United States
Consumer Product Safety Commission

February, 2025

CPSC Staff Statement on: Class-Based Qualitative Hazard Assessment of Polyhalogenated Organophosphate (PHOP) Flame Retardants

The U.S. Consumer Product Safety Commission (CPSC or Commission) contracted with ICF (Contract BPA No. 61320622A0005, Order No. 61320622F2011) to complete a class-based qualitative hazard assessment of Polyhalogenated Organophosphate (PHOP) Flame Retardants.

This statement was prepared by the CPSC staff. ICF produced the following report for CPSC staff. The statement and report have not been reviewed or approved by, and may not represent the views of, the Commission.

ICF's report consists of a main report that describes background, methods, and results from this analysis. This report is a case study that applies the 2024 *Guidance Document for Conducting Qualitative Class-Based Hazard Assessment of Organohalogen Flame Retardants* (The Guide) to conduct a qualitative class-based hazard assessment of PHOP flame retardants.[1]

Thirty-four discreet PHOP compounds were evaluated for similarity, toxicological relevance, and health effects by their structural fingerprints and by building five profiles: 1) metabolites; 2) physiologically based toxicokinetics (PBTK); 3) physicochemical properties; 4) mechanistic effects; and 5) adverse effects. Structural analogs and metabolites were sought for these PHOPs and included in the five profiles when appropriate. First, the case study outlines the process to identify analogs and metabolites, and to acquire data for all chemicals. Next, the case study discusses the five profiles for PHOPs resulting from the identification process and their respective health outcomes and conclusions.

The profile evaluations identified five low confidence chemicals for inclusion in the PHOPs subclass. ICF recommended reassigning these five chemicals from PHOPs to other subclasses. The remaining 29 PHOPs had medium to high confidence for subclass inclusion and were reviewed regarding health outcomes in this case study. Four PHOPs have abundant data available in toxicological databases and/or the literature.

The identified health effects for the PHOPs subclass include hepatic toxicity, renal toxicity, thyroid effects, developmental neurotoxicity and reproductive toxicity (influenced by thyroid changes), and the potential for carcinogenicity. These qualitative hazard assessments for chemicals in the PHOP subclass can be used to identify critical effects for derivation of quantitative toxicity reference values (TRVs), which may then be combined with quantitative class-based exposure assessments in a class-based risk assessment for the PHOP subclass.

Related reports and documentation are available on CPSC's Organohalogen Flame Retardant Chemicals Assessment Web page.[1]

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[1] Available at https://www.cpsc.gov/Business--Manufacturing/Organohalogen-Flame-Retardant-Chemicals-Assessment.

Class-based Qualitative Hazard Assessment of Polyhalogenated Organophosphate (PHOP) Flame Retardants

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Acronyms and Abbreviations

Acronym / Abbreviation	Term/Definition
ADME	absorption, distribution, metabolism, and excretion
API	application programming interface
BCIPHIPP	bis(1-chloro-2-propyl) 1-hydroxy-2-propyl phosphate
BCIPP	bis(1-chloropropan-2-yl) hydrogen phosphate
BPP	brominated phenyl phosphate
CAS RN	Chemical Abstracts Service Registry Number
cHTS	curated high throughput screening
CID	PubChem compound identifier
CPSC	U.S. Consumer Product Safety Commission
CTD	Comparative Toxicogenomics Database
DTT	Division of Translational Toxicology, of the National Institute of Environmental Health Sciences (NIEHS) (formerly DNTP – Division of the National Toxicology Program)
EPA	U.S. Environmental Protection Agency
HTS	high throughput screening
httk	high throughput toxicokinetics
ICE	Integrated Chemical Environment
MOA	mode of action
NASEM	National Academies of Science, Engineering, and Medicine
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OFR	organohalogen flame retardant
OPERA	Open (Quantitative) Structure-activity/property Relationship Application
PBTK	physiologically based toxicokinetic
PCA	principal component analysis
PHA	polyhalogenated alicycles
PHACbx	polyhalogenated aliphatic carboxylates
PHACh	polyhalogenated aliphatic chains
PHBA	polyhalogenated benzene alicycle
PHBzAF	polyhalogenated benzene aliphatics and functionalized

Acronym / Abbreviation	Term/Definition
PHB	polyhalogenated benzene
PHBAF	polyhalogenated bisphenol aliphatics and functionalized
PHC	polyhalogenated carbocycle
PHDE	polyhalogenated diphenyl ether
PHOP	polyhalogenated organophosphate
PHPhAE	polyhalogenated phenol aliphatic ether
PHPhD	polyhalogenated phenol derivative
PHPBI	polyhalogenated phthalates/benzoates/imide
PHT	polyhalogenated triazine
QSAR	quantitative structure-activity relationship
SMILES	Simplified Molecular Input Line Entry System
TCEP	tris(2-chloroethyl) phosphate
TCPP	tris(2-chloroisopropyl) phosphate
TDCPP	tris(1,3-dichloro-2-propyl) phosphate
TDBPP	tris(2,3-dibromopropyl) phosphate

1. Introduction

This case study is an application of process guide, “Guidance Document for Conducting Class-based Hazard Assessment of Organohalogen Flame Retardants” (henceforth referred to as the Guide), in response to an effort by the Consumer Product Safety Commission (CPSC) to develop and apply read-across principles to classes of chemicals under CPSC Blank Purchase Agreement (BPA) 61320622A0005, Call Order 61320622F2011, for polyhalogenated organophosphate (PHOP) flame retardants. Within this case study, principles and tools discussed in the Guide are used to address the class assignments originally suggested by the National Academies of Sciences, Engineering, and Medicine (NASEM) (NASEM, 2019), by determining if the chemicals within each subclass are grouped together similarly by their health effects or other properties suggestive of similar bioactivity instead of primarily focusing on their structure. Finally, the Guide is applied to draw conclusions on the health effects of a class, based on available data and similarity.

This case study used the 14 organohalogen flame retardant (OFR) subclasses reported by CPSC in the 2022 article titled “Development of a Flame Retardant and an Organohalogen Flame Retardant Chemical Inventory”, which included a downloadable inventory of chemicals used as flame retardants (Bevington et al., 2022). Although some of the initial steps in the case study are applied to all chemicals across all 14 subclasses of OFRs, the case study primarily focuses on the PHOP subclass as an example application of the Guide.

This case study documents the steps taken to classify and assess PHOPs by building five profiles: metabolites, physiologically based toxicokinetics (PBTK), physicochemical properties, mechanistic effects, and adverse effects. Using traditional read across principles, structural analogs and metabolites were sought for PHOPs and included in the five profiles when appropriate. The case study first outlines how analogs and metabolites were identified, and how data was acquired for all chemicals (Section 2). A brief description of quality control and chemical list management is also shared. Then, the results of the five profiles for PHOPs are reported and their respective conclusions are discussed (Section 3). Assessments of the PHOP classification and shared health effects were made and confidence in the assessments were reported (Section 4). There were several factors inhibiting high confidence in the overall qualitative hazard assignment for PHOPs and the case study ends with suggestions for next steps to increase confidence.

2. Getting Started

2.1. Quality Control

A standardized method of chemical identification and tracking must be employed to ensure quality control of the data and consistency across steps when working with many chemicals and many pieces of information connected to each chemical. The initial OFR list was subjected to standardization by applying quantitative structure-activity relationship (QSAR) ready Simplified Molecular Input Line Entry System (SMILES) workflow developed using RDKit and was implemented in Python using the in house developed library CompDesc (https://test.pypi.org/project/CompDesc/). This package mimics the QSAR ready preparation workflow developed by Mansouri et al. (2016); Mansouri et al. (2024). After standardization, prepared SMILES were then deduplicated. The resulting SMILES identifiers are referred to as “cleaned SMILES” or “cleaned structures” herein.

The cleaned SMILES for each OFR are included in the supplementary files. The CPSC’s 2022 inventory listed 488 OFRs; after mixtures and uncommonly reported congeners were removed, 258 chemicals remained. Deduplicating by cleaned SMILES resulted in 248 unique chemicals. OFRs were tracked and evaluated throughout the case study by their cleaned SMILES. The details of this process and how to manage chemical lists are described in Appendix A of the Guide.

2.2. PHOPs Initial Classification

The 14 OFR subclasses were developed and defined by NASEM by grouping chemicals via chemotypes and expert-driven predictions on the chemotype bioactivity (NASEM, 2019). The initial PHOP subclass as defined by NASEM (22 chemicals) and expanded by CPSC (by 20 additional chemicals) was composed of 42 chemicals, each containing at least one organophosphate functional group accompanied by halogenated alkyl or aryl substituents, except for one member, dimethyl {[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]methyl}phosphonate, which instead has a halogenated triazine moiety (CPSC, 2023). All members of this subclass were considered phosphates or phosphites, depending on the oxidation state of the phosphorous atom(s). Within the subclass, the halogen identities varied; some chemicals were exclusively brominated, some were solely chlorinated, while others contain both halogens.

After applying cleaned SMILES and deduplicating, the list of PHOPs was reduced from the 42 listed in CPSC’s inventory (Bevington et al., 2022) to 34 discreet compounds. The initial list of 42 PHOPs mapped to the final list of 34 unique PHOPs cleaned SMILES used for this case study are listed in Appendix A (Table A.1).

Following the Guide, the 34 discrete compounds were initially evaluated to determine if their classification was suitable prior to creating profiles, by assuring they were adequately similar to each other. Hierarchal clustering of all NASEM defined subclasses was conducted using topological RDKit fingerprint. This fingerprint is an implementation of the Daylight-like fingerprint (Daylight Chemical Information Systems Inc., 2011b) based on hashing molecular subgraphs (Daylight Chemical Information Systems Inc., 2011a, 2011b; Landrum, n.d.). Tanimoto coefficients based on fingerprint-based representations (where each molecule is encoded into a series of "bits" that indicate the presence (1) or absence (0) of specific fragments within the substance) are computed for each pair of chemicals and then visualized through a dendrogram with a hierarchical clustering and single aggregation method (Figure 1). The clustering was completed for all OFRs across all 14 subclasses because visualizing similarity among PHOPs (or any subclass) requires substances binned into other subclasses be contrasted against the subclass of interest to quantify intra- and inter-class similarity.

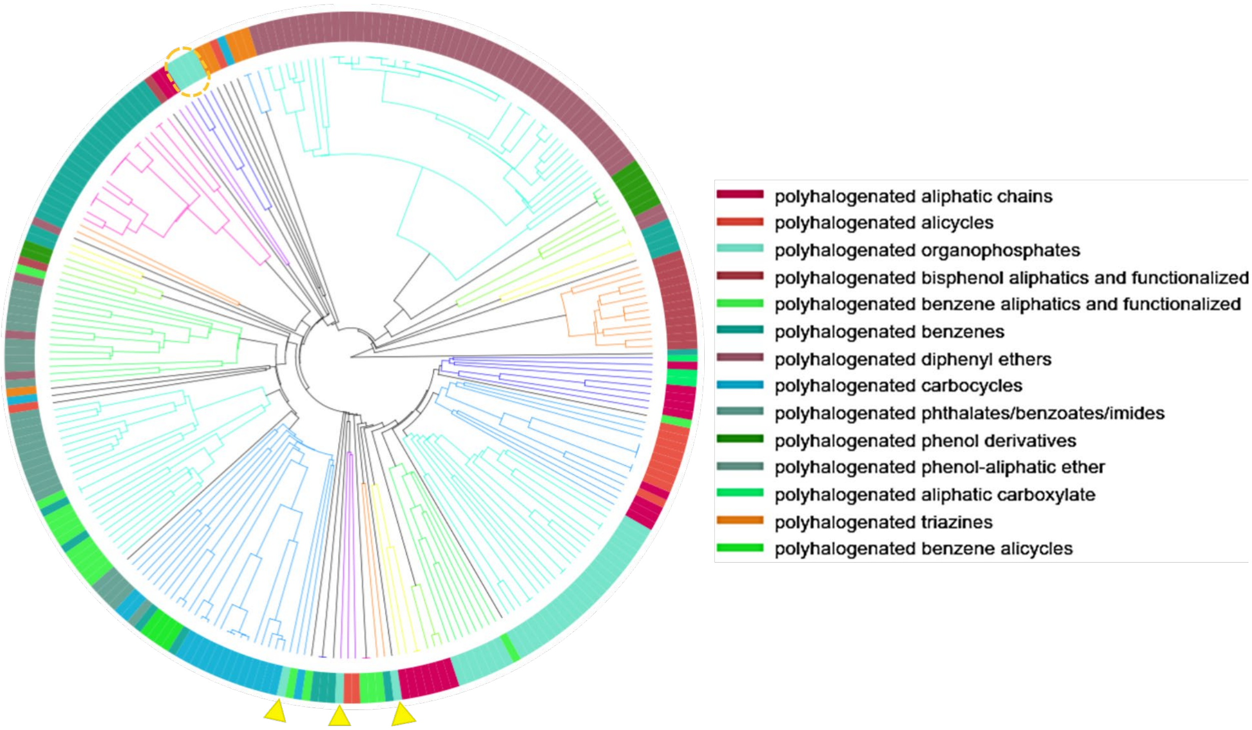

Figure 1. Dendrograms comparing structural grouping using hierarchical clustering based on topological Fingerprint and Tanimoto score of CPSC OFR subclasses.

Each subclass is defined by a unique color, and each chemical is a slice matched to a unique inner branch line. PHOPs are light blue-green in color. The orange dashed circle and yellow triangles highlight seven chemicals discussed in the surrounded text for their differences from the rest of the PHOPs.

A larger image of Figure 1 with the chemicals labeled by Chemical Abstracts Service Registry Number (CAS RN) is in Appendix B for reference. PHOPs (light blue-green) are almost all grouped together in the bottom right quadrant of Figure 1, validating the general structural similarity of the chemicals initially classified as PHOPs relative to the other subclasses. The 4 PHOP compounds appearing separate from the main PHOPs cluster are located within the orange dotted circle and are:

1. Tris(2,4,6-tribromophenyl) phosphate
2. Tris(2,4-dibromophenyl) phosphate
3. Tris(2,3-dibromophenyl) phosphate
4. Tris(2-bromo-4-methylphenyl) phosphate

All four chemicals are structurally similar to each other and are brominated phenyl phosphates (BPPs). Although the results of topological fingerprint clustering are suggestive that the BPPs are not well defined as PHOPs, these four chemicals were evaluated with the rest of the PHOPs throughout the case study since topological fingerprint alone does not mean they will behave differently physiologically.

In addition to the four BPPs, there were three PHOPs that were separated from the main cluster in Figure 1 (identified with yellow triangles). In order of furthest from the PHOPs main cluster to closest, these three PHOPs were:

1. Phosphonic acid, P-[1-[[(2-chloroethoxy)(2-chloroethyl)phosphinyl]oxy]ethyl]-, 1-[bis(2-chloroethoxy)phosphinyl]ethyl 2-chloroethyl ester
   - Commercially called Phosgard C-22-R.
   - This is the only OFR with three phosphate groups; there are 6 bisphosphate OFRs.
2. Dimethyl (4,6-dichloro-1,3,5-triazin-2-yloxy)methylphosphonate
   - This is the only PHOP with a triazine moiety.
3. 2,3-Dibromopropyl dihydrogen phosphate
   - Although the 2,3-dibromopropyl is similar to other PHOPs upon expert review, this is the smallest PHOP and the only one with a single branch, leaving the phosphate group more acidic than other PHOPs. This was clustered with chemicals in the polyhalogenated aliphatic chain (PHACh) subclass in Figure 1.

The separation of these 3 PHOPs from the PHOPs cluster is not large and does not directly suggest any differences in bioactivity. However, the small difference was enough to consider investigating each of these chemicals individually within the profiles of this case study to determine if any patterns were present.

It is also noteworthy that all 14 subclasses have reasonable intra subclass similarity. The majority of the chemicals in each subclass clustered together but with a small percentage in each subclass further away on the dendrogram, which is in agreement with NASEM's method for subclass grouping (NASEM, 2019). Polyhalogenated diphenyl ethers (PHDE) are the most tightly clustered chemicals and polyhalogenated benzene alicycles (PHBA) have the most intra subclass dissimilarity. Further analysis of intra- and inter-class clustering is out of scope of this case study, but worth pursuing for each subclass in additional applications of the Guide.

2.3. Analog Search

The 2019 NASEM report identified >1,000 analogs for the 14 subclasses of OFRs using chemotypes available in the U.S. Environmental Protection Agency's (EPA's) Chemistry Dashboard. Initial data availability of these analogs was screened as part of CPSC's scope documents and associated evidence maps. This was completed prior to the expansion of the OFR inventory list by CPSC in 2022. Searching and screening new analogs related to newly identified OFRs in the expanded list was part of the purpose and scope of the Guide and case study.

There are several databases and types of fingerprints to choose from for identifying analogs, as described in the Guide. The database choice for this case study focused on several criteria. Most importantly, the database should include most of the OFR compounds from all subclasses. The NASEM report and CPSC's scoping documents discovered that although there are some data rich OFR compounds in 11 of the 14 OFR subclass, many OFRs in each subclass only have some readily available or no data at all, especially PHOPs. This makes it important to cast a wide net for potential analogs with the end goal of finding additional data rich chemicals with sufficient similarity to the seed OFRs. To accomplish this, the chosen database should have an extensive library of chemicals to identify as potential analogs.

Another criterion was that the tool should ideally be publicly available and not proprietary unless a proprietary tool is significantly more fit-for-purpose than publicly available tools. Other considerations include time to compute and compatibility with the fingerprint choice. The fingerprint choice for the OFR analog search needed to be nonspecific enough to identify as many reasonable analogs as possible, but specific enough to sufficiently cluster each OFR subclass by structure.

We chose PubChem as the most suitable database that met the criteria for OFRs. PubChem has an extensive collection of more than 100 million chemicals, providing comprehensive coverage for most of the OFR-related substances. Additionally, PubChem has an application programming interface (API) that makes it convenient to query for a large list of chemicals such as the OFRs (NLM, 2024).

In addition, the PubChem database can be queried using specifically developed PubChem fingerprints. The fingerprints include 880 bits covering element and ring counts, chemical topology including bound types, and substructure search, but they do not directly cover functional groups or chemotypes. The PubChem fingerprints were validated to determine if they sufficiently separate the OFRs by subclass (i.e., the fingerprints were effective at identifying the OFR unique chemistry) when compared to the RDKit topological fingerprints used in Section 2.2 (Figure 1). The comparison between structural grouping using the two different fingerprints is illustrated by dendrograms in Figure 2. OFRs were clustered into their assigned subclasses less effectively with PubChem fingerprints than RDKit, but PubChem sufficiently distinguished subclasses overall. The RDKit topological fingerprints recognize structural subgraphs and provided up to 1,400 bits per chemical whereas the PubChem fingerprints do not use structure subgraphs but instead map atoms and structural substructures and had up to 120 bits per chemical. The reduced specificity of the PubChem fingerprint was favored for this case study because of its likelihood to identify more unique analogs.

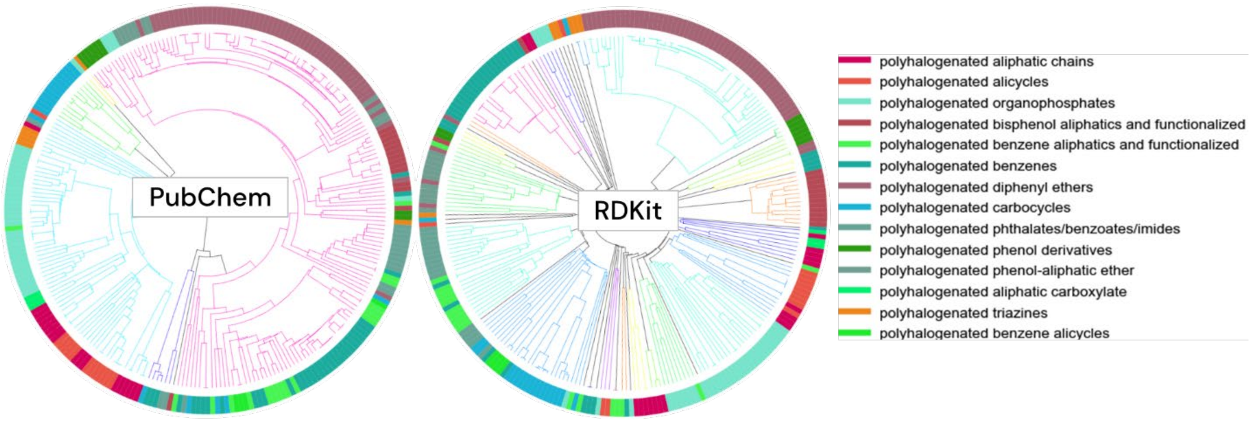

Figure 2. Dendrograms of OFR clustering by fingerprint type and color coded by subclass.

The RDKit dendrogram is the same as Figure 1.

A minimum Tanimoto cut-off of 0.85 was used to identify analogs for all 14 subclasses of OFRs. Initial use of a Tanimoto ≥0.80 produced analog list quantities that were unreasonable to evaluate, and a Tanimoto ≥0.90 did not increase potential analog quality of those identified with Tanimoto ≥0.85 but reduced the overall number of starting analogs and therefore potentially eliminated useful analogs. Tanimoto ≥0.85 gave sufficient coverage of the chemical universe without generating an unreasonable number (i.e., tens of thousands) of analogs to evaluate or clearly uninformative analogs (i.e., benzene as an analog to polyhalogenated benzene (PHB) OFRs). The result ensured a high degree of similarity between parent and analog compounds while allowing sufficient room for diversity.

Analogs were retrieved from PubChem in SMILES format and their associated PubChem compound identifiers (CIDs). For quality control of the structures, the same cleaning process used for the OFRs was applied to create QSAR ready SMILES for each analog. Duplicates were then eliminated based on the cleaned SMILES along with any mixtures and inorganic compounds. A request with the PubChem API was used to retrieve available CAS RNs and chemical names for each CID. Analogs without CAS RN were excluded from further analysis because chemicals without CAS RNs typically have limited available information, making them not useful for read across. It is important to note that a single cleaned SMILES could correspond to several CAS RNs (as demonstrated in the list of PHOPs in Table A.1), either due to different forms of the same chemical or due to obsolete CAS RNs. All CAS RNs were retained for the purpose of database searches (Section 2.5).

Table 1. Quantity of analogs identified by OFR subclass after cleaning and deduplication.

Subclass (all Polyhalogenated)	Abbreviation	Total Unique Analogs with CAS RN[1]
Organophosphates	PHOP	211
Bisphenol aliphatics and functionalized	PHBAF	843
Diphenyl ethers	PHDE	2,026
Benzene alicycles	PHBA	58
Benzene aliphatics and functionalized	PHBzAF	3,176
Phenol aliphatic ethers	PHPhAE	1,311
Alicycles	PHA	86
Phenol derivatives	PHPhD	198
Aliphatic carboxylates	PHACbx	44
Benzenes	PHB	1,730
Phthalates-benzoates-imides	PHPBI	1,354
Carbocycles	PHC	134
Triazines	PHT	13
Aliphatic chains	PHACh	327
Total		9,857

[1] All analogs that are OFRs are included in this number.

The refined analog list was compared to NASEM's analog list (NASEM, 2019) after removing analogs that were also OFRs. Of the analogs identified by NASEM across all 14 subclasses, 46% were also in the analog list for this case study (Figure 3). For PHOPs, there were 48 overlapping analogs identified of the 75 NASEM analogs and 175 newly identified analogs. Differences between the two analog lists were not unexpected because NASEM used a different process, database, and similarity score than what were used in this case study. Specifically, NASEM used the EPA Distributed Structure Searchable Toxicity (DSSTox) database (Richard, 2004; Richard et al., 2006) to find chemicals, then used a Tanimoto similarity index threshold of 0.80 (versus 0.85 used in this case study). Ultimately for PHOPs both strategies proved unfruitful for finding data rich analogs (Sections 2.5 and 3.5) to anchor PHOPs health effects for read across or add additional confidence to conclusions. However, there are data rich analogs present for other subclasses identified by both approaches.

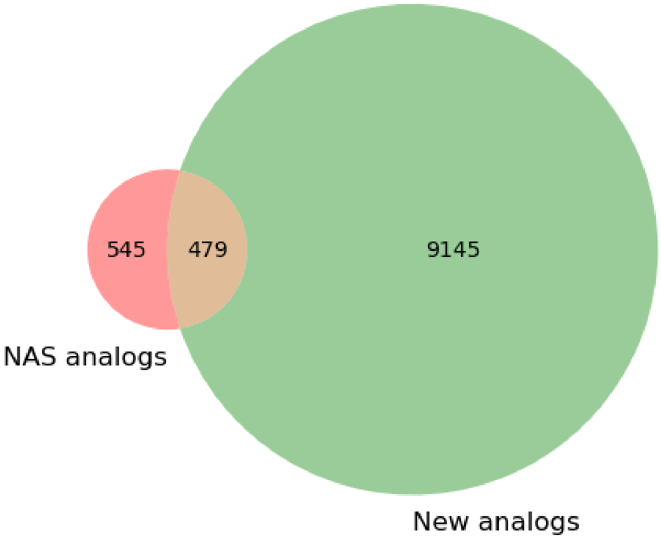

Figure 3. Venn diagram showing the number of analogs for all subclasses that are shared and not shared between the NASEM 2019 report and this case study.

The total number of analogs from the NASEM report was 1,073, but after applying cleaned SMILES and deduplicating that number reduced to 1,024. The 1,024 cleaned SMILES analogs were used to compare to the new analogs in this figure.

2.3.1. Analog Evaluation

Analogs were evaluated by goodness of fit with their parents by evaluating the distribution of their predicted physical chemical properties with their parents, in consultation with CPSC staff. Overall, the analogs were good matches to their respective parents except for their molecular weight distributions.

There were many analogs that had lower molecular weights than the OFRs. Figure 4 shows the molecular weight distributions for A) all OFRs and B) identified analogs. For all OFR subclasses, the lowest molecular weight was approximately 200 g/mol except for polyhalogenated aliphatic carboxylates (PHACbx). As noted by the yellow dashed vertical line in Figure 4B, many identified analogs had molecular weight less than 200 g/mol. The distribution difference can be explained by the overall lower molecular weight distribution of chemicals in the PubChem database (Kim et al., 2016). Upon expert review of a sampling of the lowest molecular weight analogs, many were nonhalogenated and missing important functional groups unique to the subclass. A molecular weight cutoff of 200 g/mol for the analogs was applied to remove these from further use.

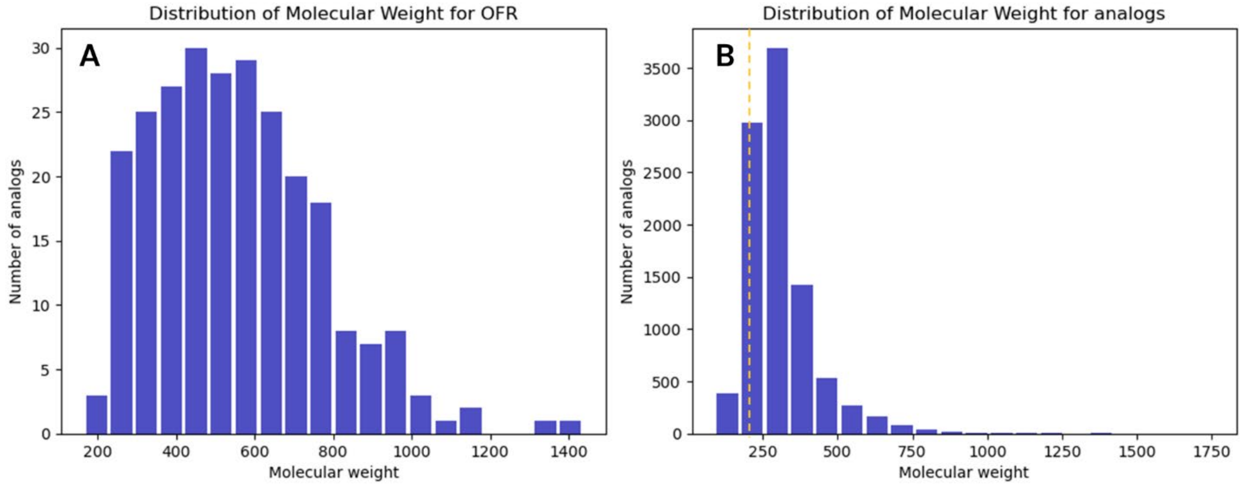

Figure 4. Molecular weight (g/mol) distribution of (A) all OFRs in the 14 subclasses and (B) all the corresponding analogs.

After review and refinement of analogs by molecular weight, data availability was assessed by referencing the evidence maps for this case study which are described in Section 2.5. Table 2 lists the analogs for PHOPs after review, refinement, removal of chemicals without any data in the evidence maps, and removal of two analogs that lacked a phosphate functional group. This refinement resulted in 16 potentially useful PHOPs analogs for class-based read across, ranging from data poor to data intermediate. The analog search and refinement did not yield data rich chemicals to aid in the read across assessment of health effects for PHOPs. The analog considered to have the most data, diethyl (2-chloroethyl) phosphonate, only had ten data hits in the evidence maps, all of which were for acute toxicity. It is likely that many of the hits across databases are duplicate hits of the same acute toxicity data and the true number of unique data hits are <10 for this analog.

In contrast to the analogs, the most data rich PHOP, tris(1,3-dichloro-2-propyl) phosphate (TDCPP), had over 10,000 database data hits across many hazard categories. There were several OFRs with at least 10-fold more data hits than the analogs. Therefore, the data rich PHOPs were considered to be better anchor chemicals for other chemicals in the subclass, when compared with PHOP analogs. Despite this, the analogs for PHOPs were evaluated in all the profiles in Section 3 of this case study in the event that they would help define or interpret PHOPs as a subclass.

Table 2. Refined PHOP analogs.

CAS RN	Cleaned SMILES	Name	Data Type[1]
10235-09-3[2]	S=P(OCCCl)(OCCCl)OCCCl	O,O,O-tris(2-chloroethyl) phosphorothioate	Acute toxicity
1070-42-4[2]	OP(OCCCl)OCCCl	Bis(2-chloroethyl) phosphonate	Acute toxicity
111712-46-0	CC(Br)(CBr)COP(=O)(OCC(C)(Br)CBr)OCC(C)(Br)CBr	Tris(2-methyl-2,3-dibromopropyl) phosphate	Mutagenicity/Genotoxicity

CAS RN	Cleaned SMILES	Name	Data Type[1]
111712-47-1	CC(Br)C(Br)COP(=O)(OCC(Br)C(C)Br)OCC(Br)C(C)Br	Tris(2,3-dibromobutyl) phosphate	Mutagenicity/Genotoxicity
111712-49-3	O=P(OCCC(Br)CBr)(OCCC(Br)CBr)OCCC(Br)CBr	Tris(3,4-dibromobutyl) phosphate	Mutagenicity/Genotoxicity
3568-51-2	CCOP(=O)(OC=C(Cl)Cl)OCCCl	Phosphoric acid, 2-chloroethyl 2,2-dichloroethenyl ethyl ester	Acute toxicity
5266-08-0	O=P(OC=C(Cl)Cl)(OCCCl)OCCCl	Phosphoric acid, bis(2-chloroethyl) 2,2-dichlorovinyl ester	Acute toxicity
3862-21-3	COP(=O)(OC)OC(Cl)C(Cl)(Cl)Cl	Dimethyl 1,2,2,2-tetrachloroethyl phosphate	Acute toxicity
3309-67-9	CCOP(=O)(OC=C(Cl)Cl)OCC(Cl)CCl	2,2-Dichloroethenyl 2,3-dichloropropyl ethyl phosphate	Acute toxicity
40806-03-9	COP(=O)(OC)OC(Br)C(Br)(Br)Br	Dimethyl 1,2,2,2-tetrabromoethyl phosphate	Acute toxicity
10419-79-1[2]	CCOP(=O)(CCCl)OCC	Diethyl (2-chloroethyl) phosphonate	Acute toxicity
111712-48-2	CC(C)(Br)C(Br)COP(=O)(OCC(Br)C(C)(C)Br)OCC(Br)C(C)(C)Br	Tris(3-methyl-2,3-dibromobutyl) phosphate	Mutagenicity/Genotoxicity
63451-45-6	O=P(OCCCl)(OCCCl)OCC(CO)(CO)COP(=O)(OCCCl)OCCCl	Phosphoric acid, 2,2-bis(hydroxymethyl)-1,3-propanediyl tetrakis(2-chloroethyl) ester	Acute toxicity
111712-45-9	CC(OP(=O)(OC(C)C(Br)CBr)OC(C)C(Br)CBr)C(Br)CBr	Tris(3,4-dibromo-2-butyl) phosphate	Mutagenicity/Genotoxicity
64050-66-4	CCCOP(=O)(OC=C(Cl)Cl)OCCCl	Phosphoric acid, 2-chloroethyl 2,2-dichlorovinyl propyl ester	Acute toxicity
64050-69-7	CCOP(=O)(OC=C(Cl)Cl)OCC(C)Cl	2-Chloropropyl 2,2-dichloroethenyl ethyl phosphate	Acute toxicity

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[1] Data type of the data hit(s) resulting from the analog evidence maps created from database downloads. The result or direction of the data is not included in the evidence maps; therefore, these data types are not conclusive of hazard.

[2] Analogs also identified by NASEM.

2.4. Metabolite Identification

Identifying metabolites of OFRs serves two purposes for class-based assessment:

1. Identifying shared metabolites among OFRs suggests similar metabolism for the parent chemicals, and therefore can strengthen grouping chemicals together within a subclass or indicate reclassification.

2. Data on some OFRs are limited (e.g., five PHOP OFRs have data). Because metabolite data may be more prolific, and toxicity of OFRs can be tied to their metabolites depending on

half-lives and metabolic mechanisms, metabolite toxicity information may be useful in class-based assessment.

Although this case study focuses its analysis on PHOPs, metabolites were identified for OFRs in all 14 subclasses simultaneously to be used for additional case studies of other subclasses. Empirically reported metabolites were identified through literature searches, and computationally predicted metabolites were identified using predictive tools. The metabolite lists obtained from these two sources were combined. The process for identifying metabolites and results is described in the following subsections.

2.4.1. Identification of Empirical Metabolites

Literature searching was built on work from previous and ongoing CPSC collaborative projects (sources 1 and 2 below). Instead of a traditional literature search, OFR metabolite references were collated from a variety of sources:

1. CPSC provided 100 references as being relevant to metabolism/metabolites that were identified in the draft Division of Translational Toxicology (DTT) systematic evidence mapping of the 14 OFR subclasses (Rooney et al., 2023).
2. Gray literature and review articles that were identified as part of the broader literature survey on previous CPSC task orders #61320621F1001, 61320621F1002, and 61320621F1003 (Tasks #12 through 14) under contract CPSC-D-17-0001.[1]
3. Tree searching, by identifying additional references cited in or citing relevant references identified in the literature obtained from sources 1 and 2.
4. Expert identification of additional key references through targeted article and database review.

References from sources 2, 3, and 4 were used to fill gaps in data-poor subclasses. References pulled from the literature survey included those tagged for absorption, distribution, metabolism, and excretion (ADME)/toxicokinetics and/or human biomonitoring after review of the full text for relevancy. Chemicals were identified as metabolites for extraction when they were:

- Used for human biomonitoring of an OFR parent chemical regardless of association with a health effect. The metabolite must have been specifically linked to a parent chemical.
- Detected (qualitatively or quantitatively) in a human, animal, and/or in vitro metabolism study of an OFR.

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[1] Supporting contractor reports are available at https://www.cpsc.gov/Business--Manufacturing/Organohalogen-Flame-Retardant-Chemicals-Assessment.

- Identified in a metabolic scheme of an OFR and determined by expert judgement to not be an intermediate, transient species. These metabolites were extracted whether they were measured or only proposed.

Metabolite information was extracted from all references and organized by subclass. The type of information extracted included:

- Identification of the parent and metabolite(s),
- The species studied,
- The matrix evaluated (e.g., urine, blood, plasma),
- Additional toxicokinetic parameters if reported (clearance, volume of distribution, half-life, and fractional urinary excretion for parent and/or metabolite), and
- Notes on the metabolic process generating the metabolite.

After the same parent-metabolite pair in the same species and matrix was identified by multiple references, additional references that did not identify novel information for this parent-metabolite pair were noted but did not undergo data extraction. When metabolites were identified only by a diagram of the chemical structure and/or metabolic process (with no chemical name or other chemical identifier), those structures were translated manually into SMILES strings. Extracted data were checked for accuracy by a secondary extractor to ensure quality control, and corrections were made as needed.

All extracted chemicals were further identified by adding SMILES strings and CAS RNs (when possible), and duplicate parent-metabolite pairs within a subclass were removed. The output was a list of metabolites for each subclass deduplicated by the available chemical identifiers. These metabolite-parent pairs were not entirely deduplicated at this stage because a SMILES string can be written many ways for the same chemical. A machine-readable list of the parent-metabolite pairs was generated to check assignments of CAS RNs and SMILES with PubChem and the EPA CompTox Dashboard. Full deduplication required standardizing SMILES into QSAR-ready cleaned SMILES generated from the same source. This standardization used the same process used for predicted metabolites (Section 2.4.2) and was applied prior to combining the empirical list with the predicted list. The process is described in Figure 5.

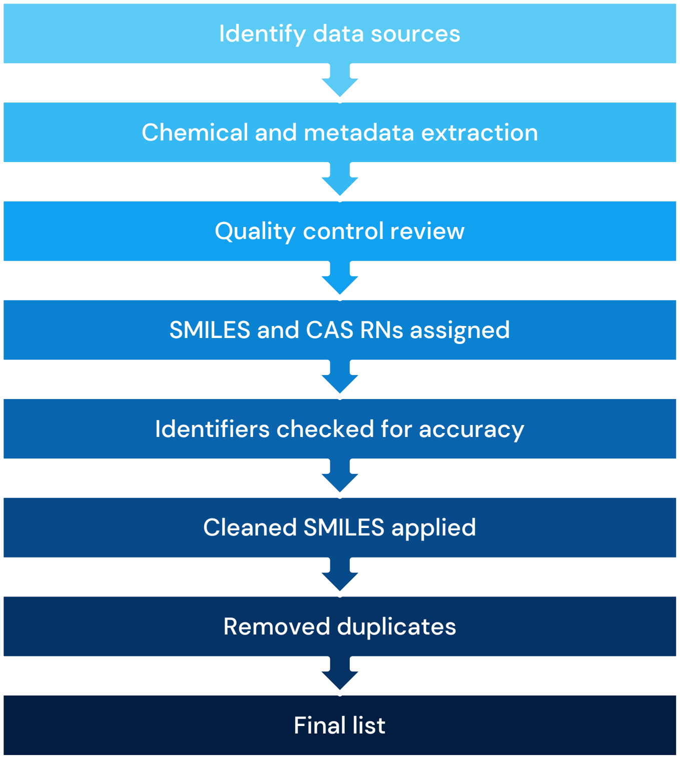

Figure 5. Process of identifying unique empirical metabolites.

After final deduplication, 44 unique metabolites were reported in the literature for PHOPs, which are listed in Table 3 by cleaned SMILES and PubChem reported name, when available. Most of the metabolism data identified in the literature consisted of qualitative identification of metabolites; relatively little information on quantitative metabolic parameters was identified, and therefore not discussed in this case study.

There were three metabolites of three parent PHOPs that were frequently reported as biomarkers in the National Health and Nutrition Examination Survey (CDC, 2024):

1. bis(1-Chloropropan-2-yl) hydrogen phosphate (BCIPP) as a biomarker of tris(2-Chloroisopropyl) phosphate (TCPP),
2. bis(1,3-Dichloro-2-propyl) phosphate as a biomarker of TDCPP, and
3. bis(2-Chloroethyl) phosphate as a biomarker of tris(2-chloroethyl) phosphate (TCEP).

Another metabolite, bis(1-chloro-2-propyl) 1-hydroxy-2-propyl phosphate (BCIPHIPP), has been increasingly reported as an additional biomarker for TCPP in recent literature (e.g., Wang et al., 2021). In at least one case, BCIPHIPP was detected in a greater percentage of urine samples than BCIPP (98% vs. 18%; Hoffman et al., 2021). Since these four metabolites are accepted as known biomarkers, they were deemed as major metabolites for the purpose of validating metabolite predictions, which is described in the next section. For reference, the last column in Table 3 notes whether the empirical metabolite was also predicted.

Table 3. Empirical metabolites of PHOPs identified by literature review.

Metabolite SMILES	Metabolite Name	Predicted
Major Metabolites[1]		
CC(CCl)OP(=O)(O)OC(C)CCl	Bis(1-Chloropropan-2-yl) hydrogen phosphate	Yes
CC(CO)OP(=O)(OC(C)CCl)OC(C)CCl	Bis(1-chloro-2-propyl) 1-hydroxy-2-propyl phosphate	Yes
O=P(O)(OC(CCl)CCl)OC(CCl)CCl	Bis(1,3-dichloro-2-propyl) phosphate	Yes
O=P(O)(OCCCl)OCCCl	Bis(2-chloroethyl) phosphate	Yes
Other Metabolites		
BrCC1CO1	1-Bromo-2,3-epoxypropane	Yes
C=C(Br)COP(=O)(O)O	NA	No
C=C(Br)COP(=O)(O)OCC(=C)Br	NA	No
C=C(Br)COP(=O)(OCC(Br)CBr)OCC(Br)CBr	NA	No
CC(CCl)OP(=O)(OC(C)CCl)OC(C)C(=O)O	2-({Bis[(1-chloropropan-2-yl)oxy]phosphoryl}oxy)propanoic acid	Yes
CC(CCl)OP(=O)(OC(C)CCl)OC(CCl)COS(=O)(=O)O	NA	No
CC(CCl)OP(=O)(OC(C)CCl)OC(CO)CCl	2-({Bis[(1-chloropropan-2-yl)oxy]phosphoryl}oxy)-3-chloropropan-1-ol	Yes
CC(CO)OP(=O)(O)OC(C)CCl	[(1-Chloropropan-2-yl)oxy][(1-hydroxypropan-2-yl)oxy]phosphinic acid	No
CC(OP(=O)(OC(CCl)CCl)OC(CCl)CCl)C(=O)O	NA	No
NC(CCC(=O)NC(CSCC(CCl)OP(=O)(OC(CCl)CCl)OC(CCl)CCl)C(=O)NCC(=O)O)C(=O)O	NA	No
NC(CCC(=O)NC(CSCCOP(=O)(OCCCl)OCCCl)C(=O)NCC(=O)O)C(=O)O	NA	Yes
O=C(O)C(CCl)OP(=O)(OC(CCl)CCl)OC(CCl)CCl	2-({Bis[(1,3-dichloropropan-2-yl)oxy]phosphoryl}oxy)-3-chloropropanoic acid	Yes
O=C(O)C1OC(OCCOP(=O)(OCCCl)OCCCl)C(O)C(O)C1O	NA	Yes
O=C(O)COP(=O)(OCCCl)OCCCl	{[Bis(2-chloroethoxy)phosphoryl]oxy}acetic acid	Yes
O=C(O)CP(=O)(OCCCl)OCCCl	NA	Yes
O=CCOP(=O)(OCCCl)OCCCl	Bis(2-chloroethyl) 2-oxoethyl phosphate	Yes
O=P(O)(O)OC(CCl)CCl	2-Propanol, 1,3-dichloro-, dihydrogen phosphate	Yes
O=P(O)(O)OCC(Br)CBr	2,3-Dibromopropyl dihydrogen phosphate	Yes
O=P(O)(OC(CO)CCl)OC(CCl)CCl	NA	Yes

Metabolite SMILES	Metabolite Name	Predicted
O=P(O)(OCC(Br)CBr)OCC(Br)CBr	Bis(2,3-dibromopropyl)phosphate	Yes
O=P(O)(OCCCl)OCC(CCl)(CCl)COP(=O)(OCCCl)OCCCl	NA	Yes
O=P(O)(OCCCl)OCC(CCl)(CCl)COP(=O)(OCCO)OCCCl	NA	No
O=P(OC(CCl)CCl)(OC(CCl)CCl)OC(CCl)COS(=O)(=O)O	NA	No
O=P(OC(CO)CCl)(OC(CCl)CCl)OC(CCl)CCl	2-({Bis[(1,3-dichloropropan-2-yl)oxy]phosphoryl}oxy)-3-chloropropan-1-ol	Yes
O=P(OCC(Br)CBr)(OCC(Br)CBr)OCC(Br)CBr	Tris(2,3-dibromopropyl) phosphate	No
O=P(OCCCl)(OCCCl)OCC(CCl)(CCl)COP(=O)(OCCCl)OCC(O)Cl	NA	Yes
O=P(OCCCl)(OCCCl)OCC(CCl)(CCl)COP(=O)(OCCCl)OCCOS(=O)(=O)O	NA	No
O=P(OCCCl)(OCCCl)OCC(CO)(CCl)CCl	NA	Yes
O=P(OCCCl)(OCCCl)OCCCl	Tri(beta-chloroethyl) phosphate	Yes
O=P(OCCCl)(OCCCl)OCCOS(=O)(=O)O	NA	No
O=P(OCCO)(OCCCl)OCC(CCl)(CCl)COP(=O)(OCCCl)OCC(O)Cl	NA	No
O=P(OCCO)(OCCCl)OCC(CCl)(CCl)COP(=O)(OCCCl)OCCCl	NA	Yes
O=P(OCCO)(OCCCl)OCC(CCl)(CCl)COP(=O)(OCCO)OCCCl	NA	No
O=P(OCCO)(OCCCl)OCC(CO)(CCl)CCl	NA	No
O=P(OCCO)(OCCCl)OCCCl	Bis(2-chloroethyl) 2-hydroxyethyl phosphate	Yes
OC(CCl)CCl	1,3-Dichloro-2-propanol	Yes
OC1C(Br)CC(Br)CC1Br	2,4,6-Tribromophenol	Yes
OC1CCC(Br)CC1Br	2,4-Dibromo-phenol	Yes
OCC(Br)CBr	2,3-Dibromo-1-propanol	Yes
OCCCl	2-Chloroethanol	Yes

NA = not available

[1] Major metabolites are established biomarkers of TDCPP, TCEP, and/or TCPP.

2.4.2. Metabolite Predictions

Several tools were evaluated to determine the best option(s) for predicting OFR metabolites by considering the following criteria:

1. **Is the tool publicly available?** Publicly available tools are ideal because they are free to use and allow for anyone to reproduce results.
2. **Is the tool regularly maintained and was it updated recently?** Tools that are not regularly maintained or updated are inclined to be less accurate and potentially have errors that would propagate through the case study analysis.
3. **Does the tool have published studies that evaluate its performance with measured data?** Validation of predictive properties is important for confidence in any predictions.
4. **Can the tool be run in batch mode?** Given that there are several hundred OFRs, it would be time prohibitive to use any tools that cannot run in batch mode.

Using this criteria, two tools were determined to be a good fit for predicting OFR metabolites: GLORYx (de Bruyn Kops et al., 2021) and the Organisation for Economic Co-operation and Development (OECD) QSAR toolbox (Schultz et al., 2018). Both tools were used to predict metabolites independently, then the results were compared and combined. Using both tools was advantageous because they differ in the type of approach used to make predictions, which prevents predictive bias that can occur from using a single tool. Additionally, this tool combination enables us to encompass both predictive methodologies: reaction-based and machine learning-based approaches.

GLORYx and the OECD toolbox were run in batch mode and predictions were made for both Phase I and Phase II metabolism. The rat liver S9 metabolism simulator, in vivo rat metabolism simulator, and observed rat in vivo metabolism simulator were chosen for the OECD toolbox settings.

The outputs of GLORYx predictions and OECD toolbox predictions were transformed into cleaned SMILES, combined, and deduplicated to 3,764 unique compounds (Figure 6). Of these, only 336 (9%) had CAS RNs, which suggested most of the predicted metabolites would not be useful for health effect determinations because there were unlikely to be data rich without CAS RNs. Moreover, only 13 of the 14 subclasses had predicted metabolites; PHBA had zero predicted metabolites. Further analysis of inter and intra subclass metabolites for any other subclass besides PHOPs was out of scope of this case study.

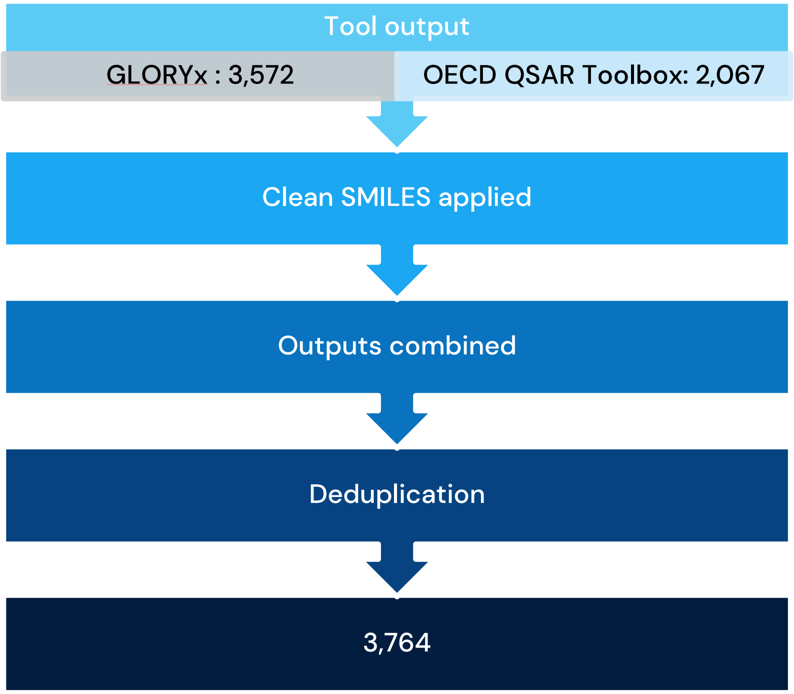

Figure 6. Diagram describing the process of obtaining from metabolite prediction tool outputs a revised list of unique predicted metabolites.

For the PHOP subclass, there were 629 total predicted metabolites with unique cleaned SMILES. Of the empirical metabolites, 66% (29) were also predicted, providing confidence in the output of the predicted tools; the overlap is listed in Table 3. The combined list of cleaned structures from GLORYx, the OECD toolbox, and the literature review was 644 unique metabolites, and all are listed in the supplemental files.

Ideally, all empirical metabolites would have also been predicted, but there are a number of reasons why some of the metabolites recorded in the literature were not predicted. Metabolites considered empirical in this work did not need to be measured in mammals and some were either proposed as part of a metabolic pathway by study authors or reported as measured in vitro. Predictions have many limitations too, most notably is that metabolite predictions often center around a single reaction originating from the parent compound, neglecting potential chain reactions among metabolites that take place in vivo. While this limitation can be mitigated in machine learning-based approaches, the effectiveness of machine learning-based approaches is contingent upon the comprehensiveness of the training set, which may be limited in quantity and diversity of chemicals and may not include the OFRs or similar chemicals. Considering the limitations of both the literature review and predictive tools, a 66% overlap of the empirically reported metabolites for PHOPs with predicted was considered reasonable support for prediction accuracy.

Several metabolites were predicted for more than one PHOP. Most notably, 2-chloroethanol was predicted for 9 PHOPs, was found empirically, and was a metabolite unique to the PHOP subclass. A more detailed analysis of shared metabolites among PHOPs is presented in Section 3.

2.5. Toxicological Database Downloads and Evidence Maps for OFRs, Analogs, and Metabolites

Determining experimental data availability for OFRs, analogs, and predicted and empirical metabolites was essential for identifying data gaps of the PHOP subclass and for which chemicals could help fill the data gaps. To assess chemical data availability of a large list of chemicals, databases containing toxicity and hazard information are the most effective source for determining data availability, because databases can be batch searched and, in most cases, the database output can be read with custom code to organize and sort the output without manual extraction efforts. A literature review is not ideal for this goal given the time and resources required to complete literature searching, screening, and then manual tagging of the information for hundreds of chemicals.

Evidence maps were used to visually report data availability. Evidence maps list the availability of toxicity data from the databases as the number of data hits for relevant study or assay types (e.g., acute toxicity, carcinogenicity), with a hit being that data was reported for the chemical, but not the directionality of the result. For OFRs in all 14 subclasses, a literature survey was compiled through CPSC task orders #61320621F1001, 61320621F1002, and 61320621F1003 (Tasks #12 through 14) under contract CPSC-D-17-0001 and evidence maps were already created and available for this case study.[2] Additional evidence maps were developed for newly identified analogs and metabolites, created using a similar process used in the literature survey for the OFRs and NASEM analogs. Detailed documentation of the approach can be found in the Literature Survey Guide[3], and additional considerations outlined in the Read Across Guide.

2.5.1. Databases

The evidence maps for analogs and metabolites in this case study pulled from eight databases developed for the OFRs from the literature survey (University of Cincinnati Risk Science Center, 2022). There are two databases from the literature survey that were not used for these additional analog and metabolite maps. First, the Danish QSAR database/models were excluded here because only experimental data were considered for evaluating data availability of the chemicals. Second, the ECHA Dossier results obtained via IUCLID were also excluded for analogs and metabolites. ECHA Dossiers require manual effort to individually search each chemical in IUCLID and it was likely that any chemicals with ECHA Dossier results also had data in other databases searched, and thus the manual effort would provide minimal returns.

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[2] Supporting contractor reports are available at https://www.cpsc.gov/Business--Manufacturing/Organohalogen-Flame-Retardant-Chemicals-Assessment.

[3] UC Risk Science Center (2022). Literature Survey Guide.

2.5.2. Database Downloads and Evidence Map Creation

Evidence maps were created in Excel for all 14 subclasses using a custom R code developed to batch search each database then stitch the results together into a custom Excel template. The evidence maps list the availability of toxicity data for the analogs and metabolites as the number of data hits, with a hit being that data was reported for the chemical, but not the directionality of the result. These maps are included as supplementary files.

The evidence maps separate chemicals with data available from chemicals with no data available into different tabs to aid visualization of chemicals with data. Since the aim of class-based read across assessment of data poor OFRs is to fill data gaps, only analogs and metabolites with toxicity data are useful to drawing conclusions for data poor OFRs. As such, the analog and metabolites lists were filtered to determine how many chemicals per subclass had data available according to the evidence maps, and therefore potentially useful in hazard assessment of OFRs. To more fully ensure that indicated available data are data that are useful for read-across and hazard assessment, only toxicity data in animal models or alternatives accepted for use in risk assessment were used to assess data availability and filter the analog and metabolite lists. Acceptable alternatives included standard genotoxicity assays or specific combinations of in vitro assays for skin sensitization (OECD, 2023). Table 4, Table 5, and Table 6 list the total number of unique analogs, predicted metabolites, and empirical metabolites identified, respectively. Due to shared analogs/metabolites between subclasses, the overall total quantity of chemicals does not equal the sum of the counts for each individual subclass.

Table 4. Quantity of analogs for each subclass with animal toxicity or accepted alternative data in the evidence maps.

Subclass (all Polyhalogenated)	Abbreviation	Total Unique	Total with Data Available
Organophosphates	PHOP	175	19
Bisphenol aliphatics and functionalized	PHBAF	825	14
Diphenyl ethers	PHDE	1957	23
Benzene alicycles	PHBA	53	0
Benzene aliphatics and functionalized	PHBzAF	3125	65
Phenol aliphatic ethers	PHPhAE	1242	5
Alicycles	PHA	75	3
Phenol derivatives	PHPhD	187	3
Aliphatic carboxylates	PHACbx	41	5
Benzenes	PHB	1680	38
Phthalates-benzoates-imides	PHPBI	1335	59

Carbocycles	PHC	115	15
Triazines	PHT	6	0
Aliphatic chains	PHACh	316	18
Total	-	9624	252

Table 5. Quantity of predicted metabolites for each subclass with animal toxicity or accepted alternative data in the evidence maps.

Subclass (all Polyhalogenated)	Abbreviation	Total Unique	Total with Data Available
Organophosphates	PHOP	629	32
Bisphenol aliphatics and functionalized	PHBAF	211	23
Diphenyl ethers	PHDE	676	17
Benzene alicycles	PHBA	76	0
Benzene aliphatics and functionalized	PHBzAF	263	10
Phenol aliphatic ethers	PHPhAE	160	18
Alicycles	PHA	234	0
Phenol derivatives	PHPhD	86	3
Aliphatic carboxylates	PHACbx	54	14
Benzenes	PHB	459	6
Phthalates-benzoates-imides	PHPBI	288	27
Carbocycles	PHC	327	10
Triazines	PHT	95	7
Aliphatic chains	PHACh	326	15
Total		3764	118

Table 6. Quantity of empirical metabolites for each subclass with animal toxicity or accepted alternative data in the evidence maps.

Subclass (all Polyhalogenated)	Abbreviation	Total Unique	Total with Data Available
Organophosphates	PHOP	43	7
Bisphenol aliphatics and functionalized	PHBAF	5	0
Diphenyl ethers	PHDE	17	2

Benzene alicycles	PHBA	10	0
Benzene aliphatics and functionalized	PHBzAF	0	0
Phenol aliphatic ethers	PHPhAE	7	1
Alicycles	PHA	27	2
Phenol derivatives	PHPhD	5	3
Aliphatic carboxylates	PHACbx	0	0
Benzenes	PHB	13	0
Phthalates-benzoates-imides	PHPBI	8	4
Carbocycles	PHC	1	1
Triazines	PHT	1	1
Aliphatic chains	PHACh	5	4
Total		138	22

3. Profiles for Evaluating Classification and Applicability of Class-based Assessment

3.1. Metabolite Profile

The methods and results for finding and predicting PHOP metabolites are described in Section 2.4. There were 644 total metabolites (predicted and empirical), 9 of which were OFRs themselves and 5 were PHOPs. Table 7 lists the 5 PHOPs that were determined to be metabolites of other PHOPs. These parent-metabolite pairs are structurally similar chemicals differing in the number of haloalkyl branches and/or phosphorus oxidation state. Table 7 includes 3 of the top 4 data rich PHOPs. Data availability is discussed in detail in the Adverse Effect Profile (Section 3.5).

Table 7. Metabolites of PHOPs that are also PHOP OFRs.

Metabolite PHOP	Parent PHOP(s)
TDCPP[1]	Tris(2-chloroethyl) phosphite
Bis(2,3-dibromopropyl) hydrogen phosphate	TDBPP[1]
2,3-Dibromopropyl dihydrogen phosphate	1. TDBPP[1] 2. Bis(2,3-dibromopropyl) hydrogen phosphate
TCEP[1]	Tris(2-chloroethyl) phosphite
Bis(1,3-dichloro-2-propyl) phosphate	1. TDCPP[1] 2. 2,2-Bis(bromomethyl)-3-chloropropyl bis(2-chloro-1-(chloromethyl)ethyl) phosphate

[1] One of the PHOP OFR with highest data availability counts
TDCPP= Tris(1,3-dichloro-2-propyl) phosphate
TDBPP = Tris(2,3-dibromopropyl) phosphate
TCEP = Tris(2-chloroethyl) phosphate

The remaining list of metabolites consisted of many small halogenated, especially chlorinated, compounds unique to PHOPs. Most of the metabolites were unique to a single parent, but some metabolites were shared among two or more parent OFRs, and these relationships were considered to evaluate PHOPs as a subclass fit for class-based hazard read across.

3.1.1. Classification of PHOPs Using Shared Metabolites

Shared metabolites between chemicals can provide insight into whether those chemicals are metabolized similarly and, importantly, if they share adverse health effects as a result of their metabolites directly. For this case study, shared metabolites among parent PHOPs were assessed using a matrix (Figure 7). An interactive Tableau is included as a supplementary to this case study (linked below Figure 7).

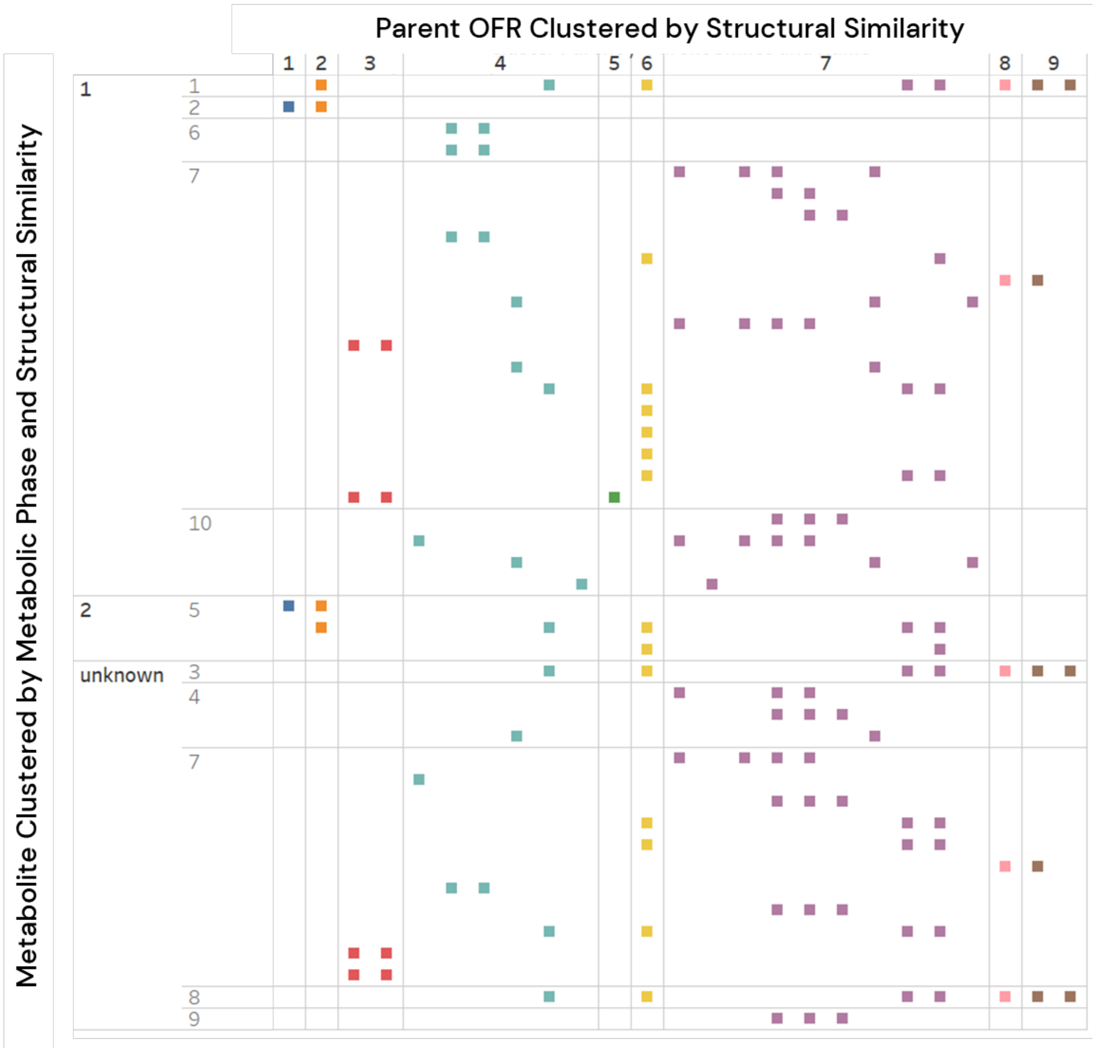


Figure 7. Matrix comparing shared metabolites among PHOPs OFRs.

OFRs are grouped by structural similarity clusters. Metabolites are grouped by metabolic phase, if known, then clustered by structural similarity. Cluster numbers serve only to differentiate clusters within OFRs and with metabolites. Shared cluster numbers do not indicate similarity between OFRs and metabolites. Metabolites not shared by any OFRs are not shown. Figure available at: https://public.tableau.com/app/profile/cpsc/viz/CPSCParentsMetabolitesVisual/MatrixDashboard

Metabolites were considered individually (i.e., specific chemical sharing multiple parents), but they were also considered by structural groups created using the same RDKit topological fingerprint clustering used for evaluating the OFRs in Section 2.2. The parent OFRs and metabolites in Figure 7 were each assigned into 9 and 10 clusters, respectively, using a hierarchical clustering based on intra-chemical similarity within the listed OFRs and metabolites. The numbered assignments for clustering were not the same between OFRs and metabolites but only relevant within the respective group (i.e., OFR group 1 is not structurally similar to metabolite group 1). The colors in Figure 7 differentiate the OFR structural groups for visual aid. The quantity of groups (9 and 10) was chosen based on chemical clustering to balance the number of groups required to differentiate chemicals without creating too many individual groupings; fewer groups would not differentiate clusters well, but more groups would put more chemicals into groups containing no other chemicals (like groups 1 and 2).

The structural similarity grouping was assigned to evaluate metabolites with similar structures in effort to indicate similar metabolism and overall hazard class among PHOPs with slightly different parent branching structures or differences in halogenation. Indeed, the large quantity of metabolites in structural group 7 imply shared structures and potential shared bioactivity among over half of all shared PHOPs metabolites.

Of all predicted and empirical metabolites, 44 were shared between at least two PHOP parents, and 25 of 34 PHOPs have shared metabolites. The most shared metabolite is 2-chloroethanol because it was predicted and empirically reported, and it was associated with 8 PHOP parents. Furthermore, it was not a metabolite for any of the other OFR subclasses, making it a unique biomarker for PHOPs. 2-Chloroethanol was assigned to its own structural cluster (metabolite group 1). Chloroacetic acid (metabolite group 8) and chloroacetaldehyde (metabolite group 3) were the second most shared metabolites, each with 7 different PHOP parents and no parents from other subclasses. These 2 were predicted metabolites and not found empirically in the literature through this case study.

Many shared metabolites were structurally clustered together in group 7 because they are small phosphates with one or two halogenated substituents. For example, 1 chloropropan-2-yl dihydrogen phosphate and 2,3-dibromopropyl dihydrogen phosphate are both in metabolite group 7. However, it is notable that the metabolites in groups 8, 9, and 10 shared similarities and overall parent clusters with metabolite group 7. For comparison, 1,3-dicholoro-2-propanol was assigned to group 10, but 2,3-dibromo-1-propanol was assigned to group 7. This highlights the limitations of hierarchical clustering using RDKit topological fingerprints, which predominantly rely on path size (i.e., the number of chemical bonds) within a set of chemicals. The lack of overall diversity within this small set of chemicals reduces the effectiveness of clustering to compare differences and emphasizes the general structural similarity among the shared metabolites.

Importantly, three of the four major empirical metabolites outlined in Table 3 were shared among multiple PHOPs. The relationships of the major empirical metabolites and their parents are listed in Table 8. These relationships are a result of both empirical literature and the output of predictive tools.

Table 8. Parents of major empirical metabolites.

Metabolite CAS RN	Metabolite Chemical Name	Parent Chemical Name(s)
789440-10-4	Bis(1-chloropropan-2-yl) hydrogen phosphate	1. Bis(2-chloro-1-methylethyl) 2-chloropropyl phosphate 2. TCPP

		3. Tetrakis(1-chloropropan-2-yl) ethane-1,2-diyl bis(phosphate) 4. 2,2-Bis(chloromethyl)-1,3-propanediyl tetrakis(1-chloro-2-propanyl) bis(phosphate)
3040-56-0	Bis(2-chloroethyl) phosphate	5. TCEP 6. Phosphoric acid, 1,2-ethanediyl tetrakis(2-chloroethyl) ester 7. Diethylene glycol bis[bis(2-chloroethyl) phosphate] 8. Phosphoric acid, 2,2-bis(chloromethyl)-1,3-propanediyl tetrakis(2-chloroethyl) ester
72236-72-7	Bis(1,3-dichloro-2-propyl) phosphate	1. TDCPP 2. 2,2-Bis(bromomethyl)-3-chloropropyl bis(2-chloro-1-(chloromethyl)ethyl) phosphate
1477495-11-6 (not shared)	Bis(1-chloro-2-propyl) 1-hydroxy-2-propyl phosphate	TCPP

TCPP = Tris(2-chloroisopropyl) phosphate
TCEP = Tris(2-chloroethyl) phosphate
TDCPP = Tris(1,3-dichloro-2-propyl) phosphate

In Section 2.2, four PHOP OFRs were structurally categorized differently than the rest of the PHOPs, denoted as the BPPs. While evaluating their metabolite profile, none of the BPPs shared metabolites with other OFRs, but three of them shared at least one metabolite with other subclasses and two of these metabolites were OFRs themselves. 2,4-Dibromophenol, an empirical metabolite and OFR in the polyhalogenated phenol derivatives (PHPhD) subclass, is a metabolite of tris(2,4-dibromophenyl) phosphate as well as twelve other OFRs in two other subclasses (PHDE and polyhalogenated phenol aliphatic ethers (PHPhAE)). 2,4,6-tribromophenol, an OFR in the PHPhD subclass, is a metabolite of tris(2,4,6-tribromophenyl) phosphate and seventeen other OFRs in three other subclasses (PHDE, PHPhAE, and PHB). Lastly, 2,3-dibromophenol is a metabolite for tris(dibromophenyl) phosphate in addition to an OFR in the PHDE subclass. Tris(2-bromo-4-methylphenyl) phosphate, the fourth BPP, had no metabolites shared with other subclasses, but its metabolites were structurally synonymous with metabolites of the other BPPs.

There were three other PHOPs that were structurally categorized as slightly different from the remaining PHOPs in Figure 1, although not as significantly as the BPPs, and therefore were individually evaluated by their metabolites to look for similarities or differences with other PHOPs. The first, Phosgard C-22-R, shared five metabolites with other PHOPs (Figure 7, OFR group 8 in pink). Three of these five metabolites were the most shared metabolites: 2-chloroethanol, chloroacetic acid, and chloroacetaldehyde. The other two were shared with bis(2-chloroethyl) 2-chloroethylphosphonate and are within metabolite structural group 7. The second PHOP, 2,3-dibromopropyl dihydrogen phosphate, was a metabolite itself, shared between its bis and tris forms. Both Phosgard C-22-R and 2,3-dibromopropyl dihydrogen are considered within PHOP's metabolic profile based on these results.

The third PHOP considered slightly different in Figure 1 is dimethyl (4,6-dichloro-1,3,5-triazin-2-yloxy)methyl phosphonate; it did not share any metabolites with PHOPs, or any other OFR subclass. Moreover, the metabolites for this chemical were structurally different from all other PHOP metabolites, with most (80%) keeping the phosphate-triazine core bond and structure intact; no other PHOPs have a triazine moiety. With respect to the metabolite profile, dimethyl (4,6-dichloro-1,3,5-triazin-2-yloxy)methyl phosphonate is not well categorized as a PHOP.

3.1.2. Metabolite Profile Conclusions

Most PHOPs were categorized well together, with 74% sharing at least one metabolite with another PHOP. Additionally, the overlap of PHOP OFRs as metabolites of each other emphasizes both the similarity of these compounds and likelihood of shared bioactivity. There were five PHOPs with metabolites that varied significantly from the remainder of the subclass: the four BPPs and dimethyl (4,6-dichloro-1,3,5-triazin-2-yloxy)methyl phosphonate. The four BPPs are best clustered together and dimethyl (4,6-dichloro-1,3,5-triazin-2-yloxy)methyl phosphonate is a singular outlier.

3.2. Physiologically Based Toxicokinetic Profile

3.2.1. PBTK Model Methods and Results

PBTK models of the OFRs, analogs, and metabolites are important for understanding similarities between the chemicals, but also for understanding the toxicological importance of the chemicals. If a chemical is excreted from the body quickly, or not distributed throughout the body at toxicologically relevant concentrations, then it is unlikely to contribute to health hazards. To understand the toxicological relevance of PHOP OFRs, analogs, and metabolites, EPA's high throughput toxicokinetics (httk) R package, version 2.3 (Pearce et al., 2017) was used to predict half-life and plasma concentration. The httk software was chosen to generate the PBTK profiles because the tool is free to the public, easy to use in batch mode with R, and is also integrated into the EPA chemicals dashboard and the National Toxciology Program (NTP) Integrated Chemical Environment (ICE).

All OFRs, analogs, and metabolites were input into httk to compare to each other. The Open (Quantitative) Structure-activity/property Relationship App (OPERA) (Mansouri et al., 2018) was used to determine the necessary input values (hepatic clearance and fraction unbound) that were not available as already measured and within the httk dataset. Default parameters for httk were used:

- Oral route
- Solve PBTK model
- Human with 70 kg body weight
- Exposure dose 1 mg/kg/dose
- 24 hours of interval exposure

- 3 days simulation
- ADME source

The distributions of the calculated whole-body half-lives and peak plasma concentrations (C_{max}) for PHOP OFRs are shown in Figures 8A and 8B, excluding two outliers (tris([2,4,6-tribromophenyl) phosphate and tris(tribromoneopentyl) phosphate) with calculated half-lives >200 hours. Values above this 200-hour threshold are less likely to be representative of experimental values as they exceeded the computable limits of httk (or OPERA for the required httk inputs). Instead, their results were considered qualitatively representative of their toxicological relevance.

The three highest half-lives within the window of Figure 8A are labeled with their corresponding PHOP name. Most PHOPs had calculated half-lives <25 hours and peak plasma concentrations between 0.04 mg/L and 1.5 mg/L which indicates that accumulation of these compounds is unlikely. However, the short half-life combined with the low plasma concentrations does indicate that for those compounds that have associated biological health effects, it is likely that the metabolites are the primary toxic agents and not the parent compounds. Interestingly, TDCPP (tris(1,3-dichloro-2-propyl) phosphate) had a high calculated half-life and plasma concentration relative to the other PHOPs, although not by any orders of magnitude. TDCPP did not differentiate from other PHOPs in the other profiles of this case study, making implications of this observation, if any, unclear.

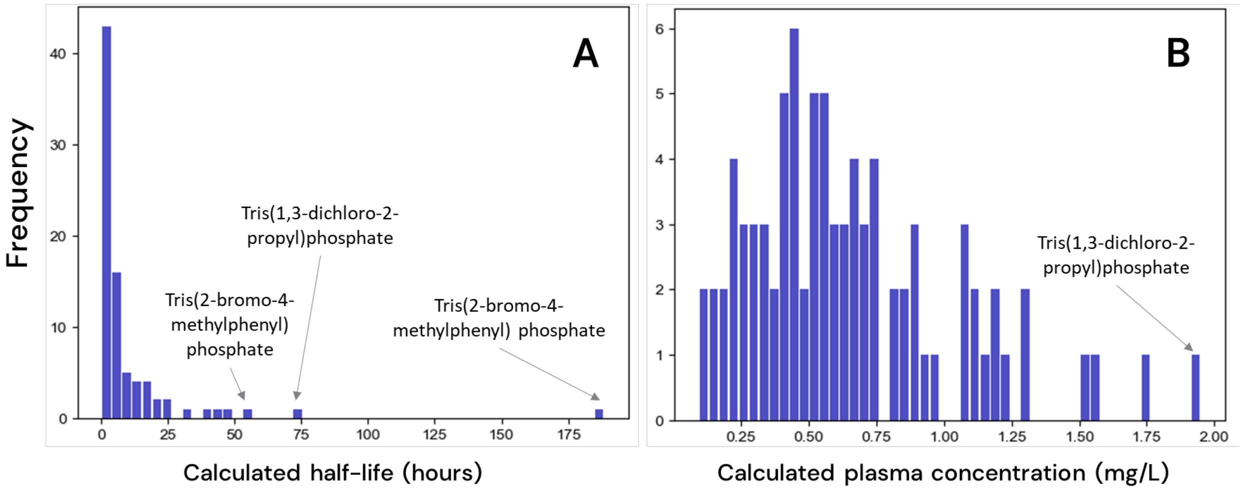


Figure 8. Calculated (A) half-life and (B) plasma concentration (C_{max}) for PHOP OFRs only.

Half-life axis maximum is set to 200 hours causing some data to not be pictured.

Figure 9 has two plots of the httk calculated half-life as a function of plasma concentration for PHOP OFRs, analogs, and metabolites. With some exceptions, most of the chemicals are within the bottom left quadrant of the graph, but do not exhibit any clear clustering pattern among each group. The PHOP OFRs and analogs mostly overlap. Metabolites have a higher range of peak plasma concentration than PHOP OFRs and analogs, and fewer compounds with long half-lives.

Since the metabolites are inherently different structures than the PHOP OFRs and analogs, with mostly smaller structures, a general difference in PBTK output was expected. The overlap between the PHOP OFRs and analogs emphasizes the toxicokinetic similarity of PHOPs and their respective analogs, which supports the analogs' viability for use in read across, if data was available.

Only seven analogs had half-lives >200 hours, and all were analogs to at least one of the four BPPs. Moreover, there were nine metabolites with calculated half-lives > 200 hours and all were metabolites of three BPPs: tris(2-bromo-4-methylphenyl) phosphate (6 metabolites); tris(2,4-dibromophenyl) phosphate (2 metabolites); and tris(2,4,6-tribromophenyl) phosphate (1 metabolite). All nine metabolites contained at least one brominated phenyl moiety bound to phosphate. Given the long calculated half-lives of the BPPs themselves, this highlights the potential toxicological significance of the BPP structure and differentiates the BPPs as a potential new subclass from the rest of the PHOPs.

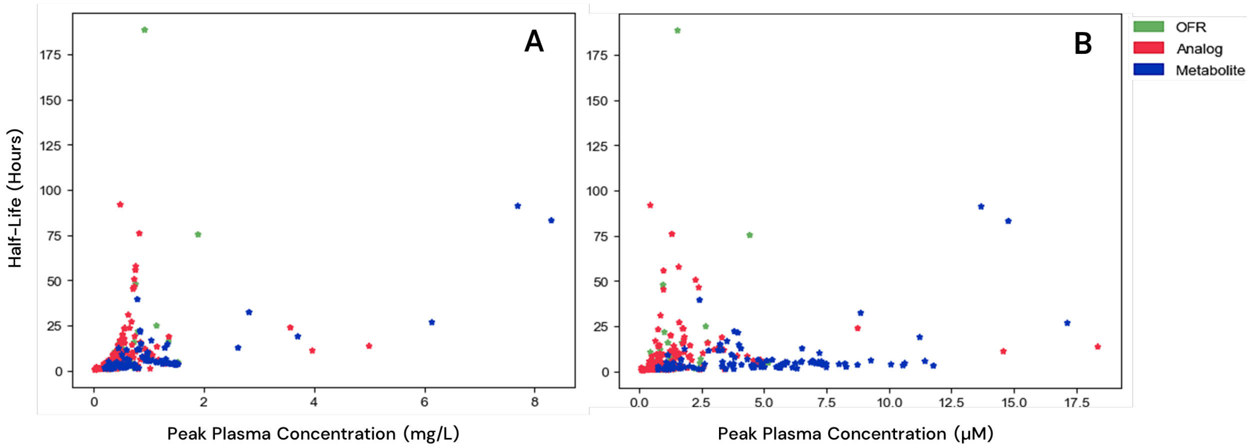


Figure 9. Calculated toxicokinetic values of the PHOPs OFRs, analogs, and metabolites.

Half-life axis maximum is set to 200 hours causing some data to not be pictured. Peak plasma concentrations displayed in A) mg/L, and B) μM.

Results of PBTK modeling require careful consideration due to its reliance on predicted data, especially when experimental pharmacokinetic data are unavailable to validate the model simulation outcomes. Route to route and inter-species extrapolations are common applications of PBTK modeling. However, factors contributing to differences between different exposure routes and species need to be well understood before these applications can be successfully implemented. Initially, physicochemical properties are required for modeling a chemical's ADME properties. The physicochemical properties for PHOPs were predicted using QSAR models, adding an additional layer of predictions that decreases confidence in accuracy of the PBTK predicted values. Despite these limitations, httk calculated values have been shown to be within 3.2-fold of in vivo observed values for 65% of a set of 349 diverse chemicals tests (Wambaugh et al., 2015). This level of accuracy provides confidence that the httk values in this case study are still useful to semi-quantitatively compare chemicals to each other as part of the overall weight of evidence for defining the PHOPs subclass.

It is crucial to acknowledge the limitations of this QSAR approach, such as the applicability domain and prediction uncertainty. Furthermore, PBTK models, such as those developed in high throughput format (e.g., with EPA's httk R package), often provide a generic model structure with simplified set of differentiation equations. This simplified structure may not fully account for all the biological interaction of chemicals in the body, including extra-hepatic clearance, individual enzymatic reactions, active transport, and crossing the blood-brain barrier. While PBTK modeling can provide a comprehensive overview of a chemical's time-tissue concentration profile, it is essential to recognize that the prediction may not be as accurate without further validation.

3.2.2. Determination of Toxicological Relevance

The httk calculations were used to narrow down the list of metabolites to those useful for read across by establishing bounds for toxicological relevance. A chemical with a very short half-life or a very low peak plasma concentration is not expected to be toxicologically relevant because it will not be present in the body for long-enough or at high-enough concentrations that are likely to cause health effects. A key caveat to this generalization applies if the chemical of interest is rapidly metabolized to a metabolite that has identified toxicities. In such cases, the metabolite would be expected to meet the criteria for being of toxicological relevance, even though the parent chemical does not. As discussed in the rest of this subsection, this was the situation for many of the PHOP OFRs.

There was limited data available that reported both internal and external PBTK data in animals or humans for any PHOPs to confirm the predicted httk results and provide experimental ranges for toxicological significance of PHOPs specific half-lives or plasma concentrations. Rat steady-state plasma concentrations were reported to be 7 µM in an International Agency for Research on Cancer study exposing rats to TCEP at 88 mg/kg-day (IARC, 1999). ICE was used to determine that 1 mg/kg-day of PHOP OFRs resulted in human steady-state plasma concentrations ranging from 0.196 – 7.5 µM, which corroborates the rat data. However, httk may have underpredicted plasma concentrations for these chemicals since the majority of PHOP OFRs had calculated concentrations less than 2.5 µM, and the highest was 5.9 µM.

Since there was essentially no PBTK data corresponding to toxicity for any PHOPs, PBPK data was reviewed for low toxicity and high toxicity compounds (acetaminophen and organophosphate pesticides) to determine a plasma concentration of toxicological relevance for the two opposing hazard categories and develop a reasonable range of toxicological relevance for PHOPs. While there are no direct correlations between PHOPs and the selected chemical types for creating these bounds, the toxicology of these groups are well understood and helpful in creating the proposed half-life and plasma concentration boundaries for PHOPs. For acetaminophen (low toxicity), a daily oral dosage of 4,000 mg for the average human

(57 mg/kg-day) results in a plasma concentration of 97 µM (14.6 µg/mL); no adverse blood levels are 20 mg/L (Agrawal & Khazaeni, 2023). In contrast, organophosphate pesticides are considered highly acutely toxic (Eddleston et al., 2008). A specific example, chlorpyrifos, is considered fatal at a plasma concentration of 4.73 µM, but non-fatal (yet still hazardous) at 1.1 µM.

PHOPs contain organophosphate functional groups but are not as acutely toxic as organophosphate pesticides, meaning a plasma concentration of organophosphate pesticides known to be hazardous is a conservative lower bound (more toxic) for potential toxicological relevance of PHOPs. Additionally, a data rich chemical such as acetaminophen is a conservative upper bound (less toxic). This information was used to inform boundary setting (bounding) for plasma concentration and half-life. Based on the weight of evidence between parent PHOP kinetics, and the kinetics of compounds known to be more or less toxic than PHOPs with data, bounds were selected that captured plasma concentrations and half-lives where toxicity occurred and where the chemical was present in the body long enough to likely cause an adverse effect. Taking this into account, PHOP metabolites were considered toxicologically relevant if their calculated plasma concentrations were greater than 1 µM and their half-lives were greater than 1 hour; except for compounds with half-lives greater than 20 hours. This upper half-life bound was selected, as continuous exposure could result in accumulation, and is toxicologically relevant regardless of plasma concentration given the extensive time they would be circulating in the body.

All four of the major PHOP empirical metabolites identified in Section 2.4.1 (BCIPP, bis(1,3-dichloro-2-propyl phosphate, bis(2-chloroethyl) phosphate, and BCIPHIPP) met the criteria for being of toxicological importance, supporting the bounds used for this determination. The full list of toxicologically relevant metabolites of PHOPs that meet the PBTK criteria, in addition to having any amount of data according to the evidence maps, are listed in Table 9. Many of the metabolites are small organic compounds with molecular weights less than 100 g/mol and two chemicals are OFRs themselves that were categorized into other subclasses by CPSC.

Table 9. Toxicologically relevant metabolites of PHOPs.

Metabolite Name	Number of Parent PHOPs	Empirical or Predicted
2,3-Dibromo-1-propanol[1]	3	Both
1,3-Dichloropropan-2-ol	3	Both
2,4-Dibromophenol[1]	1	Both
2,3-Dichloropropanal	1	Predicted
2,3-Dichloropropanoic acid	1	Predicted
2,3-Dichloro-1-propanol	2	Predicted

Metabolite Name	Number of Parent PHOPs	Empirical or Predicted
2,3-Dibromopropanal	2	Predicted
2,3-Dibromopropanoic acid	2	Predicted
2-Chloroacetaldehyde	7	Predicted
2-Chloroacetic acid	7	Predicted
3-Chloropropanoic acid	1	Predicted
3-Bromo-2,2-bis(bromomethyl)propanoic acid	2	Predicted
2-Chloropropan-1-ol	3	Predicted
Chloroacetone	3	Predicted
1,3-Dichloroacetone	2	Predicted
2-Chloroethylphosphonic acid	2	Predicted
Bromoacetaldehyde	1	Predicted
Diglycolic acid	1	Predicted

[1] Compounds identified as PHOP metabolites but are also categorized by CPSC as OFRs in other subclasses.

Upon review of the PHOP OFRs' PBTK data, three of the 34 PHOP OFRs were considered not toxicologically relevant due to their less than 1-hour half-lives:

- Bis(2-chloroethyl) vinylphosphonate
- Tris(2-chloroethyl) phosphite
- Dimethyl {[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]methyl}phosphonate

However, these three PHOPs had metabolites that were classified as toxicologically relevant. Additionally, twelve PHOP OFRs had httk calculated peak plasma concentrations less than the cutoff of 1.0 µM; however, all of these had at least one metabolite, if not several, considered toxicologically relevant. This suggests that metabolites are the active form for all PHOPs with calculated TK values outside the toxicologically relevant window. However, these results could have been influenced by httk underpredicting the peak plasma concentration for the PHOPs compounds.

To consider whether the data support the conclusion that toxicity of PHOP OFRs in general is due to their metabolites rather than the parent chemicals, Table 10 arrays the httk predictions for the four most data rich PHOP chemicals and their respective key metabolites. For TCPP, TDBPP, and TCEP the average metabolite peak plasma centration is higher than the parent PHOP, and the average metabolite half-life is toxicologically relevant. This suggests that toxicity is more likely a result of metabolites. TDCPP is the exception because the httk results indicate a longer half-life for the parent than the respective metabolite, suggesting that in this case the parent may be the active form. Overall PHOP toxicity is probably driven by the parent compound in some cases and the metabolite(s) in others. These differences may not affect the conclusion regarding whether all of the chemicals belong to the same subclass, since the original list of PHOP OFRs included some chemicals that were metabolites of other OFRs and some metabolites are shared among PHOPs (Section 3.1). TDCPP is a predicted metabolite of tris(2-chloroethyl) phosphite, for example.

Table 10. Calculated toxicokinetic values for the four PHOPs with the most data availability and their metabolites.

Parent OFR	Peak Plasma Concentration (µM)	Half-life (Hours)	Average Metabolite Peak Plasma Concentration (µM)	Average Metabolite Half-life (Hours)
TDCPP	4.4	75.2	2.6 ± 2.4	3.8 ± 3.1
TCPP	1.4	5.5	3.6 ± 2.3	3.4 ± 2.0
TDBPP	0.6	8.5	2.7 ± 0.9	9.2 ± 9.2
TCEP	2.4	4.4	4.7 ± 4.1	2.8 ± 1.6

Reported variance is standard deviation.
TDCPP = Tris(1,3-dichloro-2-propyl) phosphate
TCPP = Tris(2-chloroisopropyl) phosphate
TDBPP = Tris(2,3-dibromopropyl) phosphate
TCEP = Tris(2-chloroethyl) phosphate

Importantly, PHOPs that did not meet the toxicologically relevant toxicokinetic criteria outlined in this case study all have at least one, and often several, predicted and/or empirical metabolites that are considered toxicologically relevant. Moreover, the three metabolites with the most shared parents discussed in Section 3.1 are all considered toxicologically relevant, ranging between 7.6-13.1 µM and 1.6-5.6 hours. Given the limitations of httk discussed earlier and the lack of experimental data, confidence is low in the quantitative accuracy of the httk results for PHOPs and their metabolites, but the relative calculations are informative. Other predictive tools could be used to compare with the httk results in future work to add confidence in the case study conclusions.

3.2.3. Physiologically Based Toxicokinetic Profile Conclusions

All OFRs, analogs, and metabolites were input into httk to calculate half-lives and peak plasma concentration by using OPERA to predict any unknown necessary input values. No patterns or clear clustering was present in the data for each group. Reasonable toxicological ranges were determined using well-known PBPK and toxicity information of acetaminophen and organophosphates.

Some chemicals passed the calculable limits of httk and OPERA, resulting in half-lives that were exceedingly high (>200 hours). Tris(2,4,6-tribromophenyl) phosphate and tris(tribromoneopentyl) phosphate were the OFRs that surpassed this mark. All other chemicals above this range were analogs or metabolites for at least one of the four BPPs, clearly differentiating the PBTK profile of BPPs from other OFRs.

Most OFRs had calculated half-lives <25 hours and peak plasma concentrations between 0.04 mg/L and 1.5 mg/L, several of which did not meet the toxicological relevance criteria. However, any PHOPs that did not meet the criteria had at least one metabolite considered toxicologically relevant. Based on these estimates, it is likely that the metabolites, not parent compounds, are the primary toxicants for some PHOPs. Limitations of the PBTK profile for PHOPs exist and were discussed, and the predictions used may benefit further validation to increase accuracy.

3.3. Physicochemical Properties Profile

3.3.1. Empirical Physicochemical Properties of PHOPs

None of the PHOP OFRs had a strong experimental physicochemical profile; there were only six properties for five PHOP OFRs available in the searched databases. As a result, empirical properties were not considered for this case study since inclusion of these six individual values were unlikely to change the outcome of the physiochemical profile of the entire subclass. The entire PHOPs physicochemical profile was created using predicted values as described in the following section. Empirical properties will be considered for future-steps of class-based assessments, especially if predicted properties are outside of predictive domains. To the extent practical, the same value(s) will be used across different components of class-based assessment in further work beyond this case study.

3.3.2. Predicted Physicochemical Properties of PHOPs

There are multiple predictive models and approaches to predict physicochemical properties. For purposes of initial grouping of chemicals for hazard identification, OPERA was used to predict all properties and create the physicochemical profile for PHOPs because: 1) its training set encompasses one of the most extensive chemical datasets, and 2) it provides an applicability domain score that indicates the confidence level of the prediction. Additionally, it is integrated into the OECD QSAR toolbox and aligns with OECD guidelines defined by regulatory authorities (OECD, 2014).

Principal component analyses (PCA) were developed to visualize the relative positions of the PHOP OFRs, analogs, and metabolites relative to one another based on properties. Figure 10 shows PCA plots of PHOPs OFRs overlayed onto their analogs and metabolites with data availability in the evidence maps. Figure 10A was computed using a subset of physicochemical descriptors predicted using OPERA, and Figure 10B was based on a subset of 1D and 2D descriptors computed using RDKit. Highly correlated descriptors (pair-wise Pearson correlation score above 0.9) were removed. The chemicals are distributed similarly between Figures 10A and 10B despite the different programs and descriptors, therefore providing confidence in the observed relative positions and profiles.

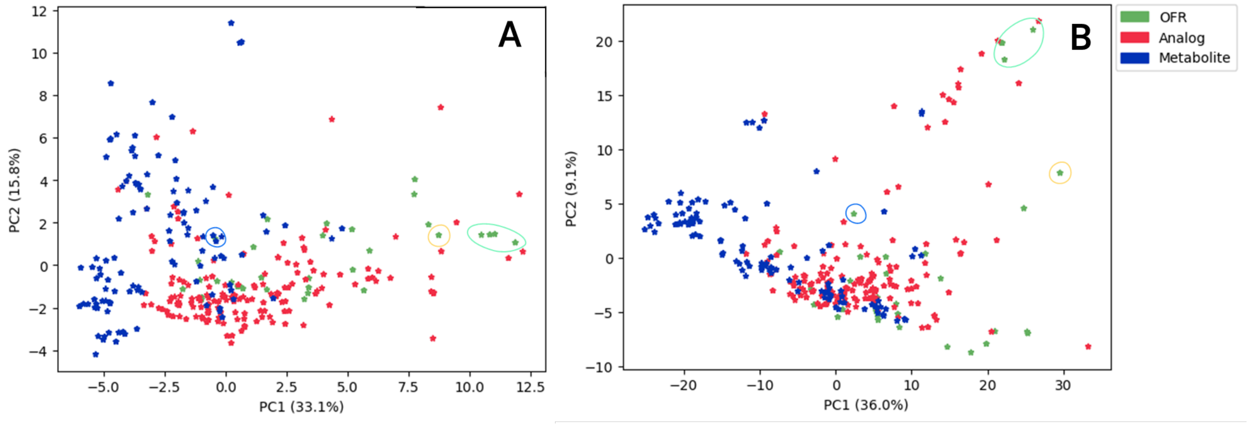


Figure 10. PCA plots of PHOP OFRs, analogs, and metabolites using (A) OPERA physicochemical descriptors and (B) RDKit 1D2D molecular descriptors.

The four BPPs are circled in bright green on both graphs. Two overlap each other in 10B. 2,4,8,10-Tetraoxa-3,9-diphosphaspiro[5.5]undecane is circled in yellow. dimethyl {[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]methyl}phosphonate is circled in blue.

The variability among chemicals in the PCA plots was large; both PCAs roughly explained 50% of the descriptor variability, meaning the properties themselves were highly variable between chemicals within a series. Analogs are positioned near their seed PHOP OFRs resulting in overlapped clusters indicating the analog physical property profile was highly similar to the PHOP's and therefore supporting them as good analogs for read across analysis if they had toxicological data. This is not unexpected given the high Tanimoto cutoff (0.85) used to retrieve them (refer to Section 2). Metabolites clustered together on the left half of the PCA plots, separately from the parent PHOPs and analogs. This correlates with the relatively smaller size of metabolites and the potential loss of functional groups compared to their parent PHOP(s) resulting from the chemical reaction creating them.

Similar to the PBTK analysis, the four OFR BPP chemicals(Tris(2,4,6-tribromophenyl) phosphate, tris(2,4-dibromophenyl) phosphate, tris(dibromophenyl) phosphate, and tris(2-bromo-4-methylphenyl) phosphate) cluster together as outliers. The clearly differentiated physical properties of the BPPs indicate that they are likely to have different ADME than the other PHOPs, and possibly a different adverse effects profile as a result.

The three other PHOPs categorized slightly differently from the remaining PHOPs using a hierarchical clustering based on a Tanimoto score computed using topological RDKit fingerprints (Section 2.2) were not differentiated on the PCA plots and were located within the mid-range of the OFRs distribution. However, dimethyl {[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]methyl}phosphonate was on the vertical outer edge of the OFRs distribution, visually obscured by blue metabolite data points (circled in blue). The other PHOP closest to the BPPs on both graphs (circled in yellow), is 2,4,8,10-tetraoxa-3,9-diphosphaspiro[5.5]undecane. Given the overall scattered distribution of the predicted properties of PHOPs, no conclusions could be made on these outlier chemicals with reasonable confidence.

3.3.3. Physicochemical Properties Profile Conclusions

PHOPs predicted physicochemical properties span a reasonably wide distribution with no distinct clusters except for the four BPPs. This observation indirectly suggests that the four BPPs will have a different toxicological profile and may not belong within the PHOP subclass. The PHOPs' wide distribution of predicted properties provides no information on generalized properties of PHOPs. The overall physicochemical clustering of the PHOPs does not have tight, distinct clusters (sans BPPs) that would suggest multiple subclasses.

The spatial distribution of OFRs and their analogs overlap on the PCA plots, indicating reasonable physical property matches between the analogs and their seed PHOPs. Metabolites distribute differently from the parent PHOPs and analogs, which is expected since metabolites are smaller and structurally discrete. Additionally, the metabolites clustered together well and have a distinguishable physicochemical profile amongst themselves, which supports grouping the parent PHOPs into one subclass.

3.4. Mechanistic Effect Profile

Multiple databases provide information on perturbations to mechanistic pathways that occur following exposure to a chemical of interest. By building a mechanistic profile, adverse effects and signaling pathways can be connected to chemical subclasses. For this case study, the databases utilized were ICE curated high throughput screening (cHTS), PubChem, and the Comparative Toxicogenomics Database (Table 11). While several of these databases are anchored in the same data, the details provided by each are different and can help build out a profile. Using chemical CAS RNs, each database was searched for all available data, with only three or four PHOPs and/or seven metabolites having useful data in any of the databases. Highly common effects, such as cytotoxicity and liver enzyme effects that can be key effects in many different chemical classes were removed for analysis, and data were then filtered to get to key pathway types. For the PHOPs, the key pathways were primarily related to hormone signaling, indicating that in this use case these chemicals are endocrine active, a conclusion that is supported by the existing in vivo data reported in the scoping document.

Table 11. Databases searched for mechanistic data.

Database	Description
NTP ICE cHTS data	Includes ToxCast and Tox21 data.
PubChem	Reports in vitro assays covering a range of mechanisms with active, inactive, inconclusive, and unspecified results.
Comparative Toxicogenomics Database (CTD)	Reports chemical-gene interactions, individually by gene and grouped by gene ontology or pathway (Kyoto Encyclopedia of Genes and Genomes). Results only include active hits and not inactive.

It should be noted, certain limitations and challenges exist when using these databases, both from the perspective of the chemical or substance of interest and for what each database can provide. One issue encountered in this case study was an overall lack of data availability for the PHOPs across the assays. This could be due to a chemical being outside of a method's applicability domain (insoluble) or because it was untested. Additionally, for this case study, the data was not extracted and analyzed to determine if a chemical suppressed or increased a specific signaling pathway or outcome, which is a step that would help to further define the mechanistic profile of the chemical subclass and is a recommended next step (Section 4.4). Even with the data rich TDCPP it was difficult to build a profile for PHOPs with high confidence because anchoring an entire subclass by a single chemical could be too restrictive or place over-weight specific mechanistic effects unique to that one chemical and not the subclass at-large. Mechanistic data that was recovered from each database is discussed in the following sections.

3.4.1. Comparative Toxicogenomics Database

Heatmaps were generated with data hits for each chemical of interest. The use of heatmaps provided a visual interpretation of the quantity of data that exists for certain endpoints. As an example of this type of visualization, Figure 11 visualizes the assays with data for any PHOP, analog, or metabolite found in the Comparative Toxicogenomics Database (CTD). TDCPP (the top row in Figure 11A and B) has the highest density of blue which indicates that TDCPP is mechanistically data rich. This supports TDCPP as a good anchor chemical for the PHOPs. Figure 11B is the same heatmap as Figure 11A but reduced to the top thirty pathways altered by PHOPs. Some overlap between the PHOPs and the metabolites (such as 2-chloroethanol, listed as ethylene chlorhydrin) shows that there are common pathways between the parent and metabolite compounds, further strengthening their association. Redox regulation, generic hormone signaling, generic metabolic pathways, and neuronal outgrowth were the primary pathways indicated in the CTD database for these chemicals, in general agreement with the in vivo data from the scoping document.

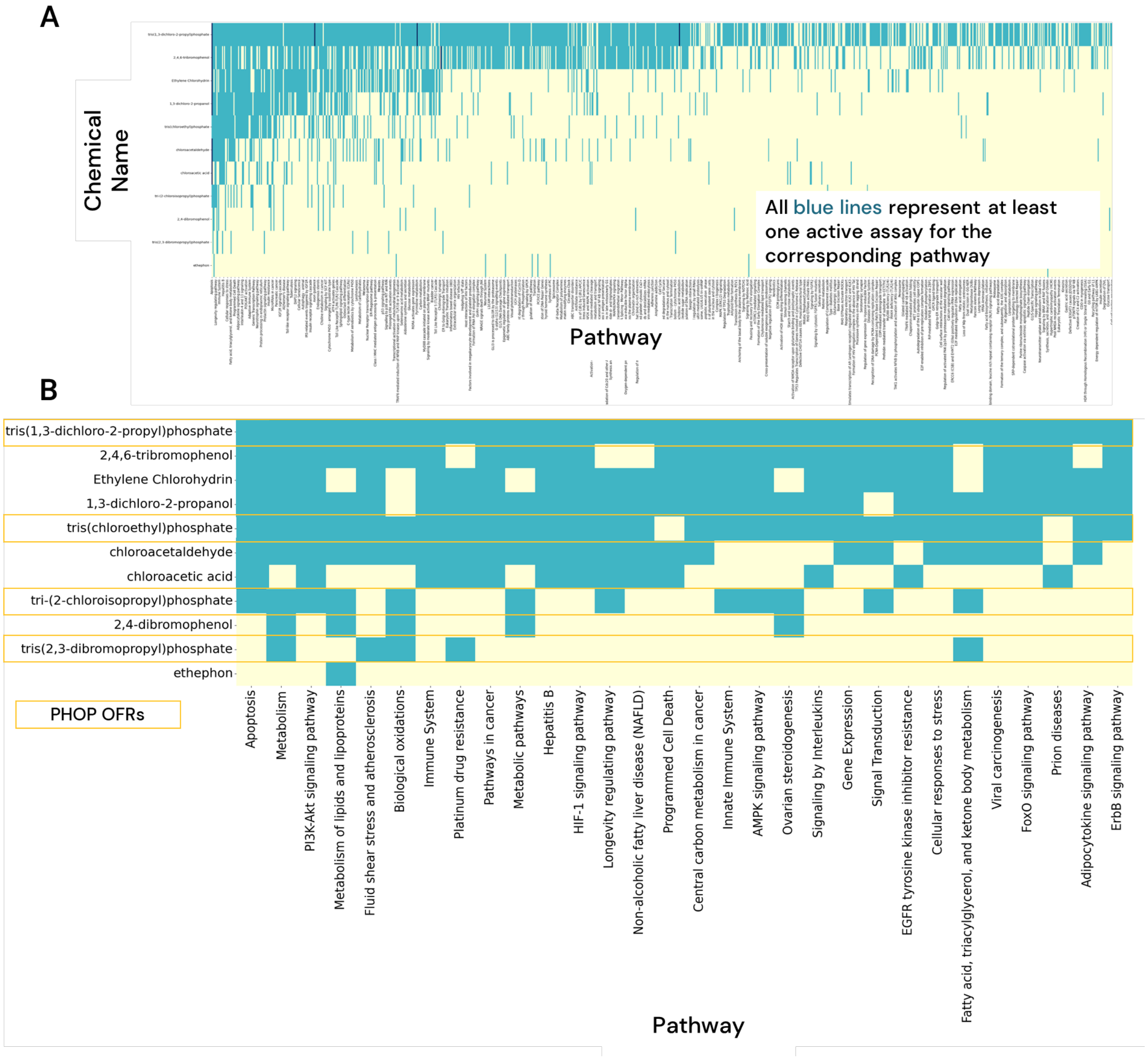


Figure 11. Heatmaps of PHOP OFRs and metabolites with data hits in CTD. A) All pathways. B) Top thirty pathways.

Blue lines indicate at least one hit (active) in a pathway. In Figure 11B PHOP OFRs are highlighted on the y-axis by yellow boxes, all other chemicals are metabolites.

3.4.2. PubChem

Within PubChem 3,928 records were found for ten PHOPs. These were then filtered for active assays, which reduced the results to 353 records for eight PHOPs. Then, assays that were not informative for a mechanism of action were also filtered out, including endpoints such as cytochrome P450 induction and generic cytotoxicity. This reduced the PubChem results to 268 records for seven PHOPs. Since some in vivo data are available on cancer and mutagenicity within NTP reports and the scoping document for this subclass, assays that targeted carcinogenicity and mutagenicity were also filtered out, resulting in 119 records for six PHOPs. Similar to those results obtained from the heatmaps in CTD, the primary pathways targeted were related to receptor activation, endocrine (steroid hormone and thyroid) effects, and inflammation/redox. For all these chemicals, there was a mix of active and inactive assays within the same target pathways, creating ambiguity for defining a subclass and targets at a macro level.

3.4.3. Integrated Chemical Environment

Data from ICE were also pulled. These data are similar to the data from PubChem since they both contain Tox21 high throughput screening (HTS) assays. However, the assays included in ICE have undergone additional curation to limit data to the highest quality. Assay curves in ICE must pass curation criteria for concentration-response curve fit and chemical quality checks for inclusion, and annotation of the mechanistic effect may be different from those provided in PubChem or CompTox Dashboard. For example, a rat cell line with a human gene insert is annotated to a human based mode of action (MOA) and therefore ICE considers it a human assay, but CompTox/Tox21 would consider it a rat assay as it is hosted within a rat cell line. More details on the curation of HTS data are available on the ICE website (NTP, n.d.). ICE had data on six PHOPs, but only four had mechanistic data. Data for the other two PHOPs were either flagged during the curation or were general cytotoxicity assays. As with CTD, most data were linked to TDCPP. Outputs similar to those from CTD were targeted: redox regulation, generic hormone signaling, and generic metabolic pathways.

3.4.4. Conclusions of Database Analyses

While PubChem had a large quantity of data initially, filtering for relevant information brought true data availability down to the same six chemicals that were found in ICE and CTD, indicating a dearth of available data for other chemicals within the PHOPs subclass. However, it is possible to filter to likely mechanistic pathways for those six chemicals, such as thyroid and estrogen receptor effects, oxidative stress responses, and aromatase (steroid-hormone metabolism) inhibition. These hits can be applied to provide more or less confidence to the adverse effects of PHOPs (Section 3.5). However, these pathways are common among multiple chemical subclasses; additional data gathering is recommended, either in evaluation of directionality of the assays for which data are available, or in further testing in relevant methods that do not yet have information. For this case study, there was not enough detailed data gathered from any database to build a mechanistic profile for PHOPs with high confidence. Table 12 provides a summary of the types of data and assays found from each of the databases.

Table 12. Summary of mechanistic database information.

Information	CTD	ICE	PubChem
Number of PHOPs with usable data[1]	4	4	6 (4 with same data as ICE)
Primary pathways indicated	• Generic hormone • Generic metabolic • Redox • Neuronal outgrowth	• Generic hormone • Generic metabolic • Oxidative stress response	• Hormone (estrogen, progesterone, androgen, thyroid) • Other generic signaling • Aromatase inhibition (hormone pathway) • Caspase

[1] TDCPP was the PHOP with the most data in all three databases.

3.5. Adverse Effect

PHOP health effects were initially searched and reported as a part of the scoping effort (NTP, 2023; CPSC, 2023). Select data sources were identified that reviewed studies of PHOPs, in particular TDCPP, TCEP, TCPP, and TDBPP. To summarize, endpoints of interest with some positive data were developmental toxicity, neurotoxicity, reproductive/endocrine toxicity (especially thyroid), carcinogenicity, and effects on the kidney and liver. However, the confidence in the effects was variable depending on the study species or number of available studies of each type. In addition, there were some inconsistencies in the observed effects, only some of which could be attributed to differences in the available test data.

Also noted in the scoping document, DTT published carcinogenicity studies in rodents for five PHOPs or their metabolites (National Cancer Institute, 1978; NTP, 1991; NTP, 2023). A wide range of tumor targets was identified, without a clear pattern, although kidney tumors were noted in rodents exposed to TDCPP and TCEP and hepatocellular adenomas were noted for TDCPP and bis(2,3-dibromopropyl) hydrogen phosphate (a metabolite of TDBPP).

A wider search for potential adverse effects from PHOPs, analogs, and/or metabolites was conducted in this case study through two avenues: evidence maps illustrating database data availability and the current results of a systematic review of OFRs currently in progress by DTT (NIEHS, 2022).

3.5.1. Evidence Map Data Availability

The method for creating evidence maps of database data availability is described in Section 2.5. Appendix Figure B.1 is a full size matrix showing binary results of evidence map data availability for all PHOPs and their analogs and metabolites for selected data types useful for read across. The evidence maps include predictive data, such as QSAR and in vitro assays such as those in ToxCast/Tox21; these were removed for assessing data availability and potential adverse effects of PHOPs in an effort to limit conclusions to experimental adverse effects data for this profile. Each row in the evidence map is a type of experimental study or assay (e.g., acute toxicity, genotoxicity) within the animal toxicity or acceptable alternative, human toxicity, quantitative hazard characterization, and qualitative hazard characterization categories. White space indicates the chemical did not have any of the selected data hits from the databases searched.

All analogs found in this case study that were not PHOPs themselves had few data hits and most of them had no data hits at all, making them poor analogs for the purpose of assisting in determining the potential health effects of PHOPs. Reviewing NASEM's analog list (Section 2.3) revealed 27 PHOP analogs not included in the PHOP analog list from this case study; the other NASEM PHOP analogs overlapped with the new analog list presented here. No data was found by DTT or in previous work for CPSC for the 27 analogs not included in this case study.

Empirical human toxicity data in the evidence maps were sparse; only TDCPP, TCEP, TCPP and the metabolites 2-chloroethanol and chloroacetaldehyde had any human data. The data richness of the chemicals was generally equivocal between the animal toxicity and qualitative hazard categories, but the former category had more overall hits and the latter presented hits for endocrine assays. Figure 12 presents the heat map for qualitative hazard characterization for PHOPs and PHOP metabolites only as a representative of the data availability, but the full heatmap in Appendix B was used for the following analysis and discussion.

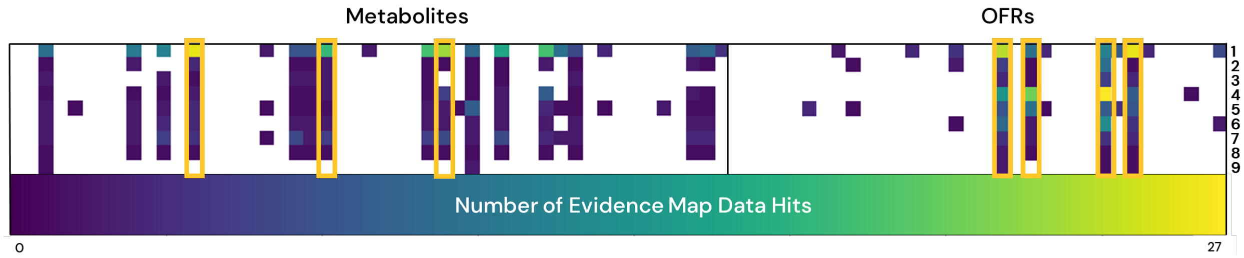


Figure 12. A heat map showing the number of data hits for qualitative hazard characterization in the evidence maps for metabolites and PHOPs.

The vertical axis is the assay or study type, with numbered rows corresponding to: 1) acute toxicity, 2) systemic repeated dose toxicity, 3) neurotoxicity, 4) carcinogenicity, 5) mutagenicity/genotoxicity, 6) reproductive or developmental toxicity, 7) irritation, 8) sensitization, and 9) endocrine disruption. White space represents zero values. Yellow boxes indicate the three metabolites and four PHOPs with the most total data hits across the evidence maps. Each column is a chemical.

There were four PHOP OFRs with the most data hits and the most categories with hits. These four are:

- TCPP
- TDCPP
- TCEP
- TDBPP

The same four PHOPs were also considered analogs to other PHOPs s (See Table 14 in Section 4). The predominant types of data for all PHOPs, but especially these, were acute toxicity, carcinogenicity, mutagenicity/genotoxicity, and reproductive or developmental toxicity. Each of the other study/assay types also had data hits for at least some PHOPs or metabolites.

The metabolites had more chemicals with data and more data availability than the analogs, making them more useful for read across. This is emphasized by the likelihood that adverse effects from many (or all) PHOPs are due to the prevalence of metabolites predicted to be more toxicologically relevant than their parent in the PBTK profile (Section 3.2).

Table 5 and Table 6 list the predicted and empirical metabolites, respectively, with data in their evidence maps by their total data hits. Metabolites with the most data were small, halogenated compounds. The three metabolites with the most data, boxed in yellow in Figure 12, are 2—chloroethanol, chloroacetic acid, and 2-chloroethylphosphonic acid (known as Ethephon, a plant growth inhibitor and organophosphate pesticide) (U.S. EPA, 2024a; U.S. EPA, 2024b; U.S. EPA, 2024c). Chloroacetic acid and 2—chloroethylphosphonic acid were not identified as metabolites in the empirical literature search and were only predicted with the OECD QSAR toolbox, reducing confidence regarding whether or not they are true metabolites and contribute to PHOP toxicity. However, they have both been characterized for toxicity and shown to be neurotoxic, affecting acetylcholinesterase activity (U.S. EPA, 1995; U.S. EPA, 2016).

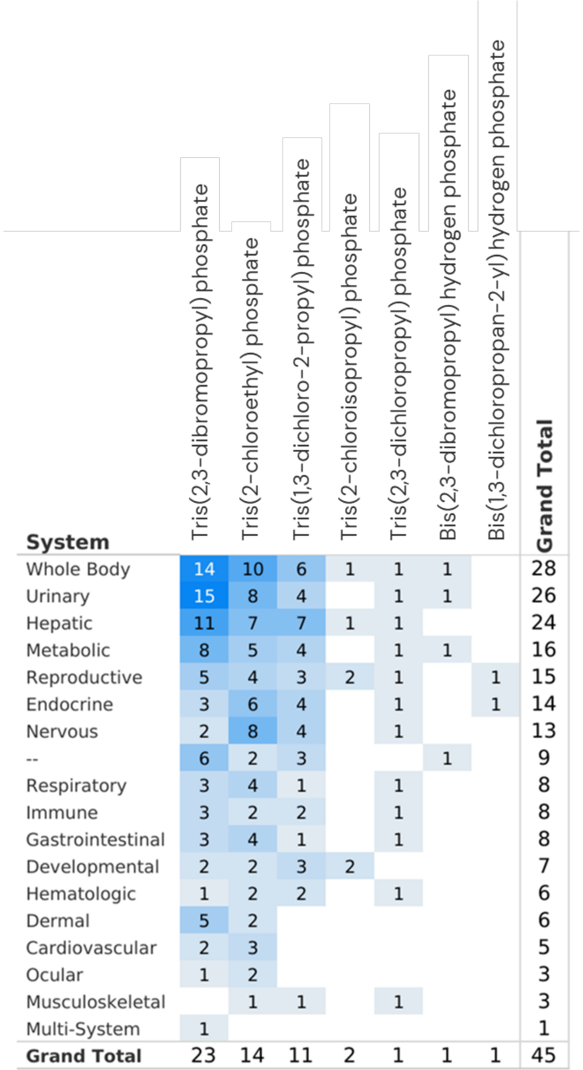
2-Chloroethanol was predicted by both GLORYx and the OECD QSAR toolbox and found empirically, and it was predicted to be a metabolite for nine PHOP OFRs (26% of the entire subclass, 30% of the subclass sans BPPs). As a result, 2-chloroethanol is likely an important contributor to understanding the adverse effects of the PHOP subclass. It can be considered a data rich chemical substance as there are several toxicity reference values reported across many databases. It is a highly hazardous substance with acute toxicity targeting several systems, especially the liver and kidneys (Hartwig & Commission, 2022; NIOSH, 2019).

The brominated metabolites had less data availability overall than the chlorinated metabolites, but there was some data available. 2,3-dibromo-1-propanol, a metabolite shared by 3 brominated parents, is a likely carcinogen (NTP, 2021). The other shared brominated metabolites had no data hits.

3.5.2. Literature Review Data Availability

The Guide describes several organizations that publish literature reviews for chemical hazard assessments, which were reviewed in the context of this case study. OFRs are widely used but have not been prioritized for literature review by the majority of public agencies. At the time of this case study, DTT had a literature review on OFRs in progress and in collaboration with CPSC (NIEHS, 2022). The data used for this case study was published in an interactive Tableau (Rooney et al., 2023) that was presented at the 2023 Society of Toxicology annual meeting. The data reported was an inventory of which effects were measured, but not the result or direction of the effect. Just like the evidence maps, the counts were not directly indicative of an effect, only suggestive. The conclusions of this case study would be best updated upon DTT's final literature extraction results, when published. Additionally, there was some expected overlap between the data hits from the evidence maps and those in the DTT literature review because some of the database data originated in literature that was caught by DTT's search. The extent of the overlap is unknown, but both methods were likely to find more unique data hits than the same data hits.

Only 12 of the 34 unique PHOPs have published studies reporting the results of testing for health outcomes according to DTT's literature review. For this case study, DTT's inventory was filtered to remove any data reported with a mixture or co-exposure or if the data were obtained from nonmammalian species. Additionally, DTT did not categorize any in vitro studies by health effect/target system. This meant that counts from in vitro studies were not helpful without further categorization of assay type or outcome and therefore these studies were also filtered out of our analysis. Figure 13 is an image of a Tableau heatmap created using DTT's data after removing mixtures, co-exposures, nonmammalian studies, and mechanistic studies.



System	Tris(2,3−dibromopropyl) phosphate	Tris(2−chloroethyl) phosphate	Tris(1,3−dichloro−2−propyl) phosphate	Tris(2−chloroisopropyl) phosphate	Tris(2,3−dichloropropyl) phosphate	Bis(2,3−dibromopropyl) hydrogen phosphate	Bis(1,3−dichloropropan−2−yl) hydrogen phosphate	**Grand Total**
Whole Body	14	10	6	1	1	1		28
Urinary	15	8	4		1	1		26
Hepatic	11	7	7	1	1			24
Metabolic	8	5	4		1	1		16
Reproductive	5	4	3	2	1		1	15
Endocrine	3	6	4		1		1	14
Nervous	2	8	4		1			13
--	6	2	3			1		9
Respiratory	3	4	1		1			8
Immune	3	2	2		1			8
Gastrointestinal	3	4	1		1			8
Developmental	2	2	3	2				7
Hematologic	1	2	2		1			6
Dermal	5	2						6
Cardiovascular	2	3						5
Ocular	1	2						3
Musculoskeletal		1	1		1			3
Multi-System	1							1
Grand Total	23	14	11	2	1	1	1	45

Figure 13. Image of the Tableau dashboard built for this case study showing the DTT literature review measured effect counts by PHOP chemical and categorized into system categories.

The dashboard was filtered to remove counts for mixtures and co-exposures, any nonmammalian species, and uncategorized mechanistic effects. A count equals one primary reference with data on that chemical and system. A single primary reference can contribute to more than one chemical-system combination, and thus the grand total row/column identify the total number of unique primary references contributing to that row/column, and not a simple sum of the counts in each row/column. The row marked “--" represents reference counts for all other health effects not represented by the systems listed. Figure available at: https://public.tableau.com/app/profile/cpsc/viz/CPSCCO-01PHOPOFRsVisual/MainDashboard

Only 8 PHOPs had reported measurements after the aforementioned criteria were applied. Of these 8, only 3 had several counts in multiple systems (the first 3 on the top, y-axis in Figure 13):

- TDCPP
- TCEP
- TDBPP

It is important to point out that data counts were subject to several biases, most notably that the percentage of repeat dose toxicological studies was high relative to other literature. This defaults to several instances of high data counts reported for systems commonly measured in repeat dose toxicity studies. This effect is noticeable in the quantity of whole body effect counts compared to other systems that are not required by repeat dose studies, such as immune system effects. The primary contributor to counts in the whole body system was for body weight measurements, which are almost always reported (regardless of change from controls) for repeat dose toxicity studies.

Data availability from the DTT literature review was examined for similar health endpoints shared among the top four categories with the most data counts per chemical, excluding whole body and unclassified effects. Table 13 shows a side-by-side comparison of the top four categories for TDCPP, TCEP, and TDBPP according to their data richness in Figure 13. Hepatic and urinary (renal) targets appear across all three chemicals, while nervous and endocrine systems have many data hits for both chlorinated chemicals. When the systems are expanded in the interactive Tableau to show specific targets within each system (not pictured), many of the endocrine system targets noted for the chlorinated chemicals are related to the thyroid. Triiodothyronine, thyroxine, and testosterone endpoints had the most data hits. Although not conclusive on outcome, the likelihood of thyroid effects (e.g., hormone level changes, follicular hyperplasia) based on data availability is consistent with the finding reported in the PHOP scoping document.

The DTT literature review included the NASEM analogs in their search for data as well. Only two analogs had data hits: BCIPP and bis(2-chloroethyl) phosphate. Both were also considered analogs found via PubChem in this case study but had no hits on the evidence maps. Between them, there were five total studies found by DTT, but only one of these was a study that was not a mixture or co-exposure. DTT’s literature search results further emphasized the lack of data rich analogs to aid in read across of the PHOPs subclass.

Table 13. Top four systems according to data counts from the DTT literature review for three PHOP chemicals with the most overall counts.

TDCPP	TCEP	TDBPP
Hepatic	Nervous	**Urinary**
Nervous	**Urinary**	**Hepatic**
Developmental, Endocrine, Metabolic, **Urinary**	**Hepatic**	Metabolic
Reproductive	Endocrine	Reproductive, Dermal

The top systems shared across all three chemicals are in blue.
TDCPP = Tris(1,3-dichloro-2-propyl) phosphate
TCEP = Tris(2-chloroethyl) phosphate
TDBPP = Tris(2,3-dibromopropyl) phosphate

3.5.3. Adverse Effect Data Conclusions

PHOPs as a subclass has four data rich anchor chemicals, while the remainder of the subclass had generally minimal adverse effects data availability from either toxicological databases or the literature. The analogs for PHOPs have limited additional information. However, there were several metabolites with data hits in the evidence maps, and the most data rich shared metabolites had toxicological profiles indicating they were toxic to the nervous, hepatic, and/or renal systems. Reproductive effects also showed up with multiple hits in both sets of evidence maps and were consistent with other data indicating endocrine effects.

Since this case study primarily investigated data availability and not outcomes, conclusions are largely based on inferences made about the likelihood of adverse effects by PHOPs. This was accomplished by assessing data availability for the PHOPs and their metabolites and considering some known reported outcomes from the PHOP scope document and other sources. From this analysis, the hepatic and urinary systems are most likely to be toxicological targets, as are various effects related to thyroid function and the nervous and reproductive systems. Moreover, PHOPs are likely to be carcinogenic.

These inferences were generally consistent with information reported in the PHOP scope document which discussed outcomes of some systematic repeated dose toxicity, developmental neurotoxicity, carcinogenicity, and endocrine disruption studies. While developmental neurotoxicity was not specifically flagged within the DTT evidence maps, both nervous system and developmental toxicity were top data hits in Table 13 for TDCPP and nervous system was in the top data hits for TCEP. Neurotoxicity and developmental toxicity assays had data hits for TDCPP, TCEP, TCPP and TDBPP in the evidence maps.

Beyond this, the PHOP scope document infers that thyroid and other endocrine targets may be relevant to PHOPs based on in vitro data from other organophosphate esters. Effects on the thyroid can also appear in other systems because the thyroid regulates various other biological processes, including developmental neurotoxicity. We hypothesize that a PHOPs hazard MOA is by affecting the thyroid function either directly or indirectly (e.g., by altering thyroid hormone clearance), and these changes result in outcomes categorized into other systems and by other studies. This hypothesis is supported by the known adverse effects associated with thyroid dysfunction in the PHOP scope document and the highly likely targets from the data availability analysis. For example, the up or down regulation of thyroid hormones can affect endometrium thickness, for which TDCPP and TCIPP both show data hits in the expanded DTT Tableau. Additionally, thyroid hormone can increase metabolism, which may help explain the existence of DTT data related to metabolism for five PHOPs (TDCPP, TCEP, TDBPP, Tris(2,3-dichloropropyl) phosphate, and bis(2,3-dibromopropyl) hydrogen phosphate).

In addition to the PHOP scope document, known toxicological outcomes of three of the four anchor chemicals (TDCPP, TCPP, and TCEP) in this case study and phosphoric acid, 2,2-bis(chloromethyl)-1,3-propanediyl tetrakis(2-chloroethyl) ester (commercially known as V6) were obtained from a document released by the EPA (U.S. EPA, 2015). EPA screened studies and characterized the hazard summary (very low hazard to very high hazard) for categories almost equivalent to the categories used in the evidence maps herein. Notably, carcinogenicity, reproductive, developmental, and repeat dose toxicities were assessed as medium or high hazard for all four chemicals. The hazard assessment did not evaluate endocrine effects separately, but the authors also discuss endocrine activity as a possible MOA, since they found thyroid data included changes in thyroid weight accompanied by other endocrine related changes (e.g., thyroid follicular hypertrophy and altered levels of hormones) in animal, in vitro, or epidemiological data. Overall, the EPA assessment is congruent with the conclusions and hypothesis from this case study.

The main limitation to the adverse effects profile is the lack of completed systematic evidence mapping for outcomes due to ongoing extraction of all the data in the evidence maps and DTT literature review beyond what has been reported to date. This, ultimately, can also become a strong line of evidence once complete. In order to build a complete adverse effects profile for PHOPs the outcomes from the data collected in the evidence maps and DTT's literature search will be extracted, reviewed, and critically analyzed. The PHOP scope document did look at outcomes, but noted a lack of consistency in study designs and findings that limits confidence. These inconsistencies have not yet been fully investigated to fully integrate the evidence. Follow-up work is recommended to complete these steps.

4. Assessment and Conclusions

4.1. Identifying Anchor Chemical(s) and Defining the Chemical Class

The first step in the assessment of PHOPs was identifying the anchor chemical(s) representing the class. These chemicals must have some data at a minimum, and ideally be data rich. None of the analogs found in this case study or by NASEM met this criterion, and therefore no analogs were useful as anchor chemicals. Instead, there were four PHOPs with relatively well-defined profiles in this case study and had the most data among PHOPs:

1. TDCPP (most data)
2. TCPP
3. TCEP
4. TDBPP (least data)

These four PHOPs are considered the anchor chemicals for this study, with TDCPP having the most data available as described in the mechanistic and adverse effect profiles.

4.2. Classifying PHOPs for Class-based Read Across

4.2.1. Outlier Identification

4.2.1.1. Brominated Phenyl Phosphates

There were four OFRs in the PHOPs subclass that were outliers in every profile and in the initial clustering using fingerprint. These four chemicals, called the BPPs in this case study, are structurally similar to each other in that they all are phosphates bound to three phenyl rings of varying bromination and methylation. They are:

1. Tris(2,4,6-tribromophenyl) phosphate
2. Tris(2,4-dibromophenyl) phosphate
3. Tris(2,3-dibromophenyl) phosphate
4. Tris(2-bromo-4-methylphenyl) phosphate

Almost all the other PHOP chemicals have primarily chlorinated or brominated alkyl functionalized phosphates. The difference in structure between these aryl groups and primarily alkyl groups appear to significantly affect the bioactivity because the BPPs had different metabolites, higher predicted toxicokinetic values (and subsequently higher toxicological relevance), and physical chemical properties that differed from all other PHOPs.

The BPPs are best classified as outside of the PHOP subclass. It is possible that they have similar bioactivity to other NASEM defined subclasses, particularly the PHDE, PHPhAE and/or PHB subclasses given their shared metabolites, and they should be reevaluated alongside those subclasses.

4.2.1.2. Other Chemicals

There were three other PHOPs that structurally differed from the main PHOPs grouping in Section 2.2. These OFRs are:

1. Phosphonic acid, P-[1-[[(2-chloroethoxy)(2-chloroethyl)phosphinyl]oxy]ethyl]-, 1-[bis(2-chloroethoxy)phosphinyl]ethyl 2-chloroethyl ester; commercially called Phosgard C-22-R.
2. Dimethyl (4,6-dichloro-1,3,5-triazin-2-yloxy)methylphosphonate
3. 2,3-Dibromopropyl dihydrogen phosphate

Phosgard C-22-R and 2,3-dibromopropyl dihydrogen phosphate were not considered different from the main group of PHOP OFRs in any of the profiles (i.e., PBTK, physicochemical, mechanistic, adverse effects). Furthermore, both were rated as high confidence for inclusion in the metabolic profile. Upon closer look, these two were differentiated structurally because they land on the extremes of size and functionality. Phosgard C-22-R has 3 phosphate groups linked together, making it larger and more phosphate rich than the other PHOPs. 2,3-Dibromopropyl dihydrogen phosphate was the least functionalized phosphate with only a single alkyl group. These size differences did not affect their properties or bioactivity with respect to profiles in this case study.

The third OFR listed as structurally different from the main PHOP group was dimethyl (4,6-dichloro-1,3,5-triazin-2-yloxy)methyl phosphonate. As mentioned previously, this is the only PHOP with a triazine moiety and, unlike other PHOPs, it does not have any alkyl functional groups. As a result, it did not share any metabolites with PHOPs, or any other OFRs from any subclass. Given the suggestive evidence in this case study, dimethyl (4,6-dichloro-1,3,5-triazin-2-yloxy)methyl phosphonate should not be considered part of the PHOP subclass and should be reevaluated with the Polyhalogenated Triazine (PHT) subclass of OFRs.

4.2.2. PHOP Subclass Similarity

After removing the BPPs and dimethyl (4,6-dichloro-1,3,5-triazin-2-yloxy)methyl phosphonate, we recommend the remaining PHOPs be grouped together as a subclass for risk assessment. At a minimum, there are no convincing reasons for differentiating the chemicals based on the five profiles evaluated in this case study. There is also direct support for grouping these together when assessing structural similarity by Tanimoto score and fingerprint, and when considering the metabolite profile results.

Using both the RDKit and PubChem fingerprints (Figure 2), the PHOPs group together closely relative to all other OFRs, emphasizing their overall structural similarity. Additionally, a Tanimoto score of ≥0.85 used for the analog search resulted in several PHOPs determined to be analogs for other PHOPs. Table 14 lists the thirteen PHOPs that were considered analogs of three anchor chemicals, linking 47% of the OFRs directly to each other structurally.

Table 14. Analogs of anchor PHOPs that are also PHOP OFRs.

Analog PHOP	Seed PHOP(s)
1. Tris(2-chloroethyl) phosphite 2. Tris(2-chloropropyl) phosphate 3. Bis(2-chloroethyl) 2-chloroethylphosphonate	TDCPP
1. Bis(2-chloro-1-methylethyl) 2-chloropropyl phosphate 2. (2-Chloro-1-methylethyl) bis(2-chloropropyl) phosphate 3. TCPP 4. Tris(3-chloropropyl) phosphate 5. Bis(1,3-dichloropropan-2-yl) hydrogen phosphate 6. Tris(2,3-dichloropropyl) phosphate 7. Tetrakis(1-chloropropan-2-yl) ethane-1,2-diyl bis(phosphate) 8. Tris(dichloropropyl) phosphate	TCEP
1. Bis(2,3-dibromopropyl) hydrogen phosphate 2. 2,3-Dibromopropylphosphate	TDBPP

TDCPP = Tris(1,3-dichloro-2-propyl) phosphate
TCPP = Tris(2-chloroisopropyl) phosphate
TCEP = Tris(2-chloroethyl) phosphate
TDBPP = Tris(2,3-dibromopropyl) phosphate

Confidence in each OFR inclusion in the PHOPs subclass was assessed for each profile. A rating scale was applied to each chemical, rating the chemical as 0 (low/no confidence) to 2 (high confidence) for each classifying factor described in Table 15. Every profile was included in the rating system as a classifier in addition to rating the chemicals by structural similarity and by their metabolite's adverse effects independent of the OFR's adverse effect profile. The sum of the confidence ratings was used to determine the overall confidence for including each OFR in the PHOPs subclass. The maximum possible confidence rating was 13 and the lowest possible confidence rating was 0.

Table 15. Confidence rating scale criteria for subclass inclusion.

Classifier	Low/no confidence (0)	Medium confidence (1)	High confidence (2)
Metabolite Profile	No shared metabolites with other PHOPs.	No shared metabolites with other PHOPs but have metabolites that are structurally similar to anchor chemicals' metabolites.	Shared metabolites with other PHOPs.
PBTK Profile	Predicted TK values of OFR or metabolites were outliers.	Predicted TK values of OFR or metabolites were within range of main distribution.	None.
Physicochemical Profile	Outliers to PCA descriptor distributions.	Qualitatively different from PCA descriptor distributions according to expert opinion.	Within the PCA descriptor distribution.
Mechanistic Profile	Not enough data hits available to evaluate confidence.	Data availability suggests similarity to TDCPP (assigned only to TCEP).	Assigned only to TDCPP because it was the only chemical with reasonable assay data availability for confidence evaluation.
Adverse Effect Profile	No or little data availability, limiting confidence evaluation; or data availability hits do not meet criteria for medium confidence.	Data hits of OFR included at least 2 of urinary, hepatic, nervous, reproductive, metabolic, or endocrine targets in DTT review; or data hits of OFR included at least 3 of the systemic repeated dose toxicity, neurotoxicity, carcinogenicity, mutagenicity/genotoxicity, reproductive/developmental, endocrine categories in evidence maps.	Known shared health effects with anchor OFR through the scoping document or other source. Anchor OFRs are rated 2 by default.
Metabolite Adverse Effect	No or little data availability, limiting confidence evaluation; or Data availability hits do not meet criteria for medium confidence.	Data hits of at least 1 metabolite included at least 3 of the systemic repeated dose toxicity, neurotoxicity, carcinogenicity, mutagenicity/genotoxicity, reproductive/developmental, endocrine categories in evidence maps.	Data hits of at least 3 metabolites included at least 3 of the systemic repeated dose toxicity, neurotoxicity, carcinogenicity, mutagenicity/genotoxicity, reproductive/developmental, endocrine categories in evidence maps.
Structural Similarity	Clear or potential PHOP outlier on fingerprint dendrograms.	Within the main PHOP grouping (within 2 chemicals distance) on fingerprint dendrograms.	Within the main PHOP grouping (within 2 chemicals distance) on fingerprint dendrograms and considered an analog of anchor chemicals.

The resulting confidence rating for each OFR in the PHOPs subclass as defined by NASEM is listed in Table 16. The four anchor chemicals have the highest rating and are the most representative of the subclass. Two additional chemicals were also rated as highly as the anchor chemicals; one is a metabolite to TDBPP, and the other had known hazards tying it to the anchor chemicals (Section 3.5). On the opposite side of the confidence spectrum are the four BPPs and the triazine containing PHOP, quantitatively confirming the same conclusions discussed previously that these five chemicals should be excluded from the PHOPs hazard assessment subclass. The remaining 25 chemicals are almost evenly distributed between medium and high confidence ratings. The medium confidence chemicals predominately lacked any data availability (mechanistic or adverse) for themselves and their metabolites. The overall confidence rating for the medium confidence rated chemicals can be improved through collection of more data (see Section 4.4).

Table 16. PHOP subclass inclusion confidence.

Confidence	Potential Rating Total	Actual Rating Total	OFR Name
Very High	10-13	10-12	• Tris(1,3-dichloro-2-propyl) phosphate[1] • Tris(2-chloroethyl) phosphate[1] • Tris(2-chloroisopropyl) phosphate[1] • Tris(2,3-dibromopropyl) phosphate[1] • Bis(2,3-dibromopropyl) hydrogen phosphate • Phosphoric acid, 2,2-bis(chloromethyl)-1,3-propanediyl tetrakis(2-chloroethyl) ester
High	8-9	8-9	• (2-Chloro-1-methylethyl) bis(2-chloropropyl) phosphate • 2,3-Dibromopropylphosphate • Bis(2-chloro-1-methylethyl) 2-chloropropyl phosphate • Bis(2-chloroethyl) 2-chloroethylphosphonate • Bis(2-chloroethyl) vinylphosphonate • Diethylene glycol bis[bis(2-chloroethyl) phosphate] • Phosphoric acid, 1,2-ethanediyl tetrakis(2-chloroethyl) ester • Tetrakis(1-chloropropan-2-yl) ethane-1,2-diyl bis(phosphate) • Tris(2,3-dichloropropyl) phosphate • Tris(2-chloropropyl) phosphate • Tris(dichloropropyl) phosphate
Medium	5-7	5-7	• Bis(1,3-dichloropropan-2-yl) hydrogen phosphate • Tris(3-chloropropyl) phosphate • 2,2-Bis(chloromethyl)-1,3-propanediyl tetrakis(1-chloro-2-propanyl) bis(phosphate) • 2,2-Bis(bromomethyl)-3-chloropropyl bis[2-chloro-1-(chloromethyl)ethyl] phosphate • 2-Bromoethyl 5-bromopentyl 2-chloroethyl phosphate • Tris(2-chloroethyl) phosphite • Tris(tribromoneopentyl)phosphate • Ethanol, 2-bromo, phosphate (3:1)

Confidence	Potential Rating Total	Actual Rating Total	OFR Name
Low	0-4	0-3	• Phosphonic acid, P-[1-[[(2-chloroethoxy)(2-chloroethyl)phosphinyl]oxy]ethyl]-, 1-[bis(2-chloroethoxy)phosphinyl]ethyl 2-chloroethyl ester • 4-Bromo-2-chlorobutyl 3-bromo-2,2-dimethylpropyl phosphate • Tris(1,3-dichloropropan-2-yl) phosphite • 2,4,8,10-Tetraoxa-3,9-diphosphaspiro[5.5]undecane, 3,9-bis[3-bromo-2,2-bis(bromomethyl)propoxy]-, 3,9-dioxide • Dimethyl {[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]methyl} phosphonate • Tris(2,4-dibromophenyl) phosphate • Tris(2,4,6-tribromophenyl) phosphate • Tris(dibromophenyl) phosphate • Tris(2-bromo-4-methylphenyl) phosphate

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[1] Anchor chemical.

4.3. Case Study Summary and Qualitative Hazard Assessment Conclusions

In this case study, the OFR subclass PHOPs, as defined by CPSC in the 2022 article titled “Development of a Flame Retardant and an Organohalogen Flame Retardant Chemical Inventory” (Bevington et al., 2022), was evaluated in a class-based read across assessment following the principles described in the Guide. Analogs were identified and were refined based on similarity by molecular weight and halogenation status, and then further refined by data availability within toxicology databases. Predictive tools and a literature search and extraction were completed to identify known and probable metabolites for OFRs. Database data availability was also determined.

All PHOPs, analogs, and metabolites were evaluated for similarity, toxicological relevance, and health effects by their structural fingerprints and by building 5 profiles (metabolite, PBTK, physicochemical properties, mechanistic effects, and adverse effects). Using the results of these profiles and the structural fingerprint comparison, 4 anchor chemicals (TDCPP, TCEP, TCPP, and TDBPP) were identified among the PHOPs and the analogs were considered not useful for read across. The confidence of each PHOP belonging in the overall PHOP subclass was then determined, and 5 OFRs were considered poor fits for the PHOP subclass. Of these five, four were abbreviated as the BPPs:

1. Tris(2,4-dibromophenyl) phosphate
2. Tris(2,4,6-tribromophenyl) phosphate
3. Tris(dibromophenyl) phosphate

4. Tris(2-bromo-4-methylphenyl) phosphate

These four OFRs were different from the other PHOPs in every profile and are therefore likely to have different health effects and biological mechanisms. Their bioactivity is more likely to be a function of the brominated phenyl moieties rather than the phosphate functional group based on evidence in this case study, and they should be reevaluated with other subclasses containing similar moieties such as PHDE, PHPhAE and/or PHB subclasses.

The fifth chemical with low confidence of subclass inclusion is dimethyl{[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]methyl}phosphonate. This OFR differed from the other PHOPs in multiple profiles. However, there was no data available for this chemical, its analogs, or its metabolites and therefore some of the low confidence in inclusion was a result of the lack of information. Dimethyl{[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]methyl}phosphonate would likely be better evaluated with other triazine containing OFRs based on the evidence available in this case study.

The 29 PHOPs of medium to high confidence for subclass inclusion were considered as the PHOPs subclass for considering health outcomes in this case study. The PHOPs subclass has four data rich OFRs with data available in toxicological databases and/or the literature.

Qualitative hazard assessment, for PHOPs, is summarized based on evidence discussed in Section 3.5 and supported by additional profiles described in the case study. The draft conclusions for the PHOPs subclass are: potential for hepatic toxicity, potential for renal toxicity, potential for thyroid effects on their own, potential for developmental neurotoxicity (influenced by thyroid changes), potential for reproductive toxicity (influenced by thyroid changes), and potential for carcinogenicity. The analogs for PHOPs provide limited additional information. However, there were multiple data rich metabolites that are toxic to the nervous, hepatic, and/or renal systems. PHOP metabolites also had several hits for reproductive effects in both sets of evidence maps that were consistent with other data indicating endocrine effects. In this draft qualitative hazard assessment, there is potential for the effects described here to occur in any of the 29 chemicals determined to be in the PHOPs subclass within this case study. In the final quantitative hazard assessment, dose-response analysis will characterize one or more of these effects for PHOPs, where possible.

There were limitations in this case study in determining the hazard and health effects of the 29 OFRs considered as part of the PHOP subclass. Most notably was that adverse effects and mechanistic effect profiles did not include conclusive, directional outcomes for the chemicals with data available. Additionally, the overall availability of experimental toxicological data on PHOPs and their metabolites was limited. As a result, the hazard conclusions determined are considered draft and subject to peer review and public comment before finalization. Additional steps are recommended in the next section to increase confidence in the conclusions presented here.

4.4. Recommendations for Next Steps

Collating all available data in a visual format allows for patterns to be discerned as to the most likely systems affected within a chemical subclass. Utilizing anchor chemicals, such as TDCPP, TCEP, TCPP, and TDBPP, and comprehensive literature and database reviews for those chemicals can reveal the most likely targeted systems and provide recommendations for further testing to address whether suspected similar chemicals within the subclass share the same health effects. For PHOPs, analysis of the DTT literature tagging revealed that hepatic, urinary (renal), endocrine and endocrine-related, and nervous system effects were most commonly evaluated for these chemicals. Review of the evidence maps of OFRs from the literature survey supports focus on these effects in addition to general carcinogenicity and acute toxicity. The initial scoping report for PHOPs, which reviewed results of the NASEM report and other selected key studies, reported thyroid, cancer, and nervous system outcomes that support the suggestive findings in the evidence maps and DTT literature review.

The natural next step is to complete a full data extraction of the evidence maps and DTT literature review studies. Pulling out the outcome level details for each study would provide clarity identifying any already measured health effects. Homogenous, accurate data extraction can be a laborious effort, and there are several methods for reducing the level of effort to accomplish this work. Using a system of prioritization and/or machine learning tools rank highest, and there are several tools for using machine learning to extract data from literature that can be evaluated for use in future work.

To further increase confidence in the targets and health effect outcomes for PHOPs as a subclass, follow-on high throughput testing of all OFRs identified as PHOPs with medium confidence or higher is recommended. The Tox21 pipeline of assays is a first choice because it is already developed to identify likely toxicity through selected assay types (Thomas et al., 2018). The specific testing pipeline could be refined by additional data analysis, and as such, definite assay specific recommendations are not going to be made here. Outside of Tox21, testing for effects such as for endocrine disruption (possibly focused on thyroid) or cholinesterase inhibition, as has been noted in both the scoping literature review and in the analysis of available data from various databases and publications, would be a logical starting point if assay prioritization is necessary.

To address developmental toxicity concerns, using available zebrafish methodologies or embryonic stem cells is also suggested; these are also available in a medium to high throughput capacity (Becker et al., 2022). High throughput testing for carcinogenicity and systemic repeated dose toxicity is more challenging, especially since the genotoxicity data for the anchor chemicals is inconsistent (see Section 3.4). Therefore, it would make sense to focus on the more accessible and established assays for evaluation of the subclass, and then investigate other endpoints as needed for a subset of chemicals.

The results of high throughput testing suggested here would allow for review of a consistent set of endpoints evaluated for the entire hypothesized subclass, substantially increasing confidence in identifying the chemicals that should be grouped together, as well as identifying shared health effects for conducting a dose-response assessment (Dasgupta et al., 2024). Furthermore, coupling this data with any extracted outcomes from the evidence maps and/or DTT literature review would bolster the understanding of PHOPs' health effects.

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Appendix A. PHOPs List

Table A.1. List of 42 PHOPs with Revised SMILES.

Count	CAS RN	Chemical Name	Cleaned SMILES
1	49690-63-3	Tris(dibromophenyl) phosphate	O=P(OC1CCCC(Br)C1Br)(OC1CCCC(Br)C1Br)OC1CCCC(Br)c1Br
2	2788-11-6	Tris(2,4-dibromophenyl) phosphate	O=P(OC1CCC(Br)CC1Br)(OC1CCC(Br)CC1Br)OC1CCC(Br)CC1Br
3	7046-64-2	Tris(2,4,6-tribromophenyl) phosphate	O=P(OC1C(Br)CC(Br)CC1Br)(OC1C(Br)CC(Br)CC1Br)OC1C(Br)CC(Br)CC1Br
4	126-72-7	Tris(2,3-dibromopropyl) phosphate	O=P(OCC(Br)CBr)(OCC(Br)CBr)OCC(Br)CBr
5	19186-97-1	Tris(tribromoneopentyl) phosphate	O=P(OCC(CBr)(CBr)CBr)(OCC(CBr)(CBr)CBr)OCC(CBr)(CBr)CBr
6	61090-89-9	2,4,8,10-Tetraoxa-3,9-diphosphaspiro[5.5]undecane, 3,9-bis[3-bromo-2,2-bis(bromomethyl)propoxy]-, 3,9-dioxide	O=P1(OCC(CBr)(CBr)CBr)OCC2(CO1)COP(=O)(OCC(CBr)(CBr)CBr)OC2
7	27568-90-7	Ethanol, 2-bromo, phosphate (3:1)	O=P(OCCBr)(OCCBr)OCCBr
8	29716-44-7	Tris(chloroethyl) phosphate	O=P(OCCCl)(OCCCl)OCCCl
9	115-96-8	Tris(2-chloroethyl) phosphate	
10	26248-87-3	Tris(chloropropyl) phosphate	O=P(OCCCCl)(OCCCCl)OCCCCl
11	1067-98-7	Tris(3-chloropropyl) phosphate	
12	26604-51-3	Tris(dichloropropyl) phosphate	CC(Cl)C(Cl)OP(=O)(OCCC(Cl)Cl)OCC(Cl)CCl
13	98923-48-9	4-Bromo-2-chlorobutyl 3-bromo-2,2-dimethylpropyl phosphate	CC(C)(CBr)COP(=O)(O)OCC(Cl)CCBr
14	13674-84-5	Tris(2-chloroisopropyl) phosphate	CC(CCl)OP(=O)(OC(C)CCl)OC(C)CCl
15	1047637-37-5	2,2-Bis(chloromethyl)-1,3-propanediyl tetrakis(1-chloro-2-propanyl) bis(phosphate)	CC(CCl)OP(=O)(OCC(CCl)(CCl)COP(=O)(OC(C)CCl)OC(C)CCl)OC(C)CCl
16	34621-99-3	Tetrakis(1-chloropropan-2-yl) ethane-1,2-diyl bis(phosphate)	CC(CCl)OP(=O)(OCCOP(=O)(OC(C)CCl)OC(C)CCl)OC(C)CCl
17	76025-08-6	Bis(2-chloro-1-methylethyl) 2-chloropropyl phosphate	CC(Cl)COP(=O)(OC(C)CCl)OC(C)CCl
18	76649-15-5	(2-Chloro-1-methylethyl) bis(2-chloropropyl) phosphate	CC(Cl)COP(=O)(OCC(C)Cl)OC(C)CCl
19	6145-73-9	Tris(2-chloropropyl) phosphate	CC(Cl)COP(=O)(OCC(C)Cl)OCC(C)Cl
20	4351-70-6	Phosphonic acid, P-[1-[[(2-chloroethoxy)(2-chloroethyl)phosphinyl]oxy]ethyl]-, 1-[bis(2-chloroethoxy)phosphinyl]ethyl 2-chloroethyl ester	CC(OP(=O)(OCCCl)C(C)OP(=O)(CCCl)OCCCl)P(=O)(OCCCl)OCCCl

21	35656-01-0	Tris(2bromo4methylphenyl) phosphate	CC1CCC(OP(=O)(OC2CCC(C)CC2Br)OC2CCC(C)CC2Br)C(Br)C1
22	13674-87-8	Tris(1,3-dichloro-2-propyl) phosphate	O=P(OC(CCl)CCl)(OC(CCl)CCl)OC(CCl)CCl
23	40120-74-9	Tris (1,3- dichloropropyl) phosphate	
24	66108-37-0	2,2-Bis(bromomethyl)-3-chloropropyl bis[2-chloro-1-(chloromethyl)ethyl] phosphate	O=P(OCC(CCl)(CBr)CBr)(OC(CCl)CCl)OC(CCl)CCl
25	6749-73-1	Tris(1,3-dichloropropan-2-yl) phosphite	ClCC(CCl)OP(OC(CCl)CCl)OC(CCl)CCl
26	78-43-3	Tris(2,3-dichloropropyl) phosphate	O=P(OCC(Cl)CCl)(OCC(Cl)CCl)OCC(Cl)CCl
27	6294-34-4	Bis(2-chloroethyl) 2-chloroethylphosphonate	O=P(CCCl)(OCCCl)OCCCl
28	84282-27-9	2-Bromoethyl 5-bromopentyl 2-chloroethyl phosphate	O=P(OCCCl)(OCCBr)OCCCCCBr
29	115-98-0	Bis(2-chloroethyl) vinylphosphonate	C=CP(=O)(OCCCl)OCCCl
30	38051-10-4	Phosphoric acid, 2,2-bis(chloromethyl)-1,3-propanediyl tetrakis(2-chloroethyl) ester	O=P(OCCCl)(OCCCl)OCC(CCl)(CCl)COP(=O)(OCCCl)OCCCl
31	53461-82-8	Diethylene glycol bis[bis(2-chloroethyl)phosphate]	O=P(OCCCl)(OCCCl)OCCOCCOP(=O)(OCCCl)OCCCl
32	33125-86-9	Phosphoric acid, 1,2-ethanediyl tetrakis(2-chloroethyl) ester	O=P(OCCCl)(OCCCl)OCCOP(=O)(OCCCl)OCCCl
33	140-08-9	Tris(2-chloroethyl) phosphite	ClCCOP(OCCCl)OCCCl
34	1373346-90-7	Dimethyl {[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]methyl}phosphonate	COP(=O)(COC1NC(Cl)NC(Cl)N1)OC
35	72236-72-7	Bis(1,3-dichloropropan-2-yl) hydrogen phosphate	O=P(O)(OC(CCl)CCl)OC(CCl)CCl
36	34432-82-1	Bis(2,3-dibromopropyl) hydrogen phosphate--ammonia (1/1)	O=P(O)(OCC(Br)CBr)OCC(Br)CBr
37	36711-31-6	Bis(2,3-dibromopropyl) phosphate, magnesium salt	
38	5412-25-9	Bis(2,3-dibromopropyl) hydrogen phosphate	
39	64864-08-0	Sodium bis(2,3-dibromopropyl) phosphate	
40	66519-18-4	Potassium bis(2,3-dibromopropyl) phosphate	
41	5324-12-9	2,3-Dibromopropylphosphate	O=P(O)(O)OCC(Br)CBr
42	125997-20-8	Phosphoric acid, mixed 3-bromo-2,2-dimethylpropyl and 2-bromoethyl and 2-chloroethyl esters	Excluded by the cleaning process because it is a mixture

NA = Not Assigned by CPSC.

Appendix B. Full Scale Figures

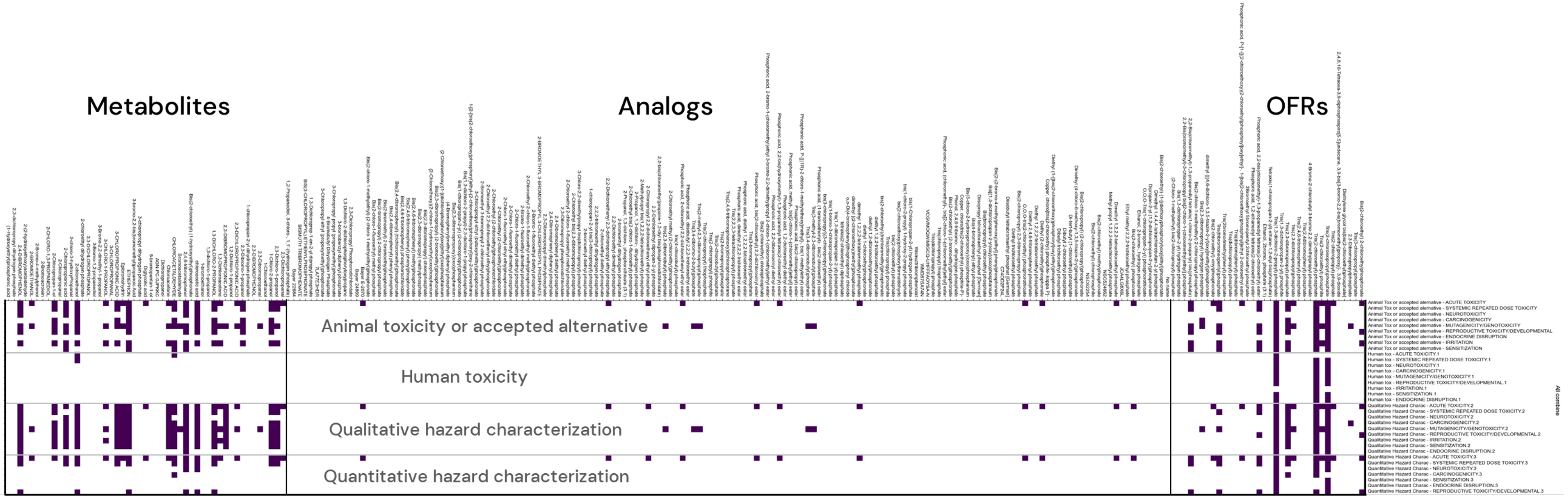

Figure B.1. A matrix showing if there are data hits available in the evidence maps for OFRs, analogs, and metabolites.

Dark purple squares indicate at least one data hit and white space indicates no data hits. Each row is a study or assay type (e.g., acute toxicity, genotoxicity) within the four categories listed horizontally: animal toxicity or acceptable alternative, human toxicity, quantitative hazard characterization, and qualitative hazard characterization. Each column is a chemical. Not all data hits are unique; qualitative and quantitative data hits may represent data from the human and/or animal toxicity.

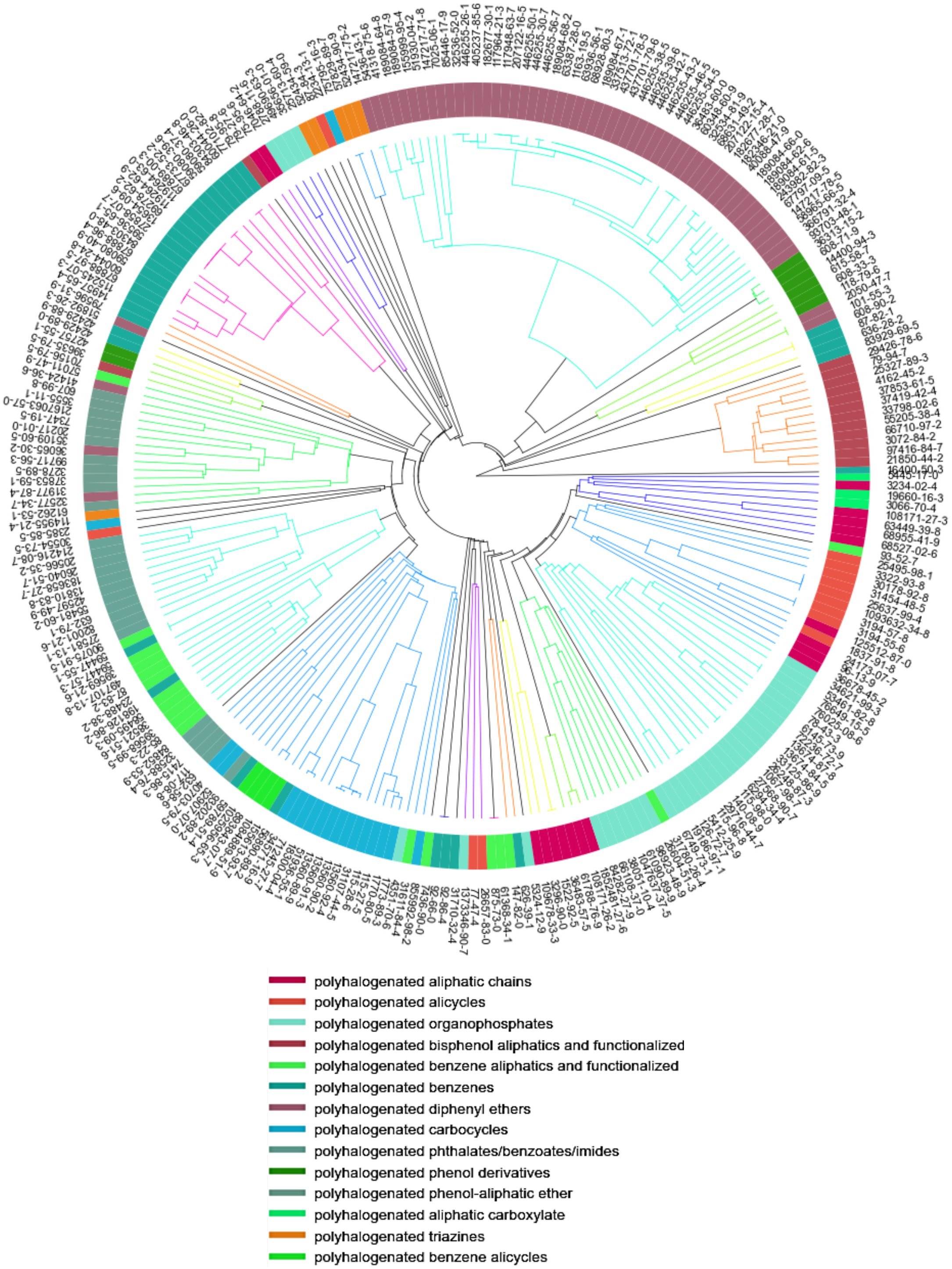

Figure B.2. Dendrogram comparing structural grouping using hierarchical clustering based on RDKit topological Fingerprint and Tanimoto score of PHOPs subclass.

Each subclass is defined by a unique color, and each chemical is a slice matched to a unique inner branch line and labeled by CAS RN.

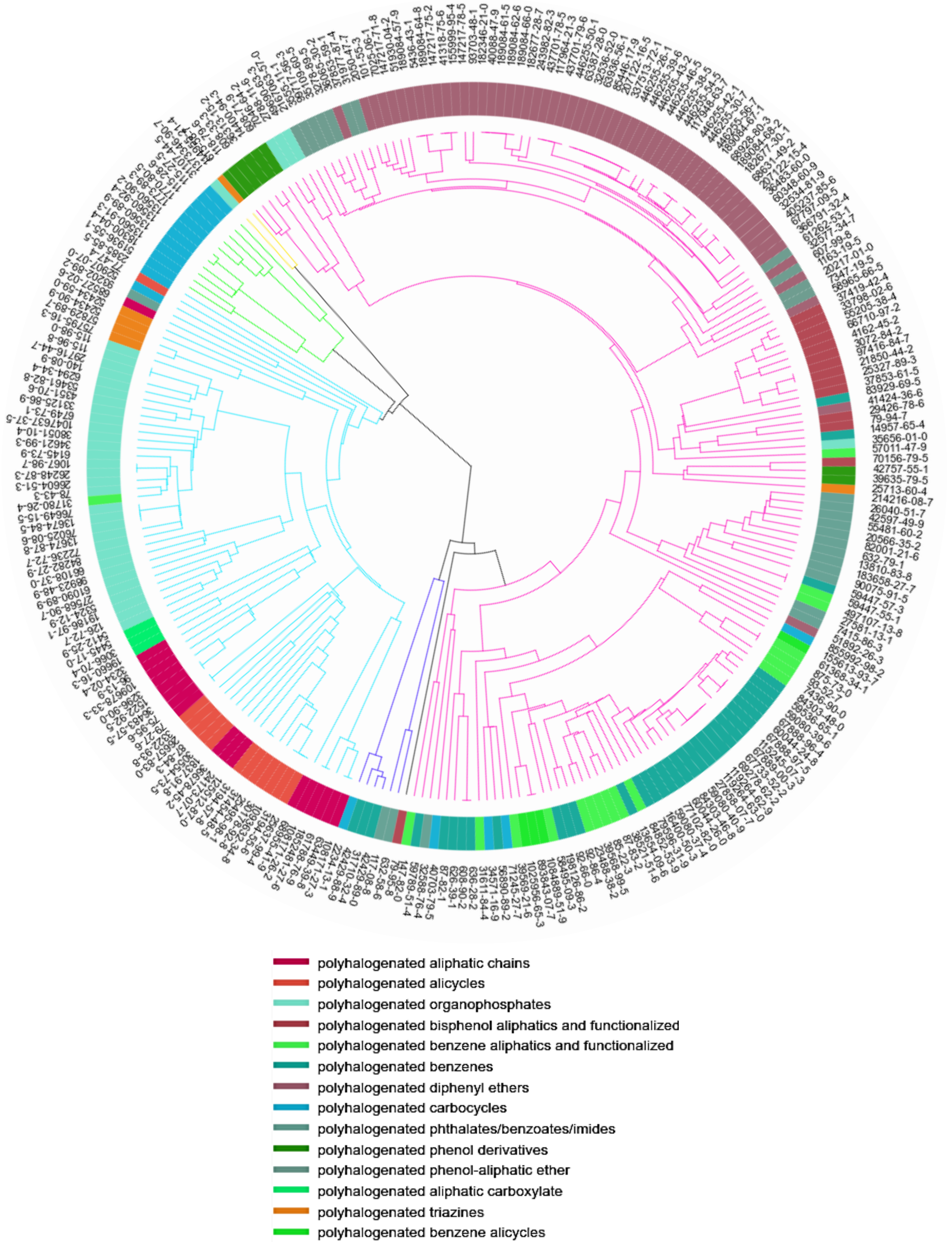

Figure B.3. Dendrogram comparing structural grouping using hierarchical clustering based on PubChem Fingerprint and Tanimoto score of PHOPs subclass.

Each subclass is defined by a unique color, and each chemical is a slice matched to a unique inner branch line and labeled by CAS RN.