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Utility of In Vitro Bioactivity as a Lower Bound Estimate of In Vivo Adverse Effect Levels and in Risk-Based Prioritization

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Abstract

Use of high-throughput, *in vitro* bioactivity data in setting a point-of-departure (POD) has the potential to accelerate the pace of human health safety evaluation by informing screening level assessments. The primary objective of this work was to compare PODs based on high-throughput predictions of bioactivity, exposure predictions, and traditional hazard information for 448 chemicals. PODs derived from new approach methodologies (NAMs) were obtained for this comparison using the 50th ($POD_{NAM,50}$) and the 95th ($POD_{NAM,95}$) percentile credible interval estimates for the steady-state plasma concentration used in *in vitro* to *in vivo* extrapolation of administered equivalent doses (AEDs). Of the 448 substances, 89% had a $POD_{NAM,95}$ that was less than the traditional POD ($POD_{traditional}$) value. For the 48 substances for which $POD_{traditional} < POD_{NAM,95}$, the POD_{NAM} and $POD_{traditional}$ were typically within a factor of 10 of each other,

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[5]Supplemental Files

Supplemental Appendix: A text file containing Supplemental Figures 1–7 and supporting text.
Supplemental File 1: A spreadsheet containing all of the *in vivo* POD values used to derive the $POD_{traditional}$.
Supplemental File 2: A spreadsheet containing all of the summary information (including BER and POD ratio values)

and there was an enrichment of chemical structural features associated with organophosphate and carbamate insecticides. When $POD_{traditional} < POD_{NAM,95}$, it did not appear to result from an enrichment of $POD_{traditional}$ based on a particular study type, (e.g. developmental, reproductive, chronic studies). Bioactivity:exposure ratios (BERs), useful for identification of substances with potential priority, demonstrated that high-throughput exposure predictions were greater than the $POD_{NAM,95}$ for 11 substances. When compared to threshold of toxicological concern (TTC) values, the $POD_{NAM,95}$ was greater than the corresponding TTC value 90% of the time. This work demonstrates the feasibility, and continuing challenges, of using *in vitro* bioactivity as a protective estimate of POD in screening level assessments via a case study.

Keywords

High-throughput screening; high-throughput toxicokinetics; threshold of toxicological concern (TTC), point of departure (POD); new approach methodologies

1 Introduction

The future of chemical risk assessment is moving towards high-throughput approaches that can provide preliminary estimates of hazard and exposure. The utilization and sharing of these new approaches and associated data internationally is imperative because each regulatory authority is addressing distinct but related challenges in chemical screening and evaluation. A commonality among these challenges is the need to prioritize chemicals for further evaluation and conduct screening-level assessments, as there are thousands of chemicals with potential human exposures but with minimal hazard information (Egeghy *et al.*, 2012; Judson *et al.*, 2009). New approach methodologies (NAMs) (ECHA, 2016; EPA, 2018a) include *in vitro* and *in silico* approaches for prediction of hazard and exposure, thereby enabling solutions to some of these regulatory challenges. NAMs for hazard evaluation can be used in high-throughput formats, and in some cases may identify chemical mechanisms of action. NAMs for exposure provide rapid estimates using limited information for more chemicals than lower-throughput models can achieve. The promise of NAMs is motivating regulatory authorities to define and adopt fit-for-purpose NAMs, and support efforts to reduce, refine, and replace resource-intensive vertebrate animal tests. International collaborative efforts that deepen understanding of NAMs and their application while preventing duplicative efforts have become a salient need.

There are several key regulatory drivers of international use of NAMs in toxicology applications. In the US, the amended Toxic Substances Control Act (TSCA) (Lautenberg, 2016) requires a risk-based screening process for prioritizing chemicals as high-priority substances for risk evaluation or low-priority sibstances for which risk evaluations are not warranted. The amended TSCA requires the U.S. Environmental Protection Agency (EPA) to develop a plan, “to promote the development and implementation of alternative test methods and strategies to reduce, refine, or replace vertebrate animal testing and provide information of equivalent or better scientific quality and relevance for assessing risks of injury to health or the environment” (EPA, 2018a). In the European Union (EU), the European Chemical Agency (ECHA) regulates chemical substances under the Registration,

Evaluation, Authorisation and Restriction of Chemicals (REACH) (Commission, 2007), that also promotes the use of NAMs as a means to increase the data availability for data poor substances (ECHA, 2016, 2017). Health Canada (HC) and Environment and Climate Change Canada (ECCC) are continuing work under the Chemicals Management Plan (CMP) to address human health and ecological concerns for approximately 4,300 prioritized substances on the Canadian Domestic Substances List (DSL) by the year 2020 (ECCC/HC, 2016a). High-throughput NAMs have been identified as a possible means to meet near-term timelines for screening assessments and to inform selection of future priorities as the program continues to evolve post-2020 (ECCC/HC, 2016b). These and other regulatory drivers underscore the need for an international discussion about how to apply NAMs in a transparent and effective way.

One aspect of prioritization and screening-level assessment strategies is a trade-off between speed and uncertainty. Though the specific implications of these strategies are likely to differ by regulatory authority, each of the aforementioned regulatory agencies are responsible for protecting human and environmental health and have traditionally relied on *in vivo* studies in efforts to achieve this mission. Despite increasing interest in prediction of human health hazard directly, rather than relying on animal models and associated extrapolation concerns, a conceptual bridge for toxicologists to understand how current practice could be augmented by incorporation of NAMs will enable greater discussion and progress. Thus, there is a clear need and opportunity to demonstrate how preliminary screening-level risk assessment using a NAM-based approach would perform when compared to traditional points of departure (PODs). Acknowledging and documenting the caveats and limitations of this comparison is central to building the confidence and insight needed to employ NAMs. Hence, the study documented here sought to use as many chemicals as possible to illustrate how the current state-of-the-science would support NAM-based screening-level risk assessment.

NAMs for exposure, bioactivity, and *in vitro* to *in vivo* extrapolation are available, for differing numbers of substances, to inform this exercise of demonstrating a risk-based screening-level assessment approach. High-throughput exposure predictions have been generated using a series of computational models under the ExpoCast project, the second version of which used a series of heuristics (Wambaugh *et al.*, 2014) including chemical use type (Dionisio *et al.*, 2015) and production volume to quantitatively predict exposure for thousands of chemicals. The US Environmental Protection Agency (EPA) Toxicity Forecaster (ToxCast) program (Kavlock *et al.*, 2012) and the interagency Tox21 project (Thomas *et al.*, 2018; Tice *et al.*, 2013) provide publicly available, high-throughput *in vitro* bioactivity information for a diverse biological and chemical space. Additionally, for this case study, researchers at the Singapore Agency for Science, Technology, and Research (A*STAR) provided high-throughput phenotypic profiling information from three cell-based toxicity models for lung, kidney, and liver toxicity for a subset of substances (Lee *et al.*, 2018; Su *et al.*, 2016). Using high-throughput toxicokinetic (httk) information and reverse dosimetry, all of these bioactivity data, i.e. the micromolar concentration of a substance that altered an assay signal *in vitro*, were transformed into administered equivalent doses (AEDs) in milligram per kilogram bodyweight per day (mg/kg/day) units within a complex process referred to as *in vitro* to *in vivo* extrapolation (IVIVE). The reverse dosimetry component of IVIVE in this case relies on the assumption that a nominal *in vitro* assay concentration

approximates an *in vivo* serum concentration using steady state kinetics, and then involves a toxicokinetic model to estimate the external exposures (in mg/kg/day units) that may have resulted in that concentration (Bell *et al.*, 2017; Jamei *et al.*, 2009; Sipes *et al.*, 2017b; Wambaugh *et al.*, 2018; Wetmore *et al.*, 2014; Wetmore *et al.*, 2015; Wetmore *et al.*, 2013; Wetmore *et al.*, 2012). A NAM-based POD, or POD_{NAM}, can be selected from the range of AEDs. This POD_{NAM} can be compared to exposure predictions to develop a bioactivity:exposure ratio (BER) to provide a risk-based context.

To understand the possible added benefit of *in vitro* bioactivity-derived POD_{NAM}, a well-known, protective *in silico* approach that can be used in the absence of *in vitro* bioactivity information, the threshold of toxicological concern (TTC), was also included for comparison to POD_{NAM} and exposure values. In addition, the POD_{NAM} can be compared to traditional *in vivo* data for these chemicals, aggregated and summarized as the traditional POD ($POD_{traditional}$). Curation of these traditional data from *in vivo* toxicity testing has provided an important resource to evaluate whether POD_{NAM} is protective relative to the $POD_{traditional}$ (understanding that the $POD_{traditional}$ itself is an approximation using animal models). Two examples of publicly-available curated traditional data include the Toxicity Reference Database (ToxRefDB) (Martin *et al.*, 2009a; Martin *et al.*, 2009b) and the Toxicity Value Database (ToxValDB) (Williams *et al.*, 2017) the latter of which aggregates summary level information from over 40 sources, including ToxRefDB; EPA sources, such as the High Production Volume Information System (HPVIS), Integrated Risk Information System (IRIS) (https://www.epa.gov/iris), Provisional Peer-Reviewed Toxicity Values (PPRTVs) (https://hhpprtv.ornl.gov/), curated data from Office of Water (OW), Office of Land and Emergency Management (OLEM), and the Office of Pollution Prevention and Toxics (OPPT); other US state and federal sources, such as the Food and Drug Administration (FDA), U.S. Geological Survey (USGS), Department of Defense (DOD), Department of Energy (DOE), and California EPA (CalEPA); and international sources, such as ECHA via eChem Portal (https://www.echemportal.org/echemportal/index.action) and EFSA via the Chemical Hazards Database (https://www.efsa.europa.eu/en/data/chemical-hazards-data), the World Health Organization (WHO), the Cosmetics Ingredients Safety (COSMOS) database (https://cosmosdb.eu/cosmosdb.v2/accounts/login/?next=/cosmosdb.v2/), Health Canada, and the Hazard Evaluation Support System (HESS) (Williams, et al., 2017). The traditional data included for derivation of a $POD_{traditional}$ included many study designs, including repeat-dose studies such as subacute, subchronic, chronic, reproductive, developmental and/or multi-generation reproduction studies, among others. Given that most of the *in vitro* bioactivity data measures disruption of a molecular target, pathway, or cellular function, rather than adverse at the tissue, organism, or population level as measured in *in vivo* toxicity studies, this work evaluates the hypothesis that the POD_{NAM} would be protective relative to the $POD_{traditional}$ across multiple study types and durations of exposure.

Several examples and case studies have considered the possibility of using high-throughput data in various regulatory decision contexts, from prioritization, to test replacement, to use in chemical-specific assessment (Browne *et al.*, 2015; Cote *et al.*, 2016; Judson *et al.*, 2014; Judson *et al.*, 2011; Kleinstreuer *et al.*, 2017; Paul Friedman *et al.*, 2016; Pradeep *et al.*, 2017; Thomas *et al.*, 2013). Progress has been made in the acceptance of NAMs for prioritization of chemicals subject to the US EPA Endocrine Disruptor Screening Program

(EDSP) (Browne, et al., 2015; Kleinstreuer, et al., 2017) and as alternatives for existing *in vivo* endocrine disruptor-related test guidelines (EFSA, 2018; USEPA, 2015). Further regulatory acceptance of NAMs is demonstrated by the development of defined approaches for assessment of skin sensitization, with the goal of developing an internationally-recognized test guideline using an integrated set of NAMs to predict human skin sensitization hazard potential (Casati *et al.*, 2018). The use of NAMs for determination of either the dose that may alter specific biological pathway activities of interest (e.g. nuclear receptor signaling) or general *in vitro* bioactivity has also demonstrated promise for prioritizing substances (Judson, et al., 2014; Judson, et al., 2011; Wetmore, et al., 2013). However, as with some *in vivo* toxicity studies, for many substances it may not be possible to identify a specific human health outcome, predominant mode-of-action (MoA), or adverse outcome pathway (AOP) based on the *in vitro* bioactivity data. Hence, screening-level assessment may require identification of a threshold dose at which no bioactivity would be observed in assays covering a broad biological space (Thomas, et al., 2013). Prioritization based on the integration of bioactivity data and predicted exposures has been suggested as a path forward for addressing the problem of thousands of chemicals with limited information for assessment by many groups, including Health Canada in their approach to the Chemical Management Plan (ECCC/HC, 2016a), academics, government scientists, and chemical industry scientists (Becker *et al.*, 2015; Embry *et al.*, 2014; Perkins *et al.*, 2017; Sipes, et al., 2017b). The retrospective case study presented herein advances the integrated use of NAMs for *in vitro* bioactivity and exposure by addressing the following questions: can the proposed workflow to derive a POD_{NAM} be shown to be broadly protective for potential application to screening-level chemical assessments independent of the biological events or adverse outcome pathways involved? Further, does *in vitro* bioactivity combined with exposure estimates provide a useful risk-informed prioritization metric?

Importantly, this work asks these questions as viewed through a multi-agency, international lens. The Accelerating the Pace of Chemical Risk Assessment (APCRA) initiative is an international cooperative collaboration of government agencies convened to address barriers and opportunities for the use of NAMs in chemical risk assessment (Kavlock, 2016; Kavlock *et al.*, 2018). This initiative includes participants from across offices of the EPA, ECHA, EFSA, the U.S. National Toxicology Program (NTP), CalEPA, Health Canada, the European Commission's Joint Research Center (JRC),the Organisation for Economic Cooperation and Development (OECD), France's INERIS, the Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS), the National Institute for Public Health and the Environment (RIVM) of the Netherlands, the Japanese Ministry of Health, Welfare, and Labour, Korea's Ministry of Environment, Singapore's Agency for Science and Technology Research (A*STAR), and the Taiwanese Safety and Health Technology Center (SAHTECH). An initial goal of this group was to directly identify and address obstacles to adoption of NAMs in regulatory decision-making, considering the geographic differences in regulatory perspectives and requirements while understanding that generation and analysis of the data for substances of concern can be shared. This first APCRA case study is the result of a collaborative discourse within APCRA that aims to evaluate how the POD_{NAM} compares to the $POD_{traditional}$ across 448 chemicals with high-throughput hazard and toxicokinetic information in the context of screening-level assessments. We evaluate whether

the POD_{NAM} can serve as a "lower bound" estimate of the $POD_{traditional}$. In addition, this case study incorporates high-throughput exposure information to examine the BER as a potential metric for prioritization. This work intends to increase confidence in NAM-based workflows that could be used in regulatory decision making by presenting a case study of how this might be applied.

2 Methods

2.1 Overview of the approach

This section gives a brief overview of the approach, as illustrated in Figure 1 and with additional details provided in subsequent sections of the Methods and the Supplemental Appendix.

First, *in vitro* bioactivity data were aggregated to develop POD_{NAM} estimates. Data were available from ToxCast for all 448 substances, and high-throughput phenotypic profiling toxicity (HIPPTox) data from A*STAR was available for 57 substances in this case study. For each ToxCast substance, a 5^{th} percentile was calculated based on the distribution of 50% maximal activity concentration (AC_{50}) values. For the HIPPTox data, a POD was defined differently (and referred to as the HIPPTox-POD). The HIPPTox-POD attempts to identify the lowest concentrations for any change in the measured cellular phenotypes of three cell models and uses EC_{10} to represent a threshold for this activity. For the HIPPTox data for kidney, liver, and/or lung toxicity, the minimum 10% effect concentration (min EC_{10}) was calculated. The intent in selecting the minimum of either the 5^{th} percentile of the ToxCast AC_{50} values or the minimum HIPPTox value was to provide a "lower bound" estimate on a bioactive concentration *in vitro*. The lower value of either the ToxCast 5^{th} percentile or the HIPPTox min EC_{10} was assumed to represent the steady state plasma concentration that was then used to calculate the administered equivalent dose (AED) values using high throughput toxicokinetic (HTTK) information from the httk R package (Pearce *et al.*, 2017). The HTTK model (built into the R package) used Monte Carlo simulation to incorporate population variability. The POD_{NAM} values used in this work correspond to the 50^{th} and 95^{th} percentile in the population distribution of steady state AED values and are referred to as the $POD_{NAM,50}$ and the $POD_{NAM,95}$, respectively.

Second, after derivation of POD_{NAM} values based on *in vitro* data, a series of comparisons to other values were made. The intersection of CASRN between ToxCast and HTTK information was used to obtain $POD_{traditional}$ from ToxValDB and sources from the case study partners, including ECHA, EFSA, and Health Canada. The POD_{NAM} was compared to the $POD_{traditional}$ to derive a POD ratio (log10 $POD_{traditional}$:POD_{NAM}) for both $POD_{NAM,50}$ and $POD_{NAM,95}$. Exposure predictions for the total U.S. population from the ExpoCast Systematic Empirical Evaluation of Models version 2 (SEEM2) framework (Wambaugh, et al., 2014) were used to derive a bioactivity:exposure ratio (BER). To understand how NAMs in this work compared to the TTC (HealthCanada, 2016; Kroes *et al.*, 2004; Patlewicz *et al.*, 2008), the $POD_{NAM,95}$ was also compared to a TTC to derive a $POD_{NAM,95}$:TTC ratio. The $POD_{traditional}$:POD_{NAM} ratios, the BER, and the $POD_{NAM,95}$:TTC ratio, expressed as logarithms in base 10, are the main metrics employed to evaluate the hypotheses in this study.

All of the data sources used in this case study, including chemical use type, high-throughput bioactivity data, HTTK, *in vivo* data, and exposure information, are summarized in Table 1, including the version and citations if applicable. Supplemental File 1 contains all of the *in vivo* POD information used, and Supplemental File 2 contains all of the values derived in this work (including BER and POD ratio). The software (written using R version 3.5.1) and all required source files are available via the US EPA GitHub repository (https://github.com/USEPA/-Examining-the-Utility-of-In-Vitro-Bioactivity) and FTP (ftp://newftp.epa.gov/COMPTOX/NCCT_Publication_Data/FriedmanPaul_K/APCRA_retrospective).

2.2 Comparison of high-throughput bioactivity, in vivo point-of-departure, and exposure information

2.2.1 Substance identification—Compilation of the data for this case study resulted in a total of 448 chemicals with the requisite *in vitro* bioactivity, high-throughput toxicokinetic, exposure prediction, and traditional animal *in vivo* toxicity values. Each CASRN from the intersection of data sources was mapped to a registered substance identifier (DTXSID) in EPA's DSSTox database through the Batch Search feature of the EPA CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard/dsstoxdb/batch_search) (Williams, et al., 2017). Mapping to DTXSID enabled mapping from substance to identifiers that indicate specific structure(s) needed for use in evaluation of enrichment of structural features and generation of TTC values. Linking data records to DTXSID promotes data interoperability and clarity on the specific chemical structures used as databases including ToxCast, ToxValDB, and HTTK databases, among others, rapidly evolve.

The substance use categories utilized in ExpoCast SEEM2 modeling (Dionisio, et al., 2015; Wambaugh, et al., 2014) and available via the Aggregated Computational Toxicology Online Resource (ACToR) were retrieved to evaluate the functional diversity of the 448 substances examined in this case study. In some cases, a substance may be associated with multiple functional uses.

2.2.2 In vitro bioactivity data—*In vitro* bioactivity data from two sources were used: ToxCast data from the US EPA ToxCast program and HIPPTox data from the A*STAR program. The details of data extraction and selection of an *in vitro* bioactivity concentration to use for *in vivo*-to-*in vitro* extrapolation of AEDs is described in detail below. Briefly, the minimum of either the 5th percentile of the filtered ToxCast AC_{50} values or the HIPPTox-POD, if available, was used as the *in vitro* bioactive concentration for each substance in the case study.

2.2.2.1 ToxCast data: The ToxCast high-throughput bioactivity data were obtained from the MySQL database, invitrodb (version 3) (EPA, 2018b), for all 448 chemicals. Only multi-concentration screening data were used, as single concentration screening data were not considered quantitatively informative of a POD_{NAM}. The structure of information in invitrodb and the R package used to maintain the database and perform curve-fitting are described in detail elsewhere (Filer *et al.*, 2017; NCCT, 2018; Watt *et al.*, 2018). The data retrieved included the AC_{50} and hit-call determination (from level 5 of invitrodb), caution flags on the curve-fitting for each AC_{50} (from level 6), and quantitative uncertainty

associated with the curve-fitting (from level 7). The caution flag and uncertainty information from levels 6 and 7 were used to filter the ToxCast dataset, with the intent of removing AC_{50} values from the dataset that originate from curve-fits that may be less informative on a quantitative basis. As the ToxCast data pipeline is a semi-automated, first-tier analysis tool for heterogeneous data, using these data on a single substance-basis presents a challenge as a subset of the potency values may be from curve fits that may be artefacts of the curve-fitting workflow. In this work, we implemented a filtering of the curves available for each substance prior to estimation of the 5th percentile on the distribution of ToxCast AC_{50} values by substance.

Level 6 caution flag information denotes curve behavior that may indicate a less quantitatively-informative AC_{50} value, such as curves based on a single active concentration, AC_{50} value lower than lowest concentration screened, borderline activity, efficacy less than 50%, and general indicators of excessive noise and overfitting. There are currently 10 possible caution flags, and the curves for the substances in this case study had zero to six flags associated with them prior to filtering (Supplemental Appendix). Level 7 uncertainty information was generated (Brown *et al.*, in prep) using bootstrap resampling to define the reproducibility of the curve fits (Watt and Judson, 2018; toxboot R package v0.2.0). Briefly, toxboot uses smooth, nonparametric bootstrap resampling to add random normally distributed noise to give a resampled set of concentration-response values. The resampled data is fit to the three ToxCast models (constant, Hill, gain-loss), repeated 1000 times, and the variables relating to model fitting parameters are stored in a Mongo database. The resulting data were used to generate point estimates, winning model, and hitcall for each of the 1000 resamples. Summary statistics (hit percent, median AC_{50}, and AC_{50} 95% confidence interval) were generated based on the toxboot resampling. Hit percent is the probability of a positive hitcall given the collection of resampled data. Filtering criteria using level 5, 6, and 7 information were as follows: curves were required to have less than 3 flags, and AC_{50} value greater than the lowest concentration screened, and a hit percent of greater than or equal to 50%.

Prior to curve filtering, for the 448 chemicals in this case study, the number of ToxCast concentration-response assay endpoints in which each substance was screened varied from 211 to 4557, with a median of 883 assay endpoints; the differences in numbers of assays screened may affect the observed positive hit rate. The filtering criteria described above reduced the total number of curves used from 54,048 to 46,735 (approximately 14% removed). The remaining curves are each associated with zero to 2 caution flags, and a median hit percent of 100, indicating that post-filtering most curves were highly reproducible (ranging 13 to 100). Following filtering, the number of positive hitcalls per substance ranged from 0 to 1351, with a median of 56 positive hitcalls per substance. Most substances (297 out of 448) had hitcall sums of less than 100, and only 36 substances had 5 or fewer positive hitcalls. One substance, phenobarbital (CASRN 50-06-6), was active in 4 out of 290 assay endpoints screened, but all of these were dropped during the filtering process. Thus, for phenobarbital, a single AC_{50} of 100 μM was used as a representative AC_{50} at the maximum concentration screened in ToxCast to derive a threshold AED. For phenobarbital, a concentration of 100 μM is actually fairly consistent with *in vitro* bioactivity from other reports that typically use phenobarbital to induce *CYP2B6 in vitro* at

concentrations ranging from the 100 μM to 2 mM (Faucette *et al.*, 2004; Hariparsad *et al.*, 2017). No cytotoxicity filtering of the ToxCast data was performed. All of the positive data in ToxCast for a given chemical, after the curve filtering described here, were included in the AC_{50} distribution. The 5^{th} percentile of that distribution was used to identify a minimum bioactive concentration for each chemical in ToxCast, regardless of the specific biological pathways involved. The effects of filtering the ToxCast AC_{50} values (Supplemental Figures 1–2) and of using the 5^{th} percentile versus the minimum AC_{50} (Supplemental Figure 3) are further described in the Supplemental Appendix.

2.2.2.2 HIPPTox data: *In vitro* bioactivity data from the high-content-imaging-based HIPPTox platform were also included for a subset of 57 chemicals examined in this case study. Three human cell lines were tested with the chemicals. They include a bronchial epithelial cell line, BEAS-2B (Lee, et al., 2018), a proximal tubule cell line, HK-2 (Su, et al., 2016), and a hepatocarcinoma cell line, HepG2. Up to 165 phenotypic readouts (Lee, et al., 2018) were measured from the images of the cell models using the cellXpress software v1.4.2 (Laksameethanasan *et al.*, 2013). For each cell model, a series of multivariate classifiers were trained to distinguish the cells treated with a chemical at seven concentrations (0.87 to 500 μM) from the cells treated with DMSO. The classifiers used multivariate phenotypic profiles constructed from all the readouts, and produced a series of classification accuracy values at all the tested concentrations (Loo *et al.*, 2007). Then, the values were fitted using a standard log-logistic model and a flat constant model. The best fitted curve was determined using the Akaike information criterion (AIC). An EC_{10} was derived from the best fitted curve for each cell model. For curves based on the flat constant model, an arbitrary large number (namely, 10^5 μM) was used. Finally, the minimum EC_{10} across the three cell models was supplied for use in this case study as the HIPPTox-POD.
Calculation of AEDs and calculation of POD_{NAM}

The minimum of the ToxCast 5^{th} percentile of the AC_{50} distribution or the HIPPTox-POD was converted to administered equivalent doses (AEDs) using the concept of reverse dosimetry and HTTK information, largely from *in vitro* experiments. The approach taken using the httk R package (v1.8) was similar to the approach used by Wetmore *et al.* (2012, 2014), as represented by the following Equation 1:

$$AED\left(\frac{\frac{mg}{kg}}{day}\right) = minimum\,bioactivity\,value(\mu M) * \frac{1\frac{\frac{mg}{kg}}{day}}{C_{ss}(\mu\mathrm{M})} \quad \text{Eq 1.}$$

Where the C_{ss} is the steady state plasma concentration estimated based on a 3-compartment steady state model assuming 100% bioavailability. Monte Carlo simulation was used to vary the pharmacokinetic parameters to represent inter-individual variability in a population. Population variability was incorporated into the first-order hepatic metabolic clearance, plasma protein binding, liver blood flow, and the rate of clearance via the kidney (Pearce, et al., 2017; Wetmore, et al., 2012). Dosing assumes oral infusion at a constant rate (Pearce, et al., 2017). More specifically, the AEDs were calculated programmatically using the "calc_mc_oral_equivalent" function in the httk R package (v1.8), with the following

options: the 95th quantile (which.quantile = c(0.95)); restrictive clearance (restrictive.clearance=T); selection of species (species='Human'); direct resampling of the population data (method='dr'); a correction for the amount of unbound chemical in whole blood versus plasma (well.stirred.correction=T); the default 3 compartment model (model='3compartmentss'); the output unit as mg/kg-bw/day (specific in httk as output.units='mg'). Although many AEDs could be calculated, the $POD_{NAM,50}$ and $POD_{NAM,95}$ were derived from AEDs that resulted from the 50th and 95th percentile, respectively, of the Monte Carlo simulation of C_{ss}. For clarity, the $POD_{NAM,95}$ is a lower AED than $POD_{NAM,50}$. Additionally, the maximum AED (max AED) achievable was calculated using the 95th percentile C_{ss} prediction and the typical maximum *in vitro* concentration screened in ToxCast of 100 μM.

2.2.3 Selection of the $POD_{traditional}$—The largest source of summarized *in vivo* point-of-departure (POD) information that was publicly available for this case study was the US EPA Toxicity Value Database (ToxValDB) (Table 1). ToxValDB includes summary study information and POD information from 40 sub-sources, including sources such as: COSMOS, ToxRefDB, HPVIS, HESS, and PPRTVs. This database is currently publicly viewable on a single-substance basis using the CompTox Chemicals Dashboard (Williams, et al., 2017), under the Hazard tab and the Point-of-departure sub-tab. Additionally, as part of the efforts of APCRA, POD information for a subset of the chemicals in this case study was contributed by collaborators from ECHA (61 substances), EFSA (46 substances), and Health Canada (29 substances), which generally increased the amount of hazard data for chemicals already in ToxValDB, but also expanded the chemical space overall by 6 chemicals.

Following this data aggregation step, several filters were applied. First, only oral exposures in units of mg/kg-bw or mg/kg-bw/day, or units that could be converted to mg/kg-bw/day values such as parts per million or parts per billion in the diet or mg/kg in the diet, were used, thereby including systemic exposures and excluding inhalation and dermal routes. The factors used to convert parts per million in diet to mg/kg-bw/day units were as follows: 0.05 (rat); 0.15 (mouse); 0.025 (dog); and, 0.03 (rabbit). Study type was not constrained to allow for inclusion of the highest number of substances in the case study; acute, chronic, developmental/reproductive, neurotoxicity and developmental neurotoxicity, and other repeat dose study designs were all included (though there were only 66 records associated with an acute exposure design, which is less than 0.3% of the 22627 total study records included). Only the following POD types were included: no observable or no observable adverse effect levels (NOEL, NOAEL) or lowest observable or lowest observable adverse effect levels (LOEL, LOAEL). The $POD_{traditional}$ was then calculated as the 5th percentile of the distribution of PODs from all sources for a given substance, in an effort to approximate a reasonable low POD value. Given that the number and distribution of POD records vary by substance, and that the majority of substances are associated with fewer than 100 POD observations, the 5th percentile was calculated using a discontinuous function with averaging between discontinuities (see type 2 for the quantile() function in the R stats package).

2.2.5 Calculation of the POD ratio—The log_{10}POD ratio indicates whether the POD_{NAM} is less than the $POD_{traditional}$. A log_{10}POD ratio of less than zero indicates that the POD_{NAM} is greater than the $POD_{traditional}$, whereas a log_{10}POD ratio of greater than zero indicates that the POD_{NAM} was less than the $POD_{traditional}$. Using POD values in log_{10}-(mg/kg-day) units, the log_{10}POD ratio is given by the difference between the $log_{10}POD_{traditional}$ and the $log_{10}POD_{NAM}$ as in Equation 2:

$$log_{10}PODratio \quad = log_{10}POD_{traditional} - log_{10}POD_{NAM}, \qquad \text{Eq. 2}$$

Where the $log_{10}POD_{NAM}$ employed may be the $log_{10}POD_{NAM,50}$ or $log_{10}POD_{NAM,95}$, resulting in log_{10}POD $ratio_{50}$ or log_{10}POD $ratio_{95}$, respectively. In this work, the log_{10}POD $ratio_{50}$ was computed for comparison with log_{10}POD $ratio_{95}$, but log_{10}POD $ratio_{95}$ was used as the primary value for further analyses study type enrichment and chemotype enrichment. As the ratios calculated in this work are on a log_{10} scale, it is important to note that the log of a ratio ($log_{10}(x/y)$) is the difference of the logs ($log_{10}(x) - log_{10}(y)$).

2.2.6 Allometric scaling of the $POD_{traditional}$—To at least partially address cross-species differences in the $POD_{traditional}$ values, a second iteration of the case study was performed using allometrically-scaled human equivalent doses for POD information from mouse, rat, guinea pig, rabbit, dog, and hamster studies. Allometric scaling was performed based on data adapted and modified from the U.S. Food and Drug Administration (FDA) guidelines (Nair *et al.*, 2016) using the following scaling factors for each species to convert mg/kg/day values to human equivalent doses: mouse (0.081), rat (0.162), guinea pig (0.216), rabbit (0.324), dog (0.541), and hamster (0.135). The POD ratio was then recalculated using these allometrically scaled PODs, per equation 3 below:

$$POD_{tradional,human} = F \times POD_{traditional,animal} \qquad \text{Eq. 3}$$

Where F is the species-specific scaling factor as indicated above.

2.2.7 Exposure data

To enable exposure comparison for the largest number of substances possible, exposure predictions from the US EPA ExpoCast program Systematic Empirical Evaluation of Models version 2 (SEEM2) model (Wambaugh, et al., 2014) were used for all 448 substances in the case study (the SEEM2 model was run *de novo* for a single substance, raloxifene hydrochloride, that did not appear in the 2014 publication). The ExpoCast SEEM2 model was calibrated to existing human exposure predictions inferred from human biomonitoring data, and further relies on production volume and four binary use categories from the ACToR use database that indicate if a substance had industrial and consumer product use, consumer produce use alone, industrial use without consumer product use, and/or use as a pesticide active or inactive ingredient. The model can be used to generate predictions for a large number of substances, but these predictions are associated with large credible intervals. From the SEEM2 model, the “US Total Exposure” median and 95th percentile on the

credible interval for the median prediction were used in calculation of the BER, as described in section 2.2.7.

Additionally, Health Canada provided exposure values from published screening level risk assessments conducted for existing substances under the Canadian Environmental Protection Act (1999) for consumer product and environmental exposures to the Canadian population; there were 18 chemicals in this case study with these values. These data were used only as a comparison to ExpoCast SEEM2 exposure values, and not in computation of the BER. Such a comparison is challenging due to differences in pathways, populations and metrics underlying the Health Canada traditional estimates and SEEM2 predictions. The Health Canada estimates used in screening level assessments for environmental media consider exposure for an *individual* from all sources, whereas screening level assessments for consumer products consider exposures for the *users* of such products on a product by product basis. Both environmental media and consumer product exposure estimates often make use of conservative assumptions. Further, the SEEM2 model prediction in the case study is based on the U.S population median and the credible interval around this median value. With these differences in mind, a comparison between the two was conducted as follows. For the Health Canada environmental exposure data, the total, or aggregate exposure from multiple media, for the 20–59 years age group were considered. For the consumer exposure data, daily exposure estimates for adults from the use of certain sentinel consumer products, where use was considered to be chronic, were examined where available (e.g. personal care products, cleaning products, textile, foam, plastics). The consumer product resulting in the highest exposure estimate was carried forward for analysis and no aggregation of exposure estimates across consumer products was performed. The highest exposure estimate from the combined data set of the selected consumer product and environmental media intakes were used for comparison to the 95th percentile on the credible interval for the median general population exposure estimate from ExpoCast SEEM2.

2.2.8 Calculation of the BER

Using the POD_{NAM} and 95th percentile on the prediction of the median exposure from ExpoCast, both in $\log_{10}$(mg/kg-day) units, the $\log_{10}BER_{95}$, is given by the difference between the $\log_{10}POD_{NAM,95}$ and the $\log_{10}ExpoCast_{95}$ prediction (Equation 4):

$$log_{10}BER_{95} = log_{10}POD_{NAM,95} - log_{10}ExpoCast_{95} \quad \text{Eq. 4}$$

2.2.9 Enrichment calculations

2.2.9.1 Chemotype enrichment: Enrichment of chemical structural features for substances for which the $\log_{10}$POD ratio$_{95}$ is less than zero makes it possible to investigate possible limitations in the NAM-based approach that might lead to POD_{NAM} values greater than $POD_{traditional}$. For this purpose, a recently developed chemotype-enrichment workflow (CTEW) was utilized, based on the ToxPrint structure feature set developed by Altamira (Altamira, Columbus, OH USA) and Molecular Networks (Molecular Networks, Erlangen, GmbH) under contract from the U.S. Food and Drug Administration (Yang *et al.*, 2015). Chemotype enrichment calculations were carried out by defining chemicals with a

$\log_{10}$POD ratio$_{95}$ of less than zero as the "positive" enriched space of interest, relative to the remaining case study set, i.e., the "negative" space. The general approach has been previously described (Strickland *et al.*, 2018; Wang *et al.*, 2019). The set of DTXSIDs corresponding to the 448 CASRN in this case study provide input to the CTEW and were used to retrieve DSSTox structures and compute a ToxPrint feature fingerprint for each structure using a Linux implementation of the CORINA software (Molecular Networks, GmbH). Of the 448 substances, 445 were mapped to a single DSSTox structure and further processed. The mixtures dipropylene glycol monomethyl ether (CASRN 34590-94-8) and abamectin (CASRN 71751-41-2) could not be mapped to a single structure, nor could the isomeric mixture 3-[(dimethoxyphosphinyl)oxy]-2-butenoic acid, methyl ester (CASRN 7786-34-7). ToxPrint chemotype (CT) enrichment statistics were evaluated for presence in the "positive" space. Enrichment was based on a computed odds ratio (OR) for each CT according to the following logic: a true positive indicates a chemical in the $\log_{10}$POD ratio$_{95}$ < 0 space contained the CT; a true negative indicates a chemical in $\log_{10}$POD ratio$_{95}$ that did not contain the CT; false positive indicates a chemical in the $\log_{10}$POD ratio$_{95}$ > 0 space that contained the CT; and, false negative indicates a chemical in the $\log_{10}$POD ratio$_{95}$ < 0 space that did not contain the CT. Quantitative metrics were used to evaluate the resultant confusion matrix and the significance of any enrichment. The Fischer's exact test (as implemented in Python, scipy.stats, alternative=greater) was used to compute the significance of the enrichments as indicated by p-value, which tends to yield greater weight to enrichments of CTs that are associated with a higher number of chemicals. To identify the most interesting associations, OR values ≥3 and p-value ≤0.05 thresholds were used to filter the CT results for significance and further examination. This statistical test does not account for activating or deactivating effects when multiple CTs are present and is only indicative of the chemical features that may lead to an underestimation of potential hazard by the $\log_{10}$POD$_{NAM,95}$.

2.2.9.2 Study type enrichment: To understand the possibility that certain *in vivo* endpoints, as represented by study types, might drive POD$_{traditional}$ values for which the corresponding POD$_{NAM}$ values were not lower, an analysis of whether certain study types included as described in Section 2.2.4, might disproportionately define the $\log_{10}$POD ratio$_{95}$ < 0 space was undertaken. The study types were programmatically reduced to: acute toxicity studies; repeat dose toxicity studies, defined by any study from 7 to 90 days in duration, including subacute and subchronic studies; chronic/carcinogenesis, defined by any repeat dose study in adult animals for greater than or equal to one year; reproductive/ developmental, defined by any study including more than one generation, including developmental, reproductive, multigeneration reproductive studies, or similar designs; and, neurotoxicity studies. Following this programmatic simplification and standardization of study type, a Fischer's exact test (R stats package) was used to indicate the significance, or p-value, of any enrichment of study type underlying the minimum POD$_{traditional}$ value for the $\log_{10}$POD ratio$_{95}$ < 0 space. Separate tests were run to understand potential enrichment of (1) reproductive/developmental studies and (2) chronic/carcinogenesis studies for the $\log_{10}$POD ratio$_{95}$ < 0 space. The confusion matrices were defined per the following logic: true positive indicated that the minimum POD$_{traditional}$ value for a given substance with $\log_{10}$POD ratio$_{95}$ < 0 was derived from a reproductive/developmental study or chronic/

carcinogenesis study; true negative indicated that the minimum $POD_{traditional}$ value for a given substance with $log_{10}POD\ ratio_{95} > 0$ was not derived from a reproductive/developmental study or chronic/carcinogenesis study; false positive indicated that the minimum $POD_{traditional}$ value for a given substance with $log_{10}POD\ ratio_{95} > 0$ was derived from a reproductive/developmental or chronic/carcinogenesis study; and, a false negative indicated that the minimum $POD_{traditional}$ value for a given substance with $log_{10}POD\ ratio_{95} < 0$ was not derived from a reproductive/developmental or chronic/carcinogenesis study. Like the CTEW described above, significance thresholds of a $p \leq 0.05$ and $OR \geq 3$ were used to determine significance of any association.

2.2.10 TTC values—The $POD_{NAM,95}$ was compared to the TTC approach that is often proposed for rapidly screening chemicals for priority (EFSA, 2012; HealthCanada, 2016; WHO, 2016). TTC values for the substances that could be associated with distinct structures were assigned using the software ToxTree [v2.6.6] (Patlewicz, et al., 2008) which implements the TTC decision-tree as described in Kroes *et al.,* 2004. The DSSTox chemical structure-data (SD) file generated within the CompTox Chemicals Dashboard was converted from V3000 to V2000 format using ACD/Spectrus DB 2017.2 and where necessary organic substances with counter ions (e.g. sodium salts) were converted to their neutral form with KNIME (v 3.2.1) and the RDKit salt stripper node. The structure file was imported into ToxTree, where the Kroes TTC decision tree was run in batch mode. The daily intake was set at > 90 μg/day for each chemical to run through the entire decision tree. A separate approach was required for organophosphates (OPs) since ToxTree does not correctly interpret the Kroes decision tree for these chemicals. First, each OP was screened using the carcinogenicity and mutagenicity rule-base by ISS within ToxTree to screen for genotoxicity alert (GA). If an OP triggered a GA then it was assigned a TTC value of 0.0025 μg/kg bw/day; otherwise, the OP was assigned the default Kroes TTC value for this class of chemicals, which is 0.3 μg/kg bw/day. Moreover, custom structural profilers built in OASIS LMC Pipeline Profiler [v1.0.53] were used to exclude benzidines, steroids and organo-silicon compounds from TTC value assignment. More recent scientific opinions related to TTC have recommended expansion of the original Kroes et al. 2004 exclusion criteria to maintain the conservative nature of the approach and/or that these compounds are not well represented in the dataset from which the TTC values were derived. Likewise, based on these opinions, carbamate substances were assigned a TTC value of 0.3 μg/kg bw/day (EFSA, 2012; HealthCanada, 2016; WHO, 2016).

3 Results

Al of the inputs and calculated metrics are summarized in Table 2.

3.1 Substance diversity

The extent of substance diversity in this case study was demonstrated using the same functional use categories that inform the ExpoCast SEEM2 exposure model. Multiple general functional use categories (Dionisio, et al., 2015; Wambaugh, et al., 2014) may be associated with a given substance. The possible functional use categories included: industrial process with no consumer use; pesticide active with no consumer use; pesticide inert;

consumer and industrial process; personal care product; flame retardant, consumer and no industrial process; pesticide active with consumer use; herbicide; colorant; fertilizer; petrochemical; food additive; and fragrance. Examination of these use categories demonstrated that substances with at least one use as a pesticide active (categories denoted as: pesticide active no consumer, pesticide active and consumer, herbicide, and/or antimicrobial) comprised nearly 70% (314/448) of the case study substances (Figure 2). This result is expected because the ToxCast Phase I chemical library was originally selected (Richard *et al.*, 2016) in part to maximize the overlap with the ToxRefDB (Martin, et al., 2009a; Martin, et al., 2009b). Hence, pesticide active ingredients represent a significant percentage of the union of the ToxRefDB and ToxCast phase 1 libraries and supply much of the POD information available from summaries of registrant-submitted toxicity studies, known as data evaluation records (DERs), from the U.S. EPA's Office of Pesticide Programs (OPP). Further, HTTK information are available largely for the ToxCast phase 1 and phase 2 chemical libraries (Pearce, et al., 2017).

3.2 BER for the 448 chemicals

The exposure predictions from ExpoCast, the POD_{NAM} estimates based on ToxCast and HIPPTox data, and the $POD_{traditional}$ information are compared and visualized in Figure 3, all on a log_{10}-mg/kg-bw/day basis. In this comparison, two estimates of the POD_{NAM} have been included: the $POD_{NAM,50}$ and the $POD_{NAM,95}$, with the $POD_{NAM,95}$ representing a lower dose and therefore more conservative estimate. For the majority of substances, the upper 95^{th} percentile on the credible interval for the median total US exposure from the ExpoCast SEEM2 model corresponded to a daily log_{10}-mg/kg-bw/day dose well below that anticipated to have bioactivity as well as the log_{10}-mg/kg-bw/day dose at which effects were observed in traditional animal studies. Even using the $POD_{NAM,95}$ estimate 95^{th} percentile estimate from ExpoCast (Figure 4A black line), only 11 substances had a log_{10}-BER_{95} of less than zero, indicating the potential for exposure to occur within the dose range that was bioactive *in vitro* (Figure 4B). Further examination of Figure 4 suggests that using the 95^{th} percentile from the credible interval for the median total US exposure, rather than the predicted median or 50^{th} percentile, significantly decreased the $log_{10}BER$ (shifting the BER_{95} values and BER_{50} values approximately 2 log_{10} orders of magnitude to the left in Figure 4A). Of course, given that the BER juxtaposes exposure and bioactivity predictions, uncertainty in the IVIVE methods applied to bioactivity can also result in a "shifting" of the BER estimate; using the $POD_{NAM,50}$ results in a $log_{10}BER_{95}$ that is "right-shifted" in comparison to the log_{10}-BER values from the $POD_{NAM,95}$, as expected since for the substances in this case study the $POD_{NAM,50}$ was 1.7 to 19-fold higher than the $POD_{NAM,95}$ (see Supplemental Appendix Figure 5 for more details). Although the BER values provide an indication of risk-based priority, the BER values may be smaller given the nature of the ExpoCast predictions, i.e. predictions that have large uncertainty may result in the prediction of high exposures at the 95^{th} percentile, and *in vitro* bioactivity data, i.e. very low AC_{50} values that were the result of permissive approaches in curve-fitting. The nature of the BER values were further explored in Figure 5 via 3 comparisons: ExpoCast versus $POD_{NAM,95}$, followed by a side-by-side comparison of ExpoCast and ToxCast *in vitro* bioactivity data for the 11 substances with $log_{10}BER_{95} < 0$ to the distribution of these data for the entire case study set of 448 substances. In Figure 5A, the 11 substances identified with low BER values

are labeled and appear to demonstrate exposures that are generally greater than the median ExpoCast (95th percentile) estimate for the 448 substances, and all the $POD_{NAM,95}$ are less than the median $POD_{NAM,95}$ value for the case study substances. This is interrogated further in panels 5B and 5C. A distribution of the 95th percentile ExpoCast prediction for all 448 chemicals is used to understand if the 11 substances with $log_{10}BER_{95}$ <0 had high exposure predictions. A similar demonstration of the AC_{50} used to calculate the POD_{NAM} is provided, where a distribution of all AC_{50} values for the 448 chemicals in the top panel is compared to AC_{50} values for the chemicals with $log_{10}BER$ <0. Several characteristics become apparent for the 11 substances with $log_{10}BER < 0$: one, that many of these substances demonstrated relatively potent *in vitro* activity; two, that the most potent $POD_{NAM,95}$ values, based on the combination of *in vitro* bioactivity and IVIVE, tended to drive lower BER values; and, three, that ExpoCast SEEM2 95th percentile estimates higher than the median in the case study seemed to contribute to lower BER values.

The performance of the ExpoCast SEEM2 model has been previously evaluated and described (Wambaugh, et al., 2014). In this case study, ExpoCast predictions for a relatively small subset of 18 chemicals were compared to manually curated values from Health Canada human health risk evaluations. The curated exposure values for these 18 chemicals had consumer product or environmental media exposure values from Health Canada assessments that could be compared to the median and 95th percentile on the credible interval for prediction of the median total US exposure from ExpoCast. The results illustrate for a limited chemical space that, as expected, the 95th percentile-ExpoCast values were within a range of the higher of either the consumer product or environmental Health Canada exposure values (Figure 6). The majority of the residuals (from the first to third quartile) for this comparison fall within ± 0.75 log_{10}, indicating that the 95th percentile ExpoCast SEEM2 values may be a reasonable estimate of exposure in the absence of more refined models. One substance in particular, catechol (CASRN 120-80-9), stands out for larger differences between ExpoCast SEEM2 and Health Canada estimates. The ExpoCast SEEM2 model relies heavily on the ACToR use database, and catechol is suggested as a food additive and as having industrial (with no consumer) use. For the Health Canada catechol exposure estimate, dietary intake represents the majority of environmental media exposure with the predominant source being the natural occurrence of catechol in foods, and conservative estimates were derived using literature on maximum concentrations found in various food groups (EC/HC, 2008). Accounting for natural occurrence in food is not included as a use type in the ExpoCast SEEM2 model which may explain the discrepancy. Two other substances, di(2-ethylhexyl) adipate [DEHA] (CASRN 103-23-1) and chlorohexidine diacetate (CASRN 56-95-1), respectively, are consumer product chemicals that demonstrated slightly higher residuals. For DEHA, the Health Canada consumer product exposure estimate used to compare to ExpoCast SEEM2 represents the highest concentration reported in body lotion although a considerable range across products was reported (0.1 to 6%) which may partly explain the higher residual when compared to ExpoCast SEEM2 which represents the median of the U.S. population. Moreover, the Health Canada exposure estimate used in the comparison is the applied dose and it is known that DEHA exhibits low dermal absorption (the screening assessment adjusted the applied dose to estimate an internal dose using a dermal absorption value of 10% for risk

characterization) (EC/HC, 2011). Likewise, the Health Canada estimate for chlorohexidine acetate is the applied dose, and it also exhibits low dermal absorption (EC/HC, 2017). The ExpoCast SEEM2 exposure estimates may be lower for poorly absorbed chemicals in near-field exposures such as topical application in part because the ExpoCast SEEM2 model was calibrated using human biomonitoring data.

3.3 POD ratio for the 448 chemicals

The POD ratio depends on the POD_{NAM} and the $POD_{traditional}$. In accounting for uncertainty from inter-individual variability, the $POD_{NAM,50}$ and $POD_{NAM,95}$ were both computed for comparison. The $POD_{NAM,50}$ for substances in this case study are 1.7 to 19 times higher than the $POD_{NAM,95}$, dependent on the substance, with the differences based on estimation of population differences in metabolic and renal clearance and/or plasma protein binding (see Supplemental Appendix, Figure 5). The $log_{10}POD$ ratio indicates whether a POD_{NAM} is lower than the estimate of a dose associated with *in vivo* effects ($POD_{traditional}$). A $log_{10}POD$ ratio < 0 means that the $log_{10}POD_{NAM}$ is greater than the $log_{10}POD_{traditional}$. The $log_{10}POD$ $ratio_{95}$ was < 0 for 48 of the 448 substances, or approximately 11% of the total (Figure 3C). Conversely, for 400 of 448 chemicals (89%), the $POD_{NAM,95}$ is less than the $POD_{traditional}$ (Figure 3). As the $log_{10}POD_{NAM,50}$ is greater than the $log_{10}POD_{NAM,95}$, the $log_{10}POD$ $ratio_{50}$ is < 0 for a higher percentage of substances in this case study (20%, or 92 of 448 substances). Further examination of the distribution of the $log_{10}POD$ $ratio_{95}$ demonstrates a range of −2.7 to 7.5, and a median of 2 (Figure 7A), indicating the median distance between the $POD_{traditional}$ and $POD_{NAM,95}$ on an arithmetic scale would be approximately 100-fold. Only three substances, all of which are organophosphate insecticides, dicrotophos (CASRN 141-66-2, DTXSID9023914), azamethiphos (CASRN 35575-96-3, DTXSID9034818), and mevinphos (CASRN 7786-34-7, DTXSID2032683), had a log_{10} POD $ratio_{95}$ of less than −2., with a $log_{10}POD$ $ratio_{95}$ values of −2.1, −2.7, and −2.2, respectively (Table 4). For the $log_{10}POD$ $ratio_{50}$, the median was 1.2 and the range was −2.9 to 7.0.

As the concentration range evaluated in the ToxCast assays may limit the upper bound of the POD_{NAM}, a comparison between the $POD_{traditional}$ and the maximum AED possible from high-throughput screening was also calculated. The maximum AED, using the 95^{th} percentile prediction for the C_{ss} and using 100 μM as the input concentration, was calculated. This maximum AED (Figure 3, Figure 7B) was based on the general assumption that no ToxCast library substances would be screened at nominal concentrations that exceeded 100 μM. For the 48 substances with a $log_{10}POD$ $ratio_{95}$ < 0, the maximum AED exceeded the minimum $POD_{traditional}$ in all cases. In contrast, for the remaining 400 substances with $log_{10}POD$ $ratio_{95}$ > 0, 60% had a maximum AED that was less than the minimum $POD_{traditional}$ (Figure 7B).

Similar to evaluation of the chemicals with $log_{10}BER_{95} < 0$, hypotheses regarding why substances demonstrated a $log_{10}POD$ $ratio_{95}$ < 0 were considered. First, the chemical domain was considered via calculation of statistical enrichment of ToxPrint chemical structure features, or chemotypes (CTs) (Strickland, et al., 2018; Yang, et al., 2015) (Table 3). Through this analysis, six CTs were identified as enriched, with OR ≥ 3 and p-value ≤ 0.05. The local balanced accuracy (BA) values (within a CT subspace) ranged from 0.57 to

0.62, with the bond:P=0_phosphate_thio CT completely contained within the $\log_{10}$POD ratio$_{95}$ < 0 space. Of the 48 substances with $\log_{10}$POD ratio$_{95}$ < 0, half (24 substances) contained one or more enriched CTs, corresponding to structural features indicative of organophosphate or carbamate related chemistries. Twenty-one of these 24 substances have clear indication of being a carbamate or organophosphate pesticide.

As ToxCast assay endpoints do not completely cover biological space, and generally include only short-term assays, the hypothesis that ToxCast data failed to identify chemicals that demonstrated critical effects in developmental/reproductive or chronic studies was tested via enrichment analysis of the study type that underpinned the minimum $POD_{traditional}$ for each substance. Using a Fisher's exact test, we failed to observe any significant enrichment of developmental/reproductive studies (grouped for this analysis, $p < 0.87$) or chronic studies (p-value < 0.69) in the $\log_{10}$POD ratio$_{95}$ < 0 space. The frequency of developmental/reproductive and chronic studies defining the minimum $POD_{traditional}$ is illustrated in the matrices in Figure 8. Though there were no significant enrichments of study type for the minimum $POD_{traditional}$ for chemicals with $\log_{10}$POD ratio$_{95}$ < 0, it is interesting to note that chronic toxicity data appeared to generate the minimum $POD_{traditional}$ more often, in general (for 272 out of 448 chemicals). However, this may be due to the fact that not all chemicals in the case study had all study types, introducing a major caveat to this analysis, such that broader inferences about the importance of study type are limited.

3.4 Comparison of $POD_{NAM,95}$ to a TTC approach

Considering that, in the absence of HTS data, one approach to screening level risk assessment might be to employ a TTC to help in prioritization (HealthCanada, 2016), the POD_{NAM} was further compared to a TTC developed using ToxTree (Patlewicz, et al., 2008; ToxTree, 2015). For the 448 substances in this case study, the following TTC values were defined: 141 substances were designated as 0.0025 μg/kg-bw/day (potential genotoxic chemical threshold), 36 substances were designated as 0.3 μg/kg-bw /day (OP or carbamate), 212 substances were designated as 1.5 μg/kg-bw /day (Cramer Class III), 29 substances were designated as 30 μg/kg-bw /day (Cramer Class I), and 5 substances were designated as 9 μg/kg/day (Cramer Class II). Twelve substances demonstrated exclusion criteria (6 steroids, 2 metals, 2 benzidines, 1 organosilicon, 1 N-nitroso), and finally, 3 substances lacked a defined structure as previously described.

The primary observation from this comparison is that the POD_{NAM} generally appeared to be greater than the TTC value, with the $POD_{NAM,95}$ greater than the TTC for 87% of the substances (389/448) and the $POD_{NAM,50}$ greater than the TTC for 92% of the substances (413/448). The median $\log_{10}POD_{NAM,95}$:TTC ratio was 2.25, suggesting that on average there is approximately a 100-fold difference between these two predictions (Figure 9A). This finding may be partly explained by the methods used to develop the TTC values for each chemical class which are analogous to the approaches used in quantitative cancer risk assessment or in the development of a reference dose. For potential genotoxic chemicals the TTC value was developed through the use of linear lose dose extrapolation to 1 in 10^6 lifetime risk-based on reference chemicals in a carcinogenicity database (Kroes, et al., 2004). For the non-cancer portion of the decision tree (Cramer classifications, OPs and

carbamates) the TTC values were developed including the application of an uncertainty factor (UF) (e.g. UF of 100 applied to the 5th percentile from a distribution of NOELs from Cramer classified substances making up a repeat dose reference database (Kroes, et al., 2004). No UFs are applied to the POD_{NAM} (or the $POD_{traditional}$) in this analysis. In addition, we failed to observe a linear relationship between the $log_{10}TTC$ value and the $log_{10}POD_{NAM,95}$ value, with considerable variability in the $log_{10}POD_{NAM,95}$ values reported within each TTC value category (Figure 9B). As the BER has been suggested as a prioritization metric, and BER is the quotient of the POD_{NAM} and the exposure prediction, we also examined how replacing $log_{10}POD_{NAM,95}$ with TTC would have affected the $log_{10}BER_{95}$ for the 11 substances with $log_{10}BER_{95} < 0$. Interestingly, for the 11 substances with $log_{10}BER_{95} < 0$, the $log_{10}TTC$ was greater than the $log_{10}POD_{NAM,95}$ for 8 substances, and only one substance (napthalene, DTXSID8020913) still had a $log_{10}BER < 0$ when using the $log_{10}TTC$ instead of the $log_{10}POD_{NAM,95}$.

3.5 Comparison of POD_{NAM} to $POD_{traditional}$ from allometrically scaled data

For the main case study, $POD_{traditional}$ data were collected from any *in vivo* toxicology study, regardless of species or strain, and then grouped to derive a 5th percentile from the distribution by chemical. In contrast, the POD_{NAM} was derived from an AED that was calculated using human HTTK parameters and *in vitro* bioactivity (mostly from human cell lines and proteins). To address the issue of combining multiple species in this analysis, we compared the $log_{10}POD_{NAM,95}$, derived from an AED calculated using human HTTK parameters, to a human equivalent dose, i.e. human $POD_{traditional}$, based on allometric scaling of the $POD_{traditional}$ data. Limiting to studies in mouse, rat, guinea pig, rabbit, dog, and hamster, 447 of the 448 chemicals could be included in this comparison. The human $POD_{traditional}$ is derived from $POD_{traditional}$ via multiplication by a factor less than one (derived based on body surface area by species) (Nair, et al., 2016). This led to 82 substances (18%) having $log_{10}POD_{NAM,95}$ higher than the human $POD_{traditional}$ (compared to 48 when using the animal-based $POD_{traditional}$), and 158 substances for which the $log_{10}POD_{NAM,50}$ was higher than the human $POD_{traditional}$ (compared to 92 when using the animal-based $POD_{traditional}$). The median human $log_{10}POD\ ratio_{95}$ was 1.33, with a range of −3.3 to 6.7 (Supplemental Appendix, Figure 7). Given that the mechanism(s) of toxicity and metabolic processes may differ considerably across species, a consideration of differences in dosing based on allometric scaling (i.e., surface area) provides limited information regarding uncertainty based on interspecies differences.

4 Discussion

Herein we present a retrospective analysis to address two key questions: (1) would using *in vitro* bioactivity data from HTS programs such as ToxCast provide a “lower bound” estimate of a POD when compared to traditional toxicology approaches? And, (2) is the BER, using *in vitro* bioactivity across a broad range of assays, a useful tool for prioritization of substances? This analysis is the largest of its kind presented to date, with information for 448 substances included. A major premise of this work is that the minimal concentration corresponding to *in vitro* bioactivity is likely to be a threshold for any specific effects or toxicities that might be observed *in vivo*. The primary conclusion of our work is that for

89% of the chemicals in this case study, the HTS approach to derivation of a $POD_{NAM,95}$ for screening and prioritization purposes produced a value less than or equal to the $POD_{traditional}$ from *in vivo* toxicology studies. Further, we found that BER may be a useful data-driven metric for prioritization that can be customized to the resources available for follow-up, i.e. different choices in calculation of the BER can be made depending on how much uncertainty is acceptable and how many substances can be further evaluated given resource constraints. The customizable decisions in the HTS approach employed herein are demonstrated in adjustments in the amount of uncertainty in (1) the IVIVE that is included in development of the POD_{NAM} and (2) the exposure predictions, highlighting that for different screening applications differing amounts of uncertainty can be included in this workflow. As demonstrated, metrics that account for more of these uncertainties (e.g., use of a 95^{th} percentile rather than a median on exposure predictions, or use of a $POD_{NAM,95}$ instead of a $POD_{NAM,50}$) can be used in a screening and prioritization application (see Table 2). The context for use of the POD_{NAM} was further examined via comparison to a TTC approach, ultimately demonstrating that there may be some advantages to combining these approaches for preliminary screening of substances for safety. The collaborative, international consideration of these issues in screening level assessments demonstrates the current state-of-the-science and presents a transparent and adaptable basis for utilization of HTS information.

A potential concern regarding the approach used in the case study might be whether it is overly conservative, i.e. whether the POD_{NAM} values are too low or the exposure predictions are too high. To begin to address such a question, it is necessary to consider the impacts of the selections and assumptions made in the current approach. First, we consider the uncertainty that we consider in the use of NAM-based exposure predictions; the ExpoCast SEEM2 model is akin to a low-tier exposure assessment tool, and grappling with uncertainty in exposure prediction is more familiar to traditional safety evaluation (EPA, ExpoBox). The exposure predictions (Wambaugh, et al., 2014) from the ExpoCast SEEM2 analysis have wide credible intervals, as demonstrated by the shifting of the $\log_{10}BER_{95}$ by approximately 2 $\log_{10}$ units based on selection of either the median or 95^{th} percentile exposure predictions. In selecting the 95^{th} percentile exposure prediction for most of the comparative analyses in this work, we are using a value that includes more of the uncertainty, and the median could be used instead depending on the number of substances that should be prioritized and the degree of certainty that is desired for a given application of these data. The fact that only 2.5% of the 448-chemical case study would have a $\log_{10}BER_{95}$ less than zero, and only 15% with a $\log_{10}BER_{95}$ less than 2, indicates that though the approach the $\log_{10}BER_{95}$ attempts to account for more uncertainty, it does not necessarily indicate a high priority for all substances in the case study. There are caveats to this conclusion in that the exposure model was informed by functional use categories, and pesticide actives, which comprised 70% of the substances with sufficient data for inclusion in this case study, were generally predicted to have lower exposure than substances associated with other use categories (Wambaugh, et al., 2014). It is possible that using a substance list that included more substances associated with uses as pesticide inerts, personal care products, or other functional uses (Dionisio, et al., 2015) that more substances would be prioritized using the BER. In comparing exposure estimates from the ExpoCast SEEM2 model and curated exposure assessments from Health

Canada, we found that the ExpoCast 95th percentile was a reasonable surrogate for 18 substances. Of these substances, the few that demonstrated larger differences (i.e., greater than 1 $\log_{10}$mg/kg-bw/day different) were likely related to reliance on different use and exposure scenarios between the ExpoCast SEEM2 model and the exposure assessments, supporting refinements in understanding of use and exposure scenario as essential for improved confidence in utilization of high-throughput exposure estimates (Biryol *et al.*, 2017; Brandon *et al.*, 2018; Dionisio *et al.*, 2018; Ring *et al.*, 2018). Moving forward with safety evaluation of substances across geographies, international collaboration and data-sharing regarding substance use categories and exposure scenarios or pathways will support progress in exposure prediction for thousands of substances.

As used in the aggregate in this case study, the *in vitro* bioactivity data was used to define a concentration range for any activity and does not necessarily support hypotheses regarding specific toxicological effects. Disruption of molecular targets as described by the POD_{NAM} is necessary, but not sufficient, for producing adverse effects. In contrast, the $POD_{traditional}$ is intended to represent a threshold for adversity; as such, we might anticipate that the POD_{NAM} would in many cases be lower than an estimate of $POD_{traditional}$. Caveats in the use of ToxCast data include the possibility that, despite an attempt to filter these data for more reliable curve-fits (see Methods and Supplemental Appendix), we have not completely eliminated results from noisy curve-fits or assay interference, thereby resulting in the possible inclusion of some AC_{50} values that are not reproducible and/or biologically meaningful. This may bias the distribution of AC_{50} values available for substances, and thus detailed examination of the *in vitro* bioactivity data may be warranted when deriving screening level assessment values or following up on specific substances identified as a priority based on the BER. The use of a point estimate for the potency value (i.e., AC_{50}) without use of confidence bounds (Watt, et al., 2018) may produce a higher AED value than using a threshold concentration based on a confidence interval. The need for applied research on quality control processes for improved filtering of any HTS data to include more reproducible and/or more reliable curves clearly emerges from this case study. Use of the 5th percentile for the ToxCast AC_{50} values per substance ameliorates some concerns with the appearance of low concentration outliers in the AC_{50} distribution (likely from less reproducible curve fits) (see Supplemental Appendix, Supplemental Figure 3), but also provides a “lower bound” estimate of a threshold concentration for bioactivity *in vitro*. The number of detailed, and thereby customizable, decisions made in determining an *in vitro* concentration for use in IVIVE is apparent.

Consideration of how much uncertainty the POD_{NAM} should include in the IVIVE approach used is needed. We assumed steady state conditions and complete bioavailability when converting the *in vitro* potency values to AED values (Wetmore, et al., 2014; Wetmore, et al., 2015; Wetmore, et al., 2013; Wetmore, et al., 2012). The assumption of steady state conditions may be fairly accurate for pharmaceuticals and may also work for some but not all of diverse, environmentally-relevant chemicals (Sipes *et al.*, 2017a; Wambaugh, et al., 2018; Wambaugh *et al.*, 2015; Wang, 2010; Wetmore, et al., 2015; Yoon *et al.*, 2014). However, beyond the assumption of steady state conditions, other assumptions in the HTTK and IVIVE approach may err on the side of lower AED predictions due to the lack of extrahepatic metabolic clearance; use of suspended hepatocyte model for capturing hepatic

metabolism; and, lack of information about bioavailability (Wetmore, et al., 2015). Available high-throughput IVIVE methods are being continuously improved and more data is being added to reduce assumptions in the modeling (e.g., estimates of bioavailability, extrahepatic clearance). For this analysis, we demonstrated how inter-individual variability can be accounted for by using the 95th percentile estimate of the C_{ss} from a Monte Carlo simulation, which may result in a relatively low estimate of the $POD_{NAM,95}$. We compared this to the $POD_{NAM,50}$ because, dependent on the screening level application, it may be desirable to exclude inter-individual variability from initial computation of the POD_{NAM}. The difference between the $POD_{NAM,50}$ and $POD_{NAM,95}$ is variable by substance, i.e. the range of potential C_{ss} values observed across a theoretical population is dependent on metabolic and renal clearance, plasma protein binding, and other features that are substance-dependent (for additional consideration, see Supplemental Appendix, Supplemental Figure 5). Ultimately, despite a number of approximations and uncertainties, the current workflow demonstrates the potential utility of the approach for calculation of the POD_{NAM} using data and tools that are currently available.

For the substances in this case study, the $POD_{NAM,50}$ and $POD_{NAM,95}$ were lower or approximately equal for the $POD_{traditional}$ 80% and 89% of the substances, respectively. The $POD_{NAM,95}$ and resultant $log_{10}POD\ ratio_{95}$ and $log_{10}BER_{95}$ were selected for further detailed analyses in this work because this represented an approach that included consideration of more uncertainty and narrowed the substances with $log_{10}POD$ ratio and $log_{10}BER$ values < 0 to clearly identify possible limitations in the NAM-based approach. One limitation evident from the CT enrichment work is that the current NAM battery in ToxCast and HIPPTox, combined with IVIVE, failed to quantitatively capture the potency of effects expected for substances that contain structural features of carbamate and organophosphate insecticides. ToxCast does contain assays responsive to acetylcholinesterase inhibitors (Padilla *et al.*, 2012; Sipes *et al.*, 2013); however, it has been previously suggested that these assays lack the ability to accurately reflect acetylcholinesterase inhibition potency (Aylward *et al.*, 2011). Additionally, often OP metabolites are more potent acetylcholinesterase inhibitors, and this would not be well-captured in assays with limited to no metabolism. For substances with the structural features of carbamate and/or organophosphate insecticides, a TTC approach like the one employed herein (which includes a separate workflow for these chemistries) may provide a more useful POD_{NAM} value that would be less than or equal to the $POD_{traditional}$.

Another potential limitation of the NAM-based approach included in this work is that the ToxCast and HIPPTox assays are short-term in duration. One hypothesis as to why a substance would demonstrate a $log_{10}POD\ ratio_{95} < 0$ was that the $POD_{traditional}$ may have been effects observed *in vivo* following exposures of longer duration or involving specific susceptible lifestages. Interestingly, the minimum $POD_{traditional}$ was not associated with a particular study type, as a surrogate for phenotype (i.e. developmental/reproductive or chronic studies), more frequently for substance with $log_{10}POD\ ratio_{95} < 0$. Conclusions from this analysis of study type are necessarily limited because not all substances in the case study included all study types. To allow for the largest data set possible, each substance included in the case study may have had traditional toxicity information available from

multiple sources and study types, and in some instances, it is possible that multiple records may correspond to the same study.

Although the *in vitro* bioactivity approach to definition of a POD_{NAM} has some associated uncertainties, in the absence of other information, the POD_{NAM} could be used as a "lower bound" or protective estimate. To reduce potential uncertainties, the data used to derive the POD_{NAM} could always be further reviewed and refined in a number of ways, at the expense of performing a more automated analysis for many substances. First, manual or semi-automated curation of ToxCast/Tox21 data to select the *in vitro* bioactivity concentration based only on assays and curve-fits with high reproducibility could be performed for substances identified as priority from automated analyses. Second, the use of metabolically-competent *in vitro* models to predict the bioactivity of parent and metabolite(s) may account for potential bioactivated toxicants (DeGroot *et al.*, 2018; Ramaiahgari *et al.*, 2017). Additional bioactivity screening using metabolically-competent *in vitro* models would be needed to understand the potential impacts of metabolism on a workflow like the one employed herein. Third, the specific data or assay data-based predictions to indicate specific potential adversities, modeling neurotoxicity, hepatotoxicity, reproductive, or developmental toxicity, in an expansion of the HIPPTox and ToxCast approaches included here, could be added. This also relates to a limitation in that not all substances in this case study were tested in all available assays in ToxCast or HIPPTox. Fourth, the addition of high data content *in vitro* assays, e.g. high-throughput transcriptomics and/or cellular phenotypic profiling data like HIPPTox, may help comprehensively cover the biological pathways possibly disrupted by the test substances. Finally, further refinement of the IVIVE approach may also improve the utility of the POD_{NAM}. Though currently the nominal media concentrations from assay endpoints are used in calculation of AED values that form the basis of the POD_{NAM}, refinement of the IVIVE modeling procedure to account for differential *in vitro* partitioning could reduce uncertainty (Fischer *et al.*, 2017).

Comparison of the *in vitro* bioactivity approach to a TTC-based approach for definition of a POD_{NAM} provides some practical insight for screening-level evaluation. When available, the POD_{NAM}, which uses data generated for a particular target substance, generally provides a more refined estimate of a POD than the TTC. Further, the POD_{NAM} values are likely to improve and change over time as more sophisticated and comprehensive HTS tools are developed. Though the TTC value is generally lower than the POD_{NAM}, there may be advantages to using POD_{NAM} and TTC in concert. In some cases, a TTC cannot be easily generated due to exclusion criteria or the presence of multiple structures in an undefined mixture, whereas a POD_{NAM} may be generated. In the fraction of cases where the $\log_{10}$TTC value was greater than the $\log_{10}POD_{NAM,95}$, the $\log_{10}$TTC could be used as a check on low POD_{NAM} values. Indeed, Health Canada recently applied the TTC approach to a group of substances amongst the remaining priorities under the CMP. A preliminary qualitative characterization of uses and exposure potential indicated that 237 of the CMP substances were good candidates for the TTC approach as exposure to the general population was expected to be limited. However, after developing quantitative exposure estimates only 89 of the 237 substances (38%) had exposures below their respective TTC values (HealthCanada, 2016). Thus, having an approach such as the use of POD_{NAM} to develop a BER may have

proven useful as an additional screening level tool, and a suite of *in silico* and *in vitro* NAMs, used collectively, provide an informed, multidimensional screening level approach.

There are several considerations when using the $POD_{traditional}$. For the $POD_{traditional}$ data themselves, toxicologists generally accept that animal studies conducted at different times, by different laboratories, with a different cohort of perhaps the same strain of animal, may yield results that are qualitatively and/or quantitatively distinct (Gottmann *et al.*, 2001; Kleinstreuer, et al., 2017; Ward *et al.*, 2017; Wolf *et al.*, 2017). Though we do not quantify this variability here, the resolution of the difference between the POD_{NAM} and $POD_{traditional}$ may be limited by the variability in the animal study results (Casati, et al., 2018) in addition to the variability in the *in vitro* bioactivity methods. A continuing challenge and necessity for comparing the results of NAM will be establishing the variability in reference set data. An additional limitation in interpreting this work is comparison of POD_{NAM}, largely from human *in vitro* data, to $POD_{traditional}$, which is based on several different mammalian species. Though allometric scaling based on differences in animal surface area was performed for comparison in this work, the value of this exercise is limited as species-specific absorption, distribution, metabolism, and excretion processes might be anticipated on a substance by substance basis. Since humans are the species of interest for this case study, POD_{NAM} based on mostly human bioactivity data were used. In the risk assessment process, an uncertainty factor of 10 might be used when considering interspecies differences.

Related issues (made apparent in this case study) in using traditional toxicology information as a reference for the NAM-based approach are the challenges of data curation and interoperability. For instance, clarification of records that summarize the same original study, or identification of specific effects observed, is limited in current publicly available databases of toxicity information (e.g., ToxValDB, ToxRefDB, eChemPortal, etc.) because of a lack of controlled semantic ontology for study features and biological effects. For international collaborations such as this one, it is not straightforward to identify the unique toxicological studies nor specific effects labeled using different terminology. Moreover, the basis for establishing the PODs from the original studies, regardless of their origin, may not be fully structured or reported, and differences in POD selection from different reviewers may arise. Consistent identification of the substance tested, and controlled vocabularies describing study designs and the reported effects, are needed to share curated data across databases, and across the world, in order to leverage the largest dataset possible for improved understanding of traditional toxicity information for regulatory toxicology. Efforts to create such a large, consistent, and reliable dataset are an ongoing interest within the APCRA initiative, among others. Improved curation and digitization of traditional toxicity information for comparison with NAMs across a broad range of endpoints and study types will require a significant investment of resources.

We have presented herein a retrospective analysis to demonstrate the utility of the BER in identifying potential priority chemicals for further evaluation, as well as the conservativism of a POD_{NAM} when compared to $POD_{traditional}$. The result bolsters confidence that decisions based on a POD_{NAM} can be health protective in screening level assessments, lending support for using approaches like this one to rapidly evaluate substances and potential needs for further screening information. Using relatively conservative assumptions of predicted

exposure and bioactivity, only 15% of the substances in this case study demonstrated a $\log_{10}BER_{95} < 2$, suggesting that this approach may provide a data-informed and reasonable approach to identifying chemicals of interest for further testing and assessment. A primary goal of the APCRA collaboration supporting this work is to identify and resolve impediments to adoption of NAMs in safety assessment. In demonstrating the state-of-the-science for generation of BER and POD ratio values, we have shown in practice for 448 chemicals a way to accelerate screening and assessment using NAMs for hazard and exposure. Further, we have identified a number of areas for refinement in this workflow to be considered in subsequent application of this NAM-based approach to regulatory toxicology questions. Ongoing work will be needed to demonstrate that a workflow like the one demonstrated herein is generalizable to substances with little to no available traditional POD information. As continuing improvements in HTS approaches and availability and interoperability of database resources are made, confidence in the utilization of NAMs for screening level assessments will be bolstered by scientific quality, relevance, and transparency.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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7 References

Aylward LL, and Hays SM (2011). Consideration of dosimetry in evaluation of ToxCast data. J Appl Toxicol 31(8), 741–51. [PubMed: 21381051]

Becker RA, Friedman KP, Simon TW, Marty MS, Patlewicz G, and Rowlands JC (2015). An exposure:activity profiling method for interpreting high-throughput screening data for estrogenic activity--proof of concept. Regul Toxicol Pharmacol 71(3), 398–408. [PubMed: 25656492]

Bell SM, Chang X, Wambaugh JF, Allen DG, Bartels M, Brouwer KLR, Casey WM, Choksi N, Ferguson SS, Fraczkiewicz G, et al. (2017). In vitro to in vivo extrapolation for high throughput prioritization and decision making. Toxicol In Vitro 47, 213–227. [PubMed: 29203341]

Biryol D, Nicolas CI, Wambaugh J, Phillips K, and Isaacs K (2017). High-throughput dietary exposure predictions for chemical migrants from food contact substances for use in chemical prioritization. Environ Int 108, 185–194. [PubMed: 28865378]

Brandon N, Dionisio KL, Isaacs K, Tornero-Velez R, Kapraun D, Setzer RW, and Price PS (2018). Simulating exposure-related behaviors using agent-based models embedded with needs-based artificial intelligence. J Expo Sci Environ Epidemiol doi: 10.1038/s41370-018-0052-y.

Browne P, Judson RS, Casey WM, Kleinstreuer NC, and Thomas RS (2015). Screening Chemicals for Estrogen Receptor Bioactivity Using a Computational Model. Environ Sci Technol 49(14), 8804–14. [PubMed: 26066997]

Casati S, Aschberger K, Barroso J, Casey W, Delgado I, Kim TS, Kleinstreuer N, Kojima H, Lee JK, Lowit A, et al. (2018). Standardisation of defined approaches for skin sensitisation testing to support regulatory use and international adoption: position of the International Cooperation on Alternative Test Methods. Arch Toxicol 92(2), 611–617. [PubMed: 29127450]

Commission E (2007). Registration, Evaluation, Authorisation and Restriction of Chemicals. In Regulation (EC) No 1907/2006 of the European Parliament and of the Council (

Cote I, Andersen ME, Ankley GT, Barone S, Birnbaum LS, Boekelheide K, Bois FY, Burgoon LD, Chiu WA, Crawford-Brown D, et al. (2016). The Next Generation of Risk Assessment Multi-Year Study-Highlights of Findings, Applications to Risk Assessment, and Future Directions. Environ Health Perspect 124(11), 1671–1682. [PubMed: 27091369]

DeGroot DE, Swank A, Thomas RS, Strynar M, Lee MY, Carmichael PL, and Simmons SO (2018). mRNA transfection retrofits cell-based assays with xenobiotic metabolism. J Pharmacol Toxicol Methods 92, 77–94. [PubMed: 29555536]

Dionisio KL, Frame AM, Goldsmith MR, Wambaugh JF, Liddell A, Cathey T, Smith D, Vail J, Ernstoff AS, Fantke P, et al. (2015). Exploring consumer exposure pathways and patterns of use for chemicals in the environment. Toxicol Rep 2, 228–237. [PubMed: 28962356]

Dionisio KL, Phillips K, Price PS, Grulke CM, Williams A, Biryol D, Hong T, and Isaacs KK (2018). The Chemical and Products Database, a resource for exposure-relevant data on chemicals in consumer products. Sci Data 5, 180125. [PubMed: 29989593]

EC/HC (2008). Screening assessment for the Challenge: 1,2-benzenediol (catechol), Chemical Abstracts Service Registry Number 120-80-9. In (Vol. http://www.ec.gc.ca/eseees/default.asp?lang=En&n=04FDC10E-1#a5. Government of Canada, Ottawa (ON).

EC/HC (2011). Screening assessment for the Challenge: Hexanedioic acid, bis(2-ethylhexyl) ester (DEHA) Chemical Abstracts Service Registry Number 103-23-1. In (Vol. http://www.ec.gc.ca/ese-ees/default.asp?lang=En&n=39958D25-1#a9 Government of Canada, Ottawa (ON).

EC/HC (2017). Updated draft screening assessment: Chlorhexidine and its Salts. In (Vol. http://www.ec.gc.ca/ese-ees/default.asp?lang=En&n=59BDF713-1#toc-071 Government of Canada, Ottawa (ON).

ECCC/HC (2016a). Chemicals Management Plan (CMP) Science Committee Objectives Paper Meeting no. 5 - Integrating New Approach Methodologies within the CMP: Identifying Priorities for Risk Assessment, Existing Substances Risk Assessment Program. In (Vol. Retrieved from http://www.ec.gc.ca/eseees/default.asp?lang=En&n=172614CE-1. Government of Canada, Ottawa (ON).

ECCC/HC (2016b). Chemicals Management Plan In (H. C. Environment and Climate Change, Ed.), Vol. https://www.canada.ca/en/health-canada/services/chemical-substances/chemicals-management-plan.html. Government of Canada, Ottawa (ON).

ECHA New Approach Methodologies in Regulatory Science, Proceedings of a scientific workshop, April 19–20, 2016 2016, Helsinki, Finland Available at: https://echa.europa.eu/documents/10162/22816069/scientific_ws_proceedings_en.pdf/a2087434-0407-4705-9057-95d9c2c2cc57. Accessed September 3, 2018.

ECHA (2017). ECHA Strategic Plan 2019–2023. In (

EFSA (2012). Scientific Opinion on Exploring Options for Providing Advice about Possible Human Health Risks Based on the Concept of Threshold of Toxicological Concern (TTC). In (Vol. 10, EFSA Journal.

EFSA (2018). Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009. In (doi: 10.2903/j.efsa.2018.5311, https://www.efsa.europa.eu/it/efsajournal/pub/5311.

Egeghy PP, Judson R, Gangwal S, Mosher S, Smith D, Vail J, and Cohen Hubal EA (2012). The exposure data landscape for manufactured chemicals. Sci Total Environ 414, 159–66. [PubMed: 22104386]

Embry MR, Bachman AN, Bell DR, Boobis AR, Cohen SM, Dellarco M, Dewhurst IC, Doerrer NG, Hines RN, Moretto A, et al. (2014). Risk assessment in the 21st century: roadmap and matrix. Crit Rev Toxicol 44 Suppl 3, 6–16. [PubMed: 25070414]

EPA, U. (2018a). Final Strategic Plan to Promote Development and Implementation of Alternative Test Methods Supporting Toxic Substances Control Act. In (Vol. 83, pp. 30167–30168. Federal Register, Washington, D.C.

EPA, U. (2018b). ToxCast & Tox21 Data from invitrodb_v3. In (Retrieved from http://www2.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data.

EPA, U. (ExpoBox). EPA ExpoBox: Exposure Assessment Tools by Tiers and Types - Screening-Level and Refined. Available at: https://www.epa.gov/expobox/exposure-assessment-tools-tiers-and-types-screening-level-and-refined. Accessed Febuary 13, 2019.

Faucette SR, Wang H, Hamilton GA, Jolley SL, Gilbert D, Lindley C, Yan B, Negishi M, and LeCluyse EL (2004). Regulation of CYP2B6 in primary human hepatocytes by prototypical inducers. Drug Metab Dispos 32(3), 348–58. [PubMed: 14977870]

Filer DL, Kothiya P, Setzer RW, Judson RS, and Martin MT (2017). tcpl: the ToxCast pipeline for high-throughput screening data. Bioinformatics 33(4), 618–620. [PubMed: 27797781]

Fischer FC, Henneberger L, Konig M, Bittermann K, Linden L, Goss KU, and Escher BI (2017). Modeling Exposure in the Tox21 in Vitro Bioassays. Chem Res Toxicol 30(5), 1197–1208. [PubMed: 28316234]

Gottmann E, Kramer S, Pfahringer B, and Helma C (2001). Data quality in predictive toxicology: reproducibility of rodent carcinogenicity experiments. Environ Health Perspect 109(5), 509–14. [PubMed: 11401763]

Hariparsad N, Ramsden D, Palamanda J, Dekeyser JG, Fahmi OA, Kenny JR, Einolf H, Mohutsky M, Pardon M, Siu YA, et al. (2017). Considerations from the IQ Induction Working Group in Response to Drug-Drug Interaction Guidance from Regulatory Agencies: Focus on Downregulation, CYP2C Induction, and CYP2B6 Positive Control. Drug Metab Dispos 45(10), 1049–1059. [PubMed: 28646080]

HealthCanada (2016). Science Approach Document: Threshold of Toxicological Concern (TTC)-based Approach for Certain Substances In (E. S. R. A. Bureau, Ed.). Government of Canada, Ottawa, Ontario.

Jamei M, Dickinson GL, and Rostami-Hodjegan A (2009). A framework for assessing inter-individual variability in pharmacokinetics using virtual human populations and integrating general knowledge of physical chemistry, biology, anatomy, physiology and genetics: A tale of ‘bottom-up’ vs ‘top-down’ recognition of covariates. Drug Metab Pharmacokinet 24(1), 53–75. [PubMed: 19252336]

Judson R, Houck K, Martin M, Knudsen T, Thomas RS, Sipes N, Shah I, Wambaugh J, and Crofton K (2014). In vitro and modelling approaches to risk assessment from the U.S. Environmental Protection Agency ToxCast programme. Basic Clin Pharmacol Toxicol 115(1), 69–76. [PubMed: 24684691]

Judson R, Richard A, Dix DJ, Houck K, Martin M, Kavlock R, Dellarco V, Henry T, Holderman T, Sayre P, et al. (2009). The toxicity data landscape for environmental chemicals. Environ Health Perspect 117(5), 685–95. [PubMed: 19479008]

Judson RS, Kavlock RJ, Setzer RW, Hubal EA, Martin MT, Knudsen TB, Houck KA, Thomas RS, Wetmore BA, and Dix DJ (2011). Estimating toxicity-related biological pathway altering doses for high-throughput chemical risk assessment. Chem Res Toxicol 24(4), 451–62. [PubMed: 21384849]

Kavlock R (2016). Practitioner Insights: Bringing New Methods for Chemical Safety into the Regulatory Toolbox; It is Time to Get Serious. Bloomberg BNA Daily Environment REport(223 B-1).

Kavlock R, Chandler K, Houck K, Hunter S, Judson R, Kleinstreuer N, Knudsen T, Martin M, Padilla S, Reif D, et al. (2012). Update on EPA’s ToxCast program: providing high throughput decision support tools for chemical risk management. Chem Res Toxicol 25(7), 1287–302. [PubMed: 22519603]

Kavlock RJ, Bahadori T, Barton-Maclaren TS, Gwinn MR, Rasenberg M, and Thomas RS (2018). Accelerating the Pace of Chemical Risk Assessment. Chem Res Toxicol 31(5), 287–290. [PubMed: 29600706]

Kleinstreuer NC, Ceger P, Watt ED, Martin M, Houck K, Browne P, Thomas RS, Casey WM, Dix DJ, Allen D, et al. (2017). Development and Validation of a Computational Model for Androgen Receptor Activity. Chem Res Toxicol 30(4), 946–964. [PubMed: 27933809]

Kroes R, Renwick AG, Cheeseman M, Kleiner J, Mangelsdorf I, Piersma A, Schilter B, Schlatter J, van Schothorst F, Vos JG, et al. (2004). Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. Food Chem Toxicol 42(1), 65–83. [PubMed: 14630131]

Laksameethanasan D, Tan R, Toh G, and Loo LH (2013). cellXpress: a fast and user-friendly software platform for profiling cellular phenotypes. BMC Bioinformatics 14 Suppl 16, S4.

Lautenberg FR (2016). Frank R. Lautenberg Chemical Safety for the 21st Century Act. In (Congress t. U., Ed.), pp. 114–182. Public Law.

Lee JJ, Miller JA, Basu S, Kee TV, and Loo LH (2018). Building predictive in vitro pulmonary toxicity assays using high-throughput imaging and artificial intelligence. Arch Toxicol 92(6), 2055–2075. [PubMed: 29705884]

Loo LH, Wu LF, and Altschuler SJ (2007). Image-based multivariate profiling of drug responses from single cells. Nat Methods 4(5), 445–53. [PubMed: 17401369]

Martin MT, Judson RS, Reif DM, Kavlock RJ, and Dix DJ (2009a). Profiling chemicals based on chronic toxicity results from the U.S. EPA ToxRef Database. Environ Health Perspect 117(3), 392–9. [PubMed: 19337514]

Martin MT, Mendez E, Corum DG, Judson RS, Kavlock RJ, Rotroff DM, and Dix DJ (2009b). Profiling the reproductive toxicity of chemicals from multigeneration studies in the toxicity reference database. Toxicol Sci 110(1), 181–90. [PubMed: 19363143]

Nair AB, and Jacob S (2016). A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm 7(2), 27–31. [PubMed: 27057123]

NCCT (2018). tcpl version 2.0. CRAN.

Padilla S, Corum D, Padnos B, Hunter DL, Beam A, Houck KA, Sipes N, Kleinstreuer N, Knudsen T, Dix DJ, et al. (2012). Zebrafish developmental screening of the ToxCast Phase I chemical library. Reprod Toxicol 33(2), 174–87. [PubMed: 22182468]

Patlewicz G, Jeliazkova N, Safford RJ, Worth AP, and Aleksiev B (2008). An evaluation of the implementation of the Cramer classification scheme in the Toxtree software. Sar and Qsar in Environmental Research 19(5–6), 495–524. [PubMed: 18853299]

Paul Friedman K, Papineni S, Marty MS, Yi KD, Goetz AK, Rasoulpour RJ, Kwiatkowski P, Wolf DC, Blacker AM, and Peffer RC (2016). A predictive data-driven framework for endocrine prioritization: a triazole fungicide case study. Crit Rev Toxicol 46(9), 785–833. [PubMed: 27347635]

Pearce RG, Setzer RW, Strope CL, Sipes NS, and Wambaugh JF (2017). httk: R Package for High-Throughput Toxicokinetics. Journal of Statistical Software 79(4).

Perkins EJ, Habib T, Escalon BL, Cavallin JE, Thomas L, Weberg M, Hughes MN, Jensen KM, Kahl MD, Villeneuve DL, et al. (2017). Prioritization of Contaminants of Emerging Concern in Wastewater Treatment Plant Discharges Using Chemical:Gene Interactions in Caged Fish. Environ Sci Technol 51(15), 8701–8712. [PubMed: 28651047]

Pradeep P, Mansouri K, Patlewicz G, and Judson R (2017). A systematic evaluation of analogs and automated read-across prediction of estrogenicity: A case study using hindered phenols. Comput Toxicol 4, 22–30. [PubMed: 30057968]

Ramaiahgari SC, Waidyanatha S, Dixon D, DeVito MJ, Paules RS, and Ferguson SS (2017). Three-Dimensional (3D) HepaRG Spheroid Model With Physiologically Relevant Xenobiotic Metabolism Competence and Hepatocyte Functionality for Liver Toxicity Screening. Toxicol Sci 160(1), 189–190. [PubMed: 29077947]

Richard AM, Judson RS, Houck KA, Grulke CM, Volarath P, Thillainadarajah I, Yang C, Rathman J, Martin MT, Wambaugh JF, et al. (2016). ToxCast Chemical Landscape: Paving the Road to 21st Century Toxicology. Chem Res Toxicol 29(8), 1225–51. [PubMed: 27367298]

Ring CL, Arnot J, Bennett DH, Egeghy P, Fantke P, Huang L, Isaacs KK, Jolliet O, Phillips K, Price PS, et al. (2018). Consensus Modeling of Median Chemical Intake for the U.S. Population Based on Predictions of Exposure Pathways. Environ Sci Technol doi: 10.1021/acs.est.8b04056.

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, and Knudsen TB (2013). Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol 26(6), 878–95. [PubMed: 23611293]

Sipes NS, Wambaugh JF, Pearce R, Auerbach SS, Wetmore BA, Hsieh J-H, Shapiro AJ, Svoboda D, DeVito MJ, and Ferguson SS (2017a). An Intuitive Approach for Predicting Potential Human Health Risk with the Tox21 10k Library. Accessed.

Sipes NS, Wambaugh JF, Pearce R, Auerbach SS, Wetmore BA, Hsieh JH, Shapiro AJ, Svoboda D, DeVito MJ, and Ferguson SS (2017b). An Intuitive Approach for Predicting Potential Human Health Risk with the Tox21 10k Library. Environ Sci Technol 51(18), 10786–10796. [PubMed: 28809115]

Strickland JD, Martin MT, Richard AM, Houck KA, and Shafer TJ (2018). Screening the ToxCast phase II libraries for alterations in network function using cortical neurons grown on multi-well microelectrode array (mwMEA) plates. Arch Toxicol 92(1), 487–500. [PubMed: 28766123]

Su R, Xiong S, Zink D, and Loo LH (2016). High-throughput imaging-based nephrotoxicity prediction for xenobiotics with diverse chemical structures. Arch Toxicol 90(11), 2793–2808. [PubMed: 26612367]

Thomas RS, Paules RS, Simeonov A, Fitzpatrick SC, Crofton KM, Casey WM, and Mendrick DL (2018). The US Federal Tox21 Program: A strategic and operational plan for continued leadership. ALTEX 35(2), 163–168. [PubMed: 29529324]

Thomas RS, Philbert MA, Auerbach SS, Wetmore BA, Devito MJ, Cote I, Rowlands JC, Whelan MP, Hays SM, Andersen ME, et al. (2013). Incorporating new technologies into toxicity testing and risk assessment: moving from 21st century vision to a data-driven framework. Toxicol Sci 136(1), 4–18. [PubMed: 23958734]

Tice RR, Austin CP, Kavlock RJ, and Bucher JR (2013). Improving the human hazard characterization of chemicals: a Tox21 update. Environ Health Perspect 121(7), 756–65. [PubMed: 23603828]

ToxTree (2015). ToxTree Prediction Module, Version 2.6.13.

USEPA (2015). Use of High-Throughput Assays and Computational Tools; Endocrine Disruptor Screening Program; Notice of Availability and Opportunity for Comment. In (Federal Register.

Wambaugh JF, Hughes MF, Ring CL, MacMillan DK, Ford J, Fennell TR, Black SR, Snyder RW, Sipes NS, and Wetmore BA (2018). Evaluating In Vitro-In Vivo Extrapolation of Toxicokinetics. Toxicological sciences : an official journal of the Society of Toxicology 163(1), 152–169. [PubMed: 29385628]

Wambaugh JF, Wang A, Dionisio KL, Frame A, Egeghy P, Judson R, and Setzer RW (2014). High throughput heuristics for prioritizing human exposure to environmental chemicals. Environ Sci Technol 48(21), 12760–7. [PubMed: 25343693]

Wambaugh JF, Wetmore BA, Pearce R, Strope C, Goldsmith R, Sluka JP, Sedykh A, Tropsha A, Bosgra S, Shah I, et al. (2015). Toxicokinetic Triage for Environmental Chemicals. Toxicological Sciences 147(1), 55–67. [PubMed: 26085347]

Wang J, Hallinger D, Murr A, Buckalew A, Lougee R, Richard AM, S. L, and T. S (2019). High-Throughput Screening and Chemotype-Enrichment Analysis of ToxCast Phase II Chemicals Evaluated for Human Sodium-Iodide Symporter (NIS) Inhibition. Environ Int. In press.

Wang Y-H (2010). Confidence Assessment of the Simcyp Time-Based Approach and a Static Mathematical Model in Predicting Clinical Drug-Drug Interactions for Mechanism-Based CYP3A Inhibitors. Drug Metabolism and Disposition 38(7), 1094–1104. [PubMed: 20368327]

Ward JM, Schofield PN, and Sundberg JP (2017). Reproducibility of histopathological findings in experimental pathology of the mouse: a sorry tail. Lab Anim (NY) 46(4), 146–151. [PubMed: 28328876]

Watt ED, and Judson RS (2018). Uncertainty quantification in ToxCast high throughput screening. PLoS One 13(7), e0196963. [PubMed: 30044784]

Wetmore BA, Allen B, Clewell HJ 3rd, Parker T, Wambaugh JF, Almond LM, Sochaski MA, and Thomas RS (2014). Incorporating population variability and susceptible subpopulations into dosimetry for high-throughput toxicity testing. Toxicol Sci 142(1), 210–24. [PubMed: 25145659]

Wetmore BA, Wambaugh JF, Allen B, Ferguson SS, Sochaski MA, Setzer RW, Houck KA, Strope CL, Cantwell K, Judson RS, et al. (2015). Incorporating High-Throughput Exposure Predictions With Dosimetry-Adjusted In Vitro Bioactivity to Inform Chemical Toxicity Testing. Toxicol Sci 148(1), 121–36. [PubMed: 26251325]

Wetmore BA, Wambaugh JF, Ferguson SS, Li L, Clewell HJ 3rd, Judson RS, Freeman K, Bao W, Sochaski MA, Chu TM, et al. (2013). Relative impact of incorporating pharmacokinetics on predicting in vivo hazard and mode of action from high-throughput in vitro toxicity assays. Toxicol Sci 132(2), 327–46. [PubMed: 23358191]

Wetmore BA, Wambaugh JF, Ferguson SS, Sochaski MA, Rotroff DM, Freeman K, Clewell HJ 3rd, Dix DJ, Andersen ME, Houck KA, et al. (2012). Integration of dosimetry, exposure, and high-throughput screening data in chemical toxicity assessment. Toxicological sciences : an official journal of the Society of Toxicology 125(1), 157–74. [PubMed: 21948869]

WHO, E. a. (2016). Review of the Threshold of Toxicological Concern (TTC) approach and development of new TTC decision tree. In (Vol. EN=1006, pp. 50 EFSA Event Report.

Williams AJ, Grulke CM, Edwards J, McEachran AD, Mansouri K, Baker NC, Patlewicz G, Shah I, Wambaugh JF, Judson RS, et al. (2017). The CompTox Chemistry Dashboard: a community data resource for environmental chemistry. J Cheminform 9(1), 61. [PubMed: 29185060]

Wolf JC, and Maack G (2017). Evaluating the credibility of histopathology data in environmental endocrine toxicity studies. Environ Toxicol Chem 36(3), 601–611. [PubMed: 27883231]

Yang C, Tarkhov A, Marusczyk J, Bienfait B, Gasteiger J, Kleinoeder T, Magdziarz T, Sacher O, Schwab CH, Schwoebel J, et al. (2015). New publicly available chemical query language, CSRML, to support chemotype representations for application to data mining and modeling. J Chem Inf Model 55(3), 510–28. [PubMed: 25647539]

Yoon M, Efremenko A, Blaauboer BJ, and Clewell HJ (2014). Evaluation of simple in vitro to in vivo extrapolation approaches for environmental compounds. Toxicology in Vitro 28(2), 164–170. [PubMed: 24216301]

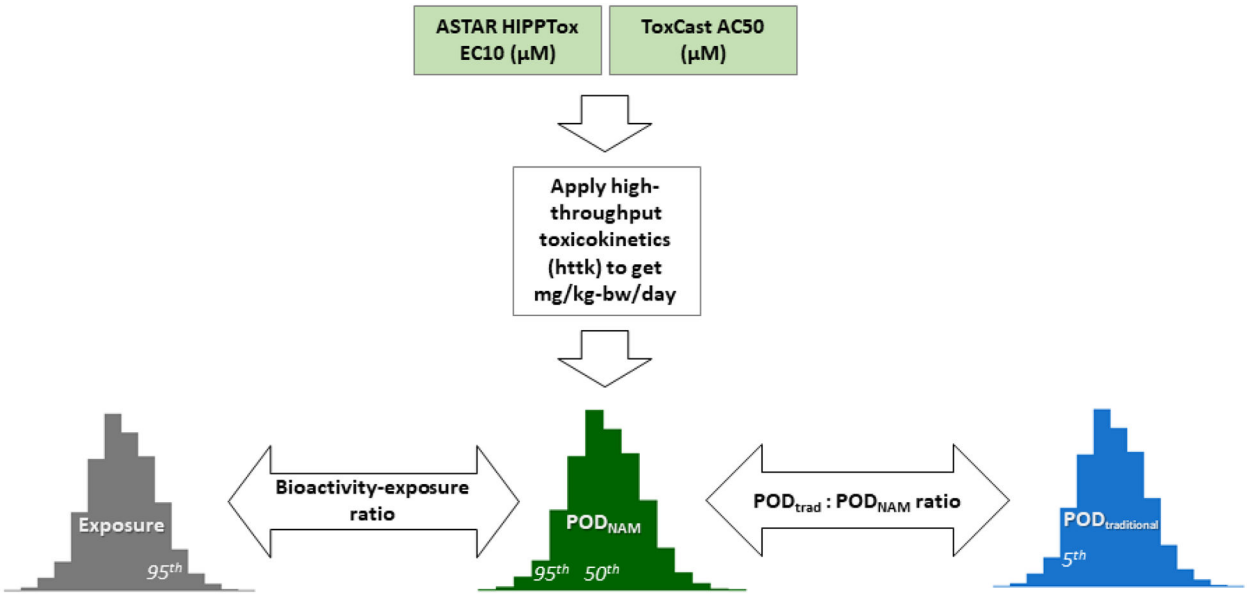


Figure 1. Overall workflow of the case study.

This case study includes 448 substances with exposure predictions, *in vitro* assay data, HTTK information, and *in vivo* hazard information. The 50th and 95th percentile from the Monte Carlo simulation of inter-individual toxicokinetic variability were used to estimate AEDs, and the minimum of either the ToxCast or HIPPTox-based AEDs were selected as the $POD_{NAM,\ 50}$ or $POD_{NAM,\ 95}$. The POD_{NAM} estimates were compared to the 5th percentile from the distribution of the $POD_{traditional}$ values obtained from multiple sources to obtain the $log_{10}POD$ ratio. The $log_{10}BER$ was obtained by comparing the POD_{NAM} estimates to exposure predictions. All values used for computation were in log_{10}-mg/kg-bw/day units

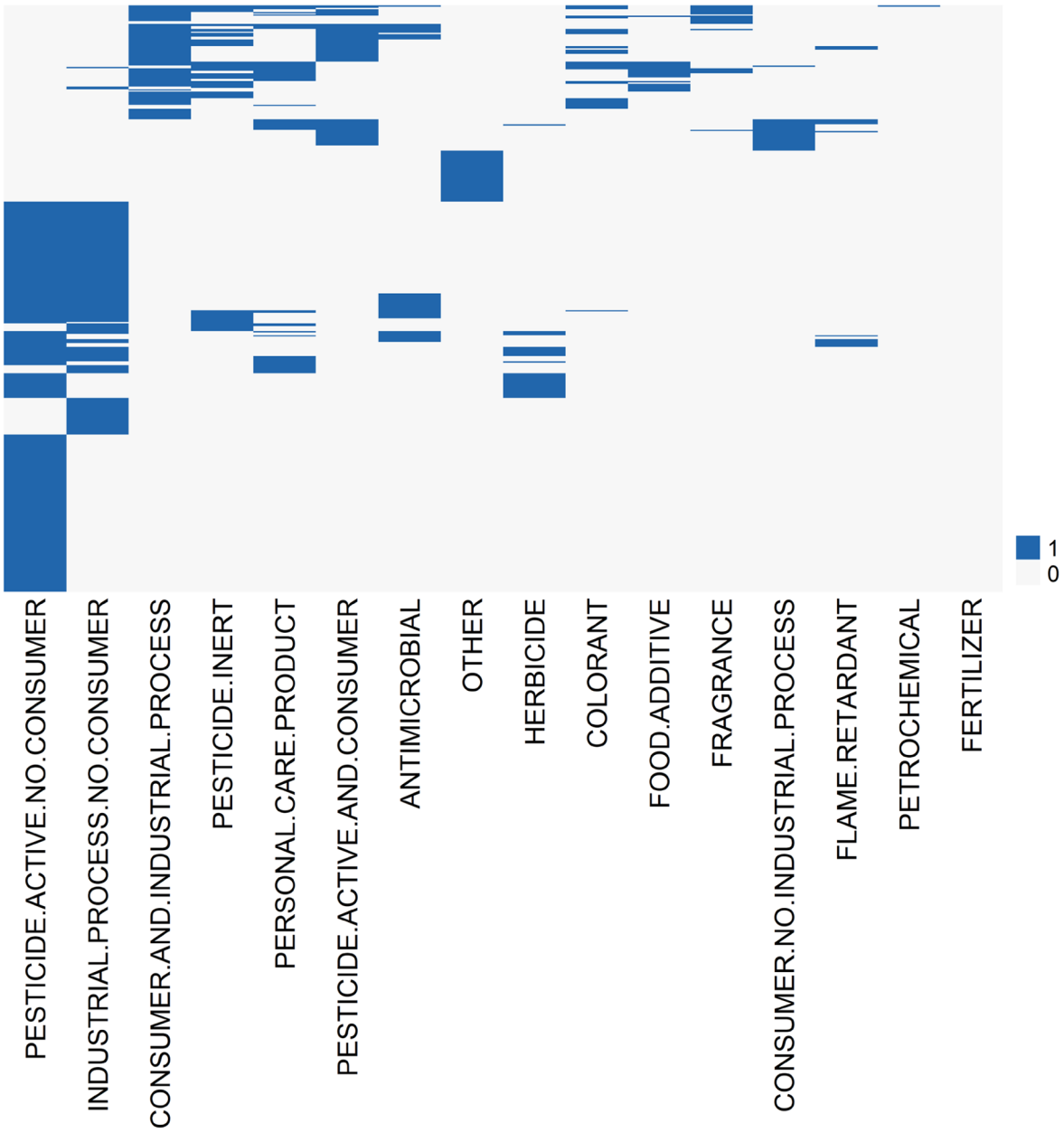


Figure 2. Substance diversity.

Generic functional use categories from ACToR for the 448 case study substances are illustrated. One substance, represented as a row in the heatmap, may be associated with multiple use categories

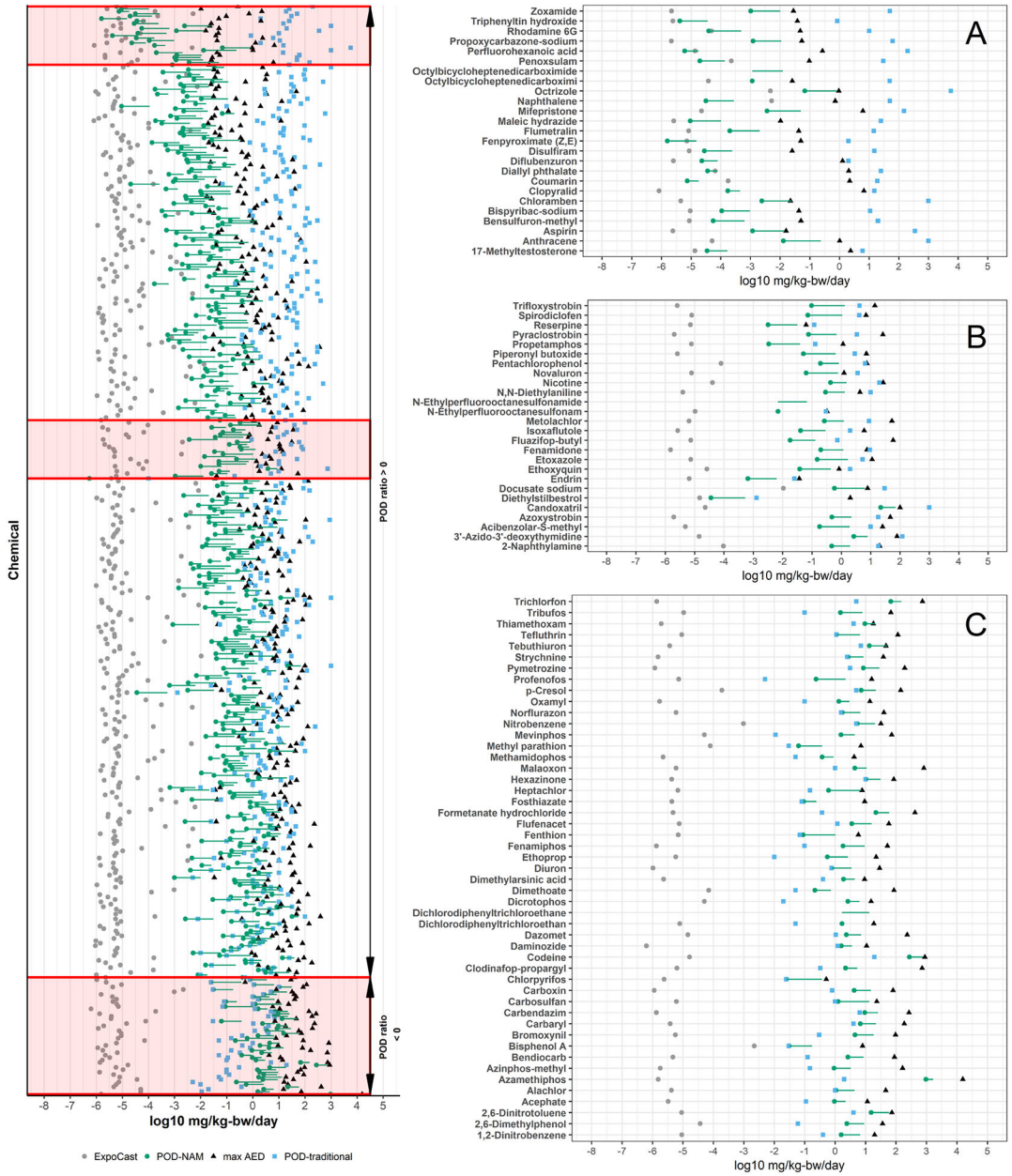
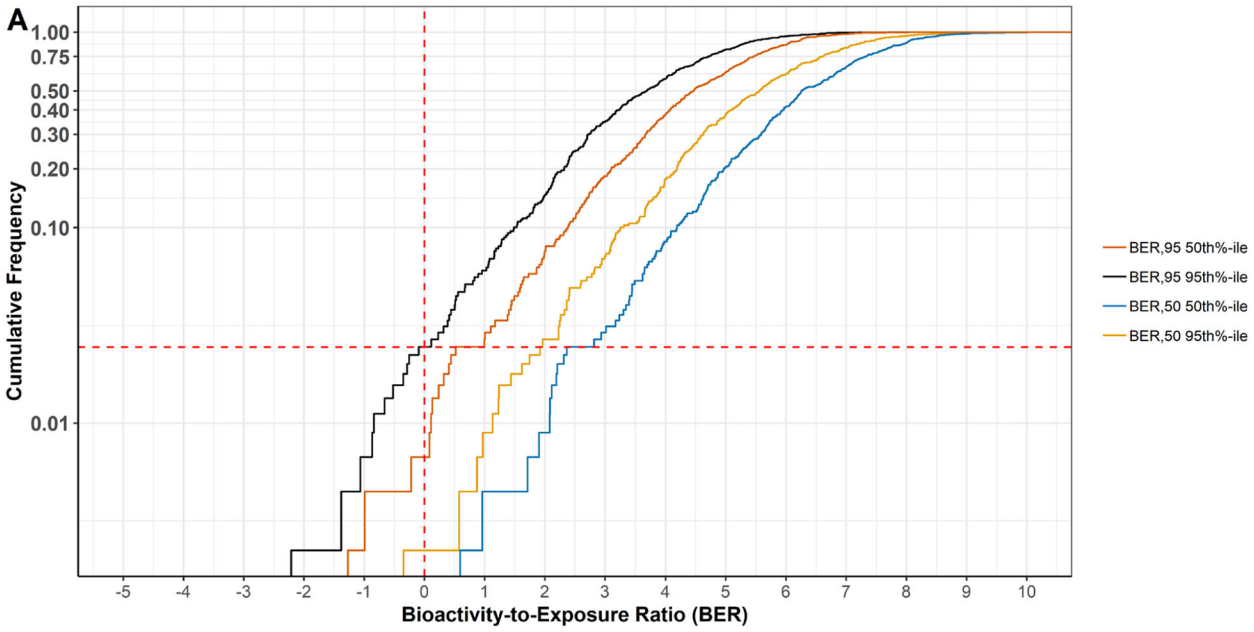


Figure 3. Comparison of the Exposure, POD_{NAM}, and $POD_{traditional}$.

Comparison of ExpoCast (gray circles), POD_{NAM} (green circles), maximum AED (black triangles), and $POD_{traditional}$ values (blue boxes) for 448 substances. The green line segment indicates the $POD_{NAM,95}$ to $POD_{NAM,50}$. Inset images A, B, and C correspond to the red boxes overlaid on the main plot. Image 3A provides a magnification on the substances with the largest log_{10}POD ratio values. Image 3B displays a sample of substances that approach the median log_{10}POD ratio. Image 3C includes all 48 substances for which the $POD_{NAM,\ 95} > POD_{traditional}$.



B

	BER, 95%-ile	log10(POD-NAM, 95th-quantile)	log10(ExpoCast 95%-ile)	Chemical Name
1	-2.21	-4.50	-2.29	Naphthalene
2	-1.38	-5.13	-3.75	Coumarin
3	-1.06	-4.70	-3.64	Penoxsulam
4	-0.87	-4.68	-3.81	Mirex
5	-0.84	-6.26	-5.42	17alpha-Ethinylestradiol
6	-0.66	-5.80	-5.14	Fenpyroximate (Z,E)
7	-0.52	-2.65	-2.13	Di-n-octyl phthalate
8	-0.35	-5.22	-4.86	Perfluorohexanoic acid
9	-0.29	-2.68	-2.39	Tributyl phosphate
10	-0.26	-4.44	-4.19	Diallyl phthalate
11	-0.09	-4.40	-4.31	Rhodamine 6G

Figure 4: Illustration of the log10-bioactivity-exposure-ratio (BER).

A) The cumulative frequency distributions for BER estimates are plotted. The BER_{95} values used the 95th percentile from the credible interval to predict the median total US population exposure from ExpoCast, whereas the BER_{50} values used the median exposure estimate. BER_{95} and BER_{50} values were calculated as the "95th%-ile" and "50th%-ile," using the $POD_{NAM,95}$ and $POD_{NAM,50}$, respectively. Orange line = BER_{95} using $POD_{NAM,50}$; black line = BER_{95} using $POD_{NAM,95}$; blue line = BER_{50} using $POD_{NAM,50}$; gold line = BER_{50} using $POD_{NAM,95}$. B) Eleven chemicals had a BER_{95}, 95th%-ile < 0, indicating overlap between the $POD_{NAM,95}$ and the 95th percentile exposure prediction. Dashed red lines indicate where BER_{95}, 95th%-ile = 0.

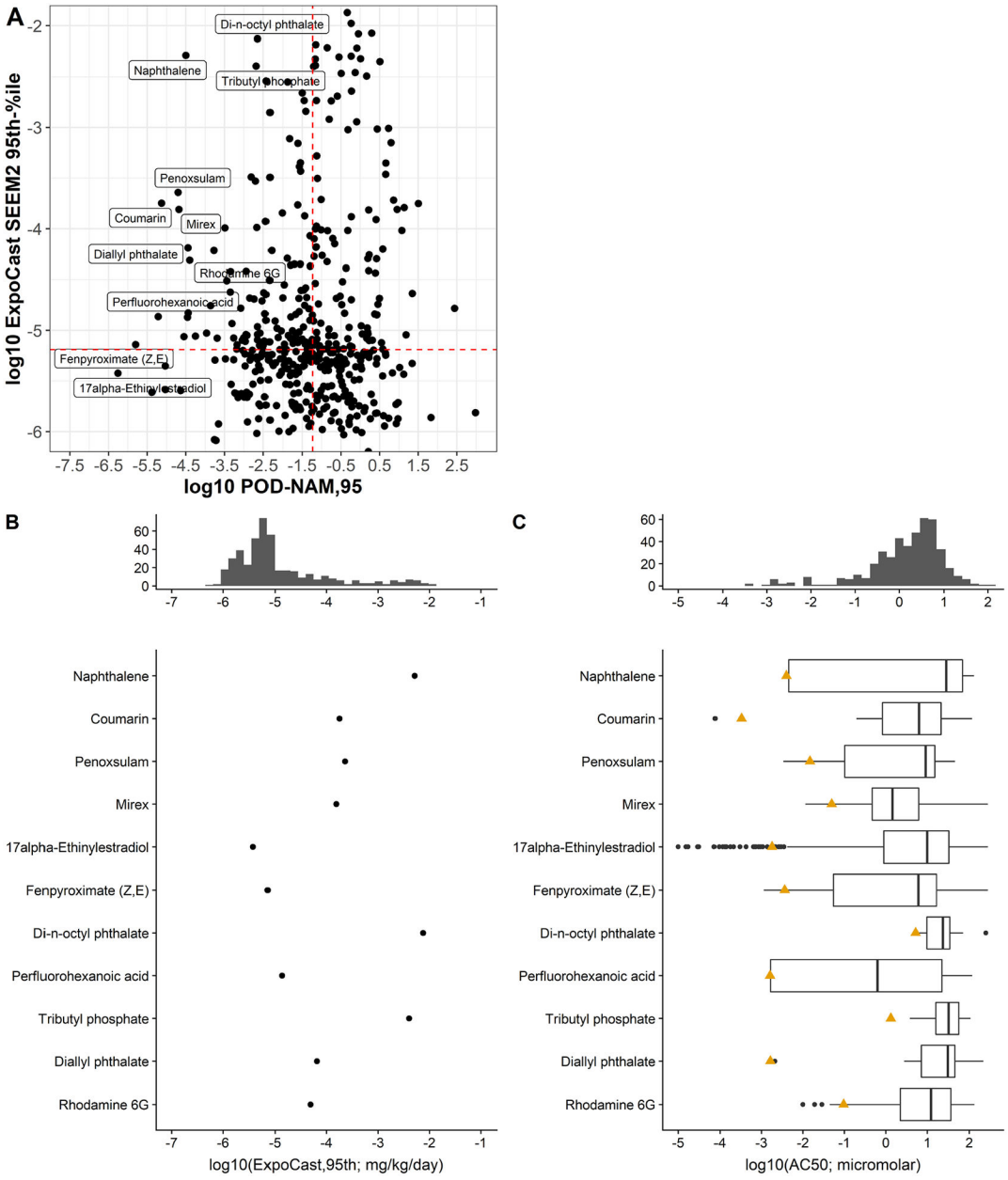


Figure 5: Exposure and *in vitro* bioactivity that defined chemicals with $log_{10}BER < 0$.

In (A), a scatterplot of log_{10} ExpoCast SEEM2 95th percentile value versus the $POD_{NAM,95}$, with dotted red lines for the respective median values. The names of the 11 substances with $log_{10}BER_{95} < 0$ are labeled. In (B) and (C), distributions of the exposure and the ToxCast AC50 data for all 448 substances are shown in the middle panels (gray histograms). Below these histograms in (B) and (C), side by side boxplots (showing the 1st quartile, median, and 3rd quartile) of the log_{10} ExpoCast SEEM2 95th percentile values and the ToxCast AC50 values are illustrated for the 11 substances with $log_{10}BER_{95} < 0$. In (C), gold triangles indicate the 5th percentile of the AC_{50} distribution.

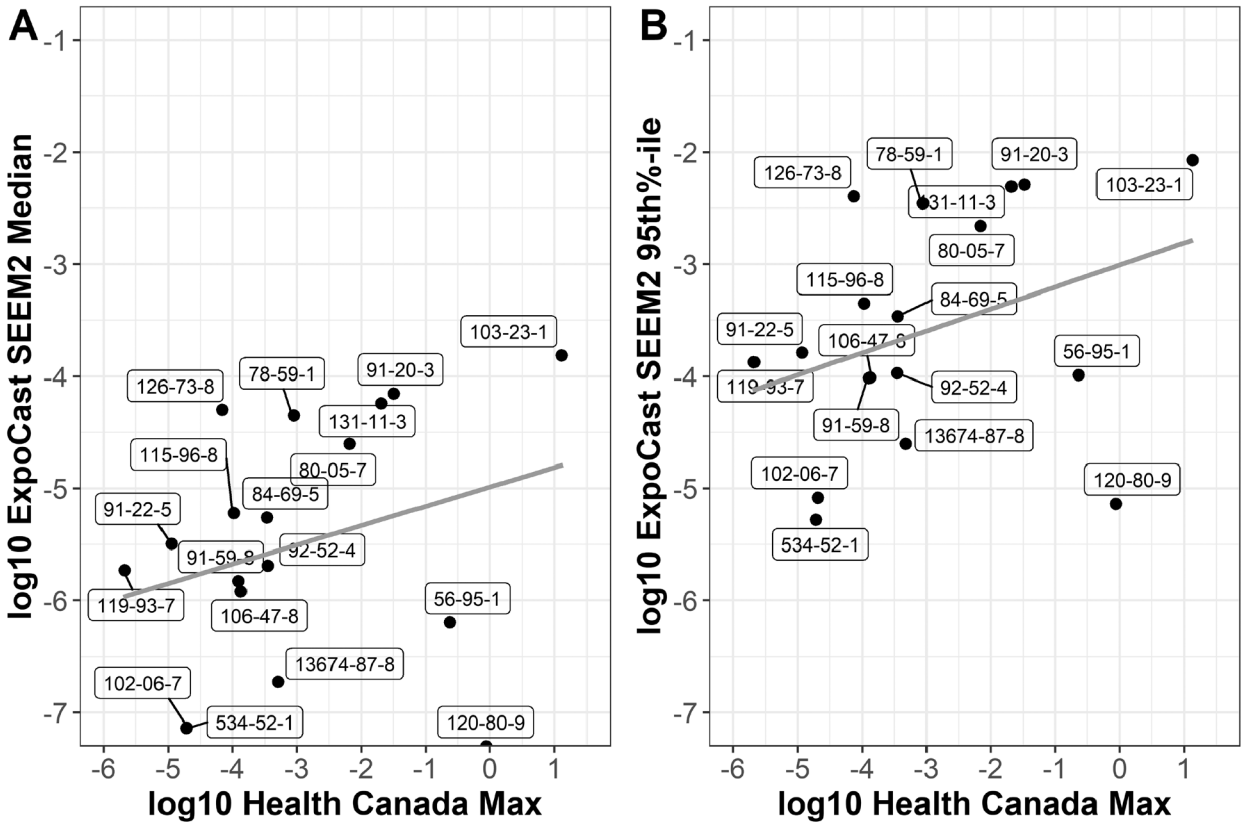
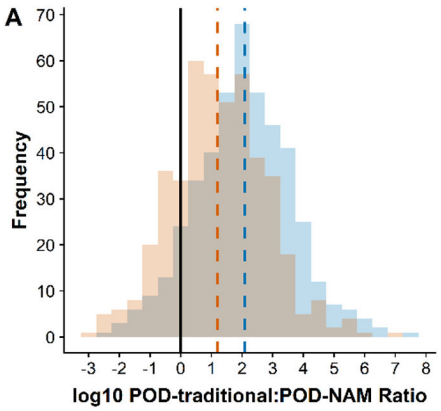


Figure 6: Comparison of Exposure Predictions from ExpoCast and Health Canada Evaluations.

The total maximum values (in $\log_{10}$-mg/kg/day units) curated from Health Canada exposure assessments for 18 substances in this case study were compared to the ExpoCast (A) median and (B) 95th percentile predictions (in log10-mg/kg/day units), respectively. CASRN for these substances are labeled. The gray line shows a linear relationship. All CASRN and substance identifiers, including substance name, can be found in Supplemental File 2.



B

Condition	Max AED < 5th-%ile POD	Max AED > 5th-%ile POD
log10 POD ratio, 95 > 0	240	160
log10 POD ratio, 95 < 0	0	48

Figure 7. Further understanding of the POD ratio distribution.

(A) The $\log_{10}$POD ratio is illustrated for the $POD_{NAM,95}$ and the $POD_{NAM,\ 50}$. The solid black line indicates where the log10-POD $ratio_{95}$ is 0. Using the more conservative (i.e., lower) $POD_{NAM,95}$, 48 of the 448 substances (10.7%) demonstrated a $\log_{10}$POD ratio < 0 (to the left of the dashed vertical line), whereas 92 of the 448 substances (20.5%) demonstrated a log10-POD ratio < 0 using the $POD_{NAM,50}$. The medians of the log10-POD ratio distributions are indicated by dashed lines for $POD_{NAM,\ 95}$ and $POD_{NAM,\ 50}$ as 2 and 1.2, respectively. (B) Maximum AED (max AED) was less than the $POD_{traditional}$ (5th-%ile POD) in 60% of the cases where the $\log_{10}$POD $ratio_{95}$ > 0 (using $POD_{NAM,\ 95}$). For the 48 chemicals with $\log_{10}$POD $ratio_{95}$ < 0, the max AED was within the range of $POD_{traditional}$.

Condition	Dev/Repro is min POD	Dev/Repro is not min POD
log10-POD ratio,95 < 0	3	45
log10-POD ratio,95 > 0	41	359

Condition	Chronic is min POD	Chronic is not min POD
log10-POD ratio,95 < 0	28	20
log10-POD ratio,95 > 0	244	156

Figure 8. Study types enriched in the $\log_{10}$POD ratio_{95} < 0 set.

The matrices used to evaluate study type enrichment are shown. Neither developmental/reproductive (grouped together) ($p = 0.88$) nor chronic ($p = 0.45$) study types appeared to be enriched in the $\log_{10}$POD $\text{ratio}_{95} < 0$ subset.

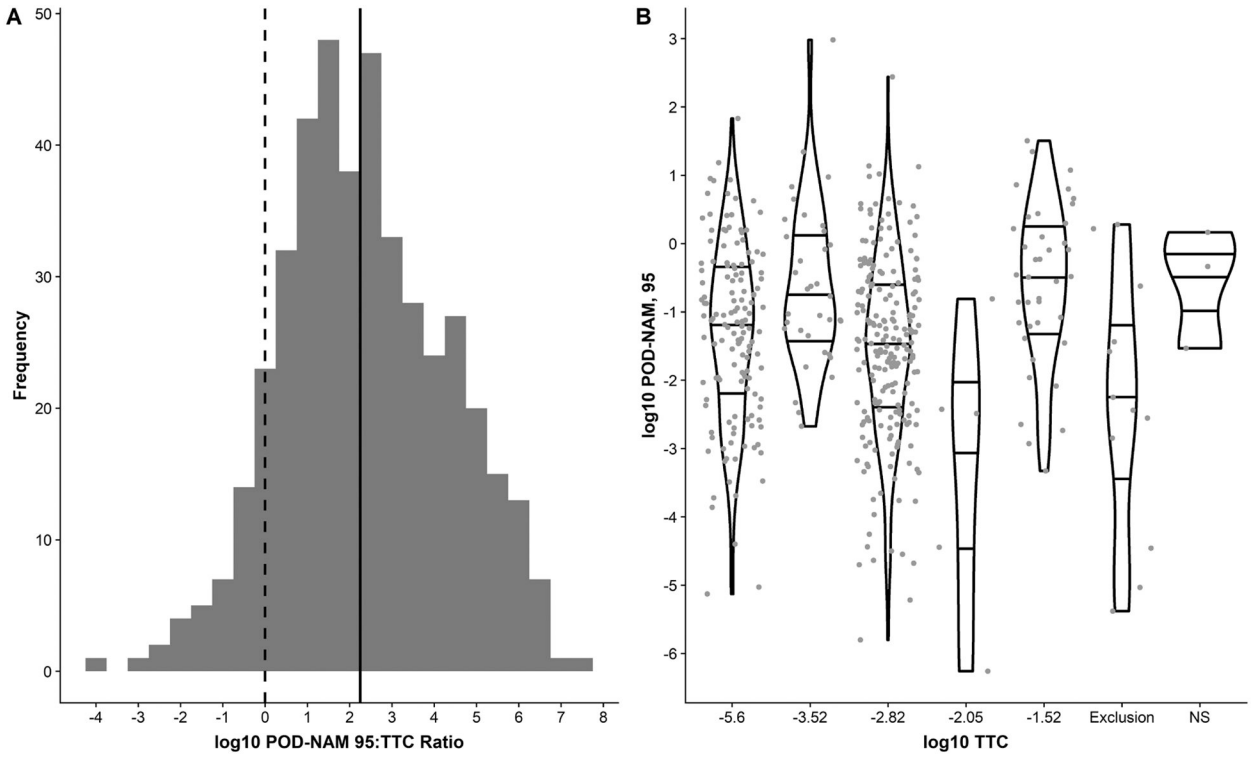


Figure 9. $POD_{NAM,95}$ compared to the TTC.

The log_{10}TTC: $POD_{NAM,95}$ ratio is illustrated for the 448 case study chemicals in (A). In (B), the log_{10} TTC value bin is compared to the $log_{10}POD_{NAM,95}$, in units of log_{10}-mg/kg/day; dots represent all points and violin plots capture the shape of the distribution.

Table 1.

Description of data sources used.

Data stream	Source	Version	Notes
Functional Use Categories	EPA's Aggregated Computational Toxicology Online Resource (ACToR)	2014	Broad use categories (Dionisio *et al.,* 2015; Wambaugh *et al.,* 2014) used in ExpoCast SEEM2 were also used to describe the functional diversity of the 448 substances in this case study.
High-throughput bioactivity data	ToxCast	Invitrodb_v3	This is the public release of invitrodb dated September 2018 (EPA, 2018). These data were fit using the ToxCast Data Pipeline approach (tcpl R package v2). The data used in this case study are available as Supplemental File X.
	In vitro phenotypic profiles of lung, kidney, and liver cell models (HIPPTox)	Performed by A*STAR for this case study	The cell models and phenotypic readouts were described previously (Lee *et al.,* 2018; Su *et al.,* 2016). All phenotypic readouts (not limited to those predictive of tissue-specific adversary effects) were used in computation of the HIPPTox-POD.
Toxicokinetics	High-throughput toxicokinetic (httk) data	Httk R package v1.8	Httk R package v1.8 is available from CRAN (https://cran.r-project.org/web/packages/httk/index.html)
In vivo PODs[a]	ToxValDB *in vivo* toxicity information	Development v5 (May 2018)	This database includes summary point-of-departure information from multiple databases (as described in text) and study types, and is public in the CompTox Chemicals Dashboard.
	ECHA	Repeated dose study results via the oral route in REACH registration dossiers	These data are publicly available at https://echa.europa.eu/
	EFSA	Published human health risk assessments in support of EU food law 158/2002	These data include PODs from multiple study types, mostly from acute, subchronic, chronic, and reproduction toxicity studies.
	Health Canada	Published risk assessments conducted for existing substances under the Canadian Environmental Protection Act, 1999	Information was retrieved based on the availability of a published risk assessment conducted under various phases of Canada's Chemicals Management Plan and earlier initiatives as well as corresponding availability of ToxCast and HTTK data. Point of departure information was extracted from oral repeat-dose studies (of various durations) as well as from developmental and reproductive toxicity studies cited within the assessments. Where possible, both the NO(A)EL and LO(A)EL for each study were collected and the basis for the effect level is described (ECCC/HC, 2016).
Exposure	ExpoCast predictions	Systematic Empirical Evaluation of Models version 2 (SEEM2)	The median and 95th percentile on the credible interval for the total US population exposure estimates were used (Wambaugh, et al., 2014).
	Health Canada	Published risk assessments conducted for existing substances under the Canadian Environmental Protection Act, 1999	Exposure estimates were extracted from the same assessments as their respective *in vivo* POD values. This included the estimated daily intakes from environmental media as well as intakes from use of certain sentinel consumer products (ECCC/HC, 2016).

[a]All in vivo POD data from source databases were concatenated and are available in Supplemental File 1.

References for Table 1.

Dionisio, K. L., Frame, A. M., Goldsmith, M. R., Wambaugh, J. F., Liddell, A., Cathey, T., Smith, D., Vail, J., Ernstoff, A. S., Fantke, P., *et al.* (2015). Exploring consumer exposure pathways and patterns of use for chemicals in the environment. *Toxicol Rep* **2**, 228–237.

ECCC/HC (2016). Chemicals Management Plan. In (H. C. Environment and Climate Change, Ed.), Vol. https://www.canada.ca/en/health-canada/services/chemical-substances/chemicals-management-plan.html. Government of Canada, Ottawa (ON).

EPA, U. (2018). ToxCast & Tox21 Data from invitrodb_v3. In (Retrieved from http://www2.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data.

Lee, J. J., Miller, J. A., Basu, S., Kee, T. V., and Loo, L. H. (2018). Building predictive in vitro pulmonary toxicity assays using high-throughput imaging and artificial intelligence. *Arch Toxicol* **92**(6), 2055–2075.

Su, R., Xiong, S., Zink, D., and Loo, L. H. (2016). High-throughput imaging-based nephrotoxicity prediction for xenobiotics with diverse chemical structures. *Arch Toxicol* **90**(11), 2793–2808.

Wambaugh, J. F., Wang, A., Dionisio, K. L., Frame, A., Egeghy, P., Judson, R., and Setzer, R. W. (2014). High throughput heuristics for prioritizing human exposure to environmental chemicals. *Environ Sci Technol* **48**(21), 12760–7.

Table 2.

Inputs and metrics.

Input or Metric	Description	Rationale
In vitro concentration used	Minimum of 5th percentile of ToxCast AC_{50} values OR the HIPPTox-POD	The goal was to use a value that represents a "lower bound" for *in vitro* bioactivity while accounting for experimental error/variability in these *in vitro* models.
$POD_{NAM,50}$	This metric uses the 50th percentile (median) from the distribution of AED values based on the *in vitro* concentration used.	In the HTTK modeling, Monte Carlo simulation was used to vary pharmacokinetic parameters to represent inter-individual variability in a population for calculation of steady state concentration (C_{ss}). Use of $POD_{NAM,50}$ results in a NAM-based POD value that is 1.7 to 19-fold higher than $POD_{NAM,95}$.
$POD_{NAM,95}$	See $POD_{NAM,50}$; this metric uses the 95th percentile (median) from the distribution.	$POD_{NAM,95}$ accounts for inter-individual variability and indicates that a lower oral dose than the $POD_{NAM,50}$ would be needed to achieve the C_{ss} indicated by the bioactive concentrations *in vitro.*
ExpoCast SEEM2 50th percentile	Using "US Total Exposure" median on the credible interval prediction.	This is a lower exposure value in mg/kg-bw/day that accounts for less uncertainty in the prediction.
ExpoCast SEEM2 95th percentile	Using "US Total Exposure" 95th percentile on the credible interval for a median exposure prediction.	This is a higher exposure value in mg/kg-bw/day, accounting for more uncertainty in the prediction. This value can be ~ 2 orders of magnitude greater than the ExpoCast SEEM2 50th percentile.
$POD_{traditional}$	5th percentile of available *in vivo* PODs, including oral NOAEL, NOEL, LOAEL, LOEL values from mammalian toxicity studies in mg/kg-bw/day.	Use of the 5th percentile is intended to represent a lower bound for *in vivo* adverse effects in the available database.
$Log_{10}POD\ ratio_{95}$	Log_{10}POD ratio using the $POD_{traditional}$ and the $POD_{NAM,95}$	This logic results in the POD_{NAM} appearing protective for the $POD_{traditional}$ 89% of the time in this case study. The median $log_{10}POD\ ratio_{95}$ was 2.
$Log_{10}POD\ ratio_{50}$	Log_{10}POD ratio using the $POD_{traditional}$ and the $POD_{NAM,50}$.	This logic results in the POD_{NAM} appearing protective for the $POD_{traditional}$ 80% of the time in this case study. The median $log_{10}POD\ ratio_{50}$ was 1.2.
BER_{95}, 95th %ile	Bioactivity:exposure ratio; bioactivity = $POD_{NAM,95}$; exposure = 95th percentile from the credible interval to predict median total US population exposure from ExpoCast SEEM2	This BER is the most protective. It includes the highest amount of uncertainty from inter-individual variability in pharmacokinetic parameters in the IVIVE and the highest amount of uncertainty in the ExpoCast SEEM2 exposure prediction. Using this BER will make more substances appear to be of greater priority for review.
BER_{95}, 50th %ile	Bioactivity:exposure ratio; bioactivity = $POD_{NAM,50}$; exposure = 95th percentile from the credible interval to predict median total US population exposure from ExpoCast SEEM2	This BER tends to be, approximately, 10 times greater than the BER_{95}, 95th %ile.
BER_{50}, 95th %ile	Bioactivity:exposure ratio; bioactivity = $POD_{NAM,95}$; exposure = 50th percentile from the credible interval to predict median total US population exposure from ExpoCast SEEM2	This BER tends to be, approximately, 10 times greater than the BER_{95}, 50th %ile and 100 times greater than the BER_{95}, 95th %ile.
BER_{50}, 50th %ile	Bioactivity:exposure ratio; bioactivity = $POD_{NAM,50}$; exposure = 50th percentile from the credible interval to predict median total US population exposure from ExpoCast SEEM2	This BER tends to be, approximately, 10 times greater than the BER_{50}, 95th %ile and 1000 times greater than the BER_{95}, 95th %ile.

Inputs and the resultant metrics used in this case study are consolidated and described, along with notes on the impact of selection of the input or metric in this analysis.

Table 3.

Chemical features enriched in the $\log_{10}$POD ratio$_{95}$ < 0 set.

ChemoType Information		Appearance of the ToxPrint			Metrics			ChemoType Information		Appearance of theToxPrint			Metrics		
Label	**ToxPrint**	**Total**	**POD ratio SO**	**POD ratio ≤0**	**BA**	**OR**	**p-value**	**Label**	**ToxPrint**	**Total**	**POD ratio ≤ 0**	**POD ratio > 0**	**BA**	**OR**	**p-value**
bond:P=O_phosphorus_oxo		18	12	6	0.62	22	7.4E-09	bond:P~N_generic		5	4	1	0.54	36	0.00055
bond:P=O_phosphate_thio		3	3	0	0.53	NA	0.0012	bond:C(=O)N_carbamate		20	6	14	0.54	3.9	0.014
bond:P~S_generic		27	13	14	0.62	10	3.5E-7	bond:CS_sulfide		53	15	38	0.61	4.3	0.00011

The enriched chemical structural features, as represented by ToxPrints, for the $\log_{10}$POD ratio$_{95}$ < 0 set. BA = balanced accuracy; OR = odds ratio; POD ratio = $\log_{10}$POD ratio$_{95}$.

Table 4.

Details on the 48 substances with $\log_{10}$POD ratio$_{95}$ < 0.

#	DTXSID	CASRN	Name	$\log_{10}$ $POD_{NAM,95}$	$\log_{10}$ $POD_{NAM,50}$	$\log_{10}$ POD $ratio_{95}$	$\log_{10}$ POD $ratio_{50}$	BER_{95}, 95th %ile	BER_{50}, 95th %ile	BER_{95}, 50th %ile	BER_{50}, 50th %ile
1	DTXSID9034818	35575-96-3	Azamethiphos	2.98	3.22	−2.68	−2.92	8.79	10.75	9.04	10.99
2	DTXSID2032683	7786-34-7	Mevinphos	0.19	0.65	−2.15	−2.61	4.49	6.19	4.94	6.65
3	DTXSID9023914	141-66-2	Dicrotophos	0.42	0.80	−2.12	−2.50	4.72	6.41	5.10	6.79
4	DTXSID4032405	23422-53-9	Formetanate hydrochloride	1.34	1.78	−1.77	−2.21	6.67	8.47	7.10	8.90
5	DTXSID4032611	13194-48-4	Ethoprop	−0.25	0.42	−1.75	−2.42	4.98	6.90	5.65	7.57
6	DTXSID3032464	41198-08-7	Profenofos	−0.63	0.34	−1.68	−2.64	4.51	6.23	5.48	7.20
7	DTXSID9024063	576-26-1	2,6-Dimethylphenol	0.39	0.96	−1.61	−2.18	4.82	6.73	5.39	7.30
8	DTXSID4020375	50-29-3	Dichlorodiphenyltrichloroethane	0.23	1.12	−1.53	−2.42	5.33	7.17	6.22	8.07
9	DTXSID9032327	22781-23-3	Bendiocarb	0.42	0.94	−1.32	−1.84	5.75	7.50	6.27	8.02
10	DTXSID3024102	22224-92-6	Fenamiphos	0.26	0.98	−1.27	−1.99	6.13	8.01	6.85	8.72
11	DTXSID1024174	78-48-8	Tribufos	0.18	0.90	−1.18	−1.90	5.15	6.90	5.87	7.62
12	DTXSID3022162	1689-84-5	Bromoxynil	0.65	1.27	−1.18	−1.79	5.90	7.72	6.51	8.33
13	DTXSID2020341	76-57-3	Codeine	2.44	2.88	−1.15	−1.59	7.22	9.19	7.66	9.63
14	DTXSID0021389	52-68-6	Trichlorfon	1.83	2.18	−1.13	−1.48	7.69	9.66	8.04	10.00
15	DTXSID6021086	23135-22-0	Oxamyl	0.12	0.47	−1.12	−1.47	5.88	8.00	6.23	8.34
16	DTXSID8023846	30560-19-1	Acephate	−0.02	0.34	−0.94	−1.30	5.46	7.23	5.83	7.59
17	DTXSID6024177	10265-92-6	Methamidophos	−0.43	−0.05	−0.87	−1.25	5.22	6.89	5.60	7.27
18	DTXSID6032354	105512-06-9	Clodinafop-propargyl	0.34	0.73	−0.83	−1.22	5.54	7.24	5.92	7.62
19	DTXSID3020122	86-50-0	Azinphos-methyl	−0.03	0.51	−0.79	−1.34	5.71	7.64	6.25	8.18
20	DTXSID0023951	5234-68-4	Carboxin	0.62	1.18	−0.72	−1.28	6.57	8.41	7.12	8.97
21	DTXSID7020508	75-60-5	Dimethylarsinic acid	0.28	0.65	−0.68	−1.05	5.91	7.58	6.28	7.95
22	DTXSID9020790	1634-78-2	Malaoxon	0.65	1.03	−0.65	−1.03	5.87	7.60	6.26	7.98
23	DTXSID7020479	60-51-5	Dimethoate	−0.67	−0.14	−0.64	−1.16	3.48	5.38	4.01	5.90
24	DTXSID3020679	76-44-8	Heptachlor	−0.22	0.85	−0.61	−1.68	4.95	6.66	6.01	7.73
25	DTXSID4024066	528-29-0	1,2-Dinitrobenzene	0.20	0.82	−0.60	−1.22	5.23	7.13	5.86	7.75

#	DTXSID	CASRN	Name	Log_{10} $POD_{NAM,95}$	Log_{10} $POD_{NAM,50}$	Log_{10} POD $ratio_{95}$	Log_{10} POD $ratio_{50}$	BER_{95}, 95th %ile	BER_{50}, 95th %ile	BER_{95}, 50th %ile	BER_{50}, 50th %ile
26	DTXSID5020528	606-20-2	2,6-Dinitrotoluene	1.18	1.78	−0.58	−1.18	6.23	8.05	6.82	8.64
27	DTXSID2032552	142459-58-3	Flufenacet	0.55	1.20	−0.47	−1.12	5.67	7.48	6.32	8.13
28	DTXSID2032637	123312-89-0	Pymetrozine	0.93	1.46	−0.44	−0.96	6.85	8.80	7.38	9.33
29	DTXSID2034962	153719-23-4	Thiamethoxam	0.98	1.33	−0.37	−0.72	6.69	8.73	7.04	9.08
30	DTXSID7024902	533-74-4	Dazomet	0.37	0.85	−0.35	−0.83	5.21	7.22	5.69	7.70
31	DTXSID1020855	298-00-0	Methyl parathion	−1.20	−0.43	−0.32	−1.10	2.90	4.73	3.67	5.51
32	DTXSID3024316	34014-18-1	Tebuthiuron	1.12	1.70	−0.28	−0.86	6.56	8.22	7.14	8.80
33	DTXSID9020247	63-25-2	Carbaryl	0.83	1.35	−0.23	−0.74	6.25	7.98	6.77	8.49
34	DTXSID4024729	10605-21-7	Carbendazim	0.97	1.40	−0.17	−0.60	6.85	8.99	7.28	9.42
35	DTXSID7021869	106-44-5	p-Cresol	0.86	1.34	−0.16	−0.64	4.58	6.33	5.06	6.81
36	DTXSID9020370	1596-84-5	Daminozide	0.21	0.56	−0.12	−0.48	6.40	8.21	6.76	8.56
37	DTXSID8020620	55-38-9	Fenthion	−1.05	0.01	−0.10	−1.17	4.10	5.95	5.16	7.02
38	DTXSID5023950	55285-14-8	Carbosulfan	0.10	1.12	−0.10	−1.12	5.31	7.04	6.33	8.06
39	DTXSID0034930	98886-44-3	Fosthiazate	−1.03	−0.62	−0.06	−0.48	4.33	6.08	4.74	6.49
40	DTXSID8024234	27314-13-2	Norflurazon	0.24	0.83	−0.04	−0.63	5.46	7.31	6.06	7.90
41	DTXSID1022265	15972-60-8	Alachlor	0.03	0.64	−0.03	−0.64	5.42	7.17	6.02	7.78
42	DTXSID6023600	57-24-9	Strychnine	0.43	0.93	−0.03	−0.53	6.25	8.14	6.74	8.63
43	DTXSID3020964	98-95-3	Nitrobenzene	0.73	1.31	−0.03	−0.61	3.74	5.67	4.32	6.25
44	DTXSID7020182	80-05-7	Bisphenol A	−1.50	−0.75	−0.02	−0.77	1.16	3.10	1.91	3.85
45	DTXSID4024145	51235-04-2	Hexazinone	1.02	1.50	−0.02	−0.50	6.38	8.07	6.86	8.54
46	DTXSID5032577	79538-32-2	Tefluthrin	0.05	0.82	−0.01	−0.78	5.09	6.91	5.85	7.68
47	DTXSID4020458	2921-88-2	Chlorpyrifos	−1.59	−0.43	−0.01	−1.17	4.02	6.19	5.19	7.35
48	DTXSID0020446	330-54-1	Diuron	−0.11	0.55	−0.01	−0.66	5.88	7.80	6.53	8.45

Substances in this table are ordered based on the log_{10}POD ratio, from smallest to largest for substances with log_{10}POD $ratio_{95} < 0$ (column in gray). Note that for 33 of the 48 substances, the log10POD $ratio_{95}$ is within one log_{10}. The full table for all substances is available as Supplemental File 2.