conducted. Over the last decade, the National Academies and others have identified three main problems with that approach.

- *Chemicals on which data are insufficient are often deemed not hazardous.* Excluding from risk assessments chemicals that have been insufficiently tested was criticized in the report *Science and Decisions: Advancing Risk Assessment* (NRC 2009). The committee that wrote that report pointed out that "with few notable exceptions (for example, dioxin-like compounds), [chemicals on which data are insufficient] are treated as though they pose no risk that should be subject to regulation" (p. 194). That assumption was characterized by the committee as a "missing default" assumption

[1]The abbreviation *OFRs* in this report refers specifically to nonpolymeric, additive organohalogen flame retardants.

[2]The FHSA defines *toxic* as applying to "any substance that has the capacity to produce personal injury or illness through ingestion, inhalation, or absorption through any body surface [15 USC § 1261(g)]" and defines *hazardous substance* as having "the potential to cause substantial personal injury or substantial illness during or as a result of customary handling or use [15 USC § (f)(1)(A)]" (CPSC 2017, pp. 10 and 11).

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in traditional regulatory risk assessment. The "no data, no risk" default assumption is used even if there are hazard predictors, such as structural similarities and biologic activity patterns analogous to those of chemicals that are known to be harmful.

- *Untested chemicals are often substituted for known hazardous chemicals.* In a regulatory system in which each chemical is evaluated separately, the responsible agency predictably moves slowly and deliberately through a sequence of discrete chemical assessments. In practice, observers have noted and described a risk of regrettable substitution (Wilson and Schwarzman 2009) in which a manufacturer responds to regulatory signals by phasing out the use of a specific chemical and replacing it with a substitute that is chemically similar and relatively untested (NRC 2014). In some cases, that practice has led to widespread introduction of substitutes about which little is known and that are not necessarily safer (Sartain and Hunt 2016).
- *Cumulative exposure and risk are often ignored.* An approach that addresses one chemical at a time does not consider cumulative risks that might be posed by exposure to multiple chemicals that act via a similar mechanism or that perturb the same biologic system. The report *Phthalates and Cumulative Risk Assessment: The Tasks Ahead* (NRC 2008) included the caution that "phthalates may not all act by the same mechanisms, and they do not have parallel dose–response curves. However, those facts do not negate the appropriateness of using general dose-addition methods in a cumulative risk assessment" (p. 9). The concern expressed by the committee that wrote that report applies to other classes of chemicals if chemicals in those classes have similar activity in biologic systems.

Ultimately, the sheer number of chemicals in use today demands a new approach to risk assessment. As articulated by NRC (2011), "the great number of chemicals of potential concern is always increasing. The vast array of chemicals that are potential environmental contaminants include… [too many] to address by the chemical-by-chemical approach of toxicity testing in animals of each health effect of concern and then predicting human risk" (p. 83).

If scientifically supportable approaches, such as read-across and evaluation of chemicals by category or class, can allow extrapolation from relatively well-studied chemicals to data-poor chemicals, the problem of regrettable substitution can begin to be addressed. Assessing chemicals as classes would also make regulatory hazard and risk assessment much more efficient. Finally, if the "no data, no risk" presumption were no longer the default, those wishing to continue manufacturing or using a chemical would have greater incentives to generate data to demonstrate safety. The movement toward a class approach to hazard or risk assessment provides the basis of the petition submitted to CPSC to ban OFRs.

PETITION TO BAN ORGANOHALOGEN FLAME RETARDANTS IN SELECTED CONSUMER PRODUCTS

OFRs have been used in various consumer products, and the petition submitted to CPSC in 2015 specified four product categories containing OFRs: infant, toddler, or children's products; upholstered furniture; mattresses; and plastic electronic casings. The petitioners argued that the chemicals as a class are toxic and that consumers are exposed to them because the chemicals "migrate out of the products regardless of how the product is used" (CPSC 2017, p. 31). Therefore, their use poses a risk to consumers.

CPSC staff investigated various aspects of the petition and concluded that OFRs constitute a broad chemical class that is defined primarily by function—to suppress combustion and increase the probability of escape from fire—rather than by any specific toxicity characteristic or chemical functional group other than a halogen. Furthermore, there are no (or too little) data on many OFRs to base a decision about toxicity. Regarding exposure, CPSC staff noted that biomonitoring data and house-dust samples show that humans are exposed to OFRs, but the data do not indicate the exposure source. Thus, one cannot link the exposure data to the products noted in the petition. CPSC staff also noted that substantial resources would be required to develop test protocols for all OFRs—protocols that would be needed to conduct a market survey to

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support regulatory action. For those and other reasons, CPSC staff recommended that the commission deny the petition. The commission voted on September 20, 2017, however, to grant the petition and directed

> staff to convene a Chronic Hazard Advisory Panel... to assess and issue a report on the risks to consumers' health and safety from the use of OFRs, as a class of chemicals, in the following products: (1) durable infant or toddler products, children's toys, child care articles or other children's products (other than children's car seats); (2) upholstered furniture sold for use in residences; (3) mattresses and mattress pads; and (4) plastic casings surrounding electronics.

Given the complexity of the task to assess the hazards posed by OFRs as a class, CPSC has asked the National Academies to develop a scoping plan for doing so.

STATEMENT OF TASK

The committee that was convened as a result of the CPSC request included experts in toxicology, epidemiology, pharmacology, computational toxicology and chemistry, and risk assessment. Biographic information on the committee is provided in Appendix A. The committee was asked to survey the hazard data available on OFRs, to identify at least one scientifically based approach to evaluate OFRs as a class for hazard assessment, and to recommend approaches for conducting research needed to evaluate OFRs under the FHSA. The verbatim statement of task is provided in Box 1-1.

BOX 1-1 Statement of Task

At the request of the Consumer Product Safety Commission (CPSC), an ad hoc committee of the National Academies of Sciences, Engineering, and Medicine (NASEM) will develop a scientifically based scoping plan to assess additive, nonpolymeric organohalogen flame retardants (OFRs) as a class for potential chronic health hazards under the Federal Hazardous Substances Act (FHSA), including cancer, birth defects, and gene mutations. In developing the plan, the NASEM committee will complete the following tasks:

1. Survey available hazard data for OFRs and identify data needed (what exists and where there are data gaps) for a Chronic Hazard Advisory Panel (CHAP) to conduct a class-level hazard assessment.[1]
2. Identify one or more approaches to scientifically assess the potential for treating OFRs as a single class for purposes of hazard assessment.
3. Provide a plan, based on information gained from tasks (1) and (2) above, that will contain recommendations on how to most efficiently and effectively conduct research needed to evaluate OFRs under the FHSA, including timeline and cost estimates for obtaining scientific information and for executing the plan. The plan will focus on evaluation of OFR toxicity.

The product of the committee's work will be a brief consensus report. The report will include methods to conduct any needed research to evaluate toxicity of OFRs as a class. NASEM will develop the plan, taking into account that the plan, when executed, will provide a hazard assessment of OFRs as a class that will be used by a CHAP, along with data on exposure and human health effects, to complete a quantitative risk assessment. To that end, CPSC needs the hazard assessment plan as envisioned by NASEM, when executed, to be able to be readily integrated with a separate quantitative exposure assessment to complete a human health risk assessment. The ultimate CPSC goal is to assess the risk to human health posed by exposure to any OFR from the four categories of consumer products.

[1]Although some scientific review will be required, the goal is to produce a plan, with costs, for a subsequent committee or panel to do the risk assessment of OFRs as a class.

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COMMITTEE'S APPROACH TO ITS TASK

To complete its task, the committee held four meetings, which included two open sessions at which the committee heard from the sponsor and interested stakeholders. In interpreting its task, the committee considered what it meant to conduct a class approach for hazard assessment. There is no consensus in the literature on exactly what constitutes a class approach, and there are few examples of the use of such an approach, although the list is growing. The committee concluded that a science-based class approach does not necessarily require one to evaluate a large chemical group as a single entity for hazard assessment. That is, an approach that divides a large group into smaller units (or subclasses) to conduct the hazard assessment is still a class approach for purposes of hazard or risk assessment. The committee also uses several terms in this report that might be unfamiliar to some readers or that have been defined in varied ways in the scientific literature. For convenience, those terms are provided in Box 1-2 and defined as used in this report.

As directed in the task statement, the committee focused on hazard assessment—one component of risk assessment—and therefore did not consider exposure in its scoping plan. However, it was asked to develop the scoping plan with the assumption that the hazard assessment will need to be integrated with a separate quantitative exposure assessment to complete a human health risk assessment. Chapter 2 considers the implications of the class-based hazard assessment for the other components of risk assessment.

ORGANIZATION OF THIS REPORT

The report is organized into three chapters and four appendixes. Chapter 2 provides the committee's scoping plan for a class approach to hazard assessment and discusses the implications of a class approach for the other components of risk assessment (dose–response assessment, exposure assessment, and risk characterization) and for efficiency and cost. Chapter 3 provides examples or case studies to illustrate various steps in the committee's scoping plan. Appendix A provides biographic information on the committee members. Appendix B provides details of the committee's class analysis of OFRs, and Appendix C provides the details of the committee's literature survey and searches. Appendix D provides details of zebrafish studies on selected OFRs discussed in Chapter 3.

BOX 1-2 Definitions of Terms Used in This Report

Bioinformatics is "conceptualizing biology in terms of macromolecules (in the sense of physical-chemistry) and then applying 'informatics' techniques (derived from disciplines such as applied maths, computer science, and statistics) to understand and organize the information associated with these molecules, on a large-scale" (Luscombe et al. 2001, p. 346).

Cheminformatics is the application of computer and informational techniques to chemistry to predict chemical and biologic properties of compounds on the basis of their chemical structure. Also known as chemoinformatics and chemical informatics.

Chemotype is a "representation that incorporates chemical structure, physicochemical properties, and biological information all together. A chemotype thus serves to link a chemical structure to a toxicity pathway" (Cherkasov et al. 2014, p. 23).

New approach methodologies (NAMs) typically represent modern approaches to toxicity testing rather than traditional laboratory animal studies, although they have been defined in various ways in the scientific literature. In this report, NAM studies encompass computational modeling, in vitro assays in human and animal cells and tissues, and toxicity testing that uses alternative animal species, such as zebrafish and nematodes. The committee acknowledges that many of these techniques have been used for decades in toxicology and are not necessarily "new"; however, the potential application of these data to regulatory toxicology is recent.

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2

Hazard Assessment Scoping Plan

The committee developed a general strategy for using a class approach for hazard assessment, which is shown in Figure 2-1. The first step is to determine whether a class approach is appropriate for the chemicals of interest. As noted in Chapter 1, answering that question might involve determining whether subclasses need to be formed if it is not possible to treat all chemicals as a single class. If a class approach is appropriate, the second step is to survey scientific literature or databases to assess the availability of toxicity data (from human, animal, in vitro, and other relevant studies) and to identify end points to investigate. If data on any chemical for a given end point are available, the next steps are to extract, evaluate, and integrate the relevant data to reach a decision regarding potential hazard that can be applied to the entire class or subclass. Whenever possible, gaps in the data on individual chemicals should be resolved by interpolation or extrapolation of data on other members of the class or subclass. This chapter discusses the key steps in further detail and provides options for managing discordant data or addressing the no-data scenario. It concludes by discussing the implications of a class approach for risk assessment and for cost and efficiency. Chapter 3 provides examples or case studies that illustrate the committee's general strategy for nonpolymeric, additive organohalogen flame retardants (OFRs).[1]

As the committee developed its scoping plan, it became clear that a multidisciplinary group is needed to execute the plan. Expertise needed includes cheminformatics, computational chemistry, computational toxicology, traditional and modern toxicology, epidemiology, and risk assessment. Furthermore, integrating the evidence at various steps will require expert judgment, and policy decisions will probably be needed to complete the assessment. For example, decisions involving what health end points to investigate, how much weight to assign a given end point, and how much uncertainty is acceptable bring value judgments into the hazard-assessment process that are beyond the scope of this report and are not discussed further here.

DETERMINE THE VIABILITY OF A CLASS APPROACH

Several methods can be used to determine whether a class approach can be applied to a chemical group to conduct a hazard or risk assessment. In this section, the committee first describes general approaches that have been used and then specifically what has been considered for flame retardants. The section concludes with the committee's strategy for determining the viability of a class approach for an OFR hazard assessment.

Past Efforts to Assess Chemicals as Classes or Categories

The scientific and regulatory community has accepted the evaluations of chemicals as groups. For example, in the 1980s, toxic equivalency factors for dioxin-like chemicals were developed on the basis of relative potency, and this allowed assessment of these chemicals as a class (Van den Berg et al. 1998). In the late 1990s, the US Environmental Protection Agency (EPA) developed a chemical-category approach under the High-Production Volume Challenge Program (65 Fed. Reg. 81686 [December 26, 2000]).

[1]The abbreviation *OFRs* in this report refers specifically to nonpolymeric, additive organohalogen flame retardants.

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Hazard Assessment Scoping Plan

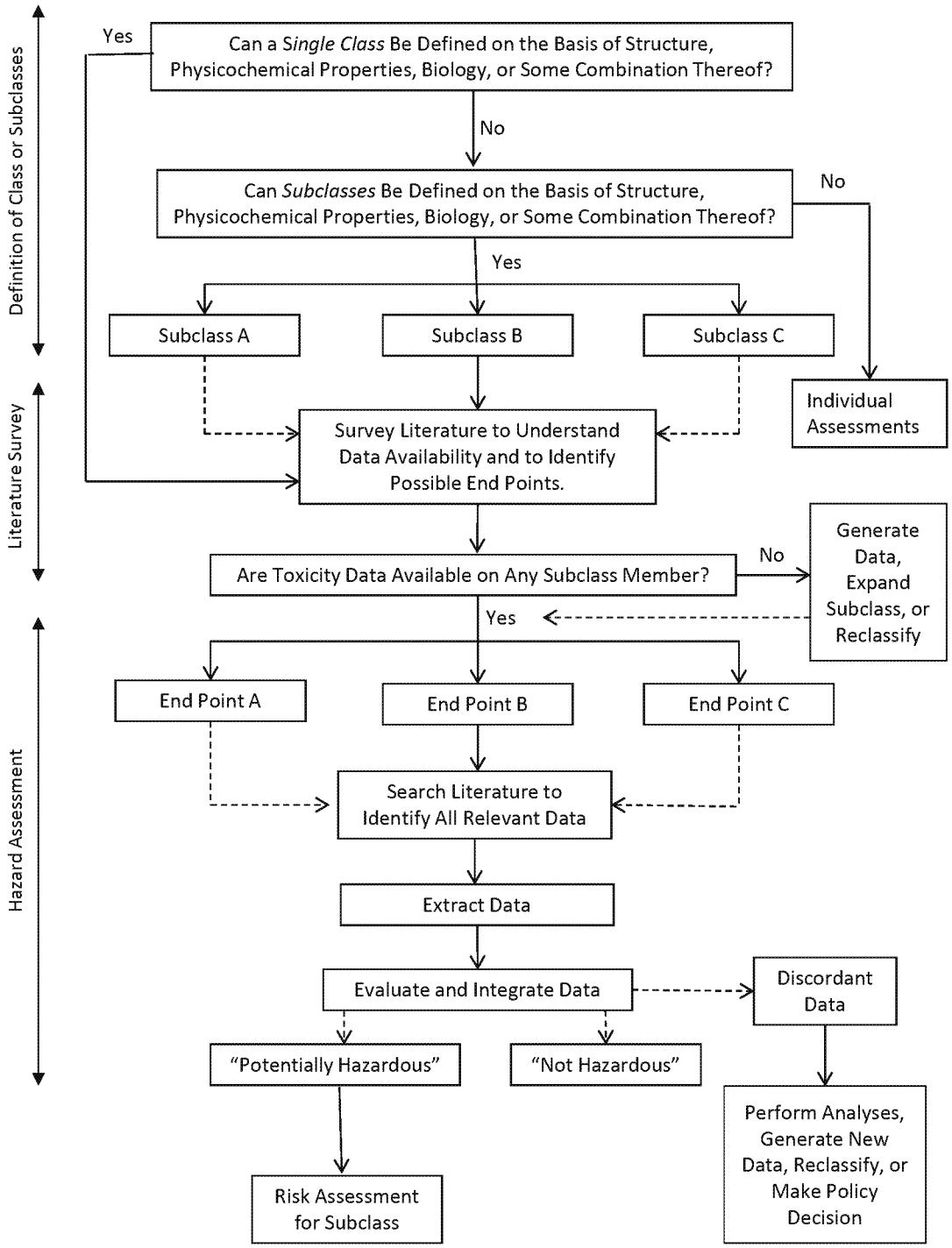

FIGURE 2-1 Scoping plan to conduct a hazard assessment for the Consumer Product Safety Commission (CPSC) by using a class approach. *Toxicity data* is an inclusive term that refers to human, animal, in vitro, and other studies for end points relevant for CPSC. *End point* is used here to refer to toxic effects that CPSC considers relevant for hazard assessment under the FHSA. *Literature* is used broadly here to refer to scientific literature and databases.

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That approach allowed some extrapolation of data on tested chemicals to similar but untested chemicals as a way to reduce animal testing. And EPA assessed the cumulative risk associated with the class of cholinesterase-inhibiting pesticides in the 2000s (71 Fed. Reg. 43740 [August 2, 2006]) and then developed a framework and guidance document for cumulative risk evaluations of pesticide classes (EPA 2016). EPA is now considering chemical categories in the New Chemicals Program of the Toxic Substances Control Act (TSCA) (Henry 2017). As of December 2017, 56 chemical categories have been defined in the TSCA program, including photo-acid generators, tracer chemicals, and perfluorinated chemicals. The Consumer Product Safety Commission (CPSC) also used a class approach to assess chemicals when it convened a chronic hazard advisory panel to evaluate several phthalates (CPSC 2014).

Agencies in other countries have explored evaluation of chemical groups. For example, Phase 2 of Canada's Chemicals Management Plan in 2011–2016 included a substance-grouping initiative that used alternative approaches, such as computational and read-across methods to screen and set priorities for chemical groups. That approach was applied to azo-based and benzidine-based substances, phthalates, and others. Health Canada and Environment and Climate Change Canada (HCECCC 2017, p. 14) set a long-term goal to "move away from substance by substance assessment approach toward priority setting on emerging classes of concern"; they envision an important role for predictive toxicology.

The European Chemicals Agency (ECHA) developed an approach to assessment of chemical categories under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation, which has allowed companies to use read-across within chemical categories for chemical assessment in lieu of testing in some situations (ECHA 2009, 2010). ECHA (2010) defines a category as "substances whose physicochemical, toxicological and ecotoxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity" (p. 21). Similarities might be based on a common functional group, a common precursor or break-down products, a constant pattern of changing potency, common constituents, or chemical classes.

The Organisation for Economic Co-operation and Development (OECD) issued guidance on grouping of chemicals in 2007 and updated it in 2014 (OECD 2014). OECD defines chemical grouping as the general approach for considering more than one chemical at a given time and provides guidance on performing analogue and category approaches. The analogue approach is used when the focus is on filling gaps in data on a single chemical whereas the category approach is used for assessments of multiple chemicals. Both approaches have rationales based on common functional groups (such as aldehyde, epoxide, ester, or specific metal ion), a common mechanism, common constituents or chemical classes or similar carbon range numbers (frequently related to substances of unknown or variable composition), likelihood of common precursors or breakdown products that result in structurally similar chemicals, or an incremental and constant change throughout the category (such as a chain-length category), as is often observed in physicochemical properties (such as boiling point range) (OECD 2014). The agency's guidance on developing categories is presented in Box 2-1.

BOX 2-1 OECD Guidance on Development of Categories under REACH

Step 1 – Identify similar substances/analogues to form a category; build category hypothesis and definition; link data for members by chemical similarities
Step 2 – Gather data from each category member, including impurities and transformation products
Step 3 – Evaluate available data for adequacy under REACH information requirements
Step 4 – Construct a matrix of data availability
Step 5 – Perform a preliminary evaluation of the category and identify data gaps
Step 6 – Propose and perform additional testing, if necessary
Step 7 – Further assessment of the category based on new test data
Step 8 – Documentation and justification of the finalized category and its rationale

Source: OECD 2014.

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The OECD guidance discusses interpolation and extrapolation of data within a category and the potential need to form subcategories when chemicals in a category do not align. The OECD guidance emphasizes the high level of uncertainty associated with extrapolation across an entire data-poor category on the basis of little information. It recommends category test plans, which are "designed to provide information to characterize the category as a whole rather than fill in every data point for every chemical in the category" (OECD 2014, p. 15). OECD points out that its approach to filling data gaps can increase efficiency and save animals and money. It also advises identifying all potential members of a category at the start and cautions that omitting chemicals because they are not widely used, not manufactured by particular companies, or not used for a stated purpose could introduce bias into the category (OECD 2014).

Individual companies and industry consortia have also explored ways to use read-across that is complemented by in vitro predictive screening data to evaluate hazard and risk of chemical groups. For example, an initiative co-funded by the European Commission and Cosmetics Europe published an approach to group evaluations of chemicals, set priorities among several groups for further evaluation, and stated an intention to continue to work together to develop case studies (Berggren et al. 2015).

Evaluation of the general approaches to forming chemical classes at various regulatory agencies and nonregulatory consortia reveals that there has been a growing understanding of the advantages and potential pitfalls of defining chemical hazard and risk in a class context. Acceptance of a class approach has also grown. Computational techniques, such as read-across, have greatly enhanced the ability to perform class-based assessments (Blackburn and Stuard 2014; Berggren et al. 2015). The approaches used by EPA, ECHA, OECD, and others to date, however, have defined chemical classes or categories narrowly. The examples above show that some classes have been defined solely by structure, others by a common metabolite, and still others by a mechanism of action. The large class of OFRs, in contrast, is defined by a combination of chemistry and functional use (flame retardant). The formation of such a class is outside the criteria defined by most US and international agencies so far.

One recent, more innovative approach to defining a class was described in a publication by researchers in the EPA Office of Research and Development and the National Toxicology Program (Patlewicz et al. 2019). Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are a large class of chemicals defined by structural features and chemical properties. The challenge was to identify a subset of PFAS for testing with the goals of supporting read-across within structure-based subgroups and capturing the diversity of the broader PFAS class. The researchers began with the DSSTox chemical library to identify chemicals that included relevant structural features, next narrowed the list to generate a library of PFAS, and then categorized the library into subclasses on the basis of structure. The investigators finally selected a set of 75 members of the class that represented 34 subclasses. Those substances are undergoing testing with an array of new approach methodologies (NAM).[2]

Past Attempts to Define Flame-Retardant Classes

There have been efforts to group OFRs in a regulatory context. For several years, EPA has been evaluating various flame retardants as "clusters" under TSCA.[3] Examples of such clusters include chlorinated phosphate esters, cyclic aliphatic bromides, brominated phthalates, and the tetrabromobisphenol A cluster. EPA justified the formation of flame-retardant clusters by saying that "grouping and evaluating flame retardants with similar characteristics together, rather than individually, will help EPA to more efficiently evaluate existing data and support more informed decisions about data gaps and needs."

The European Food Safety Authority (EFSA) published and assessed six groups of brominated flame retardants in food from 2010 to 2012. The EFSA groups included bisphenols (EFSA 2011a), phenols and derivatives (EFSA 2012a), diphenyl ethers (EFSA 2011b), alicycles (such as hexabromocy-

[2]As noted in Chapter 1, NAM studies encompass computational modeling, in vitro assays in human and animal cells and tissues, and toxicity testing using alternative animal species, such as zebrafish and nematodes.

[3]See https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/fact-sheet-assessing-risks-flame-retardants.

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clododecanes) (EFSA 2011c), and biphenyls (EFSA 2010). It also considered other emerging and novel flame retardants (EFSA 2012b).

The Danish Environmental Protection Agency (Danish EPA) applied a more systematic class approach by using cheminformatics and quantitative structure–activity relationship (QSAR) tools to group 67 brominated flame retardants (Danish EPA 2016). A commercially available structural-feature set was applied to group chemicals. The Danish analysis resulted in 15 preliminary structural classes and seven remaining substances classified as singletons (single chemicals that had mixed modes of action that were not assignable to one of the 15 classes). The agency then focused on the category of small linear and branched alkyl alcohols for further evaluation. That category included four of the initial 67 flame retardants but was expanded to include other members of the structural category, regardless of whether they were currently used as flame retardants or even had CAS numbers. The exercise to expand the initial set resulted in 62 chemicals in the category. QSAR evaluation identified structural alerts for mutagenicity and carcinogenicity. A literature review identified three members of the category on which there were relevant experimental data and found that they had demonstrated mutagenic or genotoxic effects. The Danish EPA recommended additional steps to enhance the basis of read-across in this category, specifically inclusion of additional structural analogues outside (but structurally similar to) the category, selective additional testing of several more members of the category, and further exploration of the underlying mechanisms of action.

Outside the risk-assessment context, California's Environmental Contaminant Biomonitoring Program developed a process for defining chemical classes that combined structure and functional use (Krowech et al. 2016). That approach was adopted by the California Safer Consumer Products Program (DTSC 2013). In that context, the California Office of Environmental Health Hazard Assessment conducted a hazard identification of a class of "brominated and chlorinated chemical compounds used as flame retardants" and a separate identification of "non-halogenated aromatic phosphates," which also included some flame retardants (Krowech et al. 2016, p. A222). The biomonitoring hazard identification resulted in the adoption of both classes as candidate chemicals for potential regulation in consumer products. The two class identifications, however, have not been used to conduct risk assessments.

Committee Strategy to Determine Viability of Class Approach for OFRs

OFRs have several characteristics that could define them as a single class, including some physicochemical properties, their use as flame retardants, or generation of specific combustion byproducts. Those characteristics could define them as a single class for some decision contexts but are not entirely workable for conducting a hazard or risk assessment under the CPSC regulations. The committee considered the decision context and its charge and recognized the need to perform a regulatory hazard assessment that would be followed by a risk assessment focused on exposure to the consumer. Ultimately, the committee's approach embraces the class concept, and it recommends a method to evaluate the hazard posed by OFR subclasses created on the basis of a combination of structural characteristics, physicochemical properties, and biology. The committee concludes that it is scientifically justifiable to assess OFRs by using a class approach and that extrapolation of hazard from subclass members on which there are some data to other members on which there are no data is appropriate and likely necessary to address data deficiencies.

The committee recommends that CPSC take a multistep approach to evaluating OFRs and forming subclasses. Chapter 3 illustrates how CPSC can execute this approach with details provided in Appendix B. The multistep method has the following general steps:

1) Identify and characterize a "seed" set of chemicals as a working inventory of the class.
2) Generate an "expanded" set of chemical analogues of the seed set on the basis of combined functional, structural, and predicted bioactivity information.
3) Evaluate the similarity of the seed set to the analogues to evaluate whether the OFRs are distinguishable as a single class.
4) Define subclasses for hazard evaluation.

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Optimally, all chemicals of interest would fall neatly into subclasses. However, depending on the extent of heterogeneity tolerated and how the subclasses are defined, some chemicals could be outliers. The groupings of brominated flame retardants produced by the Danish EPA, for example, identified a number of chemicals that did not fit within the classification scheme. When the present committee approached grouping of OFRs, it considered various potential classifications that would generate a number of subclasses of only one or two chemicals. After considering the advantages and disadvantages of "lumping" vs "splitting" the subclasses, the committee concluded that merging the individual (or few) chemicals into the most closely related larger subclass would be the best approach to support a hazard assessment of the chemicals. That approach is recommended because the purpose of forming chemical classes is to allow evaluation of groups of chemicals; defining the classes too narrowly would generate multiple outliers or singletons and could frustrate the entire purpose of a class approach to hazard assessment. The committee therefore recommends defining chemical classes or subclasses as broadly as is feasible for the analysis.

The committee acknowledges that there are several scientifically valid approaches to forming OFR subclasses and has discussed approaches used by other organizations or agencies, such as EFSA and the Danish EPA, in the previous section. It demonstrates its approach in Chapter 3 and provides details in Appendix B. At this stage, the committee does not find that biology can be used as a primary driver for subclass formation because the experimental data available are not adequate for doing so. That approach also diminishes the advantage of a class approach in which one can extrapolate conclusions from data-rich to data-poor chemicals (that is, data are not needed for all subclass members). However, in the future, NAM data could greatly enhance subclass formation, especially if a fit-for-purpose high-throughput in vitro system that has adequate biologic coverage is developed and validated for use with OFRs. One can imagine testing all chemicals in such a system and using the data to group the chemicals of interest to improve classification methods.

Regardless of the classification method used, the committee recommends avoiding the temptation to reclassify chemicals in an iterative fashion as chemicals are added to a class or as a new dataset becomes available. Although it is tempting to use each increment of additional information to rerun the classification and refine the subclasses, such an exercise could paralyze action and make it difficult or impossible to move forward with a hazard evaluation of the class. Reclassification might be appropriate, however, if substantive new data on several class members become available or if computational models that could substantially improve classification methods emerge.

SURVEY THE LITERATURE

Once subclasses of OFRs have been formed, the next step is to survey the literature to determine the extent, range, and nature of toxicity data (human, animal, in vitro, and other relevant studies) and to identify end points that deserve investigation (NASEM 2017a; NRC 2014). The term *literature* is used broadly here to refer to scientific literature and databases. The survey strategy should be developed in consultation with a librarian or other information specialist and should include at least two databases.

One outcome of the survey is development of an evidence table or map that provides a descriptive or visual summary of data availability (Miake-Lye et al. 2016; NRC 2014; Wang et al. 2016). Evidence tables or maps help to identify data-rich subjects on which systematic reviews and meta-analyses might be conducted. The evidence table or map can also help to identify data gaps that might need to be addressed before a class-level hazard assessment is conducted. In other cases, the evidence table or map might help to identify one or more well-studied chemicals within an OFR subclass that can be used to anchor the hazard assessment of the subclass. Well-studied chemicals can be used to identify the hazards of concern and help to set priorities for future research on less well-studied members of the subclass. It is possible that the survey will find that no data are available to support a hazard assessment for a subclass.

The survey should also help with the development of an analysis plan. It is important that the hazard assessment be conducted in a transparent and reproducible manner, and one means of achieving that is to develop an analysis plan that documents the objectives of the hazard assessment and includes a descrip-

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tion of the end points of interest and the methods used to perform the analysis. Some approaches will need to be developed during the conduct of a class-based hazard assessment; thus, an a priori description of all methods is probably not feasible. Instead, the analysis plan can be developed iteratively and updated throughout the process with the changes documented.

Specifically, the analysis plan should clearly identify the end points to be investigated and the relevant data streams—for example, experimental animal, epidemiologic, and NAM studies—that will be considered in the analysis. The analysis plan should clearly identify the type of review, such as a systematic review (IOM 2011), that will be used. When appropriate, one or more focused research questions can be developed to guide the search for data and to develop appropriate inclusion and exclusion criteria.[4] Depending on the outcome of various stages of the assessment, the analysis plan might need to be revised to document the approaches used to address data gaps and integrate the data within or across end points of interest. Ultimately, the goal of the analysis plan is to search, screen, extract, evaluate, and integrate data systematically from all relevant studies that are included in the review.

SEARCH THE LITERATURE AND EXTRACT DATA

Once the survey and analysis plan have been completed, a thorough literature search is conducted. Again, the term *literature* is used broadly here to refer to all scientific literature and databases that might contain any relevant data. The literature search differs from the literature survey; the literature survey was meant only to provide a broad understanding of data availability on various possible end points, whereas here, the goal is to identify *all* relevant studies that can potentially be used to assess a given end point for the subclass members. Different approaches to capturing the literature include systematic reviews, scoping reviews, rapid reviews, and mapping reviews (Grant and Booth 2009; Peters et al. 2015). The approach used will depend partly on data availability, timeframe, and resources, but whatever approach is selected should be transparent and reproducible and documented in the analysis plan. Specifying how the search will be conducted and documenting the results will provide assurances of the quality of the methods used both when studies are found and when they are not.

The literature search should aim to identify NAM studies in addition to traditional animal toxicity and epidemiologic studies. NAM data can be critically important in addressing human health concerns not well addressed by traditional animal toxicity studies. In drug-induced liver injury, for example, negative findings in animal studies did not predict later findings of human toxicity in clinical trials or after entry into the marketplace. The later findings led to the identification of interspecies differences—such as in immune responses and the handling of bile acids—that helped to explain some of the limitations of the animal-based predictions. The class approach proposed here would use the breadth of modern experimental and computational modeling methods to facilitate hazard assessment.

Once the relevant literature has been identified, the studies should be screened with inclusion and exclusion criteria that are specified in the analysis plan, and the data should be extracted by using consistent templates so that straightforward evaluations and comparisons can be made (NASEM 2017a; NRC 2014). The committee notes that data extraction is often resource intensive; there are often differences, for example, in chemical names, terminology, and units that further complicate the process.

EVALUATE AND INTEGRATE DATA

Once the relevant data have been extracted, the next step in the process is to evaluate and integrate them. A key concept of the class approach in contrast with the historical focus on individual chemicals is that there needs to be enough information to determine potential hazards posed by the class or subclass.

[4]This step is analogous to the problem-formulation step in a systematic review (NASEM 2017a). Problem formulation is "the process of defining the scope of a problem, formulating a question about it, and establishing the assessment parameters by which the question will be answered" (NASEM 2018, p. 38).

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The availability of epidemiologic or animal toxicity studies of at least one chemical in a subclass can provide an anchor for the hazard assessment and, if warranted, later risk-assessment steps. NAM data—for example, results of zebrafish assays, in vitro assays, and computational models—can play a useful role in demonstrating that the members of the subclass share the hazard-associated characteristics of the anchor chemical in the epidemiologic or toxicology studies (Berggren et al. 2015). For example, computational models can be used to predict whether a chemical of interest will be positive in an Ames mutagenicity assay (Hsu et al. 2016; Pandit et al. 2018). Historically, NAM data have been under-used in the absence of epidemiologic or toxicity data on individual chemicals; however, they can help to characterize the subclass and might facilitate later quantitative analyses by providing information to use, for example, in a relative-potency approach.

The committee identified three determinations for an OFR subclass: potentially hazardous, not hazardous, and discordant data. The determinations are data-dependent and could change as new data on one or more members of the subclass are acquired. The "potentially hazardous" determination is consistent with CPSC in that one has reached a decision that the chemical is "toxic" as defined in the Federal Hazardous Substances Act. In such a case, CPSC would then conduct a risk assessment to determine whether the chemical should be considered a hazardous substance. A "not hazardous" determination indicates that the chemical does not meet the definition of *toxic*. A "discordant data" determination is reached when data on individual chemicals in the subclass are too heterogeneous or inconsistent to allow a determination. Data can be discordant when experimental studies provide conflicting results, for example, when there are both positive and negative studies or when effects are seen in some studies but not in others. In such cases, analyses might be needed to determine whether the discordance resulted from differences in experimental design or models or was associated with test-chemical purity, dosing regimen, or timing of dosing or observations. A final step in the process is assignment of a confidence rating (high, medium, or low) to the determination.

The committee identified four possible scenarios that are likely to occur in the conduct of a class-based hazard assessment of OFRs in light of the case examples described in Chapter 3. Scenario 1 is a subclass that has many data-rich members on which the data are concordant. The hazard determination for the subclass should be relatively straightforward. Box 2-2 provides an example of this scenario and how it could be handled.

In Scenario 2, there are no relevant data on any subclass member that can be used to conduct the hazard assessment. The lack of data should not imply that an OFR subclass is *not* hazardous. For this scenario, the committee identified the following options:

- Option 2-1: Generate toxicity data for the subclass. The committee recommends a tiered approach that is described in Box 2-3.
- Option 2-2: Expand the analysis beyond the set of chemicals that were identified as OFRs (that is, the seed chemicals used to form the subclass). Toxicity data on structurally related chemicals could be used to inform the hazard assessment of an OFR subclass.
- Option 2-3: Reclassify the subclass so that data-poor members are distributed among other data-rich subclasses. Many OFRs have multiple functional groups and could go into multiple subclasses; reclassification might help to minimize the number of data-poor categories. Confidence in the reclassification can be increased if concordant biologic responses are seen among the members of the newly expanded subclasses, for example, if additional data show a common mechanism of action or effect.

In Scenario 3, coherent data on one or two chemicals are sufficient for evaluations but there are few or no data on the remaining members. However, the few data available might suggest that the members of the subclass have similar biologic activity, in which case the committee identified the following possible options:

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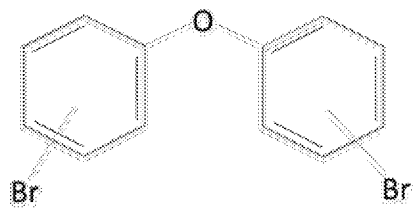
- Option 3-1: Make a science-based policy decision, for example, to classify the group as potentially hazardous on the basis of the data-rich chemicals in the subclass.
- Option 3-2: Use the data-rich chemicals to serve as an anchor as suggested above and extrapolate or interpolate to other chemicals in the group.
- Option 3-3: Generate toxicity data on data-poor group members to the extent that satisfactory confidence is gained; testing could involve NAM studies, targeted animal testing, or a combination thereof.

The committee notes that various NAMs could be used to increase confidence that group members are biologically similar for the end point of interest. That approach is similar to the one suggested for individual chemical assessments that use read-across in the report *Using 21st Century Science to Improve Risk-Related Evaluations* (NASEM 2017b) as illustrated in Figure 2-2.

BOX 2-2 An Example of a Potentially Hazardous Subclass

The committee's approach to creating subclasses described in Chapter 3 and Appendix B includes the formation of a polyhalogenated diphenyl ether (PDE) subclass. Brominated members of this subclass are generally classified as polybrominated diphenyl ethers (PBDEs) and have the general chemical structure shown below. Bromine substitution can occur at various sites on the benzene rings.



The PDE subclass has 12 members and includes decaBDE, pentaBDE, octaBDE, BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-181, BDE-183, and tetradecabromo-1,4-diphenoxybenzene. Several members have been studied extensively. Recently, the National Academies completed a systematic review of the human and animal evidence on PBDE-induced developmental neurotoxicity (NASEM 2017a). The review of the human studies evaluated effects on intelligence, attention deficit/hyperactivity disorder (ADHD), and attention-related behavioral conditions. The review focused on the PBDEs most commonly reported in human biologic samples (BDE-47, BDE-99, BDE-100, and BDE-153). A parallel review of the animal literature included any type of PBDEs and their effects on learning, memory, and attention. The animal review included BDE-47, BDE-99, BDE-153, and BDE-209 and two structurally related analogues (BDE-206 and BDE-209) that are included in the expanded list of analogues. The review posed the question, "*Is developmental exposure to PBDEs associated with effects on neurobehavioral function?*", and it synthesized the human and animal evidence and drew conclusions concerning the hazard posed by individual members of this subclass (NASEM 2017a, p. 8). For example, the National Academies concluded that with respect to effects on intelligence "there was sufficient animal and human evidence to allow the committee to conclude that BDE-47 is a potential hazard to human health". The hazard conclusions reached on the other congeners were equivalent to or weaker than the one reached for BDE-47. The present committee concludes that because the data are concordant for the well-studied members of the subclass, a designation of "potentially hazardous" can be applied to the entire subclass. The hazard assessment can be bolstered by the additional toxicology data available on the structurally related analogues. The next steps would entail completing dose–response and exposure assessments for the subclass. Completion of those steps will address whether the subclass poses a risk to specific exposed populations.

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BOX 2-3 A Tiered Approach for Assessing a Subclass on Which There Are No Relevant Toxicity Data

In some cases, the survey will reveal an absence of toxicity data on an entire OFR subclass and the need for research to fill important data gaps to support the hazard assessment. The committee recommends a tiered approach that initially relies on NAMs as defined in Chapter 1 to encompass computational modeling, in vitro assays in animal and human cells and tissues, and toxicity testing that uses alternative animal species, such as zebrafish and nematodes. The results of those studies can help to identify potential end points of interest and one or more chemicals in the subclass for targeted animal toxicity studies. The tiered approach deviates from the historical reliance of CPSC on traditional animal toxicity or epidemiologic data for a hazard assessment. Collection of the traditional data, however, can take too long and cost too much, and results from NAM studies are useful for a class-based hazard assessment. Indeed, data from in vitro assays and computational models are increasingly available (Collins et al. 2008; NASEM 2015, 2017b).

It is important to note that the hazard assessment of a subclass does not require that there be hazard data on all subclass members for an end point of interest. For example, animal toxicity data, including pharmacokinetic data, might be collected on only a small subset of chemicals of the larger subclass. As noted, identification of the chemicals to be studied in greater detail can be informed by the results of NAM studies.

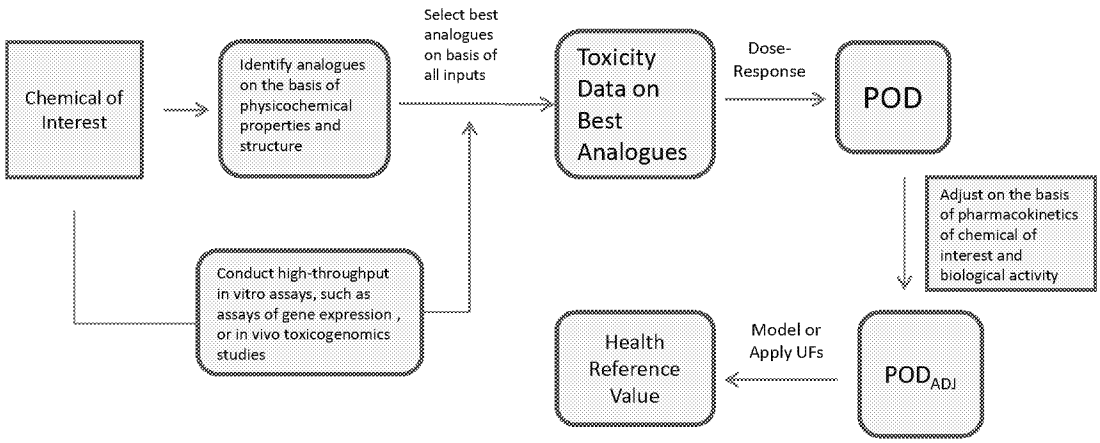

FIGURE 2-2 An illustrative example for building confidence in the derivation of health reference values when using a read-across approach. NAM studies are recommended for increasing confidence in the selection of the best analogues to use for the analysis. Abbreviation: POD, point of departure. Source: NASEM 2017b.

Scenario 4 is the most difficult to address. There are data on some chemicals in the subclass and few or no data on other group members, and the available data are too heterogeneous or inconsistent on biologic activity so that a discordant-data designation is reached. The committee identified the following possible options, which are discussed in further detail in Chapter 3:

- Option 4-1: Make a policy decision, for example, to extend the most conservative conclusion regarding hazard to the subclass.
- Option 4-2: Reclassify members to improve their biologic similarity; generate data to increase confidence that reclassification has resulted in biologically similar members.
- Option 4-3: Perform analyses to explain the discordance and allow the assessment to move forward.
- Option 4-4: Generate new data that could increase clarity and the scientific basis for a decision.

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INTEGRATING A HAZARD ASSESSMENT THAT USES A CLASS APPROACH INTO RISK ASSESSMENT

The committee has proposed a class approach to hazard assessment that represents a reinterpretation and augmentation of the traditional hazard assessment of individual chemicals. Using the proposed class approach will have implications for the later steps in the risk-assessment process—dose–response assessment, exposure assessment, and risk characterization. Although it is beyond the scope of this report to recommend how those steps should be implemented, the committee recognizes the need to integrate them with the class approach for hazard assessment.

Dose–Response Assessment

Dose–response assessments have generally been developed for a chemical by using in vivo toxicity data that adequately demonstrate the adverse effect being evaluated. Often, the analysis uses the no-observed-adverse-effect level, the lowest observed-adverse-effect level, or a benchmark dose curve-fitting approach to obtain a point of departure (POD). The POD is further adjusted by using default uncertainty factors or data-derived factors to account for interspecies extrapolation, human variability, or data deficiencies. Other extrapolation approaches have generally been applied for cancer but will not be discussed further here. Implementation of the class approach could consider several existing dose–response methods given the availability of data or potentially develop new ones.

- *Surrogate chemical.* Select or derive the appropriate dose–response value, such as an acceptable daily intake, for the most toxic chemical on which data are adequate as the subclass surrogate. Use that value in the risk characterization of all subclass members. Note that, absent data on multiple chemicals, it might not be certain that the ones on which data are available are the most toxic; NAM data might help to address this issue.
- *Multiple surrogate chemicals.* If data are available on more than one chemical in the subclass, the remainder of the subclass could be evaluated, for example, by assuming that they are similar to the most toxic chemicals in the subclass.
- *Relative potency factors or toxic equivalents.* Dose–response approaches have been implemented or proposed for a few classes of chemicals, such as polycyclic aromatic hydrocarbons and dioxins, furans, and polychlorinated biphenyls (EPA 1993, 2010; Van den Berg et al. 2006). Those approaches evaluate the toxicity of the class members compared with a selected surrogate class member, which does not have to be the most toxic. In vivo or in vitro studies have been used to characterize class members relative to the surrogate. Scalar values based on the selected study (such as receptor binding affinity relative to the surrogate) are then used to adjust the toxicity value for the surrogate chemical to provide toxicity values for each chemical. If the chemicals all act through the same molecular target, such as a single receptor or enzyme, approaches that characterize the relative interactions with that target might be feasible. If the chemicals produce the same overall effect (for example, an antiandrogenic effect) but there are several targets (for example, binding to androgen receptor or decreasing testosterone or dihydrotestosterone synthesis), evaluating the relative activity at the molecular-interaction level might not be appropriate. Alternatively, when there are multiple targets, in vivo studies that assess the apical toxicity resulting from the overall pathway might be necessary; the studies potentially could be in nonmammalian organisms that respond to that pathway. When multiple toxicity end points are evaluated for subclass members, relative potency factors or toxic equivalents could be specific to particular end point, as when different mechanisms are involved.
- *NAM studies and in vitro–in vivo extrapolation.* NAM studies can also be informative for dose–response assessments. The alternative assays often use multiple concentrations that allow estimates of such measures as the concentration that produces 50% activity (AC_{50}). They can also

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define the highest tested concentration at which no effect is observed. Negative findings might be useful in demonstrating that there is a lack of response in a pathway (as opposed to an absence of testing) or in comparing chemicals in a class. The in vitro assays provide information on media concentrations, so in vitro to in vivo extrapolation is often needed to estimate doses that are typically needed for exposure assessment. The extrapolation can be performed with a variety of pharmacokinetic analysis methods that might be associated with greater uncertainty in the estimates when more assumptions are required because of data limitations. Comparisons of predicted exposures and activity in alternative assays can provide a path for decision-making, including suggestions as to what additional data would be most valuable (Wambaugh et al. 2018). Application of these types of data and analytic approaches is a major focus of contemporary toxicology and risk assessment and reflects strong recommendations from the National Academies (NASEM 2015, 2017b).

Exposure Assessment

Several aspects of exposure assessment might need to be addressed to integrate the class approach into risk assessment.

- Availability of data on each subclass member would need to be assessed. If no data are available, predictive models could be used to estimate chemical exposures.
- Aggregate exposure by different routes (oral, dermal, and inhalation) would need to be determined in the context of expected human exposures to the products under consideration.
- Cumulative exposure to the subclass members needs to be evaluated, although this might be more important in the risk characterization than in the exposure assessment itself. Because hazard would be assessed for each subclass, each chemical in the subclass that is present in a product would be expected to contribute to the adverse outcome evaluated for that subclass.
- Exposures to members of the subclass in products not under consideration for a specific regulatory action should be included. An approach like the relative source contribution applied to drinking water risk assessments by EPA and states is a useful model (Gadagbui et al. 2012).

Risk Characterization

Use of a class approach for hazard assessment would also be expected to involve adjustments of risk characterization. Issues or approaches that seem likely to arise include the following:

- *Single subclass.* Combining dose–response and exposure assessments for a single subclass would be expected to address the combined exposure to all members of the subclass and allow evaluation of the risk of each kind of toxicity that was considered in the subclass hazard assessment. It is worth noting that even if exposures to individual chemicals are considered to be below a level of concern, consideration of the contributions from co-exposure to all members of the subclass could indicate a concern. Consideration of the cumulative and aggregate risk posed by all chemicals evaluated has a long history in site-specific risk assessments (for example, federal and state Superfund sites) or analyses that rely on relative potency factors.
- *Multiple subclasses.* When common toxicities are shared among subclasses, it might be necessary to evaluate the risks posed by all constituents of multiple subclasses. Considering the cumulative risk associated with multiple subclasses could also provide a basis for determinations related to the entire class.

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IMPROVED EFFICIENCY AND COST-EFFECTIVENESS

A class approach will likely result in increases in efficiency and decreases in cost compared with the traditional approach of evaluating individual chemicals. The magnitude of the improvements or savings will depend on several factors, including the class or subclass size, the level of confidence needed to make a decision, the number of data gaps that need to be filled, and the effect of policy decisions. Broader classes or subclasses would generally improve efficiency and reduce costs.

As discussed above, the committee encourages the use of NAM studies to fill data gaps. The costs, resources, and time required to obtain data using the alternative methods are often orders of magnitude less than would be needed for conducting traditional toxicology studies for each chemical. Adopting NAM data to evaluate hazards posed by OFRs would also further the goal of refining, reducing, and replacing animals while providing data on more chemicals in a timely manner. The use of both NAM studies and the traditional toxicology data can permit the setting of priorities for future research and thereby improve efficiency and reduce cost.

Using a class approach to hazard assessment can also expedite evaluations of new and emerging members of a subclass. That approach can also provide input to industry as alternative products are considered. For example, in the case of OFRs, a company making a decision about ingredients in its products might consider the attributes of the broadest possible class and then decide to avoid the entire class because of its environmental persistence, production of toxic halogenated compounds when burned, or potential for toxicity.

CONCLUSIONS

In this chapter, the committee has described a scoping plan that can be used to conduct a class-based hazard assessment of the OFRs. The committee acknowledges that there will be challenges with its application particularly in the regulatory setting. Approaches that extrapolate data from one chemical to another (read-across approaches) have struggled to gain regulatory acceptance. *Using 21st Century Science to Improve Risk-Related Evaluations* (NASEM 2017b), however, highlights those approaches for conducting hazard and risk assessments of data-poor chemicals and identifies them as being underused. The present committee also recognizes that there will be no relevant data on many chemicals, and CPSC will need to make difficult decisions regarding what type of data it will accept and what quantity of data will be needed to support decisions with the desired confidence. Those decisions will affect the cost and time required to generate the necessary data. If CPSC decides that only traditional rodent studies are appropriate and that some data are needed on all chemicals, data generation will be extremely expensive and take decades to complete. And although NAM data represent tremendous time and cost savings, their use in the regulatory setting is still limited even though their use has been encouraged by authoritative bodies (NASEM 2015, 2017b). Finally, the committee notes that forming classes and conducting read-across requires expertise that is not widely available (Ball et al. 2016). Although the challenges to a class approach might appear daunting, the alternative—individual assessments of hundreds of chemicals—is unrealistic. The only possible practical approach for a set of chemicals as large as the OFRs is a class approach.

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3

A Class Approach to Evaluating Organohalogen Flame Retardants: Case Studies

In Chapter 2, the committee described a scoping plan for evaluating nonpolymeric, additive organohalogen flame retardants (OFRs) as a single class for the purpose of hazard assessment.[1] This chapter first discusses creation of an inventory of OFRs and then provides analyses that show that chemicals in the inventory do not represent a single class for a regulatory hazard assessment that uses Consumer Product Safety Commission (CPSC) guidance. However, a science-based approach to creating OFR subclasses is described and recommended. The chapter then provides an example of a literature survey and identifies two case studies that are used to illustrate several steps described in the scoping plan (see Chapter 2, Figure 2-1). Because the case studies are illustrative, not all steps of the scoping plan are conducted nor are any hazard assessments performed. Furthermore, the committee does not provide a comprehensive list of all databases or tools that might be needed to assist with the analyses. The chapter concludes by discussing options for handling discordant data on subclass members and addressing a projected timeline and cost for a class approach to hazard assessment of OFRs.

CAN ORGANOHALOGEN FLAME RETARDANTS BE DEFINED AS A SINGLE CLASS?

The committee used several approaches to answer the question of whether the OFRs can be treated as a single class for hazard assessment. Details of the approaches are provided in Appendix B. As noted in Chapter 2, the main steps are as follows:

- Identify and characterize a "seed" set of chemicals as a working inventory of the class.
- Generate an "expanded" set of chemical analogues of the seed set on the basis of combined functional, structural, and predicted bioactivity information.
- Evaluate the similarity of the seed set to the analogues to evaluate whether the OFRs are distinguishable as a single class.

Box 3-1 presents an overview of the steps used by the committee to develop an OFR inventory. Additional details are provided in Appendix B.

After defining the OFR inventory, the committee identified chemically related analogues that are similar to the seed chemicals (Box 3-2). Analogues can serve several purposes other than comparison with seed chemicals, including identification of chemicals that might be used as OFRs in the future and of chemicals that might have toxicology and other data to support a class-based hazard assessment. Further details are provided in Appendix B.

When an expanded set of chemical analogues had been created, the committee analyzed the chemical space represented by the seed and analogue chemicals to determine whether the OFRs could be distinguished as a single class. The committee's analyses included the following:

- Prediction of structure–activity relationships (SARs) by using an open-source application called OPEn structure–activity/property Relationship App (OPERA v2.0) (Mansouri et al. 2016a,b;

[1]The abbreviation *OFRs* in this report refers specifically to nonpolymeric, additive organohalogen flame retardants.

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2018a,b; Kleinstreuer et al. 2018).[2] The OPERA SAR predictions showed that the seed chemicals exhibited heterogeneity in physicochemical properties, environmental fate, and toxicity end points, including estrogen and androgen receptor activities and acute oral toxicity.

- Principal component analysis (PCA) on OPERA physicochemical properties. The PCA analysis showed that the seed chemicals have similar physicochemical, toxicological and environmental fate properties that were not distinguishable from those of the expanded set of chemical analogues.[3]

When the analyses were considered collectively (see Appendix B for a detailed discussion), the committee concluded that the seed chemicals do not have a common chemical structure or predicted biologic activity. That conclusion was supported by compiling and reviewing data from the US Environmental Protection Agency (EPA) Dashboard (Williams et al. 2017) and ToxCast and Tox21 databases (Richard et al. 2016), which showed that the OFR seed chemicals had a wide array of biologic activities that varied from chemical to chemical.[4] Thus, the broad class needs to be divided into subclasses to support a regulatory hazard assessment.

BOX 3-1 An OFR Inventory

The first step taken by the committee was to identify an inventory of chemicals that have been used or proposed for use as OFRs. The chemical inventory was compiled from several sources, including documents from Eastmond (2014), the Danish Environmental Protection Agency (Danish EPA 2016), the Environment Agency of the United Kingdom (2003), the World Health Organization's International Programme on Chemical Safety (IPCS 1997), the European Food Safety Authority (EFSA 2010, 2011a,b,c, 2012 a,b), the US Consumer Product Safety Commission (TERA 2016), and US Environmental Protection Agency (EPA 2015a). The analysis identified 161 halogenated chemicals that have a functional use as a flame retardant (Appendix B). Despite the potential for overlap, few chemicals (fewer than 20) were listed in all sources; this suggests substantial heterogeneity in chemicals identified by various agencies. The next step in the process of developing an OFR inventory was to curate the chemicals by verifying their names, CAS numbers, and structures by using the US Environmental Protection Agency Dashboard and other sources. That process identified several duplicates and four mixtures that were not included in the curated inventory of 148 unique chemical structures (see list at OFR_QSAR-ready_120318.sdf; file available at www.nap.edu/25412). The curated inventory of OFRs is referred to elsewhere in this chapter as "seed" chemicals. See Appendix B for detailed discussion.

BOX 3-2 Identification of Analogues to the OFR Seed Chemicals

Chemical structures were used to identify analogues that are most similar to the OFR seed set. Automated workflows developed by using an open-source modular data analytics program (Konstanz Information Miner) were used to curate the chemical structures and identify the analogue structures (Mansouri et al. 2016a). The process consisted of identification of about 200,000 organohalogens from the US Environmental Protection Agency Distributed Structure-Searchable Toxicity (DSSTox) database (Richard 2004; Richard et al. 2006). The identified organohalogens were then compared with the OFR seed chemicals by using the chemistry development kit fingerprints and applying a Tanimoto similarity index threshold of 80% (Steinbeck et al. 2003, 2006). That step resulted in an output of 1,073 analogues that were then processed by using a quantitative structure–activity relationship-ready standardization workflow described by Mansouri et al. (2016a,b). The final analogue set is referred to in this report as the "expanded set" of OFRs. Additional details are provided in Appendix B.

[2]See prediction file: pred_OPERA_OFR.csv and list of OPERA models: OPERA2.0_models.xlsx and OFRs.xlsx. Files available at www.nap.edu/25412.

[3]See Appendix B for additional details.

[4]See OFR_ChemistryDashboard-Batch-Search_2018-12-03_17_03_39.xls and Appendix C for additional details. File available at www.nap.edu/25412.

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DEFINITION OF SUBCLASSES OF ORGANOHALOGEN FLAME RETARDANTS

The committee used cheminformatic approaches to create OFR subclasses. A public set of chemotypes[5] and methods that have been developed by Yang et al. (2015) and Richard et al. (2016) were used to identify the chemotypes present in the seed chemicals, which are listed in Figure 3-1. Using the chemotypes, the committee was able to identify several generic classes that represented the entirety of the OFR seed set (Table 3-1). Merging the biology-informed groups with the chemotypes listed in Figure 3-1 led to the formulation of 14 OFR categories for the inventory of 161 OFR chemicals (Table 3-2). Appendix B provides additional details on how the subclasses were formed and evaluated. The committee recommends that CPSC use the subclasses in Table 3-2 at least as a starting point for the class-based hazard assessment of OFRs.

SURVEY OF THE LITERATURE

When the subclasses have been formed, the committee recommends conducting a literature survey (see Chapter 2, Figure 2-1). As discussed in Chapter 2, the goal of the literature survey is to determine the extent, range, and nature of toxicity data (human, animal, in vitro, and other relevant studies) and to identify end points that deserve investigation.[6] It is distinct from the literature search performed later in the scoping plan in that it is meant to provide a broad understanding of the literature, not to identify every relevant article that might need to be retrieved and evaluated for the hazard assessment of a specific subclass.

To identify subclasses that could serve as case studies, the committee surveyed the literature in several steps. It first conducted an initial mapping exercise to evaluate the types of toxicity data available on each subgroup and then conducted several literature surveys to identify end points of possible interest. Specifically, the committee initially surveyed the literature by using bioinformatically mappable databases, which contain biologic data that can be systematically retrieved by using batch software queries for the chemicals of interest. The committee searched the following databases to determine whether they contained data for the subclass members: Comparative Toxicogenomics Database (CTD), EPA Chemical Dashboard, Hazardous Substances Data Bank (HSDB), Integrated Risk Information System (IRIS), ToxCast/Tox21, Toxicity

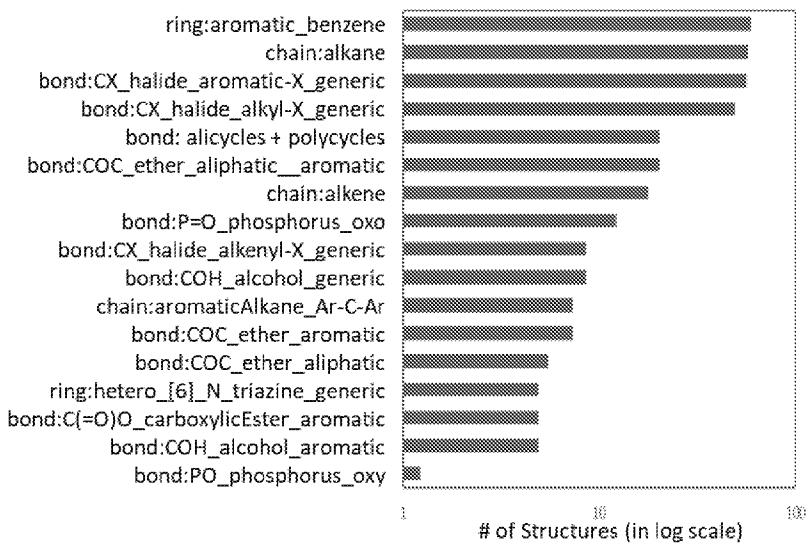

FIGURE 3-1 Substructures identified in OFR seed chemicals.

[5]ToxPrint, available at https://chemotyper.org, provides coverage of EPA and Food and Drug Administration inventories and captures chemical features important for chemical safety assessments.

[6]As noted in Chapter 2, the term *literature* is used broadly to refer to scientific literature and databases.

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TABLE 3-1 Chemotypes Identified in OFR Seed Chemicals That Have Been Associated with Predicted Biologic Activity[a]

Chemotype	Biologic Activity	Reference
Chain: aliphatic cycles	GABA receptor antagonist; AhR enzyme in steroidogenesis	Eager et al.1999
Ring: polycycles	GABA receptor modulator; ER/AR modulator	Babot et al. 2007
Bond: ether aromatic diphenyl	Aromatase enzymes in steroidogenesis; aromatase inhibition	Peters et al. 2006
Bond: alcohol aromatic phenols	Aromatase enzymes in steroidogenesis Bisphenol A: proposed for estrogen-mediated pathways by binding estrogen receptors; proposed for oxidative stress for ROS formation leading to early embryonic damage	Guo et al. 2017
Ring: aromatic_benzenes	AhR signaling	Abiko et al. 2016
Ring: hetero_triazines	Effects in steroidogenesis	Forgacs et al. 2013
Bond: aromatic carboxylic acid and derivatives	Nuclear receptor pathways (such as PPAR alpha)	Eveillard et al. 2009
Bond: P=O or P-O	Acetylcholinesterase inhibitors	Abou-Donia et al. 2016

[a]Individual OFRs that have a given chemotype might or might not demonstrate the biologic activity indicated here. Abbreviations: AhR, aryl hydrocarbon receptor; ER/AR, estrogen receptor/androgen receptors; GABA, gamma aminobutyric acid; PPAR, peroxisome proliferator activated receptor; ROS, reactive oxygen species.

TABLE 3-2 OFR Subclasses Formulated by Using Chemotypes and Predicted Biologic Activity

OFR Subclass	No. Chemicals in Subclass[a]
Polyhalogenated alicycles	17
Polyhalogenated aliphatic carboxylate	4
Polyhalogenated aliphatic chains	12
Polyhalogenated benzene alicycles	4
Polyhalogenated benzene aliphatics and functionalized	19
Polyhalogenated benzenes	19
Polyhalogenated bisphenol aliphatics and functionalized	11
Polyhalogenated carbocycles	15
Polyhalogenated diphenyl ethers	12
Polyhalogenated organophosphates (OPs)	22
Polyhalogenated phenol derivatives	7
Polyhalogenated phenol–aliphatic ether	9
Polyhalogenated phthalates/benzoates/imides	11
Polyhalogenated triazines	6

[a]Seven chemicals were categorized by using two chemotypes and included in two subclasses. This analysis was performed by using the chemicals in the OFR inventory.

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Reference Database (ToxRefDB), PubChem, and ChEMBL. The committee notes that other databases might also be surveyed, and it simply used the ones listed for illustrative purposes. Results for the OFR subclasses and analogues are shown in Figures 3-2 and 3-3 (see also Appendix C: Figures C-1, C-2, C-3, C-4, and C-5). The survey provided an indication of the amounts and types of information (data coverage) that might be available on each OFR. The mapping exercise, however, did not identify end points of interest. The details of the exercise are provided in Appendix C.

To gain an understanding of possible end points of interest, the committee next used CAS numbers to explore PubChem for toxicity data on each seed chemical. The query focused on chronic toxicity, reproductive and developmental toxicity, mutagenicity, and cancer, which were end points that were specifically listed in the committee's task statement. Other end points could be considered. The survey results were used to identify subclasses that contained members on which there was relevant toxicity information, including epidemiology, traditional mammalian toxicity, and new approach methodologies (NAM) studies.[7] One objective in the selection of the two case studies was to illustrate how high-throughput, alternative species, and other NAM data could be used to inform a hazard assessment (Box 3-3). Two relatively data-rich OFR subclasses—polyhalogenated organophosphates (OPs) and polyhalogenated bisphenol aliphatics—were selected to serve as case studies to illustrate various aspects of the hazard-assessment scoping plan.

SEARCH OF THE LITERATURE

After the literature survey and the creation of an analysis plan, the literature is searched to identify all relevant data associated with the subclass members and the end points of interest.[8] As noted at the beginning of this chapter, the committee did not attempt to complete all steps of its scoping plan and so did not create an analysis plan. It also did not conduct comprehensive, systematic literature searches; the searches were conducted so that the committee could illustrate steps of its scoping plan, and relevant literature could have been missed. The committee did, however, search multiple databases for toxicity data in vertebrates on each member of the subclasses chosen for the case studies.[9] The searches were intended to gather the available literature on the two subclasses and to illustrate the process that could be used (see Appendix C for details). For the two selected subclasses, the committee searched for English-language, peer-reviewed articles in PubChem and PubMed by CAS number, chemical name and synonyms, and outcomes of interest by using the following search terms:

- Toxicity OR reproductive toxicity OR developmental toxicity.
- Genotoxicity OR mutagenicity.
- Cancer OR carcinogenicity.

The search found a few well-studied chemicals in both classes that could help to anchor the hazard assessment of other subclass members. Each subclass also contained chemicals on which there were few or no relevant traditional (mammalian) toxicity data. An additional PubMed literature search conducted in November 2018 used the search terms "zebrafish" and "flame retardants" and retrieved 118 publications. The committee also considered two publications that reported results of zebrafish assays that were used to screen the large ToxCast chemical library, which included polyhalogenated OPs (Truong et al. 2014; Reif et al. 2016). The committee illustrates the use of zebrafish data in the following case studies because such data were available on several OFRs. Their use is not a statement that zebrafish data will be the best or most useful NAM data to use in all cases.

[7]Completion of the scoping plan does not require that a subclass have each of the data streams noted.

[8]As noted in Chapter 2, the term *literature* is used broadly to refer to scientific literature and databases.

[9]The committee recognizes that OFRs have also been evaluated in invertebrate animal models. For example, the effect of flame retardants on feeding, larval development, reproduction, and motor activity have been evaluated in the nematode *Caenorhabditis elegans* (Behl et al 2016; Xu et al 2016).

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FIGURE 3-2 Histogram of data availability for the seed chemicals. Each bar indicates the data coverage in the eight database sources for each subclass.

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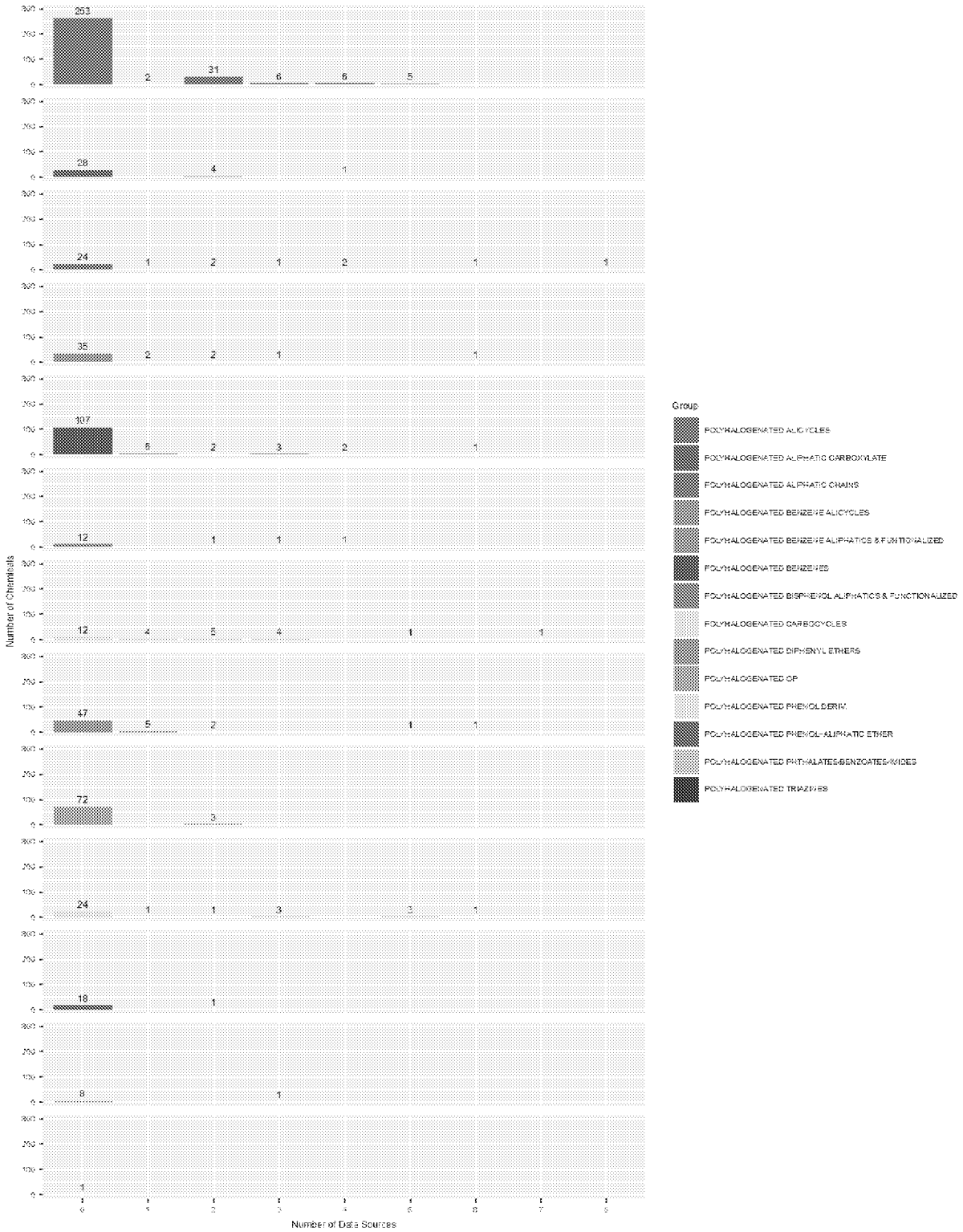

FIGURE 3-3 Histogram of data availability for the expanded set of chemicals. Each bar indicates the data coverage in the eight database sources for each subclass.

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A Class Approach to Hazard Assessment of Organohalogen Flame Retardants

BOX 3-3 Use of NAM Data in Regulatory Decision-Making

Several organizations, including the National Academies (NASEM 2017) and the European Chemicals Agency (ECHA 2016), have suggested that NAM data can be used in the context of hazard assessment. The use of nontraditional data has been suggested as an addition to the traditional data to bolster confidence in the hypothesis of common mechanisms or effects or as an additional data stream when traditional toxicology data are too sparse to support a hypothesis of common effects among group members confidently (NASEM 2017). Nontraditional methods can also increase understanding of the toxicokinetics or toxicodynamics of a substance.

Using NAM approaches to probe mechanisms might be especially beneficial for members of a given OFR subclass on which there are few or no data, especially because comparable NAM datasets can be generated for subclass members efficiently and cost effectively. In that case, using screening systems that assess toxicologic responses (such as gene expression) globally or high-throughput screening batteries that cover known toxicity mechanisms or other relevant mechanisms might be useful. Higher-order models, such as zebrafish, might also be used for testing hypotheses of biologic similarity, especially if the model has the biologic machinery that is critical for the mechanism in question (NASEM 2017). For example, the zebrafish is gaining traction as a vertebrate model system in developmental toxicology, and support for inclusion of these data in chemical assessments is increasing as the research literature grows (Lieschke and Currie 2007; Planchart et al. 2016). The expanding zebrafish research base is providing a better understanding of the strengths, similarities, and limitations of the zebrafish in developmental-toxicology research.

NAM data can also help to identify biologic targets of interest in relation to an OFR. For example, some polyhalogenated bisphenol aliphatic OFRs activate some estrogen and peroxisome-proliferator receptors (ERα and PPARγ) and act as both androgen and progesterone receptor antagonists (Li et al. 2010; Riu et al. 2011). Those data, combined with emerging in vitro–to–in vivo extrapolation methods, might also be used to set testing priorities among chemicals for future study.

Some NAM assays have important limitations. For example, high-throughput assays, such as those in Tox21 and ToxCast, typically have insufficient metabolic capability, have chemical solubility concerns, and offer incomplete biologic coverage. Furthermore, Tox21 or ToxCast assays have few thyroid-specific assays, and some biologic responses occur only at high concentrations that might suggest a cytotoxic response. The committee notes that integration of information from traditional studies and NAM data requires the application of value judgments (NRC 2014) that are beyond the scope of this report.

CASE STUDY 1: POLYHALOGENATED ORGANOPHOSPHATES

The committee identified 22 chemicals in the polyhalogenated OP subclass, which are listed in Table 3-3. Chemical structures for several subclass members are shown in Figure 3-4.

As noted above, literature searches were performed by using each chemical name, CAS number, and relevant toxicity search terms. Studies of genotoxicity, carcinogenicity, and reproductive and developmental toxicity were also sought from authoritative compilations from the European Chemicals Agency (ECHA), EPA, Environment Canada and Health Canada, the World Health Organization's International Programme on Chemical Safety, and the National Toxicology Program (NTP). The committee found that Tox21 or ToxCast data, rodent reproductive or developmental toxicity data, or zebrafish data on several members of the polyhalogenated OP subclass were available. Some epidemiologic data on several subclass members were available. Genotoxicity data are summarized in Table 3-4. As a subclass, these chemicals have discordant data; that is, some members have been negative in the Ames assay, and others have been positive.

The results of chronic mammalian bioassays are presented in Table 3-5. A metabolite of TDBPP, 2,3-dibromo-1-propanol, has been shown to be carcinogenic in rodents (Eustis et al. 1995). TDBPP has been identified as probably carcinogenic in humans (Group 2A) by the International Agency for Research on Cancer (IARC 1999b) and reasonably expected to be a human carcinogen by NTP (2016) on the basis of animal carcinogenicity data (Reznik et al. 1981). TCEP causes benign and malignant tumors at various organ sites in rats and mice (NTP 1991). A carcinogenicity study with TCPP is underway at NTP.

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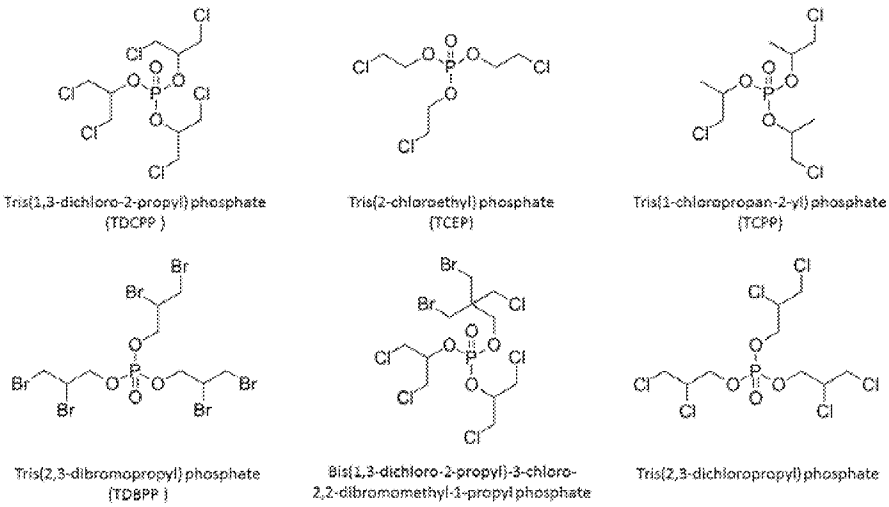

FIGURE 3-4 Representative members of the polyhalogenated organophosphate subclass.

TABLE 3-3 Members of the Polyhalogenated Organophosphate Subclass

Chemical Name	Abbreviation	CAS No.
Tris(1,3-dichloro-2-propyl) phosphate	TDCPP	13674-87-8
Tris(2-chloroethyl) phosphate	TCEP	115-96-8
Tris(1-chloropropan-2-yl) phosphate	TCPP	13674-84-5
Tris(2,3-dibromopropyl) phosphate	TDBPP	126-72-7
Bis(1,3-dichloro-2-propyl)-3-chloro-2,2-dibromomethyl-1-propyl phosphate		66108-37-0
Tris(tribromo-neopentyl) phosphate (Tris[1,1,3-tribromo-2,2-dimethylpropyl] phosphate)	TTBNPP	19186-97-1
2,2-Bis(chloromethyl)-1,3-propanediyl bis(bis(2-chloroethyl) phosphate)		38051-10-4
Tris(2,3-dichloropropyl) phosphate		78-43-3
Tris(2-chloropropyl) phosphate		6145-73-9
Tetrakis(2-chloroethyl) ethane-1,2-diyl bis(phosphate)		33125-86-9
Bis(2-chloroethyl) vinylphosphonate		115-98-0
Bis(2,3-dibromopropyl) phosphate		5412-25-9
Tris(2-chloroethyl) phosphite		140-08-9
Phosphonic acid, (1-(((2-chloroethoxy)(2-chloroethyl)phosphinyl)oxy)ethyl)-, 1-(bis(2-chloroethoxy)phosphinyl)ethyl 2-chloroethyl ester		4351-70-6
Bis(2-chloroethyl) (2-chloroethyl)phosphonate		6294-34-4
Tris(1,3-dichloropropan-2-yl) phosphite		6749-73-1
Oxydiethylene tetrakis(2-chloroethyl) bisphosphate		53461-82-8
2,4,8,10-Tetraoxa-3,9-diphosphaspiro[5.5]undecane, 3,9-bis[3-bromo-2,2-bis(bromomethyl)propoxy]-, 3,9-dioxide		61090-89-9
Tris(2,4-dibromophenyl) phosphate		49690-63-3
Phenol, 2,4,6-tribromo-, phosphate		7046-64-2
Polyhalogenated OP + triazines (phosphonic acid, *P*-[[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]methyl]-, dimethyl ester)	TPN1	114955-21-4*
Polyhalogenated OP + triazines (phosphonic acid, (4,6-dichloro-1,3,5-triazin-2-yl)-, diethyl ester (9CI))	TPN2	1373346-90-7*

*CAS no. from SciFinder.

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TABLE 3-4 Genotoxicity Data on the Polyhalogenated Organophosphate Subclass

Chemical	Experimental Results
TDCPP	Mostly negative in in vitro gene-mutation assays in bacteria and yeasts; positive in some *Salmonella typhimurium* strains when tested with metabolic activation; negative in assays of point mutations, sister chromatid exchange, and unscheduled DNA synthesis; mixed results in mouse lymphoma assay, chromosomal-aberration assay, and transformation assay; and negative in in vivo tests of sex-linked recessive lethal mutations in *Drosophila*, unscheduled DNA synthesis in rats, formation of micronuclei in polychromatic erythrocytes of mice, and chromosomal aberrations in mice (Environment Canada and Health Canada 2016).
TCEP	Not mutagenic in bacteria in absence of metabolic activation; equivocal results with metabolic activation (IARC1999a). EPA (2009) found overall evidence to be negative.
TCPP	Negative in Ames assays; equivocal results in mouse lymphoma assays, unscheduled DNA synthesis assays, and comet assays; positive in in vitro mouse lymphoma assay when tested with metabolic activation; negative in in vivo micronucleus tests and comet assays; equivocal or negative in in vivo assays of unscheduled DNA synthesis and chromosomal aberrations; and in vivo micronucleus tests in mice and rats had positive results only in male mice (Environment Canada and Health Canada 2016).
TDBPP	Mutagenic (van Beerendonk et al. 1994; de Boer et al. 1996, 2000). Mutagenic in *S. typhimurium* and in V79 Chinese hamster lung cells; positive in assays of sister chromatid exchanges and morphologic transformation in mouse and hamster embryo cells; binds covalently to proteins and DNA and causes DNA strand breaks in mammalian cells in vitro and in vivo; mutagenic, clastogenic, and recombinogenic in *Drosophila melanogaster*; induces bone-marrow micronuclei in mice and hamsters, liver micronuclei in rats, and gene mutations in mouse kidney in vivo (IARC 1999b).
Bis(1,3-dichloro-2-propyl)-3-chloro-2,2-dibromomethyl-1-propyl phosphate	Mutagenic in rat dominant lethal assay (Litton Bionetics, Inc. 1977).
Bis(2,3-dibromopropyl) phosphate	Evidence of mutagenicity (IPCS 1995a).

TABLE 3-5 Polyhalogenated Organophosphate Chronic Toxicity Studies

Chemical	Experimental Results
TDCPP	In a 2-year carcinogenicity study in Sprague-Dawley rats (Stauffer Chemical Co. 1981): male rats, significant increase in renal cortical adenomas, testicular interstitial cell tumors, and hepatocellular adenomas; female rats, significant increase in renal cortical adenomas, hepatocellular adenomas, and adrenal cortical adenomas.
TCEP	Benign and malignant tumors occurred in various organs in rats and mice (NTP 1991); tumors were found in kidneys, liver, forestomach, and hematopoietic system of mice (Takada et al. 1989).
TCPP	90-day and 2-year oral-carcinogenicity studies are being conducted by NTP.
TDBPP[a]	Reasonably anticipated to be a human carcinogen (NTP 2016); probably carcinogenic to humans (IARC 1999b).
Bis(2,3-dibromopropyl) phosphate	Rats developed papillomas and adenocarcinomas of the tongue, esophagus, and forestomach; adenocarcinomas of the intestine; and hepatocellular adenomas [neoplastic nodules] and carcinomas (Takada et al. 1991).

[a]The TDBPP metabolite 2,3-dibromo-1-propanol is carcinogenic in rodents (Eustis et al. 1995).

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A third outcome, developmental toxicity, was also identified as an end point of interest in this case study, and the committee chose this end point to illustrate evaluation and integration of data in the scoping plan (Figure 2-1). The committee emphasizes that a hazard assessment of the OPs would consider mutagenicity, carcinogenicity, and developmental toxicity and might also consider neurotoxicity, hepatotoxicity, and other systemic effects; that is, the overall class-based hazard assessment would consider multiple end points of interest. The following subsections discuss the various data streams for developmental toxicity and overall classification for this specific end point.

Developmental Toxicity Data on Polyhalogenated Organophosphates

The committee's analysis considered three main data streams: epidemiologic, traditional mammalian, and zebrafish studies. Exposures were considered relevant if they occurred during development. Additional toxicity data could also be considered. For example, Farhat et al. (2013) indicated that exposure of chicken embryos to TDCPP lowered free thyroxine (T4) concentrations in the blood and resulted in malformations, including changes in head-to-bill lengths. Similarly, frog (*Xenopus tropicalis*) embryos exposed to TCPP or TDCPP developed multiple malformations (Zhang et al. 2016).

Epidemiologic Data

Despite their relatively short half-lives, polyhalogenated OPs likely contribute to continuous exposure concentrations in humans (Dishaw et al. 2014; He et al. 2018; Phillips et al. 2018). Polyhalogenated OPs are hydrolyzed to diester metabolites that undergo urinary excretion (Cequier et al. 2015), and the metabolites have been used as biomarkers of OP flame-retardant exposure of people (Dodson et al. 2014; Hoffman et al. 2014; Cequier et al. 2015; Romano et al. 2017). However, not all OP metabolites can be attributed to the polyhalogenated OP flame retardants; some nonhalogenated OP flame retardants, such as triphenyl phosphate (TPHP), are metabolized to some of the same metabolite diesters as the polyhalogenated OPs (Cequier et al. 2015; Wei et al. 2018).

Some OP flame-retardant metabolites—including diphenyl phosphate (DPHP), bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), isopropylphenyl phenyl phosphate (ip-PPP), and 1-hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate (BCIPHIPP)—are nearly ubiquitous in human samples (Ospina et al. 2018; Doherty et al. 2019; Wang et al. 2019), but few epidemiologic studies have examined associated health outcomes. A few small studies of reproductive-age cohorts have supported the potential of some metabolites to be associated with changes in sex and thyroid hormones (Meeker and Stapleton 2010; Preston et al. 2017), but results were imprecise and inconsistent. Disruption of sex and thyroid hormones can adversely affect fertility, birth outcomes (Carignan et al. 2017, 2018; Messerlian et al. 2018), and neurodevelopment (Sarles et al. 1987; Castorina et al. 2017). In two prospective studies of exposure during pregnancy, OP flame retardants were associated with reduced language, cognition, and working memory in the children (Castorina et al. 2017; Doherty et al. 2019), but the specific implicated metabolites differed between the two studies. Table 3-6 provides a summary of the findings of the epidemiologic studies.

Mammalian Toxicity Studies

Developmental toxicity data were available on four of the polyhalogenated OPs (see Table 3-7). Although the four do not appear to be teratogenic, one study of TDCPP reported effects on sexual behavior in male rats exposed during development. Oral exposure (28 consecutive days after birth) of neonatal male rats to TDCPP suppressed male sexual behavior and reduced testis size (Kamishima et al. 2018). In contrast, oral exposure of pregnant Long-Evans rats to TDCPP or TCEP from gestational day 10 to weaning was not associated with thyrotoxicity or developmental neurotoxicity in offspring (Moser et al. 2015).

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TABLE 3-6 Available Human Epidemiologic Data on Organophosphate Flame Retardants[a]

Study Design	Sample, Exposure Period[b], Chemical	Outcome	Results	Reference
Neurodevelopment				
Prospective cohort (n = 227)	Prenatal urine 2001-2005 DPHP ip-PPP *BDCIPP* *BCIPHIPP*	Cognition at age 3 years	ip-PPP associated with lower cognition, fine motor, and expressive language.	Doherty et al. 2019
Prospective cohort (n = 310)	Prenatal urine 1999-2000 DPHP ip-PPP *BDCIPP* *BCIPHIPP*	Cognition, behavior age 7 years	No association between ip-PPP and *BDCIPP* metabolite (DPHP) and working memory, IQ deficits.	Castorina et al. 2017
Cross-sectional (n = 72)	Age 3-5 years wristband 2012-2013 Sum OPFR	Behavior, social skills	Sum OPFR associated with externalizing problems (aggression).	Lipscomb et al. 2017
Reproduction				
Cross-sectional (n = 50)	Dust 2002-2007 **TDCPP** TPP	Semen quality	**TDCPP** and TPP associated with alterations in thyroid, sex hormones.	Meeker and Stapleton 2010
Cross-sectional (n= 33)	Urine 2003-2004 DPHP *BDCIPP*	Semen quality, sex hormone, thyroid hormone	*BDCIPP* and DPHP associated with altered sperm quality and higher total T3. *BDCIPP* associated with higher TSH.	Meeker et al. 2013
Prospective cohort (n = 201)	Preconception urine 2005-2015 Sum OPFR	Semen quality, fertility	Decreased oocyte fertilization, embryo quality.	Carignan et al. 2018
Prospective cohort (n = 211)	Preconception urine 2004-2015 Sum OPFR DPHP ip-PPP *BDCIPP*	Pregnancy, implantation, live birth	Sum OPFR, DPHP, ip-PPP associated with decreased implantation, pregnancy.	Carignan et al. 2017
Retrospective cohort (n = 220)	Preconception urine 2005-2015 Sum OPFR DPHP ip-PPP *BDCIPP*	Semen quality	OPFR not associated with semen measures.	Ingle et al. 2018

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Prospective cohort (n = 179)	Preconception urine 2005-2015 Sum OPFR DPHP ip-PPP *BDCIPP*	Pregnancy loss	DPHP associated with pregnancy loss; ip-PPP and *BDCIPP* not associated with loss.	Messerlian et al. 2018
Prospective cohort (n = 349)	Prenatal urine 2002-2005 DPHP ip-PPP *BDCIPP* *BCIPHIPP*	Gestational age, birthweight	*BDCIPP,* ip-PPP associated with early delivery, especially of female infants; DPHP and *BCIPHIPP* not associated with reproductive outcomes.	Hoffman et al. 2018
Prospective cohort (n = 23)	Prenatal urine 2015 DPHP *BDCIPP*	Miscarriage, birthweight	DPHP, *BDCIPP* not associated with miscarriage, birthweight, but study was small.	Feng et al. 2016
Thyroid				
Cross-sectional (n = 51)	Urine 2010-2011 DPHP	Thyroid hormone concentrations	DPHP associated with increase in total TT4, especially among women, but not associated with other thyroid hormones.	Preston et al. 2017

[a] Polyhalogenated OPs are identified by boldface, and halogenated metabolites are identified by italic type.
[b]Exposure period for prospective studies of perinatal exposures in relation to later reproductive-developmental outcomes. No study period listed for cross-sectional studies concurrently measuring exposure and outcome.
Abbreviations: DPHP, diphenyl phosphate; ip-PPP, isopropylphenyl phenyl phosphate; BDCIPP, bis(1,3-dichloro-2-propyl) phosphate; BCIPHIPP, 1-hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate; OPFR, organophosphate flame retardant; TDCPP, tris(1,3-dichloro-2-propyl) phosphate; TPP, triphenyl phosphate; TSH, thyrotropin; TT4, total thyroxine.

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TABLE 3-7 Developmental Toxicity of Polyhalogenated Organophosphates

Chemical	Experimental Results
TDCPP	No developmental toxicity or thyrotoxicity in rats exposed during gestation and weaning (Moser et al. 2015).
	Suppression of sexual behavior and reduced testes in male rats exposed neonatally (Kamishima et al. 2018).
	NOAEL of 100 mg/kg-day in rats (significant increase in rate of resorption, significant decrease in fetal viability index, retarded skeletal development); maternal toxicity observed (NOAEL, 25 mg/kg-day) (Stauffer Chemical Co. 1978).
	NOAEL of 200 mg/kg-day in rats (based on fetal mortality); no effects on neurodevelopment at 200 mg/kg-day or below; maternal toxicity observed (NOAEL, 100 mg/kg-day) (Tanaka et al. 1981).
TCEP	No developmental toxicity or thyrotoxicity in rats exposed during gestation and weaning (Moser et al. 2015).
	NOAEL of 100 mg/kg-day in rats for maternal toxicity and developmental toxicity (Kawashima et al. 1983); teratogenic and neurobehavioral effects were evaluated.
TCPP	Two-generation reproductive toxicity study in rats found LOAEL of 1,500 mg/k-day based on an increased number of runts (TNO Quality of Life 2007); preliminary range-finding study had similar results.
	Study in Wistar rats found NOAEL of 1,000 mg/kg-day; a few cases of missing 13th ribs and cervical ribs; no maternal toxicity (Kawasaki et al. 1982).
TDBPP	No teratogenic effect in rats (IPCS 1995a; IARC 1999b).

Abbreviations: LOAEL, lowest observed-adverse-effect level; NOAEL, no-observed-adverse-effect level.

Zebrafish Studies

Toxicity data on four of the polyhalogenated OPs (TDCPP, TCEP, TCPP, and TDBPP) were available from the zebrafish model system. Depending on the polyhalogenated OP, chemical exposure of zebrafish resulted in malformations or behavioral changes, although contradictory (negative) studies have also been reported (Table 3-8). Study details and key findings from the studies are presented in Appendix D (Table D-1). Other studies of zebrafish in which teratogenic or developmental neurotoxic effects were not assessed were also reviewed and are summarized in Appendix D (Table D-2).

Several laboratories conducted zebrafish studies with multiple polyhalogenated OPs that allowed more direct comparisons between subclass members. Exposure concentrations of polyhalogenated OPs varied among chemicals. Noyes et al. (2015) reported that TDBPP (3.3 μM) and TDCPP (10 μM) had overt toxicity thresholds, and none was found for TCEP or TCPP up to 100 μM. A similar trend was observed for neurotoxicity with effect thresholds at 0.56 μM for TDBPP, 3.14 μM for TDCPP, 31.4 μM for TCEP, and 100 μM for TCPP (Noyes et al. 2015). McGee et al. (2012), Du et al. (2015), and Alzualde et al. (2018) likewise noted greater toxicity of TDCPP than of TCEP or TCPP. Differences in responses were also seen among the four chemicals. Dishaw et al. (2014) found that TCEP, TCPP, and TDBPP were not teratogenic, whereas TDCPP was teratogenic. Noyes et al. (2015) concluded that TCEP and TCPP exhibited similar neurotoxicity, but the neurotoxicity of TDCPP differed from that of TCEP and TCPP. Dach et al. (2019) observed hypoactivity as an effect of TCPP and TCEP at 30 μM 96 and 120 h after fertilization and detected no malformations up to this test concentration. In adult fish, TDCPP and TCEP were found to undergo metabolism through a dechlorination pathway, but TDCPP had a longer half-life in tissues than TCEP (Wang et al. 2017). The data also indicate that differences among the chemicals regarding neurotoxic, teratogenic, and developmental toxicity depend on the exposure period.

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TABLE 3-8 Zebrafish Teratology and Developmental Neurotoxicity Studies of Polyhalogenated Organophosphates

Chemical	Studies of malformations	Studies of developmental neurotoxic effects or altered locomotor activity
TDCPP	Increased incidence found by Alzualde et al. 2018; Behl et al. 2015[a]; Dishaw et al. 2014; Fu et al. 2013; Godfrey et al. 2017; Li et al. 2018; McGee et al. 2012; Truong et al. 2014[a]; Wang et al. 2015a,b,c; Yu et al. 2017.	Increased incidence found by Cheng et al. 2017; Dishaw et al. 2014; Jarema et al. 2015; Li et al. 2018; Noyes et al. 2015; Oliveri et al. 2015, 2018; Reif et al. 2016.
TCEP	Increased incidence found by Alzualde et al. 2018; Wu et al. 2017. Negative results reported by Behl et al. 2015; McGee et al. 2012; Truong et al. 2014.	Increased incidence found by Alzualde et al. 2018[b]; Dach et al. 2019; Dishaw et al. 2014; Jarema et al. 2015; Noyes et al. 2015; Sun et al. 2016. Negative results reported by Reif et al. 2016.
TCPP	Negative results reported by Dach et al. 2019; Dishaw et al. 2014; McGee et al. 2012; Noyes et al. 2015.	Increased incidence reported by Dach et al. 2019; Dishaw et al. 2014; Noyes et al. 2015.
TDBPP	Negative results reported by Dishaw et al. 2014.	Increased incidence found by Dishaw et al. 2014.

[a]Malformations associated with increased mortality.
[b]Effects associated with systemic toxicity.

TABLE 3-9 Summary of Experimental Evidence of Developmental Effects in Mammals and Zebrafish Associated with Polyhalogenated Organophosphates

	Mammalian		Zebrafish	
Chemical	Teratogenic	Developmental Neurotoxicity	Teratogenic	Developmental Neurotoxicity or Altered Locomotor Activity
TDCPP	–	–	+	+
TCEP	–	–	+	+
TCPP	–	Not determined	–	+
TDBPP	–	Not determined	–	+

Hazard Assessment of the Polyhalogenated Organophosphates

After data evaluation, the next step in the scoping plan is integration of the analyses for each end point of interest, for example, mutagenicity, carcinogenicity, and developmental toxicity. Regarding developmental toxicity, the committee considered the best-studied chemicals in the polyhalogenated OP subclass (Table 3-9). Oral exposure of pregnant rats to TDCPP, TCEP, TCPP, or TDBPP did not produce teratogenic or developmental neurotoxicity in their offspring. However, studies of the same chemicals in zebrafish found positive results for teratogenic effects of TDCPP and TCEP and developmental neurotoxicity for all four; this suggests developmental effects. A TDCPP metabolite, BDCIPP (Hoffman et al. 2017), has not been associated with changes in working memory or IQ deficits in children (Castorina et al. 2017).

The developmental toxicity data on the four chemicals are discordant. Several options for handling the discordant data are discussed after the next case study.

CASE STUDY 2: POLYHALOGENATED BISPHENOL ALIPHATICS

The committee identified 11 chemicals in the subclass of polyhalogenated bisphenol aliphatics (Table 3-10). The subclass members have bisphenol A as the core structure.

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TABLE 3-10 Polyhalogenated Bisphenol Aliphatics

Chemical Name	Abbreviations	CAS No.
Tetrabromobisphenol A (phenol, 4,4'-(-methylethylidene) bis[2,6-dibromo-])	TBBPA	79-94-7
Tetrachlorobisphenol A	TCBPA	79-95-8
Tetrabromobisphenol A bis(2-hydroxyethyl) ether (ethanol, 2,2'-[(1-methylethylidene) bis[(2,6-dibromo-4,1-phenylene)oxy]]bis-)	TBBPA-BHEE TBBPA-OHEE	4162-45-2
Tetrabromobisphenol A bis(dibromopropyl ether) (benzene, 1,1'-(1-methylethylidene) bis[3,5-dibromo-4-(2,3-dibromopropoxy))	TBBPA-BDBPE TBBPA-DBPE	21850-44-2
Tetrabromobisphenol A BME (benzene, 1,1'-(1-methylethylidene) bis[3,5-dibromo-4-methoxy)	TBBPA-BME TBBPA-DME	37853-61-5
3,3',5,5'-Tetrabromobisphenol A bispropionate (phenol, 4,4'-(1-methylethylidene) bis[2,6-dibromo-, dipropanoate (9CI))	TBBPA-BP	37419-42-4
Tetrabromobisphenol A bis(2-hydroxyethyl) ether bis(acrylate) (2-propenoic acid, 1,1'-[(1-methylethylidene)bis[(2,6-dibromo-4,1-phenylene)oxy-2,1-ethanediyl]] ester)	TBBPA-BHEEBA	66710-97-2
Tetrabromobisphenol A diglycidyl ether (oxirane, 2,2'-[(1-methylethylidene) bis[(2,6-dibromo-4,1-phenylene)oxymethylene]]bis-)	TBBPA-BGE	3072-84-2
Phenol, 4,4'-(1-methylethylidene)bis[2,6-dibromo-, 1,1'-diacetate	TBBPA-BOAc	33798-02-6
Tetrabromobisphenol A diallyl ether (benzene, 1,1'-(1-methylethylidene)bis[3,5-dibromo-4-(2-propen-1-yloxy)-)	TBBPA-BAE	25327-89-3
2,2,6,6-Tetrabromo bisphenol A diacrylate (2-propenoic acid, 1,1'-[(1-methylethylidene)bis(2,6-dibromo-4,1-phenylene)] ester)	TBBPA-BA	55205-38-4

TBBPA is the base structure of this subclass, and TCBPA is the chlorinated analogue (Figure 3-5). Ten of the bisphenol A-based OFRs contain bromines in the 3,3',5,5' positions. There is a comprehensive traditional toxicology database on TBBPA. TBBPA and TCBPA are active against several biological targets in in vitro assay systems. Other members of the subclass have various R group substitutions on the phenolic carbons, and these substitutions might alter targets of activity in in vitro studies in contrast with TBBPA. In mammals and other vertebrates, conjugation with glucuronic acid or sulfate on the TBBPA and TCBPA phenolic rings leads to (or is predicted to lead to) relatively rapid excretion. Metabolism of other subclass members might occur on the phenolic substitutions and possibly lead to metabolic and toxicologic divergence.

TBBPA and related subclass members have been assessed by several authoritative bodies (IPCS 1995b; ECB 2006; Environment Canada and Health Canada 2013; EFSA 2011a; NTP 2014; EPA 2015b). As noted above, the committee searched for additional traditional toxicology data and NAM data that might be incorporated to facilitate bridging from the well-studied chemicals to the poorly studied ones. PubChem, SciFinder, and ChemID plus were searched along with the European Food Safety Authority, ECHA, EPA, Environment Canada and Health Canada, and International Programme on Chemical Safety Web sites. Primary searches were restricted to sources indexed by CAS number. Additional focused searches were conducted for identified biologic targets of subclass members to locate comparative data on at least two subclass members. Genotoxicity data are provided in Table 3-11. As a subclass, these chemicals have been negative in the Ames assay and generally lack structural alerts that suggest a mutagenic potential. Data on the subclass from mammalian subchronic and chronic studies are presented in Table 3-12. Only one subclass member, TBBPA, has been tested for carcinogenicity.

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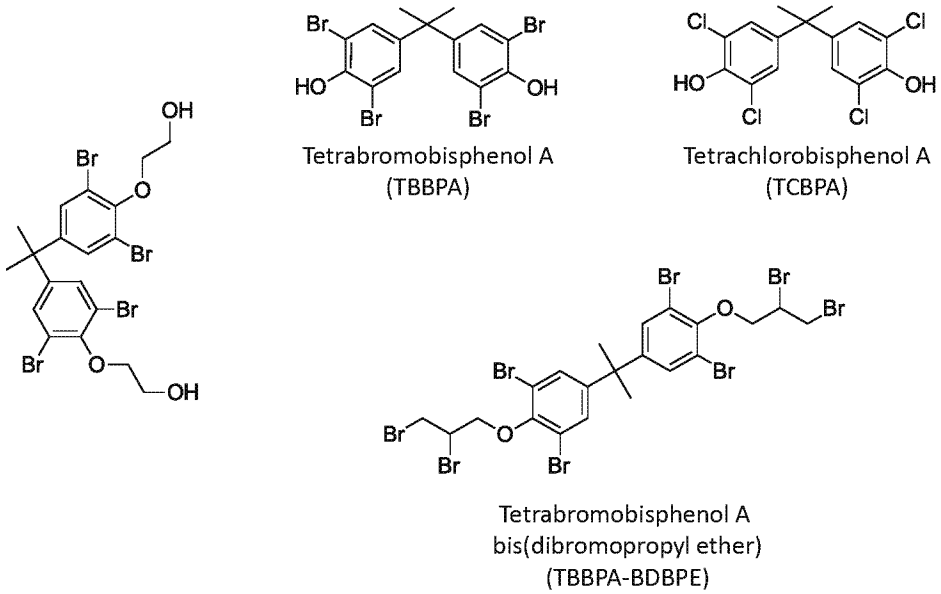

FIGURE 3-5 Representative members of polyhalogenated bisphenol aliphatics.

TABLE 3-11 Genotoxicity Data on Polyhalogenated Bisphenol Aliphatics

		Alerts[a]	
Chemical	Experimental Results	Ames	In Vivo Clastogenicity
TBBPA	Ames (-)[b] In vivo cytogenicity (-)[b] In vitro DNA damage (+/-)[c] In vivo DNA damage (-)[c] In vivo mutation (+)[c]	No	No
TCBPA	No data	No	No
TBBPA-BHEE	Ames (-)[d]	No	Yes
TBBPA-DBPE	Ames (+/-)[e] Clastogenicity (-)[e]	Yes	Yes (aliphatic halogen)
TBBPA-BME	No data	No	No
TBBPA-BP	No data	No	No
TBBPA-BHEEBA	No data	No	Yes
TBBPA-BGE	Ames (-)[f] Clastogenicity (-)[f]	Yes	Yes (epoxide)
TBBPA-BOAc	No data	No	No
TBBPA-BAE	Ames (-)[e]	No	No
TBBPA-BA	No data	No	No

Note: (+), positive results; (-), negative results; (+/-), conflicting results.
[a]Toxtree, http://toxtree.sourceforge.net/ames.html; http://toxtree.sourceforge.net/mic.html.
[b]NTP (2014).
[c]IARC (2018).
[d]IPCS (1995b).
[e]EPA (2015c).
[f]ECHA (2018a).

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TABLE 3-12 Subchronic and Chronic Toxicity Studies of Polyhalogenated Bisphenol Aliphatics

Chemical	Experimental Results
Subchronic Studies	
TBBPA	Rats: NOAEL, 100 mg/kg-day; ↓T4 (no change in T3 or TSH), ↑liver weight; ↓spleen weight (NTP 2014). Mice: NOAEL, 100 mg/kg-day; ↑liver weight, ↓spleen weight, ↓kidney weight, histopathologic changes in kidney (NTP 2014).
TBBPA-BHEE	Non-GLP 28-day study: NOAEL, 1,000 mg/kg-day (IPCS 1995b).
TBBPA-DBPE	90-day GLP gavage study in F344N rats and B6C3F$_1$ mice: no toxicity reported at doses up to 1,000 mg/kg-day in rats or 2,000 mg/kg-day in mice (highest doses tested) (NTP 2017).
TBBPA-BGE	28-day GLP/guideline oral study in Wistar rats: NOAEL, 300 mg/kg-day; at 1,000 mg/kg-day, rats had ↓body weight, ↑liver weight, centrilobular hypertrophy, ↑alanine aminotransferase, aspartate aminotransferase, bile acids (considered treatment-related but not adverse) (ECHA 2018a).
Chronic Studies	
TBBPA	Gavage studies in B6C3F$_1$/N mice and Wistar Han [Crl:WI(Han)] rats (NTP 2014). Male mice: significant increases in incidence of hepatoblastoma. Female mice: no significant increases in tumor incidence. Male rats: no significant increases in tumor incidence. Female rats: significant increase in incidence of adenocarcinoma of the uterus; several rats had malignant mixed Müllerian tumor, a rare uterine tumor; significant increase in incidence of adenoma, adenocarcinoma, or malignant mixed Müllerian tumor (combined) of the uterus.

Abbreviations: NOAEL, no-observed-adverse-effect level; GLP, good laboratory practices; T4, thyroxine; T3, triiodothyronine; TSH, thyroid-stimulating hormone.

As with the first case study, the hazard assessment of the polyhalogenated bisphenol aliphatics would consider multiple end points of interest. To provide another example of data evaluation and integration, the committee focused its evaluation of this OFR subclass on its ability to alter thyroid hormone homeostasis in adults or neonates and to produce morphologic or behavioral developmental effects. The committee considered epidemiologic, mammalian toxicity, and zebrafish data and considered exposures relevant if they occurred during development. The following subsections discuss the various data streams and their evaluation and integration for the specific end points noted.

Epidemiologic Studies

Two studies have investigated the polyhalogenated bisphenol aliphatics in relation to health outcomes in human samples (Table 3-13). One small study of mother–infant pairs at delivery suggested that TBBPA might be associated with higher free T4 in serum but not other endocrine markers in peripartum women; the effect was not observed in their infants (Kim and Oh 2014). A cross-sectional study of adolescents did not find contemporary TBBPA concentrations to be associated with attention, working memory, or thyroid hormone concentrations; however, studies of neurodevelopment ideally investigate exposure during earlier windows of brain development, such as the prenatal period or early childhood, rather than contemporary exposure during adolescence (Kiciński et al. 2012). The current epidemiology literature contributes little to hazard assessment, but assays of TBBPA and other subclass members could complement cohort studies with stored prenatal or early life samples.

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TABLE 3-13 Summary of Human Epidemiologic Studies of Polyhalogenated Bisphenol Aliphatics

Study Design	Chemical	Outcome Measured	Results	Reference
Human infants (n = 38, 26 with congenital hypothyroidism and 12 typical)	TBBPA	Thyroid	TBBPA higher in infants than mothers, but the two correlated; TBBPA exposure was associated with higher maternal free thyroxine, higher thyroid peroxidase antibody micro AB, and thyroid-stimulating immunoglobulin; interpretation of study results difficult due to small, hypothyroid sample.	Kim and Oh 2014
Cross-sectional study of adolescents (age 13–17 years) (n = 515)	TBBPA	Attention, motor development, thyroid	Contemporary TBBPA was not associated with attention, working memory, motor function, or thyroid hormone concentrations.	Kiciński et al. 2012

TABLE 3-14 Mammalian Developmental Toxicity Studies of Polyhalogenated Bisphenol Aliphatics

Chemical	Experimental Results
TBBPA	Prenatal studies in rodents: no effects in standard studies at doses up to 10,000 mg/kg-day (ECB 2006[a]).
	Two-generation rat study: NOAEL for fertility, fecundity, and developmental changes was 1,000 mg/kg-day; ↓T4 at LOAEL of 100 mg/kg-day; and ↓T3 at 1,000 mg/kg-day (reported in NTP 2014[b]).
	Negative in uterotrophic assay by both oral and subcutaneous routes in mice (Ohta et al. 2012). Positive in mice by intraperitoneal injection (Kitamura et al. 2005).
	EPA (2015c): moderate evidence of developmental effects.[c]
	Developmental toxicology studies in rats found no significant developmental effects; NOAEL of 1,000 mg/kg-day (Cope et al. 2015).
TBBPA-DBPE	Prenatal developmental toxicity study (rats, oral, according to OECD Guideline 414): fetal NOAEL of 1,000 mg/kg-day; maternal NOAEL of 300 mg/kg-day (↓food consumption, ↓body weight) (ECHA 2018b).

[a]Primary references are MPI Research (2001), Noda et al. (1985), and Velsicol Corporation (1978).
[b]Primary reference is ACC (2002).
[c]Primary references are Goldenthal et al. (1978), Noda et al. (1985), MPI Research (2001), ACC (2002), Darnerud (2003), Tada et al. (2006), and Saegusa et al. (2009).

Mammalian Toxicity Studies

In subchronic studies, TBBPA has been shown to decrease circulating T4 concentrations with no change in either triiodothyronine (T3) or thyroid-stimulating hormone (TSH) concentrations, increase liver weight, and decrease spleen weight in rats and mice (NTP 2014). Additional evidence from animal studies suggests that exposure to TBBPA can alter thyroid homeostasis in rodents (Lilienthal et al. 2008; Van der Ven et al. 2008; Cope et al. 2015). Lai et al. (2015) proposed that the thyroid effects are mediated by induction of UGT1A that results in increased T4 catabolism. Sanders et al. (2016) reported decreased serum T4 concentration and increased hepatic and uterine expression of Thra gene that encodes TRα in rats 24 h after 5 days of oral administration of TBBPA. They also noted UGT1A was upregulated in the liver and uterus after TBBPA administration; however, they concluded that the mechanism for changes in T4 is uncertain. TBBPA can displace T4 from its plasma transport protein (Hamers et al. 2006) and inhibits binding of T3 to the thyroid receptor TRβ1 and binding of T4 to transthyretin (Meerts et al. 2000; Sun et al. 2009). Meerts et al. (2000) found that TCBPA inhibits T4 binding to transthyretin but with less potency than TBBPA. Despite effects on thyroid metabolism, the polyhalogenated bisphenol aliphatic subclass, however, has minimal developmental effects in mammals (Table 3-14).

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Zebrafish Studies

TBBPA is toxic to zebrafish embryos, and there is a database on TBBPA-related effects on thyroid hormone concentrations and actions in zebrafish that suggest that the zebrafish assay might be a useful model for assessing similarities and differences within the structural class. For example, Chen et al. (2016) reported an increase in the expression of UGT genes after TBBPA exposure, which they note could affect T4 metabolism and then lead to neurobehavioral changes. Zebrafish studies of TBBPA, TCBPA, TBBPA-BHEE, TBBPA-BDBPE, and TBBPA-BME are available. Effects of this subclass on thyroid and development (including behavior) are summarized in Appendix D (Tables D-3 and D-4). Additional zebrafish studies are summarized in Table D-5.

Class Hazard Assessment Based on Effects on Thyroid Homeostasis

The committee's initial evaluation considered TBBPA, which is the best-studied chemical in the polyhalogenated bisphenol aliphatic subclass (Table 3-15). TBBPA can alter thyroid homeostasis so as to result in inconsistent changes in T3 and T4 concentrations. The data are discordant between rodent and zebrafish. Additional in vitro, zebrafish, and other thyroid-homeostasis studies of the polyhalogenated bisphenol aliphatics could provide confidence that thyroid function can serve as a key end point for hazard classification.

Class Hazard Assessment Based on Developmental Toxicity

The committee's evaluation initially considered the best-studied chemicals in the polyhalogenated bisphenol aliphatic subclass (Table 3-16). Each chemical has been studied in zebrafish, and the results are discordant. The rodent developmental toxicity studies of two subclass members have also resulted in mixed findings. Overall, the data on the four best-studied chemicals in the subclass are discordant for this specific end point. Analysis of other end points might support a hazard assessment of the subclass. The next section discusses the committee's recommendations for dealing with discordant data.

TABLE 3-15 Summary of Evidence on TBBPA and Changes in Thyroid Homeostasis

Species	Finding	Reference
Human	TBBPA exposure was associated with higher maternal free T4, thyroid peroxidase antibody micro AB and thyroid-stimulating immunoglobulin.	Kim and Oh (2014)
Human	TBBPA was not associated with attention, working memory, motor function, or thyroid hormone concentrations.	Kiciński et al. (2012)
Rat	↓T4 (no change in T3 or TSH)	NTP (2014)
Rat	Two-generation rat study: NOAEL for fertility, fecundity, and developmental changes was 1,000 mg/kg-day; ↓T4 at LOAEL of 100 mg/kg-day; and ↓T3 at 1,000 mg/kg-day.	NTP (2014)
Zebrafish	↑ T4; ↓ T3.	Zhu et al. (2018)
Zebrafish	Interference in thyroid homeostasis.	De Wit et al. (2008)

TABLE 3-16 Summary of Evidence on Developmental Effects in Mammals and Zebrafish Associated with Polyhalogenated Bisphenol Aliphatics

	Mammals[a]	Zebrafish[b]	
Chemical	Developmental toxicity	Teratogenesis	Locomotor Activity
TBBPA	+/–	+	+/–
TBBPA-DBPE	+/–	–	–
TBBPA-BHEE	Not determined	+	+
TBBPA-BME	Not determined	–	Not determined

[a]See Table 3-14.
[b]See Appendix D, Table D-4.

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ADDRESSING DISCORDANT DATA

Both case studies had discordant data on developmental toxicity between species (for example, rodent vs zebrafish), within a species, or both. Table 3-17 presents the options that could be considered when discordant data are identified: performing analyses, collecting new data, reclassifying the subclass, or making policy decisions. The approaches listed in Table 3-17 for the various options are offered as examples and are not meant to constitute a comprehensive list of all approaches that might be useful or appropriate.

Analyses

One possible explanation for discordant data within an OFR subclass might be differences in pharmacokinetics. For example, an analysis of the pharmacokinetic data on two members of the polyhalogenated bisphenol aliphatic subclass (TBBPA and TBBPA-DBPE) showed different bioavailabilty in rodents after oral exposure. Unlike TBBPA, TBBPA-DBPE is poorly absorbed from the gastrointestinal tract (Hakk et al. 2000; Kuester et al. 2007; NTP 2017). That observation might partly account for the lack of effects seen in rodent developmental toxicity studies. Metabolism data could also help to identify chemicals within a subgroup that share metabolic intermediates and, by extension, toxicity profiles.

Another approach to resolving discordant data is to explore the mechanisms of action of subclass members. One method of collecting mechanistic data is to collect NAM data on subclass members (Box 3-3). Such data can help to identify OFR biologic targets of interest. Mechanistic data have many possible applications, including the following:

- Inform additional approaches to grouping the chemicals. For example, several members of the polyhalogenated OP subclass are structurally similar to some nonpolyhalogenated OP counterparts used as agrochemicals. The nonhalogenated OPs are associated with diverse toxicity mechanisms, including cholinesterase inhibition, endocrine disruption, and neurotoxicity. In vitro assays and other NAM data could be used to evaluate whether some polyhalogenated OPs inhibit acetylcholinesterase and could therefore be associated with cholinergic neurotoxicity.
- Determine the most appropriate animal models for human risk assessment.
- Identify analogues, including nonhalogenated chemicals, that have a common mechanism of action. Data on those chemicals can help to inform the risk assessment of the subclass.

TABLE 3-17 Options and Approaches for Handling Discordant Data

Options	Approach	Advantages	Potential Pitfalls
Perform analyses	Evaluate data quality, pharmacokinetics, or specific physicochemical properties, such as log P; investigate doses, metabolites, exposure windows, or other features that could explain discordance.	Potential to understand discordance and move forward.	Might not reveal clear reason for discordance.
Generate new data	Conduct NAM studies, targeted animal studies, or new evaluations of epidemiologic samples.	Can increase clarity and scientific basis of decision.	Is time-consuming, is expensive, and could still be discordant.
Reclassify	Divide or merge classes or identify individual "outlier" chemicals.	Refines the subclass.	Presents a potential for repeated reclassifications and could result in individual chemical assessments.
Make policy decisions	Impute any hazard identified within the class to chemicals on which there are no data.	Provides a health-protective default and incentivizes data generation.	Could encounter statutory and regulatory limitations.

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It is not uncommon for different responses to occur among studies that use different species (such as rats, mice, and zebrafish), different strains within a species (such as strains of rats or mice), or different exposure windows. Assessing the human relevance of different species or strains can be useful for understanding which data would be expected to be more predictive of a selected effect, such as changes in thyroid hormones or mutagenicity. No single strain or species is expected to be predictive of all human health effects of interest, and nonmammalian species are increasingly recognized as providing reasonable predictions of selected effects in humans. However, using nonmammalian species can pose additional challenges. For example, both case studies used zebrafish data, which often were discordant. One possible explanation of discordance between zebrafish data and rodent data is that ex vivo exposure of zebrafish embryos or larvae to the parent OFR might not reflect in vivo metabolism that might occur in the rodent species. Further testing with OFR metabolites might be necessary for appropriate comparisons and yield concordance of data among species. A possible explanation for discordance among zebrafish data might be that different study protocols were used to expose the zebrafish to OFRs. Although a standardized OECD guideline (Test No. 236: Fish Embryo Acute Toxicity Test) was published in 2013, many laboratories do not use it. As a result, there are variations in developmental toxicity study designs, especially regarding the exposure window. There are also challenges in using zebrafish data, including extrapolation of water concentrations to doses that are suitable for human health assessment, but there has been progress in addressing this challenge in recent studies in which total chemical mass (based on exposure concentration and volume) relative to the mass of embryo immersed in the exposure solution is calculated. Those values can then be compared with human exposure values to determine whether a hazard would be expected to be associated with an environmentally relevant exposure.

In assessing discordant data, it is important to consider study design and implementation, which could influence observed results and might explain the discordance. For example, studies can have different powers to detect outcomes depending on the numbers of subjects or the exposure periods in reproductive-developmental studies. The doses or concentrations used in studies also can influence outcomes, and assessment of chemical purity can be important to ensure that effects do not arise from a contaminant.

Collection of New Data

Chapter 2 (Box 2-3) outlined a tiered approach for the collection of new data when there are no relevant data on any member of a subclass. That tiered approach could also be applied to the discordant-data scenario. However, the assessment that resulted in the conclusion of discordant data should have illuminated data gaps that should be useful in directing the data-collection effort.

Reclassification of the Subgroup

As mentioned above, mechanistic and pharmacokinetic data might suggest that a subgroup formed primarily on the basis of chemical structure needs to be parsed into two or more smaller subgroups. In some cases, chemicals that have multiple chemotypes are included in more than one subclass, and these chemicals might be the source of discordant data in one of the subclasses. Removing the chemical from the subclass might be appropriate then. As mentioned in Chapter 2, however, the committee advises that reclassification should be performed judiciously to obviate a never-ending cycle of reclassification.

The committee also recognizes that alternative approaches have been used to generate OFR subclasses. For example, a study conducted by the Danish EPA (2016) grouped 67 brominated flame retardants on the basis of their chemical structures and then made QSAR predictions of selected environmental and health effects. Despite the existence of alternative classification schemes, the committee recommends that CPSC begin its analyses with the subclasses described earlier in this chapter.

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Policy Decisions

The class approach relies on using data on tested chemicals to draw inferences about the potential hazard associated with class members that have not been tested. That approach is scientifically supported most strongly when many of the available data support a single conclusion (for example, when a specific toxicity is observed). When class members on which there are data appear to yield discordant findings on an end point, a key question is how to evaluate class members on which there are no data. Several inferences would be possible from the discordant findings, although they will have greater uncertainty than if the findings were concordant. Inferences would include the idea that the class members on which there are no data are similar in toxicity to class members on which there are data—for example, similar to the most toxic chemical or similar to a distribution of observed findings. Each inference could be considered in developing a hazard assessment of the class that would use policy choices to provide appropriate protection of public health. Ideally, the approach would create incentives for stakeholders to collect additional data and reduce the uncertainty in analyses.

PROJECTED TIMELINE AND COSTS

Traditional hazard-assessment methods take years and are too expensive to cover all chemicals under production. The committee has proposed a class-based hazard assessment of OFRs as an efficient alternative to traditional approaches. The proposed class-based process conveys time and cost savings in that one is no longer conducting separate hazard assessments of each individual chemical, and traditional hazard-assessment methods can be complemented by new approaches that take advantage of existing data (read-across) and require less de novo testing.

The next steps in completing class-based hazard assessments of the OFR subclasses will involve literature surveys and data-mapping for relevant toxicity end points. That process will likely require months for a research team to complete. The goal of the initial step is to evaluate whether well-studied subclass members can be used to anchor the assessment. When no such anchor chemical can be identified, it will be necessary to obtain minimal datasets on some subclass members. The committee's initial literature survey exercise suggests that this could be the case for several OFR subclasses. For subclasses on which there are adequate data, there might be a need to create one or more chronic hazard advisory panels (CHAPs) that have specific chemical and end-point expertise. On the basis of CPSC's experience with the CHAP that was formed to address phthalates, completion of that step could take several years.

CONCLUSIONS

This report proposes the use of emerging cheminformatic approaches to systematically expanding and refining the chemical and toxicologic information traditionally considered in assessing possible health hazards posed by a structurally or functionally related chemical class. The larger information base might reveal biologic activities of concern, such as effects on thyroid hormone homeostasis or neurodevelopment at one or more biologic organizational levels (for example, cells, zebrafish, laboratory mammals, and humans). The choices of which health end points are most important, how choices are made in the presence of uncertainty, and the relative importance of health end points require value judgments in the hazard-assessment process. Therefore, the committee recommends that CPSC consider providing both philosophic and regulatory-policy guidance to any CHAP that is charged with carrying out a class-based hazard assessment that uses the approaches described in this document.

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Appendix A

Biographic Information on the Committee to Develop a Scoping Plan to Assess the Hazards of Organohalogen Flame Retardants

David C. Dorman (*Chair*) is a professor of toxicology in the Department of Molecular Biosciences of North Carolina State University. The primary objective of his research is to provide a refined understanding of chemically induced neurotoxicity in laboratory animals that will lead to improved assessment of potential neurotoxicity in humans. Dr. Dorman's research interests include neurotoxicology, nasal toxicology, pharmacokinetics, and cognition and olfaction in military working dogs. He has served as a member or chair of several National Academies committees, including the Committees on Emergency and Continuous Exposure Guidance Levels for Selected Submarine Contaminants, the Committee to Evaluate Potential Health Risks from Recurrent Lead Exposure to DOD Firing Range Personnel, the Committee to Review EPA's Draft IRIS Assessment of Formaldehyde, the Committee to Review the IRIS Process, and the Committee on Endocrine-Related Low-Dose Toxicity. He received his DVM from Colorado State University. He completed a combined PhD and residency program in toxicology at the University of Illinois at Urbana-Champaign and is a diplomate of the American Board of Veterinary Toxicology and the American Board of Toxicology.

Hugh A. Barton *(retired)* was an associate research fellow with Pfizer, Inc. He specializes in the use of physiologically based pharmacokinetic and mechanistic pharmacodynamic modeling to address low-dose, interspecies, and inter-route extrapolations in estimating risks. Dr. Barton is a past president of the Biological Modeling Specialty Section and the Risk Assessment Specialty Section of the Society of Toxicology. He was a member of the National Academies Committee on Inorganic Arsenic. Dr. Barton received his PhD in toxicology from the Massachusetts Institute of Technology.

Karen Blackburn is a Victor Mills Society Research Fellow at Procter and Gamble Co. Her primary expertise is risk assessment to support safe human exposures to environmental contaminants and consumer products with an emphasis on development of novel approaches. Before working at Procter and Gamble, Dr. Blackburn was a toxicologist at the US Environmental Protection Agency. She received a PhD in physiology and biophysics from the University of Cincinnati.

John Bucher is a senior scientist in the National Toxicology Program (NTP) at the National Institute of Environmental Health Sciences (NIEHS). He has held multiple leadership positions within NIEHS and most recently retired as the NTP associate director. His specific research interests include characterization of the toxic and carcinogenic potential of various chemicals, mixtures, and physical agents and issues related to improving research and analysis tools and assays for those purposes. Additional activities include guidance in the development of systematic review procedures and their application in the environmental health sciences. Dr. Bucher is a fellow of the Collegium Ramazzini and a recipient of the Doerenkamp-Zbinden Foundation Award for Animal Protection in Science and numerous NIH awards. He recieved a PhD in pharmacology from the University of Iowa and is a diplomate of the American Board of Toxicology.

Julie L. Daniels is a professor in the departments of epidemiology and maternal and child health at the University of North Carolina at Chapel Hill. Her research focuses on prenatal environmental and nutritional exposures that might affect children's growth, neurodevelopment, and overall health. She has created a platform for

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studying early life exposure to brominated and organophosphate flame retardants, persistent organic pollutants, and long-chain fatty acids in relation to children's health in the Pregnancy, Infection, and Nutrition Study. Dr. Daniels is an associate editor of *Environmental Health Perspectives* and a member of the Autism Research Program Panel of the Department of Defense and US Army Medical Research and Material Command. She received her PhD in epidemiology from the University of North Carolina at Chapel Hill.

Jennifer L. Freeman is an associate professor in the School of Health Sciences of Purdue University. Her research interests are the underlying genetic and epigenetic mechanisms of toxicity of environmental stressors with a focus on pesticides, metals, radiation, and emerging contaminants. Her studies are investigating the developmental origin of health and disease pathogenesis with a specific focus on neurologic disorders, reproductive dysfunction, cardiovascular function, and cancer. She received a PhD in environmental toxicology and molecular cytogenetics from the University of Illinois at Urbana-Champaign.

Kamel Mansouri is lead computational chemist at Integrated Laboratory Systems. Previously, he was an investigator at ScitoVation. In 2013, he joined the National Center for Computational Toxicology at the US Environmental Protection Agency as an ORISE postdoctoral fellow. He has worked on several projects involving quantitative structure-activity relationship (QSAR) modeling, cheminformatics, and data-mining and has collaborated and led projects in the QSAR field. Dr. Mansouri obtained an engineering degree in analytic chemistry from the University of Tunis, Tunisia, an MS in cheminformatics from the University of Strasbourg, France, and a PhD in computational chemistry from the University of Milano Bicocca, Italy.

Carmen Messerlian is a research scientist at the Harvard T.H. Chan School of Public Health. Her research focuses on the effects of environmental chemicals on fertility, pregnancy, and human development. She is working on the Environment and Reproductive Health Study, which involves a prospective preconception cohort established to evaluate environmental and dietary determinants of fertility in couples attending the Massachusetts General Hospital Fertility Center in Boston. She is investigating the effects of phthalates and other emerging chemicals and their mixtures on ovarian reserve, time to pregnancy, pregnancy loss, preterm birth, birthweight, and child development. Her goal is to understand how exposure to environmental chemicals in the preconception and prenatal periods influences a couple's ability to achieve conception, maintain pregnancy, and deliver healthy offspring. Before her research career, she worked on maternal and child public-health strategies for municipal, provincial, and global health programs. Dr. Messerlian received her PhD in epidemiology from McGill University.

David M. Reif is an associate professor of biological sciences at North Carolina State University and a resident member of the Bioinformatics Research Center. His research focuses on the complex interactions between human health and the environment through the integrated analysis of high-dimensional data from diverse sources, including epidemiologic studies, high-throughput screening of environmental chemicals, and model organism data. Dr. Reif was previously a principal investigator with the US Environmental Protection Agency's National Center for Computational Toxicology, where he led several statistical and bioinformatic efforts with federal, academic, and industry partners. He served on the National Academies Committee on Predictive-Toxicology Approaches for Military Assessments of Acute Exposures. Dr. Reif received his PhD in human genetics and MS in statistics from Vanderbilt University and his BS in biology from the College of William and Mary, where he was a Monroe Scholar.

Gina M. Solomon is a principal investigator at the Public Health Institute in Oakland, California, and a clinical professor of medicine at the University of California, San Francisco. She served as the deputy secretary for science and health at the California Environmental Protection Agency from 2012 to 2017. Dr. Solomon's work has spanned a wide array of fields, including children's environmental health, reproductive toxicity, cumulative effects, and the use of novel data streams to screen chemicals for toxicity. Her work has also focused on exposure science in relation to air pollutants, pesticides, mold, and metals in soil, and on the health effects of climate change. She was involved in the aftermath of Hurricane Katrina, the Gulf oil spill, and the Chevron Richmond explosion and fire, and she successfully spearheaded regulations to improve refinery safety in California. Dr. Solomon has served on multiple boards and committees of the National Academies, the US Envi-

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ronmental Protection Agency (EPA) Science Advisory Board, and the National Toxicology Program Board of Scientific Counselors. She also serves on the EPA Board of Scientific Counselors Chemical Safety for Sustainability subcommittee. Dr. Solomon received her MD from Yale and completed her MPH and her residency and fellowship training in internal medicine and occupational and environmental medicine at Harvard.

Chihae Yang is the chief scientific officer of Altamira LLC and managing director and CEO of Molecular Networks GMbH. She is also a visiting professor in the Department of Chemical and Biological Engineering at Ohio State University. Her research interests are in molecular informatics, computational modeling and simulation, and development of chemoinformatics software. Dr. Yang was an ORISE fellow at the US Food and Drug Administration, where she was involved in the design and implementation of the Chemical Risk Estimation and Evaluation System. She is a former board member of the American Society of Cellular and Computational Toxicology. She received her PhD in chemistry from Ohio State University.

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Appendix B

Methodologic Details of Analyses to Evaluate Feasibility of Class Approach and to Define Subclasses

In Chapter 3, the committee presented a reproducible, multifaceted approach for developing subclasses of nonpolymeric, additive organohalogen flame retardants (OFRs) that considered function, chemical structure, and predicted biological activity. The main steps used by the committee can be summarized as (1) identifying and characterizing a "seed" set of chemicals as a working inventory of the class; (2) generating an "expanded" set of chemical analogues to the seed set on the basis of combined functional, structural, and predicted bioactivity information; (3) evaluating the cheminformatic similarity of the seed set to the analogues to evaluate whether the OFRs are distinguishable as a single class; and (4) defining subclasses for hazard evaluation. The goal of that modular approach was to assess the empirical justification for treating a given set of chemicals as a "class" to be evaluated en masse or as a set of subclasses to be evaluated in groups. The committee followed those steps to develop a scientifically sound approach that, although based on the collected seed set of OFRs, is independent of the number of initial structures and thus repeatable and can be applied to future lists of chemicals that might be used as OFRs. This appendix describes the methods used to develop the committee's classification scheme and is organized according to steps described above.

STEP 1. IDENTIFY AND CHARACTERIZE A "SEED" SET OF CHEMICALS

A list of chemicals that have been used as OFRs was compiled from various documents that list OFRs (Table B-1). The sources were Eastmond (2015), the Environmental Protection Agency of Denmark (Danish EPA 2016), the Environment Agency of the United Kingdom (2003), the International Programme on Chemical Safety (IPCS 1997), the European Food Safety Authority (EFSA 2010, 2011a,b,c, 2012a,b), the Consumer Product Safety Commission (TERA 2016), and the US Environmental Protection Agency (EPA 2015). In the present analysis, 161 chemicals that have been used as "flame retardants" were identified. Despite the fair amount of overlap between sources, only a few (<20) chemicals were listed in all sources; this suggests substantial heterogeneity in the chemical space identified by various groups. The committee emphasizes that this inventory is not necessarily comprehensive, and additional OFRs in commerce might exist.

To define the chemical space of OFRs (known OFRs and structural analogues), the committee initially examined and curated the structures of the chemicals identified in the collected inventory of OFRs. First, the names, CAS numbers, and structures in the initial list of 161 OFRs were verified and checked for consistency by using the US Environmental Protection Agency (EPA) Dashboard and other sources. Then, the chemical structures were normalized and deduplicated to generate quantitative structure–activity relationship (QSAR)-ready structures by using a standardization workflow described in Mansouri et al. (2016a,b) and developed in the Konstanz Information Miner (KNIME), an open-source data analytics, reporting, and integration platform (Berthold et al. 2009). Figure B-1 shows the QSAR-ready standardization workflow process.

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Two entries (CAS numbers: 52907-07-0 and 13654-09-6) were identified as mixtures and were not included in the present analyses. An additional 11 entries (CAS numbers: 32534-81-9, 59447-55-1, 855992-98-2, 3194-55-6/CMS201444, 3194-55-6/CMS201445, 3194-55-6/CMS202190, 3194-55-6/CMS220023, 3194-55-6/CMS220024, 3194-55-6/CMS220025, 3194-55-6/CMS220026, and 3194-55-6/CMS220027) were considered duplicate structures.

That process resulted in 148 unique chemical structures in the OFR seed set (see list at OFR_QSAR-ready_120318.sdf). The chemical space of the curated set of 148 seed chemicals was characterized by generating QSAR predictions with an open-source application called OPEn structure–activity/property Relationship App (OPERA) (v2.0) (Mansouri et al. 2016b, 2018), which is freely available from the National Institute of Environmental Health Sciences Github[1] and EPA's CompTox Dashboard.[2] The predictions considered physicochemical properties, environmental fate, and toxicity end points, including estrogen and androgen receptor activities (Mansouri et al. 2016a, 2017), and acute oral toxicity (Kleinstreuer et al. 2018).[3] In addition to predictions, OPERA provides applicability domains and accuracy estimates for each prediction. More information about OPERA outputs can be found on the EPA CompTox Dashboard and OPERA's QSAR model reporting format (QMRF) reports that are registered and validated by the European Commission's Joint Research Center to be OECD-compliant.[4]

TABLE B-1 Sources Used to Identify Chemicals in the OFR Inventory[a]

Data Sources	Description	No. Chemicals	Year
Eastmond	Flame retardants	90	2015
Danish EPA	Brominated flame retardants	66	2016
UK ENV	Flame retardants	68	2003
IPCS	Flame retardants	61	1997
EFSA	Brominated flame retardants in food	79	2010-2012
CPSC	OFR exposure assessment	5	2016
EPA	Flame retardants	9	2015
OFR Inventory		161[a]	—

[a]Some chemicals appear in more than one inventory. See OFR_Categories_OFR-inventory_02-15-2019.xlsx. File available at www.nap.edu/25412.

Abbreviations: CPSC, Consumer Product Safety Commission; Danish EPA, Environmental Protection Agency of Denmark; EFSA, European Food Safety Authority; EPA, US Environmental Protection Agency, IPCS, International Programme on Chemical Safety; UK ENV, UK Environment Agency.

[1]See https://github.com/NIEHS/OPERA.

[2]See https://comptox.epa.gov/dashboard.

[3]See: prediction file: pred_OPERA_OFR.csv and list of OPERA models: OPERA2.0_models.xlsx and OFRs.xlsx. Files available at www.nap.edu/25412.

[4]See https://qsardb.jrc.ec.europa.eu/qmrf/.

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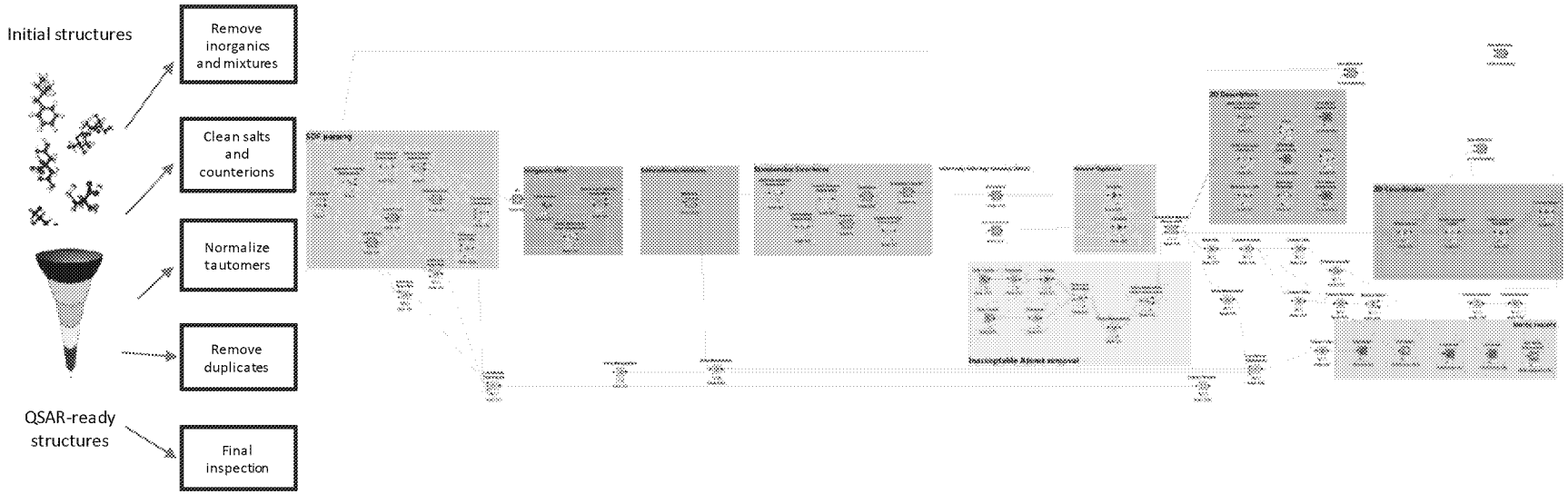

FIGURE B-1 Standardization of structures to provide QSAR-ready structures amenable to modeling: desalted, stereochemistry-stripped, tautomers and nitro groups standardized, valence corrected, structures neutralized when possible, and duplicates removed, among other steps. Figure available at www.nap.edu/25412.

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STEP 2. GENERATE AN "EXPANDED" SET OF CHEMICAL ANALOGUES ON THE BASIS OF COMBINED FUNCTIONAL, STRUCTURAL, AND PREDICTED BIOACTIVITY INFORMATION

To identify the analogues that are structurally similar to the OFR seed set, the committee developed an automated KNIME workflow to identify all organohalogens (about 200,000 structures) from the EPA Distributed Structure-Searchable Toxicity (DSSTox) database (Richard 2004; Richard et al. 2006) (Figure B-2).

The chemicals were then compared with the 148 OFR seed structures to determine the list of most similar OFR analogues. The chemistry-development kit (CDK) fingerprints were used with a Tanimoto similarity-index threshold of 80% (Steinbeck et al. 2003, 2006). That step resulted in an output of 1,073 analogues, which were then analyzed by using the QSAR-ready standardization workflow shown in Figure B-2[5] (Mansouri et al. 2016a,b).

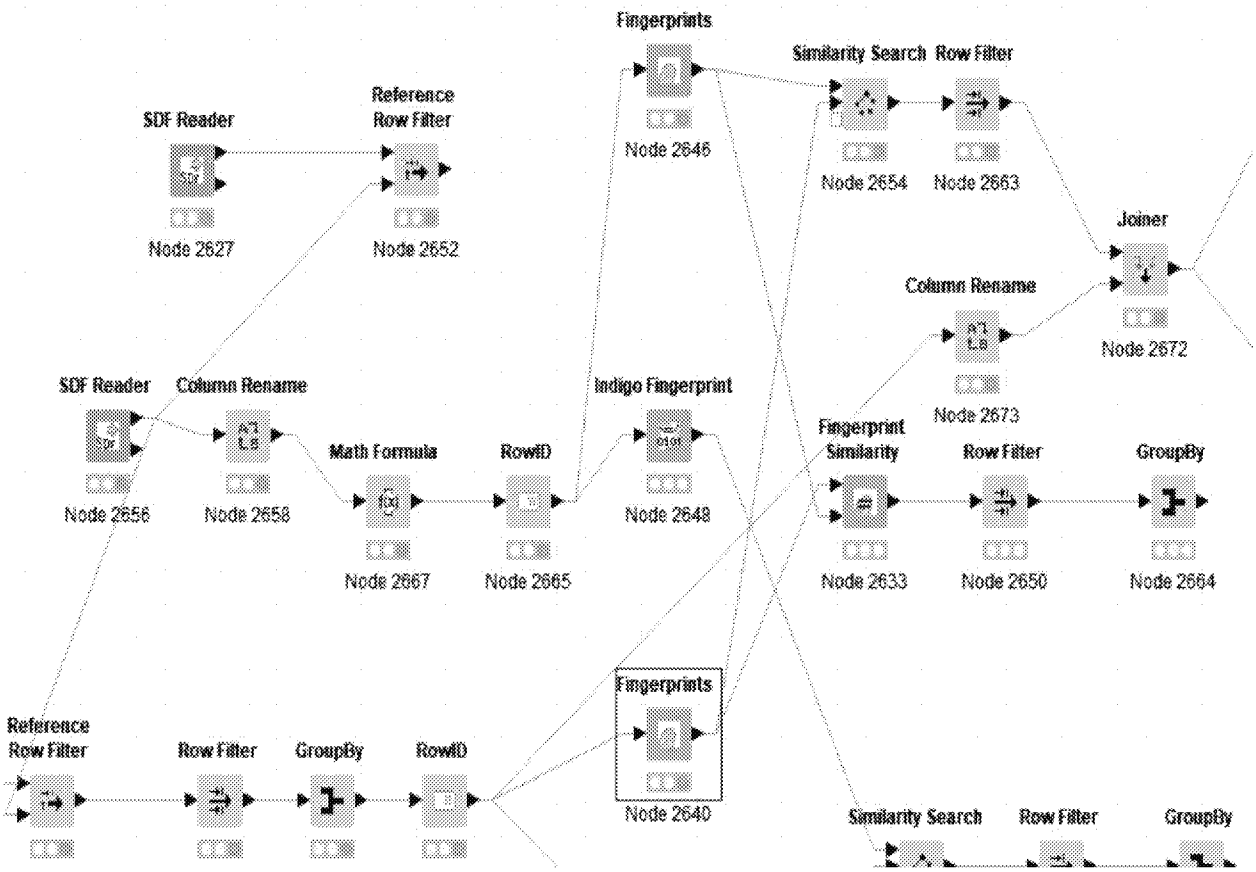

FIGURE B-2 KNIME workflow used to identify organohalogens from DSSTox and determine analogues of OFR seed chemicals.

[5]See OFR.knwf. File available at www.nap.edu/25412.

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The final set of analogue compounds used for comparative analysis (the expanded set) consisted of 1,073 unique structures.[6] Procedures described earlier were then repeated on the expanded set of OFR analogues, including collection of data by running OPERA predictions[7] and retrieval of data on these chemicals from the EPA Dashboard.[8]

STEP 3: EVALUATE THE CHEMINFORMATIC SIMILARITY OF THE SEED SET TO THE ANALOGUES TO EVALUATE WHETHER THE OFRs ARE DISTINGUISHABLE AS A SINGLE CLASS

Comparing OFR Seed Set to Analogues: Unsupervised Analyses

Two unsupervised methods were used to compare features of the OFR seed set with those of the expanded set (analogues): principal components analysis (PCA) of OPERA physicochemical properties and ToxPrint Chemotype Enrichment. PCA is a statistical procedure that uses a number of orthogonal transformations to convert a set of observations of possibly correlated variables into a set of new uncorrelated variables called principal components. The transformations are defined in such a way that the first principal components encode the largest possible variance (condensing the information on the original variables). The results of a PCA are usually discussed in terms of component loadings and scores; the loadings represent the weights of the original variables required to get the scores of the components (Pearson 1901). ToxPrint is a publicly available database of over 700 chemotypes that were developed from a chemical space on which some toxicity data were available (Yang et al. 2015). Chemotypes represent structural fragments expressed with imbedded atomic, electronic, and physicochemical properties. A chemotype can be used to represent a generic query feature (for example, chain:alkene) or a feature informed by biologic activity or as a component of an SAR model as described in this section. Most of the chemotypes in ToxPrint are not structural alerts; rather, more than 90% of them represent generic query features that can help to search and cluster substructures.

PCA of OPERA physicochemical properties. Figure B-3 shows the samples (OFRs) plotted on the basis of their coordinates (scores) for the first two components with the highest accumulated variance among all components encoding the information present in the 45 original OPERA variables. The lengths of the blue lines (loadings) show the importance of each of the 45 OPERA variables. The directions in which the chemicals (OFRs shown as red stars and analogues shown as green dots) are plotted show the similarity according to each specific variable.[9] However, because PCA is a multivariate analysis, the PCA plot shows the interactions and the similarities between the samples (chemicals) in a multivariate fashion. Apart from the cluster of mostly volatile analogue structures that have different vapor-pressure properties, the OFR seed set and the expanded set have similar OPERA-predicted physicochemical, toxicologic, and environmental-fate properties. Also, the first two components encoded less than 50% of the variance, and this indicates the high variability within the properties of the analyzed structures.

ToxPrint Chemotype Enrichment. A second unsupervised analysis was performed by using Toxprint Chemotype Enrichment (Richard et al. 2016) to identify the most enriched Toxprint Chemotypes in the OFR seed and expanded sets. The OFR seed set was found to consist mostly of aromatic chemicals. The analogues contained various types of chemical structures, both aromatic and aliphatic enrichment sites (Figure B-4). Furthermore, several ToxPrint chemotypes are highly enriched in both the seed list and the analogues.

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[6]See OFR_Analogs_QSAR-ready_120318.sdf. File available at www.nap.edu/25412.

[7]See pred_OPERA_OFR_Analogs_120318.csv. File available at www.nap.edu/25412.

[8]See OFR_Analogs_ChemistryDashboard-Batch-Search_2018-12-03_17_05_44.xls. File available at www.nap.edu/25412.

[9]List of abbreviations provided in the supplemental material OPERA2.0_models.xlsx. File available at www.nap.edu/25412.

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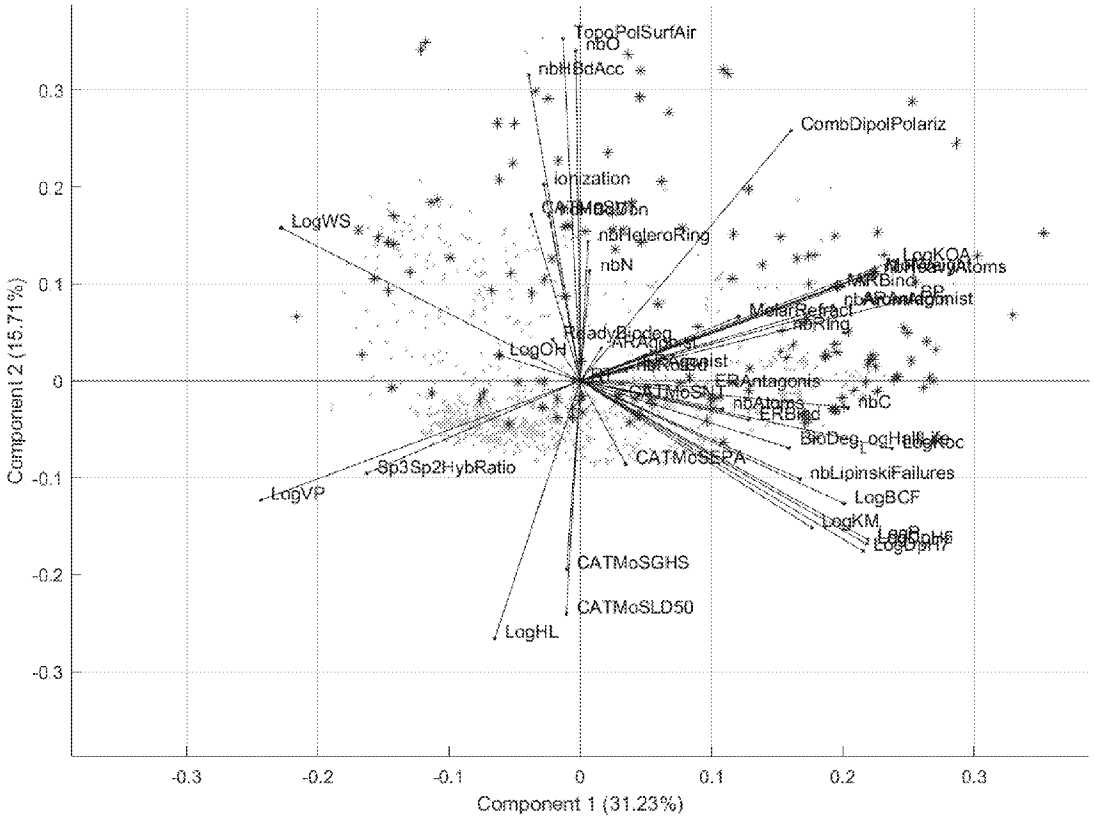

FIGURE B-3 Principal components analysis of the seed OFRs (shown as red stars) and analogues (shown as green dots). Loadings are shown as blue projections.

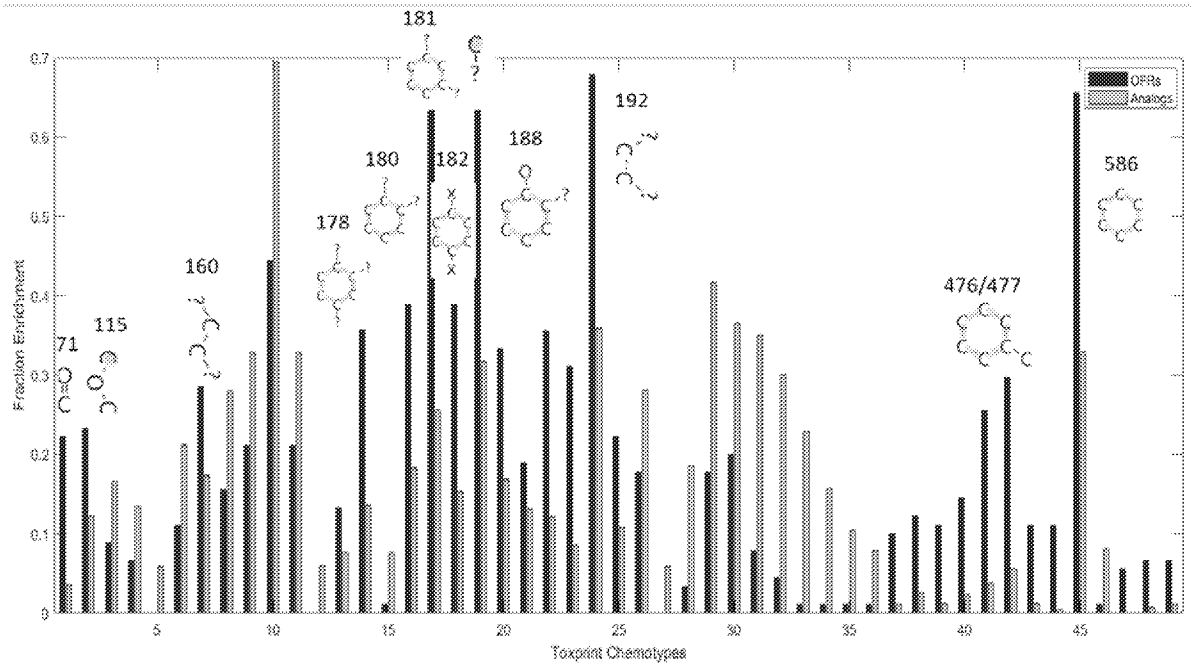

FIGURE B-4 Enrichment sites of OFRs from seed list and expanded set of analogues.

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Comparing OFR Seed Set with Analogues: Supervised Analyses

The goal of supervised analyses was to determine whether a selected set of properties can differentiate the OFR seed set from the analogues accurately. Two approaches, Machine Learning Classification and Supervised PCA, were used.

Machine Learning Classification. The well-known, reliable k-nearest-neighbors (kNN) coupled to genetic algorithms (GAs) were used to find the optimal subset of molecular descriptors that differentiate the OFR seed set and analogues (Todeschini 1989). GA begins with an initial random population of chromosomes, which are binary vectors that represent the presence or absence of molecular descriptors. An evolutionary process is then simulated to optimize a defined fitness function, and new chromosomes are obtained by coupling the chromosomes of the initial population with genetic operations, such as crossover and mutation (Ballabio et al. 2011; Leardi and González 1998). Classification-balanced accuracy (BA) was used as the fitness function and calculated in a five-fold cross-validation procedure. To adapt that method for the present purpose, the committee used OPERA properties and CDK descriptors in the model. Results of the analyses show that the variable selection techniques were able to classify the OFR set and the analogue chemicals with up to 80% balanced accuracy when the highest selected descriptors were used.

Supervised PCA. Following the supervised GA learning techniques, the committee used results on the descriptors and conducted an additional supervised PCA analysis. Specifically, the highest selected descriptors were used in this supervised PCA analysis to judge visually whether these descriptors were able to differentiate the two groups of chemicals. Figure B-5 shows the output from this supervised PCA analysis.

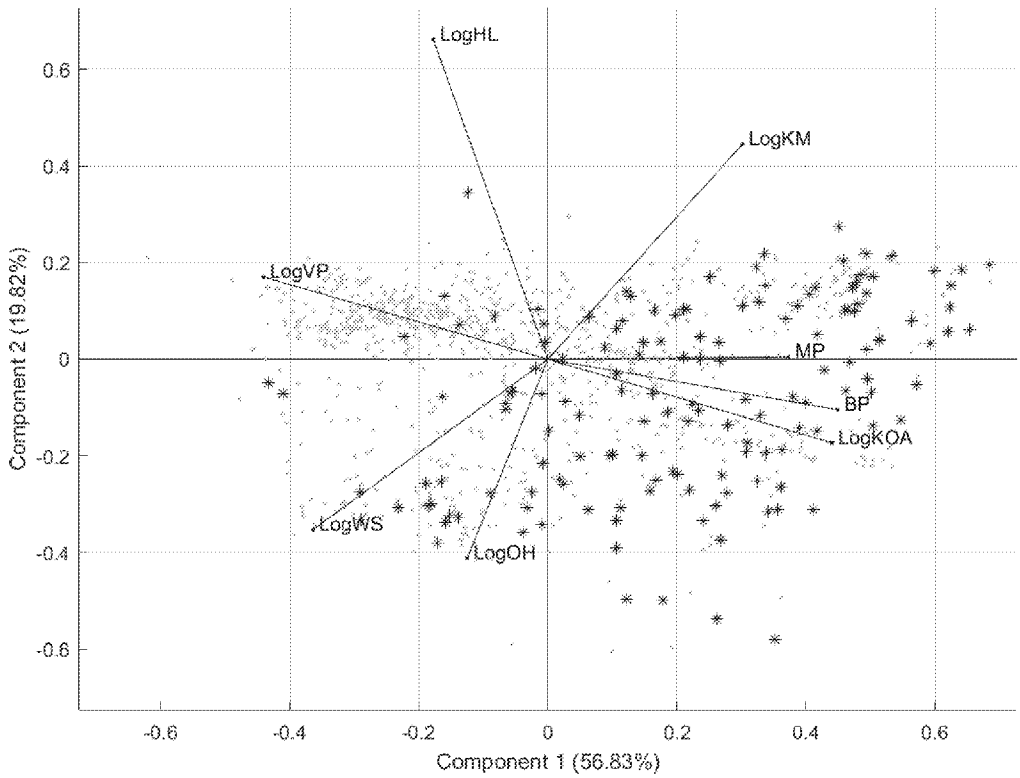

FIGURE B-5 Principal component analysis that used the highest selected descriptors that resulted from the GA procedure: OFRs (shown as red stars) and analogues (shown as green dots). Loadings are shown as blue projections.

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Results of this PCA show that the highest selected descriptors separate analogue chemicals (expanded set) from the OFR seed set; this explains the high balanced accuracy that was reached in the GA-kNN procedure. However, most of the analogue compounds are separated by vapor pressure (LogVP), soil adsorption (LogKM), and water solubility (LogWS). That finding demonstrates that analogues that easily separate from the OFR seed set do not share physicochemical and environmental properties with the chemicals in the seed set. However, the fact that many analogues are not easily separable from the seed set chemicals suggests that the analogues share properties with the OFR seed set.

Conclusion

The analyses show that many existing organohalogen chemicals, although never used as flame retardants, share properties with the known OFRs and might have the potential of being used for the same purpose. Thus, the seed list of OFR chemicals can be separated only on the basis of use category and cannot be considered to be a scientifically defined class by itself on the basis of the structural features or on chemical properties. Consequently, to study the toxicologic and biologic properties, one can categorize the OFRs into subclasses that share functional groups.

STEP 4: DEFINE SUBCLASSES FOR HAZARD EVALUATION

The chemical space of the OFR inventory was profiled in more detail to identify major chemical groups. ToxPrint Chemotypes (Yang et al. 2015; Richard et al. 2016), available in ChemoTyper,[10] were used initially to characterize the 148 structures in the seed set. The full inventory of 161 chemicals was covered by 143 (of 729 possible) chemotypes that are available in ToxPrint.

Some of the major ToxPrint chemotypes encountered in the OFR inventory in this study are listed in Figure B-6. These chemotypes roughly identify the top-level, large categories within the OFR chemical space. They include polyhalogenated analogues of benzenes, aliphatic chains, alicycles and carbocycles (polycycles), organophosphorus chemicals, aromatic and aliphatic ethers, phenols and their derivatives, and aromatic carboxylic esters (phthalates). These observations suggest a subclass approach based on the generic ToxPrint features as chemical classes.

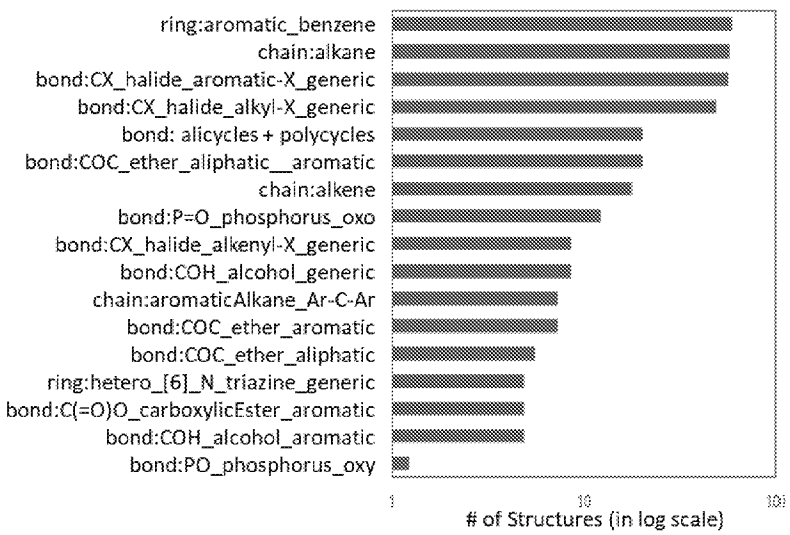

FIGURE B-6 Major chemotypes found in OFR seed set.

[10]See http://chemotyper.org.

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The committee's approach was to develop large commonly occurring core features of the OFR chemicals to represent each group that can also be associated with a predicted biologic activity whenever possible. Using expert judgment, the committee grouped the OFR inventory into eight structural classes on the basis of predicted biologic activity (such as GABA receptors, aromatase activity, and ER/AR modulators) (see Table 3-1). Merging the biology-informed groups with the chemotypes identified in Figure B-6 led to the formulation of 14 OFR categories for the inventory of 161 OR chemicals (Table B-2). Seven OFR chemicals were placed into two subclasses on the basis of their predicted biologic activity and chemical structures. The OFR subclasses were used to support the case studies described in Chapter 3.

TABLE B-2 Fourteen OFR Subclasses Formulated on the Basis of Chemotypes and Predicted Biologic Activity

OFR Subclass	No. Chemicals	CAS No. of Chemicals
Polyhalogenated alicycles	17	25495-98-1; 25637-99-4; 3194-55-6; 3194-57-8; 134237-50-6; 134237-51-7; 134237-52-8; 678970-17-7; 678970-16-6; 678970-15-5; 169102-57-2; 138257-19-9; 138257-18-8; 3322-93-8; 77-47-4; 87-84-3; 1837-91-8
Polyhalogenated aliphatic carboxylate	4	3066-70-4; 5445-17-0; 5445-19-2; 19660-16-3
Polyhalogenated aliphatic chains	12	52434-59-0; 1522-92-5; 3296-90-0; 3234-02-4; 96-13-9; 109678-33-3; 85535-84-8; 71011-12-6; 85535-85-9; 63449-39-8; 75-95-6; 79-27-6
Polyhalogenated benzene alicycles	4	1084889-51-9; 893843-07-7; 1025956-65-3; 155613-93-7
Polyhalogenated benzene aliphatics and functionalized	19	168434-45-5; 23488-38-2; 39569-21-6; 87-83-2; 85-22-3; 38521-51-6; 58495-09-3; 31780-26-4; 84852-53-9; 497107-13-8; 59447-55-1; 34571-16-9*; 855993-01-0*; 855992-98-2*; 147-82-0; 57011-47-9; 61368-34-1; 93-52-7; 39568-99-5
Polyhalogenated benzenes	19	608-90-2; 87-82-1; 84303-46-8; 60044-26-0; 67733-52-2; 67889-00-3; 69278-62-2; 59080-40-9; 13654-09-6; 36355-01-8; 92-66-0; 92-86-4; 115245-07-3; 60044-24-8; 59080-37-4; 77102-82-0; 16400-50-3; 67888-96-4; 59080-39-6
Polyhalogenated bisphenol aliphatics and functionalized	11	66710-97-2; 55205-38-4; 37853-61-5; 37419-42-4; 3072-84-2; 33798-02-6; 79-94-7; 25327-89-3; 21850-44-2; 4162-45-2; 79-95-8
Polyhalogenated carbocycles	15	13560-89-9; 51936-55-1; 13560-92-4; 34571-16-9*; 855993-01-0*; 855992-98-2*; 2385-85-5; 18300-04-4; 115-28-6; 1773-89-3; 1770-80-5; 115-27-5; 31107-44-5; 40703-79-5; 52907-07-0
Polyhalogenated diphenyl ethers	12	1163-19-5; 32534-81-9; 60348-60-9; 32536-52-0; 58965-66-5; 5436-43-1; 207122-16-5; 189084-67-1; 41318-75-6; 189084-64-8; 68631-49-2; 207122-15-4
Polyhalogenated organophosphates	22	114955-21-4*; 1373346-90-7*; 126-72-7; 19186-97-1; 115-96-8; 13674-84-5; 13674-87-8; 38051-10-4; 66108-37-0; 78-43-3; 6145-73-9; 33125-86-9; 49690-63-3; 7046-64-2; 5412-25-9; 53461-82-8; 61090-89-9; 140-08-9; 6749-73-1; 4351-70-6; 6294-34-4; 115-98-0
Polyhalogenated phenol derivatives	7	118-79-6; 608-71-9; 615-58-7; 42757-55-1; 39635-79-5; 70156-79-5; 25713-60-4*
Polyhalogenated phenol-aliphatic ethers	9	3278-89-5; 31977-87-4; 35109-60-5; 37853-59-1; 61262-53-1; 3555-11-1; 607-99-8; 26762-91-4; 20217-01-0
Polyhalogenated phthalates/benzoates/imides	11	32588-76-4; 183658-27-7; 90075-91-5; 82001-21-6; 20566-35-2; 26040-51-7; 7415-86-3; 55481-60-2; 632-79-1; 117-08-8; 57011-47-9
Polyhalogenated triazines	6	52434-90-9; 57829-89-7; 75795-16-3; 25713-60-4*; 114955-21-4*; 1373346-90-7*

*An asterisk indicates that the chemical occurs in more than one category.

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The categories were confirmed by comparison with unsupervised clustering techniques. The committee used 148 unique structures from the OFR seed set and applied an agglomerative nesting (clustering) procedure that used ToxPrint Chemotypes as the independent variables. ToxPrint Chemotypes that were found in at least two structures were then used to generate a distance matrix by calculating Jaccard distances (distances between clusters were calculated by using the average linkage method). The cluster-analysis results are in good agreement with the OFR chemotype categories that the committee generated.

To validate whether the OFR categories designed with the committee's approach can group chemicals of interest adequately, the 14 OFR categories were coded in CSRML[11] and implemented with the public ChemoTyper application. Figure B-7 shows the current OFR-categories-20192501.xml[12] in ChemoTyper to search against the "expanded set (1,073)" with highlighting to show structural features matched with the OFR categories.[13]

The organohalogen structures that are not covered by the OFR categories include organohalides with only one halogen, cyclic rings smaller than five, spiro polycyclic rings, halogens directly attached to phosphorus, or trivalent phosphorus. In accord with the supervised analyses in the preceding section, the chemical space covered by the expanded set is similar to that in the seed set in that the OFR categories cover nearly 80% of the set of 1,073.

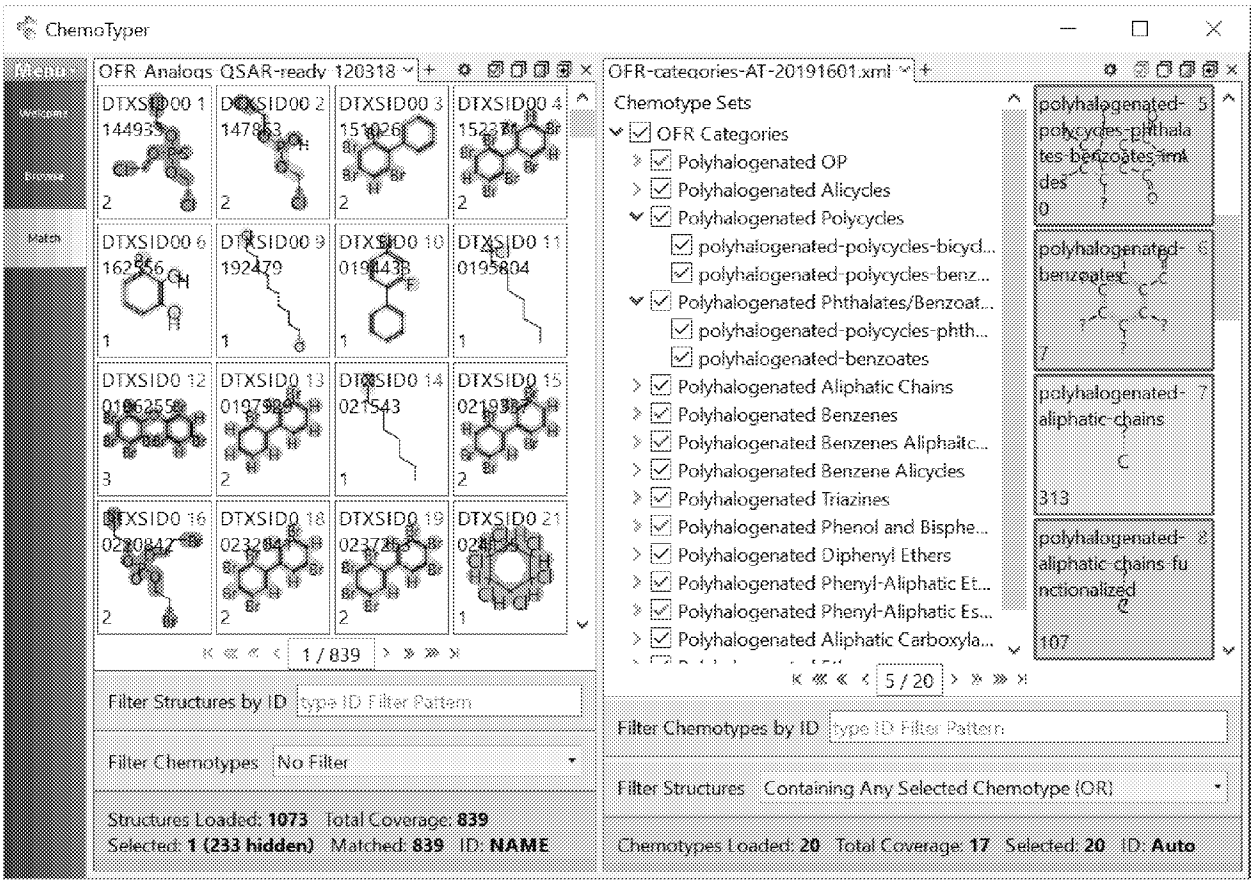

FIGURE B-7 Screenshot of OFR categories and matching structures in ChemoTyper.

[11]See OFR-categories-20192501.xml. File available at www.nap.edu/25412.
[12]File available at www.nap.edu/25412.
[13]Downloadable from https://chemotyper.org.

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Appendix C

Methodologic Details of Literature Surveys and Searches

The committee conducted several exercises associated with the literature surveys and searches that are described in Chapter 3. This appendix provides the methodologic details of those exercises.

ASSESSING THE AVAILABILITY AND ACCESSIBILITY OF BIOLOGIC DATA

The committee performed several analyses to identify case studies on the basis of available biologic data. It assessed and compared the coverage and distribution of data on the inventory of organohalogen flame retardants (OFRs) and the expanded set of chemicals. In this context, *coverage* is defined as the availability of data on given chemicals or chemical subclasses in bioinformatically mappable databases (Figures C-1, C-2, and C-3). The use of publicly available databases is a reproducible example of querying accessible data on a generic chemical set. The analysis is likely biased toward high-throughput and other new approach methodologies (NAM) data. The case examples described in Chapter 3 built on this initial survey of data availability by including additional published data on the two subclasses of interest.

The results that support the analyses are provided in a supplemental file, 20190118_DataGroup-Split.csv.[1] The chemical lists used were those generated to create the inventory and the expanded chemical set.[2] For the data mapping, CAS registry numbers, rather than SDF (structural) information, were used. The seven chemicals assigned to two groups are plotted twice in preceding data-survey figures. Each data resource was queried separately, as detailed below.

- *Comparative Toxicogenomics Database (CTD).* Chemicals were queried in the CTD. Results of the batch queries were output in JSON format and read into R as a text file. The text file was searched for the expression "Object not found"; negative results correspond to a chemical's existence within the database. Chemical presence in the database was recorded as a binary variable.
- *Environmental Protection Agency (EPA) Chemical Dashboard.* In the EPA Chemical Dashboard, chemicals can be categorized into three groups: not in database, untested in database, and tested in database. Chemicals were first queried for the first category, not in database. Source code from the URL was extracted and searched for the expression "Found 0 results"; positive results indicated that a chemical is not in database. URLs for the remaining chemicals were then Web-scraped, and resulting content was queried for bioactivity settings. For four possible bioactivity settings, text was searched for the expression "is-disabled". If all four sections of the Web page are disabled, the chemical is considered untested in database; otherwise, it is tested in database.
- *ToxNet.* The Hazardous Substances Databank and the Integrated Risk Information System were searched by using an ID tag that corresponded to each database's specific chemical ID. If a chemical ID does not exist, the chemical is considered untested.
- *ToxCast and Tox21.* Data in the ToxCast database were downloaded from ftp://newftp.epa.gov/comptox/High_Throughput_Screening_Data/ToxcastdatareleaseSept2018/INVITRODBV3_20181017.zip. CAS numbers were searched against the 161 chemicals by using the R expression NAS Casrn %in% ToxCastCASRN.

[1]The supplemental table lists all chemicals mapped to data in Figures C-1, C-2, and C-3. File available at www.nap.edu/25412.

[2]See OFR_Categories_OFR-inventory_02-15-2019.xlsx and OFR_Analogs.xlsx. Files available at www.nap.edu/25412.

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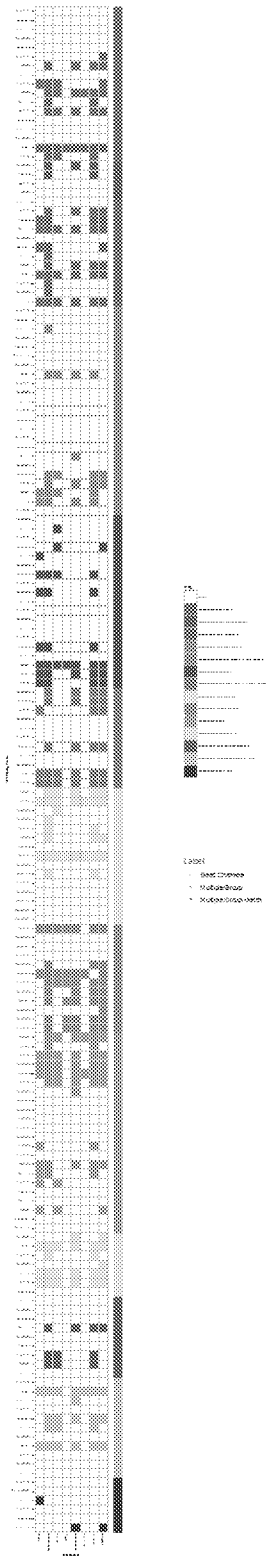

FIGURE C-1 Explicit mapping of data availability for inventory chemicals. Each chemical (row) is colored if data from a particular source (column) are available. The sidebar colors indicate chemotype classes. Figure available at www.nap.edu/25412.

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Appendix C

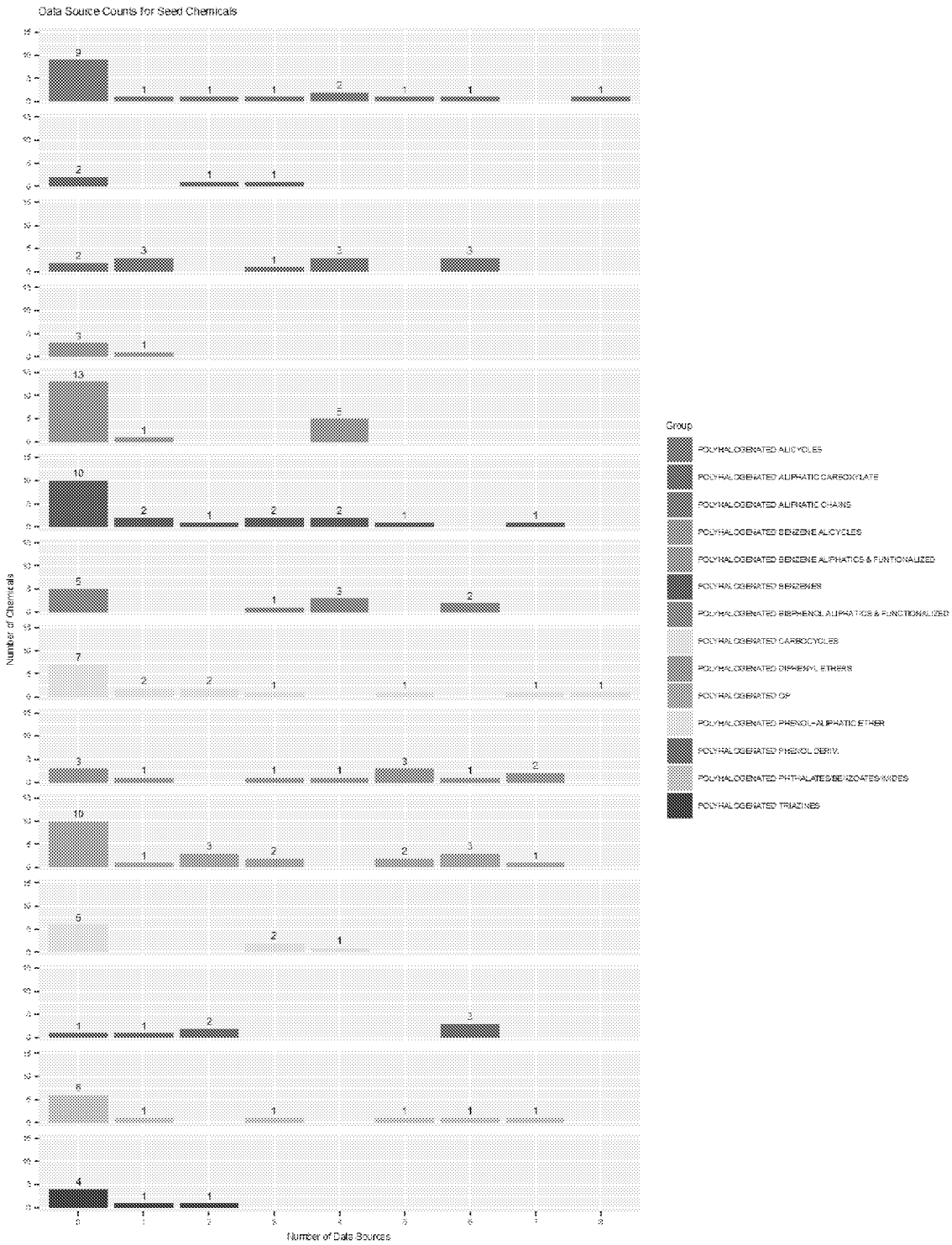

FIGURE C-2 Histogram of data availability for inventory chemicals. Each group (chemotype class) is plotted according to the frequency of chemicals in that class on which data are available.

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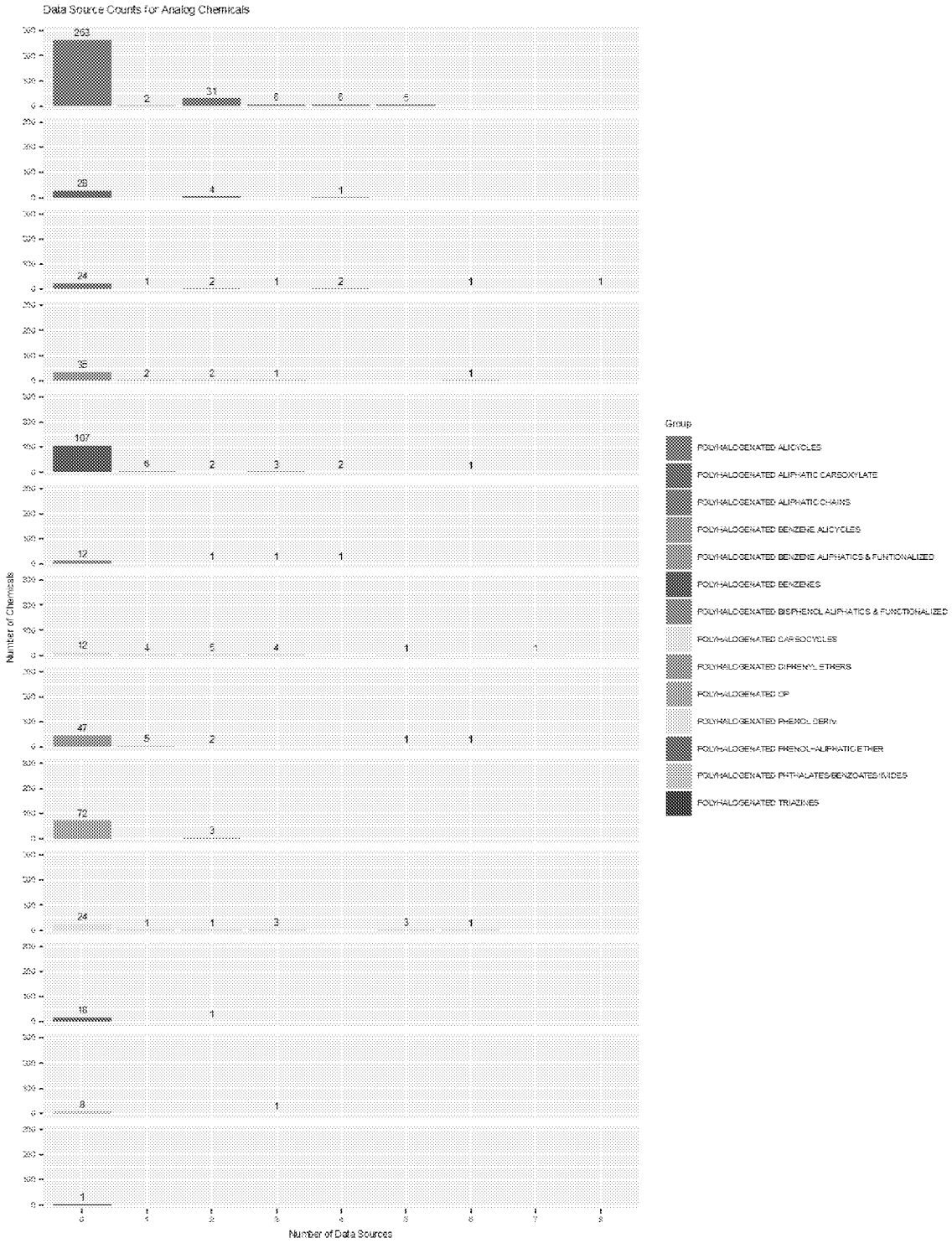

FIGURE C-3 Histogram of data availability for the expanded set of chemicals. Each chemical (row) is colored if data from a particular source (column) are available. The sidebar colors indicate chemotype classes.

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- *Toxicity Reference Database (ToxRefDB).* Data from ToxRefDB were downloaded from ftp://new-ftp.epa.gov/comptox/High_Throughput_Screening_Data/Animal_Tox_Data/toxrefdb%20(1).zip. CAS numbers were searched against the 161 chemicals by using the R expression NASCasrn %in% ToxRefDBCASRN.
- *ChEMBL.* CAS numbers are converted to ChEMBL identifiers. If a ChEMBL ID exists, the chemical is considered tested in ChEMBL.
- *PubChem.* For PubChem, chemicals can be categorized into three groups: not in database, untested in database, and tested in database. Chemicals were first queried for the first category, not in database. Source code from the URL was extracted and searched for the word "term" that exists in the resulting URL when the chemical is not in database. For the remaining chemicals, PubChem IDs are extracted and searched by using rpubchem::get.aid.by.cid(). That function returns assay IDs for tested chemicals, so chemicals with no associated assays are considered untested in database; otherwise, they are tested in database.

SEARCHING THE TOX21 AND TOXCAST DATA

Figures C-4 and C-5 provide a summary of Tox21 and ToxCast data, respectively. The following methods were used to query for those data.

- *Tox21 data.* The Tox21 Activity Profiler (https://sandbox.ntp.niehs.nih.gov/tox21-activity-browser/) was accessed on Nov. 19, 2018 to retrieve activity data on chemicals that were tested in Tox21 from the list of 161 chemicals in the OFR inventory. Activity data on 40 chemicals with acceptable analytic chemistry data or those still under analytic analysis were available for 43 toxicity end points. The log10-transformed point-of-departure (POD) activity results are presented as a heatmap. Chemicals judged to be inactive or inconclusive are represented as light gray. Dark gray in a cell indicates that the chemical was not tested in that assay. Green indicates activity, with the approximate POD concentration indicated in the key. The column and row arrangement on the heatmap is based on the results of hierarchic clustering (dendrograms). The clustering of columns is based on chemical-structure similarity (defined by Leadscope® structural fingerprints and Tanimoto coefficient); the clustering of rows is based on activity similarity according to Euclidean distance. The chemicals are also annotated on the basis of "ToxScore", "userClust", and "chemClust" (the three rows above the heatmap). The userClust is the chemical-structure category; the ToxScore is the sum of log10-transformed POD values, and chemClust is the chemical groupings based on chemical-structure similarity within a chemical class.
- *ToxCast data.* Activity data from ToxCast assays were accessed on Nov 19, 2018 (https://figshare.com/articles/ToxCast_and_Tox21_Summary_Files/6062479, version 2). Activity data were retrieved for 39 compounds on which there were acceptable analytic-chemistry data or those still under analysis of the 161 chemicals in the OFR inventory for 171 assay end points. The log10-transformed POD activity results are presented as a heatmap. Chemicals judged to be inactive or inconclusive are represented as light gray. Dark gray in a cell indicates that the chemical was not tested in the assay. Green indicates activity, with the approximate POD concentration indicated in the key. The column and row arrangements on the heatmap are based on the results of hierarchic clustering (dendrograms). The clustering of columns is based on chemical-structure similarity (defined by Leadscope® structural fingerprints and Tanimoto coefficient); the clustering of rows is based on activity similarity according to Euclidean distance. The chemicals are also annotated on the basis of "ToxScore", "userClust", and "chemClust" (the three rows above the heatmap). The userClust is the chemical-structure category, the ToxScore is the sum of log10-transformed POD values, and chemClust is the chemical groupings based on chemical-structure similarity within a chemical class.

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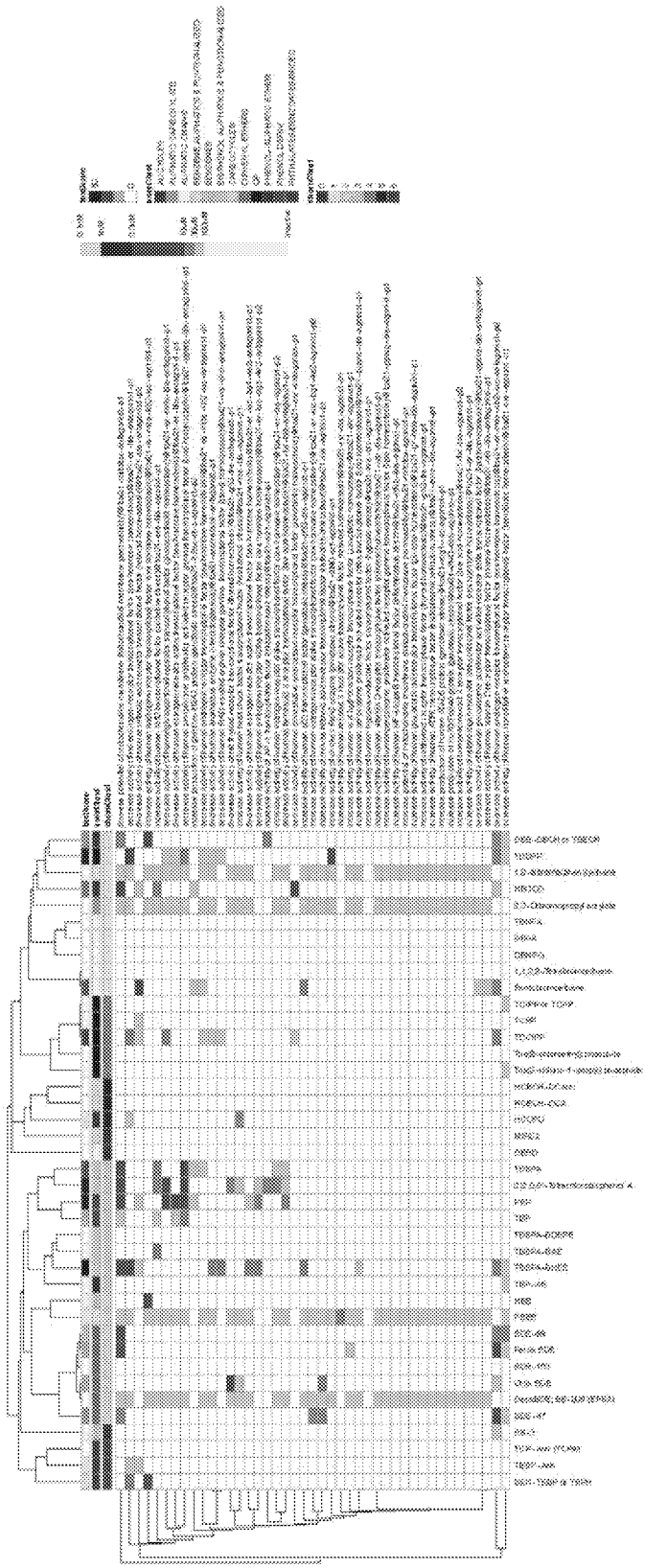

FIGURE C-4 Heatmap that represents availability of Tox21data on the chemicals in the OFR inventory. Figure available at www.nap.edu/25412.

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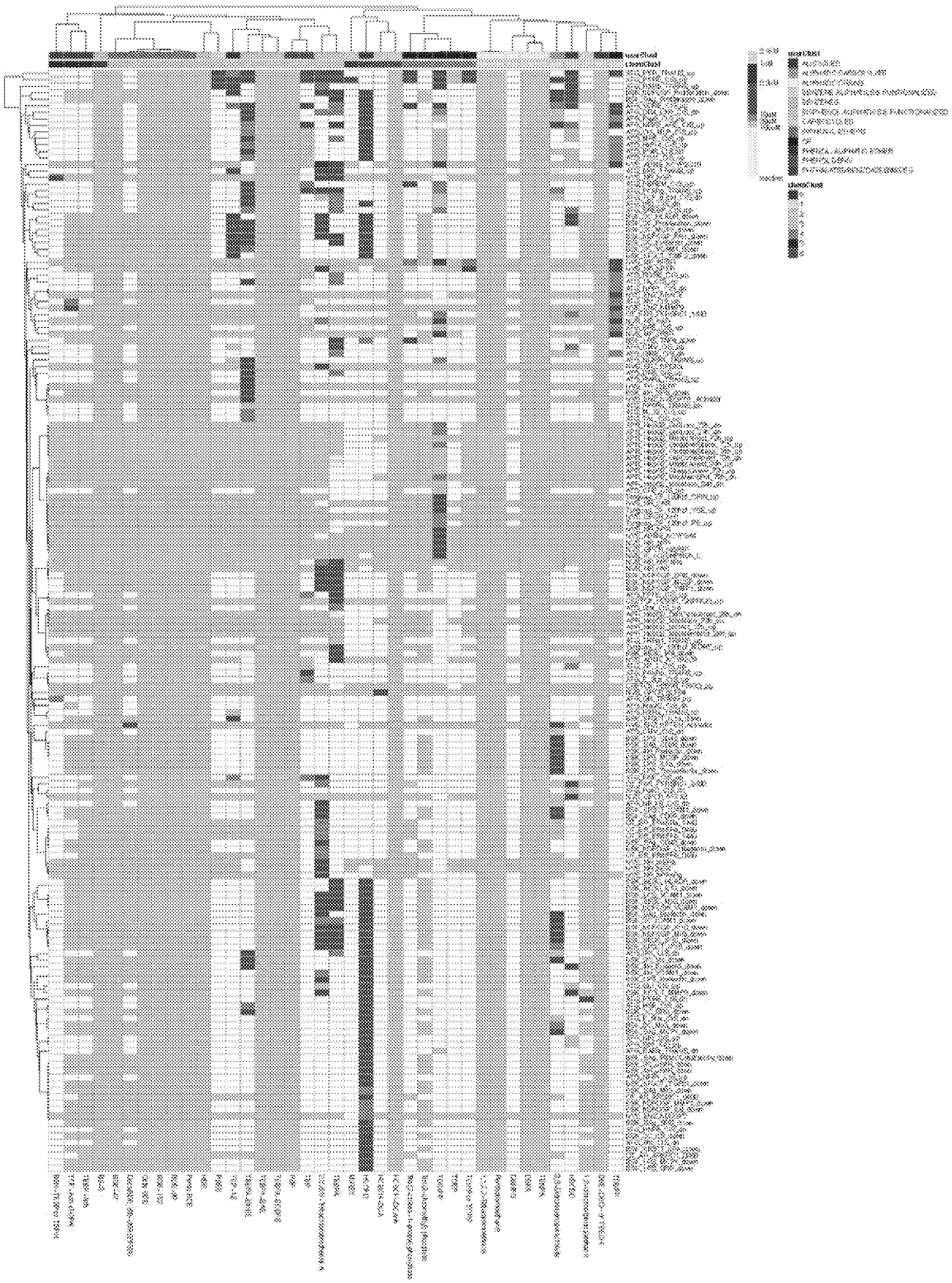

FIGURE C-5 Heatmap that represents availability of ToxCast data on the chemicals in the OFR inventory. Figure available at www.nap.edu/25412.

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LITERATURE SEARCHES

Literature searches described in Chapter 3 for the two case studies depended on searches for English language, peer-reviewed literature in two databases: PubMed and PubChem. The searches were designed and performed by a librarian trained in systematic review methods. The searches used the following terms:

Chemical-specific terms for polyhalogenated bisphenol aliphatics

- "Tetrabromobisphenol A bis(2-hydroxyethyl) ether bis(acrylate)" OR "Bis(p-acryloxyethoxy) tetrabromobisphenol A" OR "Tetrabromobisphenol A bis(2-hydroxyethyl)" OR "Ethoxylated Tetrabromo Bisphenol A Diacrylate" OR "66710-97-2" OR "TBBPA-BHEEBA"
- "2,2',6,6'-Tetrabromobisphenol A diacrylate" OR "Tetrabromobisphenol A diacrylate" OR "55205-38-4" OR "TBBPA-BA"
- "Tetrabromobisphenol A bismethyl ether" OR "tetrabromobisphenol A dimethyl ether" OR "37853-61-5" OR "TBBPA-BME"
- "37419-42-4" OR "TBBPA-BP"
- "Tetrabromobisphenol A Diglycidyl Ether" OR "3072-84-2" OR "TBBPA-BGE"
- "4,4'-Isopropylidenebis(2,6-dibromophenyl) diacetate" OR "33798-02-6" OR "TBBPA-BOAc"
- "Tetrabromobisphenol A" OR "79-94-7" OR "TBBPA"
- "Tetrabromobisphenol A diallyl ether" OR "25327-89-3" OR "TBBPA-BAE"
- "Tetrabromobisphenol A dibromopropyl ether" OR "Tetrabromobisphenol A bis(dibromopropyl ether)" OR "21850-44-2" OR "TBBPA-BDBPE"
- "Ethoxylated tetrabromobisphenol A" OR "Tetrabromobisphenol A bis(ethoxylate)" OR "4162-45-2" OR "TBBPA-BHEE"
- "Tetrachlorobisphenol A" OR "Tetrachlorodian" OR "79-95-8" OR "TCBPA"

Chemical-specific search terms for polyhalogenated organophosphates

- "Tris(2,3-dibromopropyl) phosphate" OR "TDBPP" OR "126-72-7"
- "19186-97-1"
- "Tris(2-chloroethyl) phosphate" OR "Trichlorethyl phosphate" OR "2-Chloroethanol phosphate" OR "115-96-8"
- "Tris(1-chloropropan-2-yl) phosphate" OR "Tris(1-chloro-2-propyl) phosphate" OR "Tris(2-chloro-1-methylethyl) phosphate" OR "13674-84-5"
- "Tris(1,3-dichloro-2-propyl)phosphate" OR "Tris(1,3-dichloroisopropyl)phosphate" OR "TDCPP" OR "13674-87-8"
- "2,2-Bis(chloromethyl)-1,3-propanediyl bis(bis(2-chloroethyl) phosphate)" OR "38051-10-4"
- "Bis(1,3-dichloro-2-propyl)-3-chloro-2,2-dibromomethyl-1-propyl phosphate" OR "2,2-Bis(bromomethyl)-3-chloropropyl bis(2-chloro-1-(chloromethyl)ethyl) phosphate" OR "K6UU3AT81T" OR "66108-37-0"
- "Tris(2,3-dichloropropyl) phosphate" OR "2,3-Dichloro-1-propanol phosphate" OR "78-43-3"
- "Tris(2-chloropropyl) phosphate" OR "Tris(beta-chloropropyl) phosphate" OR "6145-73-9"
- "Tetrakis(2-chloroethyl) ethane-1,2-diyl bis(phosphate)" OR "Ethylene bis(bis (2-chloroethyl)phosphate)" OR "33125-86-9"
- "Vinifos" OR "Bis(2-chloroethyl) vinylphosphonate" OR "Fyrol Bis beta" OR "115-98-0"
- "Tris(2,4-dibromophenyl)phosphate" OR "49690-63-3"
- "Phenol, 2,4,6-tribromo-, phosphate" OR "7046-64-2"
- "Bis(2,3-dibromopropyl) phosphate" OR "Bis(2,3-dibromopropyl) hydrogen phosphate" OR "5412-25-9"

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- "Tris(2-chloroethyl)phosphite" OR "Ethanol, 2-chloro-, phosphite (3:1)" OR "2-Chloroethanol phosphite (3:1)" OR "140-08-9"
- "Phosgard c-22R" OR "Phosgard c-22R (monsanto)" OR "4351-70-6"
- "Bis(2-Chloroethyl) (2-Chloroethyl)Phosphonate" OR "Bis(2-chloroethyl) 2-chloroethylphosphonate" OR "6294-34-4"
- "Tris(1,3-dichloropropan-2-yl) phosphite" OR "2-Propanol, 1,3-dichloro-, phosphite (3:1)" OR "Tris(2-chloro-1-(chloromethyl)ethyl) phosphite" OR "6749-73-1"
- "Oxydiethylene tetrakis(2-chloroethyl) bisphosphate" OR "Diethylene glycol tetra(2-chloroethyl) phosphate" OR "53461-82-8"
- "UASQAKNFTHVEDR-UHFFFAOYSA-N" OR "3,9-Bis(3-bromo-2,2-bis(bromomethyl)propoxy)-2,4,8,10-tetraoxa-3,9-diphosphaspiro(5.5)undecane 3,9-dioxide" OR "3,9-bis [3-bromo-2,2-bis(bromomethyl)propoxy]-2,4,8,10-tetraoxa-3" OR "61090-89-9"
- "114955-21-4"
- "1373346-90-7"

Specific search terms for outcomes of interest

- Toxicity OR Reproductive Toxicity OR Developmental Toxicity
- Genotoxicity OR Mutagenicity
- Cancer OR Carcinogenicity

Results (count) from the PubChem and PubMed searches are presented in Tables C-1 and C-2, respectively.

TABLE C-1 Number of Results of the PubChem Database Search

Query	Results*
Polyhalogenated bisphenol aliphatics	
"66710-97-2"[CompleteSynonym]	0
"55205-38-4"[CompleteSynonym]	0
"37853-61-5"[CompleteSynonym]	5
"37419-42-4"[CompleteSynonym]	0
"3072-84-2"[CompleteSynonym]	3
"33798-02-6"[CompleteSynonym]	0
"79-94-7"[CompleteSynonym]	688
"25327-89-3"[CompleteSynonym]	0
"21850-44-2"[CompleteSynonym]	2
"4162-45-2"[CompleteSynonym]	2
"79-95-8"[CompleteSynonym]	62
Polyhalogenated organophosphates	
"126-72-7"[CompleteSynonym]	342
"19186-97-1"[CompleteSynonym]	0
"115-96-8"[CompleteSynonym]	309
"13674-84-5"[CompleteSynonym]	89
"13674-87-8"[CompleteSynonym]	128
"38051-10-4"[CompleteSynonym]	1
"66108-37-0"[CompleteSynonym]	1
"78-43-3"[CompleteSynonym]	10

(Continued)

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A Class Approach to Hazard Assessment of Organohalogen Flame Retardants

TABLE C-1 Continued

Query	Results*
"6145-73-9"[CompleteSynonym]	31
"33125-86-9"[CompleteSynonym]	0
"115-98-0"[CompleteSynonym]	11
"49690-63-3"[CompleteSynonym]	2
"7046-64-2"[CompleteSynonym]	0
"5412-25-9"[CompleteSynonym]	15
"140-08-9"[CompleteSynonym]	12
"4351-70-6"[CompleteSynonym]	0
"6294-34-4"[CompleteSynonym]	4
"6749-73-1"[CompleteSynonym]	0
"53461-82-8"[CompleteSynonym]	0
"61090-89-9"[CompleteSynonym]	0
"114955-21-4"[CompleteSynonym]	0
"1373346-90-7"[CompleteSynonym]	0

*Total references as found in PubChem. Not necessarily related to toxicity. These are total records. The results have not been deduplicated. For a combination of chemical and toxicity, see the PubMed results in Table C-2.

TABLE C-2 Number of Results of the PubMed Database Search

No.	Query Terms and Searches	Results*
Polyhalogenated bisphenol aliphatics: Search Terms		
1	"Tetrabromobisphenol A bis(2-hydroxyethyl) ether bis(acrylate)" OR "Bis(p-acryloxyethoxy)tetrabromobisphenol A" OR "Tetrabromobisphenol A bis(2-hydroxyethyl)" OR "Ethoxylated Tetrabromo Bisphenol A Diacrylate" OR "66710-97-2" OR "TBBPA-BHEEBA"	NA
2	"2,2',6,6'-Tetrabromobisphenol A diacrylate" OR "Tetrabromobisphenol A diacrylate" OR "55205-38-4" OR "TBBPA-BA"	NA
3	"Tetrabromobisphenol A bismethyl ether" OR "tetrabromobisphenol A dimethyl ether" OR "37853-61-5" OR "TBBPA-BME"	NA
4	"37419-42-4" OR "TBBPA-BP"	NA
5	"Tetrabromobisphenol A Diglycidyl Ether" OR "3072-84-2" OR "TBBPA-BGE"	NA
6	"4,4'-Isopropylidenebis(2,6-dibromophenyl) diacetate" OR "33798-02-6" OR "TBBPA-BOAc"	NA
7	"Tetrabromobisphenol A" OR "79-94-7" OR "TBBPA"	NA
8	"Tetrabromobisphenol A diallyl ether" OR "25327-89-3" OR "TBBPA-BAE"	NA
9	"Tetrabromobisphenol A dibromopropyl ether" OR "Tetrabromobisphenol A bis(dibromopropyl ether)" OR "21850-44-2" OR "TBBPA-BDBPE"	NA
10	"Ethoxylated tetrabromobisphenol A" OR "Tetrabromobisphenol A bis(ethoxylate)" OR "4162-45-2" OR "TBBPA-BHEE"	NA
11	"Tetrachlorobisphenol A" OR "Tetrachlorodian" OR "79-95-8" OR "TCBPA"	NA
12	"toxicology"[MeSH Terms] OR "toxicity"[Title/Abstract] OR "toxicity"[Text Word] OR "developmental toxicity"[Title/Abstract] OR "developmental toxicity"[Text Word] OR "reproductive toxicity"[Title/Abstract] OR "reproductive toxicity"[Text Word]	NA
13	"toxicogenetics"[MeSH Terms] OR "toxicogenetics"[Title/Abstract] OR "toxicogenetics"[Text Word] OR "genotoxicity"[Title/Abstract] OR "genotoxicity"[Text Word] OR "mutagens"[MeSH Terms] OR "mutagenicity"[Title/Abstract] OR "mutagenicity"[Text Word]	NA
14	"neoplasms"[MeSH Terms] OR "cancer"[Title/Abstract] OR "neoplasms"[Title/Abstract] OR "tumors"[Title/Abstract] OR "cancer"[Text Word] OR "neoplasms"[Text Word] OR "tumors"[Text Word] OR "carcinogens"[Title/Abstract] OR "carcinogens"[Text Word] OR "carcinogenicity"[Title/Abstract] OR "carcinogenicity"[Text Word] OR "carcinogenic"[Title/Abstract] OR "carcinogenic"[Text Word] OR "carcinogens"[MeSH Terms]	NA
Polyhalogenated bisphenol aliphatics: Searches		
15	#1 AND #12	20
16	#1 AND #13	0
17	#1 AND #14	7
18	#2 AND #12	0

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19	#2 AND #13	0
20	#2 AND #14	0
21	#3 AND #12	2
22	#3 AND #13	0
23	#3 AND #14	0
24	#4 AND #12	0
25	#4 AND #13	0
26	#4 AND #14	0
27	#5 AND #12	0
28	#5 AND #13	0
29	#5 AND #14	1
30	#6 AND #12	0
31	#6 AND #13	0
32	#6 AND #14	0
33	#7 AND #12	291
34	#7 AND #13	4
35	#7 AND #14	40
36	#8 AND #12	1
37	#8 AND #13	0
38	#8 AND #14	0
39	#9 AND #12	6
40	#9 AND #13	0
41	#9 AND #14	1
42	#10 AND #12	2
43	#10 AND #13	0
44	#10 AND #14	1
45	#11 AND #12	25
46	#11 AND #13	0
47	#11 AND #14	5
Polyhalogenated organophosphates: Search Terms		
1	"Tris(2,3-dibromopropyl) phosphate" OR "TDBPP" OR "126-72-7"	NA
2	"19186-97-1"	NA
3	"Tris(2-chloroethyl) phosphate" OR "Trichlorethyl phosphate" OR "2-Chloroethanol phosphate" OR "115-96-8"	NA
4	"Tris(1-chloropropan-2-yl) phosphate" OR "Tris(1-chloro-2-propyl) phosphate" OR "Tris(2-chloro-1-methylethyl) phosphate" OR "13674-84-5"	NA
5	"Tris(1,3-dichloro-2-propyl)phosphate" OR "Tris(1,3-dichloroisopropyl)phosphate" OR "TDCPP" OR "13674-87-8"	NA
6	"2,2-Bis(chloromethyl)-1,3-propanediyl bis(bis(2-chloroethyl) phosphate)" OR "38051-10-4"	NA
7	"Bis(1,3-dichloro-2-propyl)-3-chloro-2,2-dibromomethyl-1-propyl phosphate" OR "2,2-Bis(bromomethyl)-3-chloropropyl bis(2-chloro-1-(chloromethyl)ethyl) phosphate" OR "K6UU3AT81T" OR "66108-37-0"	NA
8	"Tris(2,3-dichloropropyl) phosphate" OR "2,3-Dichloro-1-propanol phosphate" OR "78-43-3"	NA
9	"Tris(2-chloropropyl) phosphate" OR "Tris(beta-chloropropyl) phosphate" OR "6145-73-9"	NA
10	"Tetrakis(2-chloroethyl) ethane-1,2-diyl bis(phosphate)" OR "Ethylene bis(bis(2-chloroethyl)phosphate)" OR "33125-86-9"	NA
11	"Vinifos" OR "Bis(2-chloroethyl) vinylphosphonate" OR "Fyrol Bis beta" OR "115-98-0"	NA
12	"Tris(2,4-dibromophenyl)phosphate" OR "49690-63-3"	NA
13	"Phenol, 2,4,6-tribromo-, phosphate" OR "7046-64-2"	NA
14	"Bis(2,3-dibromopropyl) phosphate" OR "Bis(2,3-dibromopropyl) hydrogen phosphate" OR "5412-25-9"	NA
15	"Tris(2-chloroethyl)phosphite" OR "Ethanol, 2-chloro-, phosphite (3:1)" OR "2-Chloroethanol phosphite (3:1)" OR "140-08-9"	NA

(Continued)

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A Class Approach to Hazard Assessment of Organohalogen Flame Retardants

TABLE C-2 Continued

16	"Phosgard c-22R" OR "Phosgard c-22R (monsanto)" OR "4351-70-6"	NA
17	"Bis(2-Chloroethyl) (2-Chloroethyl)Phosphonate" OR "Bis(2-chloroethyl) 2-chloroethylphosphonate" OR "6294-34-4"	NA
18	"Tris(1,3-dichloropropan-2-yl) phosphite" OR "2-Propanol, 1,3-dichloro-, phosphite (3:1)" OR "Tris(2-chloro-1-(chloromethyl)ethyl) phosphite" OR "6749-73-1"	NA
19	"Oxydiethylene tetrakis(2-chloroethyl) bisphosphate" OR "Diethylene glycol tetra(2-chloroethyl)phosphate" OR "53461-82-8"	NA
20	"UASQAKNFTHVEDR-UHFFFAOYSA-N" OR "3,9-Bis(3-bromo-2,2-bis(bromomethyl)propoxy)-2,4,8,10-tetraoxa-3,9-diphosphaspiro(5.5)undecane 3,9-dioxide" OR "3,9-bis[3-bromo-2,2-bis(bromomethyl)propoxy]-2,4,8,10-tetraoxa-3" OR "61090-89-9"	NA
21	"114955-21-4"	NA
22	1373346-90-7"	NA
23	"toxicology"[MeSH Terms] OR "toxicity"[Title/Abstract] OR "toxicity"[Text Word] OR "developmental toxicity"[Title/Abstract] OR "developmental toxicity"[Text Word] OR "reproductive toxicity"[Title/Abstract] OR "reproductive toxicity"[Text Word]	NA
24	"toxicogenetics"[MeSH Terms] OR "toxicogenetics"[Title/Abstract] OR "toxicogenetics"[Text Word] OR "genotoxicity"[Title/Abstract] OR "genotoxicity"[Text Word] OR "mutagens"[MeSH Terms] OR "mutagenicity"[Title/Abstract] OR "mutagenicity"[Text Word]	NA
25	"neoplasms"[MeSH Terms] OR "cancer"[Title/Abstract] OR "neoplasms"[Title/Abstract] OR "tumors"[Title/Abstract] OR "cancer"[Text Word] OR "neoplasms"[Text Word] OR "tumors"[Text Word] OR "carcinogens"[Title/Abstract] OR "carcinogens"[Text Word] OR "carcinogenicity"[Title/Abstract] OR "carcinogenicity"[Text Word] OR "carcinogenic" [Title/Abstract] OR "carcinogenic"[Text Word] OR "carcinogens"[MeSH Terms]	NA
Polyhalogenated organophosphates: Searches		
26	#1 AND #23	78
27	#1 AND #24	46
28	#1 AND #25	49
29	#2 AND #23	1
30	#2 AND #24	0
31	#2 AND #25	0
32	#3 AND #23	69
33	#3 AND #24	9
34	#3 AND #25	31
35	#4 AND #23	311
36	#4 AND #24	63
37	#4 AND #25	219
38	#5 AND #23	87
39	#5 AND #24	7
40	#5 AND #25	17
41	#6 AND #23	0
42	#6 AND #24	0
43	#6 AND #25	0
44	#7 AND #23	0
45	#7 AND #24	0
46	#7 AND #25	0
47	#8 AND #23	3
48	#8 AND #24	2
49	#8 AND #25	1
50	#9 AND #23	311
51	#9 AND #24	63
52	#9 AND #25	218
53	#10 AND #23	1
54	#10 AND #24	0
55	#10 AND #25	1

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56	#11 AND #23	0
57	#11 AND #24	0
58	#11 AND #25	0
59	#12 AND #23	0
60	#12 AND #24	0
61	#12 AND #25	0
62	#13 AND #23	0
63	#13 AND #24	0
64	#13 AND #25	0
65	#14 AND #23	14
66	#14 AND #24	9
67	#14 AND #25	3
68	#15 AND #23	0
69	#15 AND #24	0
70	#15 AND #25	0
71	#16 AND #23	185
72	#16 AND #24	19
73	#16 AND #25	95
74	#17 AND #23	4
75	#17 AND #24	3
76	#17 AND #25	4
77	#18 AND #23	2
78	#18 AND #24	0
79	#18 AND #25	2
80	#19 AND #23	0
81	#19 AND #24	0
82	#19 AND #25	0
83	#20 AND #23	0
84	#20 AND #24	0
85	#20 AND #25	0
86	#21 AND #23	0
87	#21 AND #24	0
88	#21 AND #25	0
89	#22 AND #23	0
90	#22 AND #24	0
91	#22 AND #25	0

*The PubMed results have not been deduplicated.
Abbreviation: NA, not applicable.

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Appendix D

Summary of Zebrafish Studies

Chapter 3 provided two case examples that illustrate the committee's scoping plan for evaluating nonpolymeric, additive organohalogen flame retardants (OFRs) as a single class for the purpose of hazard assessment. Two OFR subclasses, the polyhalogenated organophosphates and the polyhalogenated bisphenol aliphatics, were selected as the case examples. Each example considered zebrafish data. This appendix provides summaries of studies identified by the committee.

Tables D-1 and D-2 provide summaries of zebrafish studies of the polyhalogenated organophosphates: Table D-1, data from developmental-toxicology studies, including changes in behavior; and Table D-2, data from other types of toxicology studies.

Tables D-3 through D-5 provide summaries of zebrafish studies of the polyhalogenated bisphenol aliphatics: Table D-3, data on the effects of tetrabromobisphenol A (TBBPA) on thyroid homeostasis in zebrafish; Table D-4, data from studies of the effects of polyhalogenated bisphenols on zebrafish development or behavior; and Table D-5, data from other types of toxicology studies.

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TABLE D-1 Summary of Zebrafish Studies That Evaluated Teratogenic or Developmental Neurotoxic Effects after Exposure to a Polyhalogenated Organophosphate Flame Retardant

Chemical	Life Stage at Exposure	Life Stage Analyzed	Concentrations	Outcomes Assessed	Results	Reference
TDCPP	[1] 5.25–96 hpf; [2] 0.75–96, 2.25–96, 5.25–96, 10–96, and 24–96; [3] 0.75–2, 2.25–5, 5.25–10, 10–24 and 24–48 hpf	Larval (96 hpf)	0.05–50 μM; 0.5–9 μM	Mortality, malformations, global DNA methylation.	Overt toxicity at <50 μM and 100% mortality at 50 μM at 96 hpf; no change in mortality in second set of exposure periods; exposure during 0.75–2 hpf (cleavage period) most susceptible; delays in remethylation of genome at 2 hpf but not at 10 or 24 hpf. Exposure to 3 μM TDCPP at 0.75–96 hpf resulted in a significant increase in mortality and developmental abnormalities.	McGee et al. (2012)
TDCPP	0.75–96 hpf	Embryonic (4 hpf) or larval (96 hpf)	0.2, 1, 3 μM	Developmental toxicity, microarray and qRT-PCR for transcript expression and protein expression, proteomics.	Increase in mortality at 96 hpf in 1- and 3-μM treatment groups; 17 genes with altered expression at 4 hpf in 3-μM treatment group; qRT-PCR and Western blot analysis confirmed transcript- and protein-expression changes in 8 target genes; proteomics on 96 hpf larvae in 0.2,- 1-, and 3-μM treatment groups showed 15 proteins with altered expression.	Fu et al. (2013)
TDCPP	2–120 hpf	Embryonic (behavior 18–28 hpf) or larval (96 and 120 hpf)	100, 300, 600, 900 μg/L	Locomotor activity, imaging of *Tg(Huc*-GFP) line, transcript expression, immunofluorescence, ACh concentration, and AChE activity.	Decreased hatching rate (96 hpf), decreased survival (120 hpf), and increased malformations (120 hpf) significant at 900 μg/L; decrease in body length (120 hpf) at 600 and 900 μg/L; embryonic (24 hpf) hyperactivity at 300, 600, and 900 μg/L; reduction in neuron-specific expression (120 hpf) at 900 μg/L; decreased transcript expression (120 hpf) of *elavl3* at 300, 600, and 900 μg/L and *ngn1* at 900 μg/L; hyperactivity (120 hpf) in dark period at 300 and 600 μg/L and hypoactivity in light period at 900 μg/L; reduction in length of dorsal and ventral axon from secondary motor neuron at 900 μg/L; up-regulation of transcript expression of *a1-tubulin*, *shha*, and *nestrin2* (120 hpf) at 900 μg/L; reduced ACh concentration (120 hpf) at 900 μg/L and reduced AChE activity (120 hpf) at 600 and 900 μg/L.	Cheng et al. (2017)

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TABLE D-1 Continued

Chemical	Life Stage at Exposure	Life Stage Analyzed	Concentrations	Outcomes Assessed	Results	Reference
TDCPP	4–96 hpf	Larval (96 hpf)	1–1,000 µM	Lethality, behavior, hepatotoxicity, cardiotoxicity.	NOAEL of 3 µM (48 hpf) and 2 µM (96 hpf); EC_{50} of 4.11 µM (48 hpf) and 3.08 µM (96 hpf); LC_{50} of 8.29 µM (48 hpf) and 6.53 µM (96 hpf); teratogenic index of 2.02 (48 hpf) and 2.12 (96 hpf); no cardiotoxicity or hepatotoxicity; no change in behavior.	Alzualde et al. (2018)
TDCPP	4.5–144 hpf or 0–28 dpf	6 or 28 dpf	19 µg/L (1% of LC_{50})	Swim bladder and body size, transcript expression, locomotor activity.	Decreased length at 28 dpf; increase in fish without anterior swim bladder at 28 dpf; increase in transcript expression at 6 dpf of *ttf-1*, *sp-a*, *sp-c*, and *tpo* and at 28 dpf of *sp-c*; no alterations in locomotor activity at 6 dpf.	Godfrey et al. (2017a)
TDCPP	6–120 hpf	Larval (144 hpf)	0.04–120 µM	Lethality, hatching, malformations.	Point of departure at 8.9 µM with dependence on mortality.	Behl et al. (2015)
TDCPP	6–120 hpf	Embryonic (24 hpf) and larval (120 hpf)	0.0064, 0.064, 0.64, 6.4, 64 µM	Behavior.	24 hpf change in photomotor response at 64 µM.	Reif et al. (2016)
TDCPP	6–120 hpf	Embryonic (24 hpf) and larval (120 hpf)	0.0064, 0.064, 0.64, 6.4, 64 µM	Malformations and behavior.	Mortality and delayed progression at 64 µM; hypoactivity at 24 hpf at 64 µM; at 120 hpf, hyperactivity at 0.64 µM in dark stimulation, hypoactivity at 64 µM in dark acclimation, and hyperactivity at 6.4 µM in light phase.	Noyes et al. (2015)
TDCPP	6–120 hpf	Embryonic (24 hpf) and larval (120 hpf)	0.0064, 0.064, 0.64, 6.4, 64 µM	Malformations and behavior.	Developmental defects at 64 µM.	Truong et al. (2014)
TDCPP	6–120 hpf	Larval (120 hpf)	5, 50, 500 µg/L	Behavior, AChE and BChE activity, LC3 immunofluorescence, measurement of acidic vesicular organelles, and gene and protein expression.	No significant effects on hatching or survival; increase in malformations in highest treatment group; decreased swimming speed in highest treatment group in dark periods; down-regulation of transcripts of *mbp*, *syn2a*, and *a1-tubulin* at 50 and 500 µg/L and up-regulation of *gap-43* at 500 µg/L; down-regulation of protein expression of *mbp* in all treatments, of *syn2a* at 50 and 500 µg/L and of *a1-tubulin* at 500 µg/L; no changes in AChE or BChE activity; increase in LC3 expression in brain and up-regulation of *atg5* and *map1lc3b* at 50 and 500 µg/L and of *becn1* and *atg3* at 500 µg/L.	Li et al. (2018)

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TDCPP	5–120 hpf	Larval (144 hpf)	3 or 6 µM	Locomotor activity.	Hypoactivity in light and dark.	Oliveri et al. (2018)
TDCPP	6–144 hpf	Larval (144 hpf)	0.56–5.6 µm	Morphology and behavior.	Overt toxicity threshold of 10 µM; teratogenic; neurobehavioral effect threshold of 3.14 µm.	Dishaw et al. (2014)
TDCPP	6–120 hpf	Larval (150–154 hpf)	0.04–120 µM	Locomotor activity.	Hyperactivity in the light phase and hypoactivity in the dark phase.	Jarema et al. (2015)
TDCPP	5–144 hpf	144 hpf and 12 wks	0.3 and 3 µM	Larval locomotor assay and adult behavioral test battery.	Increased movement at 0.03 µM in the dark phase; fish exposed at 0.3 µM swam faster only in the novel environment test.	Oliveri et al. (2015)
TDCPP	2 hpf up to 6 mo	Larval (120 hpf), adults (6 mos)	4, 20, 100 µg/L	Locomotion, AChE activity, neurotransmitter levels, and gene and protein expression in larval fish; AChE activity, neurotransmitter levels, and gene and protein expression in adult brain tissue.	No significant effects on hatching, malformations, survival, or growth rates at 5 dpf; up-regulation of *gap-43* in 5-dpf larvae; no change in protein levels of *a1-tubulin* or *mbp* in 5-dpf larvae; no changes in dopamine, serotonin, or AChE activity in larvae; no locomotor changes in larvae; growth inhibition in all treatments in both adult sexes; down-regulation of *a1-tubulin* and *mbp* in 20- and 100-µg/L treatment groups (transcript and protein), down-regulation of *syn2a* in 100-µg/L treatment group (transcript/protein not assessed), and up-regulation of *gap-43* (transcript/protein not assessed) in 100-µg/L treatment group in adult female brain; down-regulation of *a1-tubulin* in 20-µg/L (transcript only) and 100-µg/L (transcript and protein) treatment groups and up-regulation of *gap-43* (transcript/protein not assessed) in 100-µg/L treatment group in adult male brain; dopamine and serotonin reduced in all treatment groups in female brain, but no changes in adult male brain or in AChE activity in both sexes.	Wang et al. (2015a)
TDCPP	1 mo old for 240 d (~8 mo)	Adults ~9 mos of age (F0), larval progeny at 3 or 5 dpf (F1)	0.5 or 5 µg/L	Bioconcentration, life-history traits, and transcript levels of brain and liver in adult females; developmental toxicity in larvae; transcript levels in F1	BCF were 26 for 0.5-µg/L and 317 for 5-µg/L treatments in females and 45 for 0.5-µg/L and 42 for 5-µg/L treatments in males; 2.8 ng/g at 0.5 µg/L and 11 ng/g at 5 µg/L in F1 eggs; significant decrease in body mass and	Yu et al. (2017)

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TABLE D-1 Continued

Chemical	Life Stage at Exposure	Life Stage Analyzed	Concentrations	Outcomes Assessed	Results	Reference
					length in females in 5-µg/L treatment; females, down-regulation of *gh* in both treatments in brain and down-regulation of *ghra*, *ghrb*, *igf1*, and *igf1ra* in both treatments and of *igf1rb* at higher treatment in liver; decrease in survival in 3- and 5-dpf F1 larvae in higher treatment group; decreased heart rate in 3-dpf larvae in both treatment groups; decreased body length in 5-dpf larvae in both treatment groups; in 5 dpf larvae: down-regulation of *gh* and *igf1* in offspring of higher treatment group.	
TDCPP	2 hpf through sexual maturation	Adults (age not specified)	4, 20, 100 µg/L	Developmental toxicity of F1; fecundity; plasma hormone concentrations; GSI, transcript expression of brain, gonad, and liver; gonad histology.	Increase in malformations in F1 larvae from 20- and 100-µg/L treatment groups with no change in hatching, survival, or growth; decreased weight of adult females and males in all treatment groups; decreased GSI in males in highest treatment group and increased GSI in females in 20- and 100-µg/L treatment groups; decreased egg production in 20- and 100-µg/L treatment groups; E2 and T increased in 20- and 100-µg/L treatment groups in females; in brain, *fshβ* was up-regulated in highest treatment group in both sexes, *lhβ* and *gnrh2* up-regulated in females in 100-µg/L treatment group, and *cyp19β* up-regulated in all treatment groups in females and in 20- and 100-µg/L treatment groups in males; in liver, *erβ* and *vtg3* were up-regulated in females in 20-and 100-µg/L treatment groups and *era* and *vtg1* in 100-µg/L treatment group in females, whereas *vtg1* was up-regulated in 20-and 100-µg/L treatment groups and *erβ* and *vtg3* in 100-µg/L treatment group in males; in gonads, in females *3β-hsd* was up-regulated in all treatment groups, *fshr*, *star*, *Activin-βA2*, and *ActRIIA* were up-regulated in the	Wang et al. (2015b)

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					20- and 100-µg/L treatment groups, and *lhr* was up-regulated only in the highest treatment group, whereas in males *3β-hsd* was down-regulated in all three treatment groups, *17β-hsd* and *Activin-βA2* were down-regulated in the 20- and 100-µg/L treatment groups, and *ActRIIA* was down-regulated and *cyp19α* up-regulated only in the highest treatment group; in females in the 20- and 100-µg/L treatment groups, the percentage of primary oocytes was decreased and the percentage of late/mature oocytes and atretic oocytes was increased; in males, the percentage of spermatogonia was increased in the 20- and 100-µg/L treatment groups.	
TDCPP	Adult 4 mo old for 3 mo	Adult (7 mos, F0), progeny aged 5 or 10 dpf (F1)	4, 20, 100 µg/L	Developmental toxicity in larvae, thyroid end points in F0 and F1, ROS in F1.	Decreased hatching rate at 20 and 100 µg/L; growth inhibition, increased malformations, and decreased survival at 100 µg/L; T4 (at 20 and 100 µg/L) and T3 (at 100 µg/L) reduced in adult females; T4 reduced in eggs and 5-dpf larvae from 100- µg/L females and in 10-dpf larvae from 20- and 100-µg/L females, whereas T3 reduced in 100-µg/L group; 5-dpf larvae showed decreased *mbp* in 20-µg/L (transcript only) and 100-µg/L (transcript and protein) groups, *α1-tubulin* in 20-µg/L (protein only) and 100-µg/L (transcript and protein) groups, and *syn2a* in 20- and 100-µg/L groups (protein only); 10-dpf larvae had decreased *mbp* in 20-µgL (protein only) and 100-µg/L (transcript and protein) groups, decreased *syn2a* in 20-µgL (protein only) and 100-µg/L (transcript and protein) groups, decreased *α1-tubulin* in 20- and 100-µg/L groups (transcript and protein), and increased *gap-43* in 100-µg/L group (transcript only); 5-dpf larvae had decreased dopamine and GABA in 20- and 100-µg/L groups and decreased serotonin in 100-µg/L group; 10-dpf larvae had decreased	Wang et al. (2015c)

(Continued)

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TABLE D-1 Continued

Chemical	Life Stage at Exposure	Life Stage Analyzed	Concentrations	Outcomes Assessed	Results	Reference
					dopamine and histamine in 20- and 100-µg/L groups and decreased GABA and serotonin in 100-µg/L group; no change in AChE activity in larvae; swimming speed reduced in 100-µg/L group at 5 and 10 dpf; ROS was increased in 100-µg/L group at 5 and 10 dpf.	
TCEP	5.25–96 hpf	Larval (96 hpf)	0.05–50 µM	Malformations.	No overt toxicity up to 50 µM.	McGee et al. (2012)
TCEP	2–120 hpf	Larval (120 hpf)	50, 250, 1,250, 6,250 µg/L	Locomotor, gene transcript expression, AChE activity.	Hypoactivity at 6,250 µg/L; no change in AChE activity; down-regulation of *gfap* expression at 1,250 and 6,260 µg/L, of *mbp* expression at 50, 250, and 1,250 µg/L, and of *shha* and *syn2a* at 1,250 µg/L.	Sun et al. (2016)
TCEP	3–120 hpf	Embryonic, larval through 120 hpf	2,85, 28.5, 285, 14,250, 28,500 µg/L	LC_{50}, morphology, gene transcript expression.	72-h LC_{50}: 3,748 µg/L; increased mortality at 28.5 µg/L and above; developmental delay and malformations at 14,250 and 28,500 µg/L; increased transcript expression of *vtg2*, *ncoa1*, *ncoa2*, *ncoa3*, *er1*, and *er2b* at 2.85, 28.5, and 285 µg/L, of *er2a* at 28.5 and 285 µg/L, and of *pgr*, *vtg1*, and *vtg4* at 2.85 and 285 µg/L .	Wu et al. (2017)
TCEP	4–96 hpf	Larval (96 hpf)	1–1,000 µM	Lethality, behavior, hepatotoxicity, cardiotoxicity.	NOAEL of 400 µM at 48 and 96 hpf; EC_{50} 521 µM at 48 hpf and 415 µM at 96 hpf; LC_{50} >1,000 µM at 48 hpf and 977 µM at 96 hpf; teratogenic index >1.92 at 48 hpf and 2.35 at 96 hpf; no cardiotoxicity or hepatoxicity; hypoactivity only at highest concentration at which systemic toxicity was observed.	Alzualde et al. (2018)
TCEP	24, 48, 72, 96, 120 hpf	Embryonic (24, 48 hpf), larval (72, 96, 120 hpf)	0.3, 1, 3, 10, 30 µM	Lethality, malformations, photomotor behavior.	No malformations; hypoactivity at 96 and 120 hpf at 30 µM.	Dach et al. (2019)
TCEP	6–120 hpf	Larval (144 hpf)	0.04–120 µM	Lethality, hatching, malformations.	No adverse effects observed.	Behl et al. (2015)
TCEP	6–120 hpf	Embryonic (24 hpf), larval (120 hpf)	0.0064, 0.064, 0.64, 6.4, 64 µM	Behavior.	No behavioral changes.	Reif et al. (2016)
TCEP	6–120 hpf	Embryonic (24 hpf), larval (120 hpf)	0.0064, 0.064, 0.64, 6.4, 64 µM	Malformations, behavior.	No mortality or delayed progression at 24 hpf; hyperactivity at 24 hpf at 0.0064 and 0.064 µM; hypoactivity at 120 hpf at 64 µM in dark acclimation and light phase.	Noyes et al. (2015)
TCEP	6–120 hpf	Embryonic (24 hpf), larval (120 hpf)	0.0064, 0.064, 0.64, 6.4, 64 µM	Malformations, behavior.	Mortality at 0.0064 µM.	Truong et al. (2014)

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TCEP	6–120 hpf	Larval (150–154 hpf)	0.04–120 µM	Locomotor activity.	No overt developmental toxicity; decreased activity down to 12 µM.	Jarema et al. (2015)
TCEP	6–144 hpf	Larval (144 hpf)	10–100 µM	Morphology, behavior.	Overt toxicity threshold NA; not teratogenic; neurobehavioral effect threshold 31.4 µM.	Dishaw et al. (2014)
TCPP	5.25–96 hpf	Larval (96 hpf)	0.05–50 µM	Malformations.	No overt toxicity up to 50 µM.	McGee et al. (2012)
TCPP	24, 48, 72, 96, 120 hpf	Embryonic (24, 48 hpf), larval (72, 96, 120 hpf)	0.3, 1, 3, 10, 30 µM	Lethality, malformations, photomotor behavior.	No malformations; hypoactivity at 96 and 120 hpf at 30 µM.	Dach et al. (2019)
TCPP	6–120 hpf	Embryonic (24 hpf), larval (120 hpf)	0.0064, 0.064, 0.64, 6.4, 64 µM	Malformations, behavior.	No mortality or malformations at 24 or 120 hpf; no behavioral change at 24 hpf; at 120 hpf, hypoactivity at 64 µM in dark acclimation and light phase.	Noyes et al. (2015)
TCPP	6–144 hpf	Larval (144 hpf)	10–100 µM	Morphology, behavior.	Overt toxicity threshold NA; not teratogenic; neurobehavioral effect threshold 100 µm.	Dishaw et al. (2014)
TDBPP	6–144 hpf	Larval (144 hpf)	0.1–1 µM	Morphology, behavior.	Overt toxicity threshold 3.3 µM; not teratogenic; neurobehavioral effect threshold 0.56 µM.	Dishaw et al. (2014)

Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; AhR, aryl hydrocarbon receptor; dpf, days post-fertilization; BChE, butyrylcholine esterase; EC_{50}, effect concentration at which 50% of the population is affected; ER, estrogen receptor; GABA, γ-aminobutyric acid; GR, glycocorticoid receptor; hpf, hours post-fertilization; LC_{50}, lethal concentration at which 50% of the population is killed; mpf, months post-fertilization; MR, mineralocorticoid receptor; NA, not available; NOAEL, no-observed-adverse-effect level; NOEL, no-observed-effect level; PPARα, peroxisome proliferator-activated receptor alpha; qRT-PCR, quantitative real-time polymerase chain reaction; TCEP, tris(2-chloroethyl) phosphate; TCPP, tris(1-chloro-2-propyl) phosphate; TDBPP, tris(2,3-dibromopropyl) phosphate; TDCPP, tris(1,3-dichloro-2-propyl) phosphate; TRα, thyroid-hormone receptor alpha.

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TABLE D-2 Summary of Additional Zebrafish Studies after Exposure to a Polyhalogenated Organophosphate Flame Retardant

Chemical	Life Stage at Exposure	Life Stage Analyzed	Concentrations	Outcomes Assessed	Results	Reference
TDCPP	0.75–2 hpf	Embryonic (2 hpf)	2 µM	Whole-genome bisulfite sequencing for methylation.	Chromosome-specific alterations in cytosine methylation.	Volz et al. (2016)
TDCPP	0.75–4, 6, 8, 10, 12, and 24 hpf	Embryonic (4, 6, 8, 10, 12, and 24 hpf)	1.56, 3.12 µM	Transcriptomics; immunohistochemistry; hemoglobin staining; pericardial area and cardiac assessments; ocular area and pigmentation assessments.	Most sensitive developmental toxicity stage 2-3-hpf window; minimal effects on transcriptome at lower concentration; higher concentration altered expression of genes associated with gastrulation and mesoderm development and differentiation, decreased hemoglobin, increased pericardial area, and decreased ocular area and pigmentation.	Dasgupta et al. (2018)
TDCPP	2–96 hpf	Larval (96 hpf)	Not stated	96-h LC_{50}, 96-h cardiotoxicity EC_{50}.	96-h LC_{50} 0.418 mg/L; 96-h EC_{50} for pericardial edema 1.65 mg/L.	Du et al. (2015)
TDCPP	4–120 hpf	Larval (120 hpf)	[1] 0.8, 4, 20, 100, 500 mg/L; [2] 0.02, 0.2, 2 mg/L	Developmental toxicity, transcript expression.	Survival and hatching rate decreased at 20 mg/L and greater; concentration-dependent alterations in transcript expression of genes associated with AhR, PPARα, ER, TRa, GR, and MR receptor networks.	Liu et al. (2013a)
TDCPP	6–96 hpf	96 hpf	1.25–10 mg/L	96-h LC_{50}.	1.9 mg/L.	Godfrey et al. (2017b)
TDCPP	9–14 dpf	Larval (14 dpf)	0.5 µmol/L	Lipid staining in trunk.	Demonstrated obesogenic effects.	Kopp et al. (2017)
TDCPP	1 wk old through 4 mo old	Adult (4 mo)	0.05, 0.5, 5 µg/L	Fecundity, plasma hormone concentrations, GSI, transcript expression in brain, gonad, and liver.	Dose-dependent reduction in egg production with significant decrease at 5 µg/L; decrease in length and body weight in females in 0.5- and 5-µg/L treatment groups and decrease in GSI in 5-µg/L treatment group; no changes in gonad histology or hormone concentrations in both sexes; no changes in HPGL axis transcript expression of *fshβ* and *lhβ* in the brain, of *cyp19a*, *activin-βa2*, or *3βhsd* in gonad, or in *vtg1* in liver in both sexes; in GH/IGF axis, down-regulation of *gh* in brain and *igf1* in liver in all three treatment groups in males; in GH/IGF axis, down-regulation of *gh* in brain, *igf1* in ovary, and *igf1*, *igf2a*, and *igf2a* in liver in all three treatment groups and down-regulation of *igf2b* in ovary in highest treatment group.	Zhu et al. (2015)
TDCPP	1 mo old for 120 d (4 mo)	Adult females (5 mo)	0.05, 0.5, 5 µg/L	Fish morphology and gene expression associated with muscle and bone.	Significant morphologic changes and decreased muscle density in the 5-µg/L treatment group; down-regulation of *myf5* and *myog* and up-regulation of *bmp2b* and *bmp4* in the 5-µg/L treatment group.	Zhu et al. (2018)
TDCPP	Adult 4 mo old for 7 d	Adult (4 mo)	229 µg/L (1/20 96-h LC_{50}—high), 45.81 µg/L (1/100 96-h LC_{50}—low)	Biochemical, transcript expression, and SCGE in liver.	In females, ROS and CAT increased after low treatment, and GSH decreased and Mn-SOD increased after low treatment and decreased after high treatment; in males, ROS increased and GSH decreased after both treatments and Mn-SOD and Cu/Zn-SOD decreased after high treatment; transcript expression of genes related to the defense system increased in both sexes after low treatment and decreased in both sex after high treatment; decrease in transcript expression in females (except an increase in	Chen et al. (2018)

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					cyclin 1A) and an increase in transcript expression in males (except a decrease in cyclin B) after low treatment occurred in genes associated with cell-cycle regulation; expression of genes associated with cell-cycle regulation decreased after high treatment for most genes in both sexes (except an increase in chik2 in females); fen1 (DNA repair) decreased in females and increased in males after low treatment; after high treatment, rpa3 decreased in both sexes and fen1 decreased in males; after low treatment, most apoptosis-related genes increased in both sexes (except a decrease in bax in females); after high treatment, most apoptosis-related genes decreased in both sexes (except increases in bcl-2 and bax in females); DNA damage increased after both treatments in both sexes.	
TDCPP	Adult 4 mo old for 14 d	Adult 4 mo (each sex)	0.04, 0.2, 1 mg/L	Plasma hormones and gene transcription.	E2 and T increased in males and females at 1 mg/L; 11-KT decreased in males at 0.04, 0.2, and 1 mg/L; *cyp17* and *cyp19a* expression increased at 1 mg/L in males and females; *vtg* increased at 1 mg/L in males and decreased at 0.2 and 1 mg/L in females.	Liu et al. (2012)
TDCPP	Adults 4–5 mo old for 21 d	Adult (4–6 mo)	0.04, 0.2, 1 mg/L	Fecundity, plasma hormones and gene transcription of brain and gonads.	Egg/female spawning and fertilization success decreased at 1 mg/L, and spawning events per female and hatchability decreased at 0.2 and 1 mg/L; in females, E2 increased at 1 mg/L, T decreased at 0.2 and 1 mg/L, 11-KT decreased at 0.2 mg/L, and *vtg* increased at 0.2 and 1 mg/L; in males, E2 increased at 1 mg/L, T decreased at all three concentrations, 11-KT decreased at 0.04 mg/L, and vtg increased in all three treatment groups; transcription levels of HPG-associated genes were sex- and tissue-dependent.	Liu et al. (2013b)
TDCPP	Adult 4 mo old for up to 22 d	Adult (4 mo)	Not stated	Accumulation.	Half-life in five tissues <6.5 h but 9 h in roe; steady state by 14–19 d.	Wang et al. (2017a)
TDCPP	Adult 5 mo old for up to 19 d	Adult (5 mo)	1/150 96-h LC_{50} (low), 1/30 96-h LC_{50} (high)	Metabolism in liver.	Dechlorination pathway.	Wang et al. (2017b)
TDCPP	Adult 5 mo old for 4 d	Male adult (5 mo)	1 mg/L	Transcriptomics in liver; confirmation of hepatoxicity biomarkers, and histologic examination of transgenic larvae for liver toxicity.	Up-regulation of genes associated with endoplasmic reticulum stress and toll-like receptor pathway indicating hepatic inflammation; histologic evaluation showed increase in infiltrated neutrophils, hepatic vacuolization, and apoptosis with increase in liver size.	Liu et al. (2016)
TCEP	2–96 hpf	Larval (96 hpf)	Not stated	96-h LC_{50}, 96-h cardiotoxicity EC_{50}.	96-h LC_{50}, 202 mg/L; 96-h EC_{50} for pericardial edema, 179 mg/L.	Du et al. (2015)
TCEP	Adult 4 mo old for up to 22 d	Adult (4 mo)	Not stated	Accumulation.	Half-life in six tissues, <6.5 h; steady state by 3 d.	Wang et al. (2017a)

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TABLE D-2 Continued

Chemical	Life Stage at Exposure	Life Stage Analyzed	Concentrations	Outcomes Assessed	Results	Reference
TCEP	Adult 5 mo old for up to 19 d	Adult (5 mo)	1/150 96-h LC_{50} (low), 1/30 96-h LC_{50} (high)	Metabolism in liver.	Dechlorination pathway.	Wang et al. (2017b)
TCPP	2–96 hpf	Larval (96 hpf)	Not stated	96-h LC_{50}, 96-h cardiotoxicity EC_{50}.	96-h LC_{50}, 13.5 mg/L; 96-h EC_{50} for pericardial edema, 22.8 mg/L.	Du et al. (2015)

Abbreviations: dpf, days post-fertilization; EC_{50}, effect concentration at which 50% of the population is affected; hpf, hours post-fertilization; LC_{50}, lethal concentration at which 50% of the population is killed; mpf, months post-fertilization; TCEP, tris(2-chloroethyl) phosphate; TCPP, tris(1-chloro-2-propyl) phosphate; TDCPP, tris(1,3-dichloro-2-propyl) phosphate.

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TABLE D-3 Effects of Tetrabromobisphenol A on Thyroid Homeostasis in Zebrafish

Life Stage at Exposure	Life Stage Analyzed	Concentrations	Outcomes Assessed	Results	Reference
1–72 hpf	Larva (72 hpf)	0.01, 0.1, 1 µM	Transcript expression of *trβ*.	No change in transcript expression of *trβ*.	Lu et al. (2018)
1–96 hpf or 96 h started after hatching	Larva (96 hpf or 96 h after hatching)	10, 25, 50, 75% of 96-h LC_{50} or 96-h EC_{50}	96-h LC_{50}, 96-h EC_{50} (hatching); transcript expression of *tg*, *ttr*, *tshβ*, *trα*, *trβ* for both exposures.	1–96 hpf: LC_{50} 5.27 mg/L and EC_{50} 1.09 mg/L; up-regulation of *trα* (75%) and down-regulation of *tshβ* (75%); in larvae (96 h post-hatch), up-regulation of *trα* (75%), *ttr* (75%), and *tshβ* (10, 25, 50, 75%).	Chan and Chan (2012)
2–120 hpf	Larva (120 hpf)	100, 200, 300, 400 µg/L	Transcript expression, histology.	Up-regulation of *trα* (100, 200 µg/L) and down-regulation of *tpo* (100, 200, 300 µg/L); no change in *trb*, *dio1*, *dio2*, *dio3*, *tsh*; linked to changes in ocular development and behavior.	Baumann et al. (2016)
2–122 hpf	Larva (122 hpf)	0.15, 0.3, 0.6, 1.2, 2.4, 4.8 µM	Morphology, transcript expression of *thr*, *er*, *ar*, *ahr* pathways' potential to dock *thrα*.	Delay in embryogenesis at 0.6 µM and above; down-regulation of expression of *ccnd1*, *ar*, *thrα*, *er2a*, *er2b*.	Liu et al. (2018)
2–144 hpf	Larva (144 hpf)	50, 100, 200, 400 µg/L	Survival, morphology, thyroid hormone, transcript expression, acetylcholinesterase activity, behavior.	Increased T4; decreased T3; up-regulation of *tshβ, tg*; down-regulation of *ttr*, *trβ*; decreased swimming activity.	Zhu et al. (2018)
4.5–144 hpf or 0–28 dpf	Larva (6 or 28 dpf)	13 µg/L (1% of LC_{50})	Swim bladder, body size; transcript expression, locomotor activity.	No significant changes in body size or locomotor activity; no changes in expression of thyroid-related genes	Godfrey et al. (2017a)
Adult	Adult (14-d exposure)	0.75, 1.5 µM	Transcriptomics and proteomics of liver.	Interference of thyroid, vitamin A homeostasis; oxidative stress response and cellular metabolism pathways.	De Wit et al. (2008)
Adult; juvenile (1–42 d post-hatch)	Adults (30-d exposure); juvenile [42 dph (~45 dpf)]	0.023, 0.047, 0.094, 0.188, 0.375, 0.75, 1.5, 3, 6 µM (no 3- or 6-µM treatments of juveniles)	Adults: observed behavior, reproduction, histology of gonads and thyroid; juveniles: growth, development, survival, histology of gonads and thyroid.	Adults: abnormal adult behavior (3 and 6 µM) within 24 h, including reduced respiration and stress leading to euthanasia for ethical reasons; reduction in egg number in all chemical-treated groups; fertilization not affected; hatching decreased in all but 0.375-µM group (0.023-1.5 µM); increase in previtellogenic oocytes at 1.5 µM; early oocyte atresia in all treatment groups; thyroid tissue similar. Juveniles: increased female characteristics at 1.5 µM with no changes in other measures.	Kuiper et al. (2007)
NA	NA	1, 3, 10 µM	Used a species-specific reporter system based on fusion of LBD trα to GAL4 DNA-binding domain to measure displacement of T3.	Displaced T3 from trα.	Fini et al. (2012)

Abbreviations: dpf, days post-fertilization; dph, days posthatching; mpf, months post-fertilization; NA, not available; T3, triiodothyronine; T4, thyroxine.

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TABLE D-4 Effects of Polyhalogenated Bisphenols on Zebrafish Development or Behavior

Chemical	Life Stage at Exposure	Life Stage Analyzed	Concentrations	Outcomes Assessed	Results	Reference
TBBPA	2–120 hpf	Larva (120 hpf)	100, 200, 300, 400 µg/L	Behavior, histology	Changes in *trb*, *dio1*, *dio2*, *dio3*, *tsh* were not linked to changes in ocular development or behavior.	Baumann et al. (2016)
TBBPA	2–122 hpf	Larva (122 hpf)	0.15, 0.3, 0.6, 1.2, 2.4, 4.8 µM	Morphology	Delay in embryogenesis at ≥0.6 µM.	Liu et al. (2018)
TBBPA	2–144 hpf	Larva (144 hpf)	50, 100, 200, 400 µg/L	Morphology, behavior	Decreased swimming activity.	Zhu et al. (2018)
TBBPA	3–120 hpf	Larva (24, 48 hpf, transcript expression, enzyme activity; 48 hpf, heart beat; 28 dpf, survival)	0.75, 1.5, 3 µM	Mortality, malformation	100% mortality at 1.5, 3 µM; decreased heart rate, edema of the trunk, tail malformations.	McCormick et al. (2010)
TBBPA	4–96 hpf	Larva (96 hpf)	1–1,000 µM	Lethality, behavior, hepatotoxicity, cardiotoxicity	NOAEL 1.5 µM at 48 hpf, 1 µM at 96 hpf; EC_{50}, 1.81 µM at 48 hpf, 1.48 µM at 96 hpf; LC_{50}, 3.26 µM at 8 hpf, 1.90 µM at 96 hpf; teratogenic index, 1.8 at 48 hpf, 1.28 at 96 hpf; cardiotoxicity, arrhythmia/ventricular failure; no hepatoxicity; no change in behavior.	Alzualde et al. (2018)
TBBPA	4–96 hpf	Embryo (24, 48 hpf) larva, (96 hpf)	0.05, 0.1, 0.5, 1 mg/L	Survival, morphology	Decreased survival (96 hpf) at 0.5, 1 mg/L; increased malformations (96 hpf) at 0.5, 1 mg/L; blood flow disorder (24 hpf) at 0.1, 0.5, 1 mg/L; spawn coagulation (24 hpf) at 0.5, 1 mg/L; increased pericardial edema (48 hpf) at 0.5, 1 mg/L.	Yang et al. (2015)
TBBPA	4.5–144 hpf or 0-28 dpf	Larva (6, 28 dpf)	13 µg/L (1% of LC_{50})	Swim bladder and body size; locomotor activity	No significant changes in body size or locomotor activity.	Godfrey et al. (2017a)
TBBPA	24, 48, 72, 96, 120 hpf	Embryo (24, 48 hpf), larva (72, 96, 120 hpf)	0.03, 0.1, 0.3, 1, 3 µM	Lethality, malformations, photomotor behavior	Malformation, mortality at 3 µM at all time points; no effects on behavior at nonlethal concentrations.	Dach et al. (2019)
TBBPA	6–120 hpf	Embryo (24 hpf), larva (120 hpf)	0.0064, 0.064, 0.64, 6.4, 64 µM	Behavior	24 hpf, change in photomotor response at 64 µM,	Reif et al. (2016)
TBBPA	6–120 hpf	Larva (144 hpf)	0.04–120 µM	Lethality, hatching, malformations	Point of departure at 4.6 µM with dependence on mortality,	Behl et al. (2015)
TBBPA	6–120 hpf	Embryo (24 hpf), larva (120 hpf)	0.0064, 0.064, 0.64, 6.4, 64 µM	Malformations, behavior	Greatest teratogenic effects of all chemicals tested at 24, 120 hpf; significant hyperactivity at 24 hpf; at 120 hpf, hypoactivity in both dark stimulatory and acclimation phases.	Noyes et al. (2015)
TBBPA	6–120 hpf	Embryo (24 hpf), larva (120 hpf)	0.0064, 0.064, 0.64, 6.4, 64 µM	Malformations, behavior	Mortality at 6 µM with no defects.	Truong et al. (2014)
TBBPA	6–120 hpf	Larva (150–154 hpf)	0.04–120 µM	Locomotor activity	Acute exposure changed behavior, but developmental exposure resulted in no behavioral change.	Jarema et al. (2015)
TBBPA	6–168 hpf	Larva (168 hpf)	1.25, 2.5, 5, 10, 20 mg/L	Mortality, malformations, behavior	LC_{50}, 1.45 mg/L; EC_{50}, 0.99 mg/L; fin malformations, pericardial edema at 2.5 mg/L; decreased spontaneous movement with movement ceasing at 10 mg/L.	Usenko et al. (2016)

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TBBPA	8–48 or 48–96 hpf	Embyro (48 hpf), larva (96 or 120 hpf)	5, 10 µM	Malformations, behavior, apoptosis, motor neuron development, muscle fiber patterning	Increased mortality, morphologic alterations at higher concentration at earlier exposure window; no morphologic alterations at 5 µM; behavior at 120 hpf showed hypoactivity for earlier exposure period at 5 µM; increase in apoptotic cells, delayed motor neuron development, loose muscle fibers.	Chen et al. (2016)
TBBPA-BHEE or TBBPA-OHEE	6-168 hpf	Larva (168 hpf)	1.25, 2.5, 5, 10, 20 mg/L	Mortality, malformations, behavior	LC_{50}, 2.2 mg/L; EC_{50}, 1.85 mg/L; fin malformations and pericardial edema at 10 mg/L;decreased spontaneous movement with movement ceasing at 10 mg/L.	Usenko et al. (2016)
TBBPA-BDBPE or TBBPA-DBPE	6-120 hpf	Embryo (24 hpf), larva (120 hpf)	0.00064, 0.0064, 0.064, 0.64, 6.4 µM	Malformations, behavior	No mortality or malformations at 24, 120 hpf; no behavioral change at 24, 120 hpf.	Noyes et al. (2015)
TBBPA-BME or TBBPA-DME	3-120 hpf	Larva (24, 48 hpf, transcript expression, enzyme activity; 48 hpf heartbeat; 28 dpf, survival)	1, 5, 10 µM	Mortality, malformations	No mortality; some edema and hemorrhage, but less than after exposure toTBBPA.	McCormick et al. (2010)

Abbreviations: dpf, days post-fertilization; dph, days posthatching; EC_{50}, effect concentration at which 50% of the population is affected; hpf, hours post-fertilization; LC_{50}, lethal concentration at which 50% of the population is killed; mpf, months post-fertilization; TBBPA, tetrabromobisphenol A; TBBPA-BHEE, tetrabromobisphenol A bis(2-hydroxyethyl) ether; TBBPA-BDBPE, 3,3′,5,5′-tetrabromobisphenol A bis(2,3-dibromopropyl) ether; TBBPA-BME, tetrabromobisphenol A bismethyl ether; T4, thyroxine; T3, triiodothyronine.

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TABLE D-5 Additional Zebrafish Studies of Polyhalogenated Bisphenol

Chemical	Life Stage at Exposure	Life Stage Analyzed	Concentrations	Outcomes Assessed	Results	Reference
TBBPA	1–72, 1–96, 1–120 hpf	Larva (72, 96, 120 hpf)	0.01–1,000 µg/L	Transcript expression of *vtg, cyp19a, cyp19b*	No changes in transcript expression.	Wang et al. (2011)
TBBPA	1–144 hpf; 21 d in 2-mo-old males	Larva (144 hpf), 2-mo-old males (21 d)	0.5, 1.0, 1.5 mg/L	Lethality, hatching rate	Less lethal than TCBPA with 100% mortality at 1.5 mg/L by 144 hpf; LC_{50} 144 hpf, 1.24 mg/L; hatching delayed at 0.5, 1, 1.5 mg/L; increased hemorrhage, edema at 1.5 mg/L; adults, no change in mortality or vtg.	Song et al. (2014)
TBBPA	1? –192 hpf	Embryo (48 hpf), larva (96, 144, 192 hpf)—note: possible discrepancy in hpf in methods and dpf reported in results (1, 3, 5, 8 dpf)	0.1, 0.4, 0.7, 1.0 mg/L	Biochemical assays (Cu/Zn-SOD, CAT, GPx), transcript expression (cat, cu-zn-sod, gpx1a), apoptosis, histology	At 192 hpf: decreased survival at 0.7, 1 mg/L, decreased hatching at 1 mg/L, increased malformations at 0.4, 0.7, 1 mg/L, decreased length at 0.7, 1.0 mg/L; decreased Cu/Zn-SOD, CAT, Gpx1a activity at 0.4 (3, 5, 8 dpf), 0.7 (3, 5, 8 dpf), 1 mg/L (1, 3, 5, 8 dpf); decreased expression of Cu/Zn-SOD 0.1 (5 dpf), 0.4 (3 dpf), 0.7 (3, 8 dpf), 1 mg/L (1, 3, 5, 8 dpf); dcreased expression of CAT at 0.1 (3 dpf), 0.7 (1 dpf), 1 mg/L (1, 3, 5, 8 dpf); decreased expression of GPx1a at 1 mg/L (1, 3, 5, 8 dpf); increase in apoptosis at 5 dpf in brain, heart, tail; 1 mg/L led to decrease in myocardial cells and heart linearization.	Wu et al. (2015)
TBBPA	2–48 hpf	Embryo (48 hpf)	1, 10, 100, 1,000 µg/L	Embryo toxicity	Lowest effect concentration 1,000 µg/L; lack of spontaneous movement and decline in heart rate.	Carlsson and Norrgren (2014)
TBBPA	2–96 hpf	Larva (96 hpf)	Up to 10 mg/L	Lethality, *vtg1* expression	LC_{50} at 96 h, 5.27 mg/L; EC_{50} at 96h, 1.09 mg/L (hatching rate); 75% of EC_{50} = 61.2-fold increase in *vtg1* expression.	Chow et al. (2013)
TBBPA	2–96 hpf	Larva (96 hpf)	0.002, 0.01, 0.05, 0.25, 0.75, 1.5 mg/L	Lethality; SOD, LPO, Hsp70	Lethality at concentrations >0.75 mg/L; superoxide dismutase, lipid peroxidation increased with increasing concentration.	Hu et al. (2009)
TBBPA	6–96 hpf	Larva (96 hpf)	0.625–5 mg/L	96-h LC_{50}	1.3 mg/L.	Godfrey et al. (2017b)
TBBPA	120 hpf for 30 min	Larva (121 hpf)	2.5, 5, 10, 20 mg/L	Zebrafish neuromast cells	Decreased P1, OC neuromast hair cells in dose-dependent manner.	Park et al. (2016)
TBBPA	NA	NA (72-h cell exposure)	5 µM	Proteomic analysis of zebrafish liver cells	Protein related to folding. NADPH production.	Kling and Förlin (2009)
TBBPA	NA	NA	10^{-9}–10^{-5} M	Ligands of estrogen receptors and/or peroxisome proliferator activated receptors	PPARγ ligand (same at TCBPA).	Riu et al. (2011)
TCBPA	1–144 hpf; 21 d in 2-mo-old males	Larva (144 hpf), 2-mo-old males (21 d)	0.5, 1.0, 1.5 mg/L	Lethality, hatching rate	More lethal than TBBPA with 100% mortality at 1 mg/L by 120 hpf, 1.5 mg/L by 96 hpf; LC_{50} 144h, 0.75 mg/L; hatching delayed at 1.5 mg/L; increased hemorrhage, edema at 1 and 1.5 mg/L; adults, increased mortality at 1.5 mg/L; no change in vtg.	Song et al. (2014)

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TCBPA	NA	NA	10^{-9}–0^{-5} M	Ligands of estrogen receptors and/or peroxisome proliferator activated receptors	PPARγ ligand (same as TBBPA).	Riu et al. (2011)

Abbreviations: dpf, days post-fertilization; dph, days posthatching; Hsp, heat-shock protein; LC_{50}, lethal concentration at which 50% of the population is killed; LPO, lipid peroxidation; mpf, months post-fertilization; NA, not available; TBBPA, tetrabromobisphenol A; TCBPA, tetrachlorobisphenol A; vtg, vitellogenin.

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