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Evaluating the Impact of Uncertainties in Clearance and Exposure When Prioritizing Chemicals Screened in High-Throughput Assays

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Abstract

The toxicity-testing paradigm has evolved to include highthroughput (HT) methods for addressing the increasing need to screen hundreds to thousands of chemicals rapidly. Approaches that involve in vitro screening assays, in silico predictions of exposure concentrations, and pharmacokinetic (PK) characteristics provide the foundation for HT risk prioritization. Underlying uncertainties in predicted exposure concentrations or PK behaviors can significantly influence the prioritization of chemicals, though the impact of such influences is unclear. In the current study, a framework was developed to incorporate absorbed doses, PK properties, and in vitro dose–response data into a PK/pharmacodynamic (PD) model to allow for placement of chemicals into discrete priority bins. Literature-reported or predicted values for clearance rates and absorbed doses were used in the PK/PD model to evaluate the impact of their uncertainties on chemical prioritization. Scenarios using predicted absorbed doses resulted in a larger number of bin misassignments than those scenarios using predicted clearance rates, when comparing to bin placement using literature-

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Notes

The authors declare the following competing financial interest(s): Daniel Chang is employed by the Chemical Computing Group in Montreal, Canada, the publisher of the Molecular Operating Environment (MOE) software.

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b00374. Details regarding development of the clearance mechanism and rate models, along with model output and validation; literature-reported and predicted values for clearance and absorbed doses including reference citations for refs 40, 41, 43–80; tabular comparisons among scenarios using literature-reported or predicted parameters to estimate chemical activity; confusion matrices for each predictive scenario; sensitivity plots and scores for several chemicals representative of their respective chemical categories (PDF)

reported values. Sensitivity of parameters on the model output of toxicological activity was examined across possible ranges for those parameters to provide insight into how uncertainty in their predicted values might impact uncertainty in activity.

Graphical abstract

INTRODUCTION

Traditional toxicity testing offers the advantage of incorporating both hazard identification and dose–response assessment as part of the risk assessment process.¹ This testing paradigm involves a suite of in vivo animal studies that aids in gaining a mechanistic understanding of toxic outcomes occurring in a whole biological system² under well-controlled experimental conditions. Pharmacokinetic (PK) properties (absorption, distribution, metabolism, and elimination (ADME)) for the chemical(s) being tested are characterized within the dosing regimen, and chemical-specific PK data are often available.³ Similarly, traditional exposure assessment is conducted for one chemical at a time to identify its sources, fate, transport processes, or concentrations of exposure.⁴ Combining data on hazard, exposure, and dose–response can help characterize the extra risk of health effects in the population for individual chemicals,⁵ thereby supporting the establishment of exposure guidance levels for regulatory purposes.

Traditional toxicity testing and dose–response assessment, however, involve the use of large numbers of animals and require large investments in time and cost.^{6,7} As a result, in vivo dose–response data are unavailable for the vast majority of environmental chemicals. In addition, hundreds to thousands of new environmental chemicals are produced on an annual basis,^{8,9} rendering it impractical to conduct risk assessment based solely on animal toxicity testing. One strategy offering an alternative to animal toxicity testing is the utilization of highthroughput (HT) in vitro assays as a rapid, cost-efficient means to screen thousands of chemicals across hundreds of pathwaybased toxicity end points¹⁰ and to aid in chemical prioritization for more extensive in vivo testing.¹¹ A complementary vision to that of toxicity testing in the 21st century calls for an equally critical need to evaluate a large number of chemicals rapidly, and with fewer financial resources, in the field of exposure science.¹²

HT risk prioritization aims to integrate the new paradigms of in vitro assays that identify hazard,¹³ in silico models that estimate exposures,¹⁴ and quantitative structure–activity relationship (QSAR) based PK models that describe ADME properties to screen and prioritize data-poor environmental chemicals.¹⁵ However, chemicals that are active in vitro may not reach an in vivo molecular target or may interact with the target at concentrations insufficient to elicit an adverse toxicological response. The in vivo concentration at the target tissue is determined by intake doses and ADME properties.¹⁶ Incorporating available data and identifying critical factors that influence exposure potential and ADME behaviors will increase confidence of in silico models attempting to estimate such characteristics, and refine HT in vitro testing results.

In the current study, a framework was developed to aid the HT risk prioritization process through its integration of absorbed doses, ADME properties, and in vitro dose–response data into a PK/pharmacodynamic (PD) model. Chemicals involved in acetylcholinesterase (AChE) inhibition were used as a case study because available exposure and ADME data for several of these chemicals were available and sufficient for examining the impact that uncertainties in these influential variables might have when predicting chemical toxicological activity. While in vivo dose–response data are available for several chemicals presented here, this is often not the case, and our purpose was not to compare in vitro and in vivo dose–response results. Rather, the framework applies to cases in which in vivo dose–response data relevant to a particular biological end point is lacking. Thus, we caution that interpretation of the case study results should not be used for contemporary regulatory purposes.

The first objective of this study involved demonstrating the utility of the proposed framework by placing chemicals into discrete priority bins, based on the chemicals' activity, to allow more room for prediction error, rather than rank-ordering by activity alone. For this objective, activity was predicted using literature-reported absorbed doses and clearance values in the PK/PD model to form a “reference” scenario. The second objective involved evaluating the impact of uncertainties in predicted absorbed dose and clearance values on chemical prioritization by comparing chemical placement into bins using these predicted values to their bin-placement in the reference scenario.

METHODS

Chemical Selection

In a previous study,¹⁷ 10 out of 30 active chemicals identified in the ToxCast AChE inhibition in vitro assay were found to be unable to reach AChE in brain due to limited exposure or their ADME properties. Of the remaining 20 active chemicals, five were excluded from the current study: (1) two pharmaceuticals (SSR241586, SSR150106) with no exposure or ADME information, (2) mercuric chloride, which is a metal salt with ADME properties that are difficult to predict using traditional cheminformatics methods,¹⁸ and (3) gentian violet and 1-benzylquinolinium, which are pan-assay interference compounds capable of exhibiting signs of in vitro activity due to reasons other than binding to the molecular target.¹⁹

The previous study¹⁷ also identified 22 possible false negatives out of chemicals considered inactive in the same assay, based on their structural similarities to active chemicals. Since the analysis here required in vitro dose–response data, which is lacking for most inactive chemicals, only five of the 22 false negatives were carried over to the current study. These five chemicals included three inactive parents of active metabolites (chlorpyrifos, malathion, and chlorpyrifos-methyl) and two known AChE inhibitors (aldicarb and dichlorvos) for which in vitro dose–response data were available. Finally, five chemicals identified as AChE inhibitors in another study²⁰ were added, resulting in a total of 25 chemicals of interest (Figure 1). Several of these 25 chemicals have been banned (e.g., carbofuran) or cancelled, are no longer registered (e.g., mevinphos and bendiocarb), or are designated for restricted use (e.g., oxamyl and methomyl). Because the purpose of this case study was not to conduct contemporary risk assessment, however, these banned or canceled chemicals were retained in order to provide a larger set of chemicals for demonstrating the utility of the framework.

Approach Overview

A framework was developed for chemical prioritization based on in vivo activities predicted using in vitro dose–response data and average plasma concentration. While this dose metric may not be appropriate for traditional risk assessment (e.g., peak plasma concentration might be more relevant to AChE inhibition for *N*-methyl carbamates), it is the best surrogate for internal target tissue dose when evaluating data-poor chemicals using the framework. The framework begins by identifying an active chemical from an in vitro assay of interest and determining the type/category of that chemical through a series of standardized queries (Figure 2a). The 25 chemicals selected for the case study included 13 active parents with no known active metabolites, three active metabolites and their respective inactive parents, and three other active metabolites and their respective active parents (Figure 1). After the chemical category is determined using the provided workflow (Figure 2a), the required chemical-specific model parameters can be identified on the basis of the category to which that chemical belongs (Figure 2b). Ranking of chemicals based on their in vivo activities then proceeds through the following steps:

1. Obtain values of required parameters from literature whenever possible, or otherwise use in silico models to estimate their values
2. Use the PK model to predict the average chemical concentration (mg/L) in plasma (C_{avg}), followed by use of the PD model to predict activity for each chemical
3. Place chemicals into priority bins based on their relative activities

The six active metabolites are also present in the environment, and so they were first evaluated in a similar manner as the 13 active parent-only chemicals (Figure 2a). Specifically, AChE inhibition activity for those 19 chemicals (13 active parents and six active metabolites) was evaluated on the basis of absorbed doses for those chemicals only. Inhibition activity of the six parents that generate an active metabolite was evaluated on the basis of the absorbed dose of the parent chemical alone and generation of the metabolite. An additional six groupings that included absorbed doses for parents and their active metabolites (e.g., co-exposure to malathion and malaoxon, both present in the environment),

along with generation of the metabolite after parental biotransformation, resulted in a final of 31 chemicals/groupings for ranking (Figure 1).

The underlying uncertainty in model inputs and parameters can influence predictions of C_{avg} and subsequent activity. The degree of this influence was examined using 11 scenarios composed of combinations of literature-reported and predicted values for three variables (absorbed dose, clearance rate, and stoichiometric yield), compared to a reference scenario using literature-reported values. Three scenarios involved literature-reported absorbed doses, predicted clearance rates, and stoichiometric yield from a parent to an active metabolite set to 5%, 50%, or 90%. Two scenarios involved literature-reported clearance rates and stoichiometric yields, along with predicted absorbed doses at the 50th (median) and 95th population percentiles. Six scenarios involved combinations of the absorbed doses predicted at the two population percentiles, along with predicted clearance rates using the three stoichiometric yield percentages. The following sections provide additional details regarding development of the PK/PD model, methods used to determine absorbed doses and clearance rates, and approaches used to discretize chemicals into priority bins for scenario comparisons.

Developing the PK/PD Model

In the PK model, plasma concentrations of parent chemicals were predicted based on their absorbed doses and clearance rates according to the following equation

$$\frac{dC_p}{dt} = \frac{1}{V_b} \left[dose_p - \frac{Q_1 Cl_{int} C_p}{Q_1 + Cl_{int}} - (K_e C_p) \right] \quad (1)$$

where C_p is the plasma concentration of the parent chemical (mg/L), V_b is the blood volume (L), $dose_p$ is the absorbed dose rate of the parent chemical (mg/h), Q_1 is hepatic blood flow (L/h), Cl_{int} is intrinsic hepatic clearance of the parent chemical (L/h), and K_e is urinary clearance rate (L/h). In this model, V_b was set to 5 L,²¹ and Q_1 was set to 71.4 L/h.²² Urinary clearance rate is a product of the glomerular filtration rate (GFR), set to 122 mL/min,²³ and fraction of chemical unbound in plasma (f_u), which was estimated from ADMET Predictor (Simulations Plus, Inc., Lancaster, CA). Cl_{int} is a function of the Michaelis Menten kinetics equation V_{max}/K_m . To predict plasma concentrations for active metabolites based on their absorbed doses (if present in the environment), generation from parental metabolism, and clearance rates, the following additional equation was used

$$\frac{dC_M}{dt} = \frac{1}{V_b} \left[dose_M + stoich_M \frac{Q_1 Cl_{int} C_p}{Q_1 + Cl_{int}} - (Cl_M C_M) \right] \quad (2)$$

where C_M is the plasma concentration of the metabolite (mg/L), $dose_M$ is the absorbed dose rate of the metabolite if present in the environment (mg/h), and Cl_M is the total clearance rate of the metabolite (L/h). $stoich_M$ is the stoichiometric yield of the active metabolite moiety generated from overall hepatic metabolism of the parent chemical, according to the following equation:

$$stoich_M = \frac{MW_M}{MW_P} f_M \quad (3)$$

In the above equation, MW_M and MW_P are the molecular weights of the parent compound and active metabolite, respectively, and f_M is the fraction of total hepatic metabolism of the parent compound that produces the active metabolite.

For each chemical/grouping, the PK model was run for 7 days with daily single exposure to ensure that a pseudo steady state was reached for plasma concentrations, followed by integration over time and dividing the integrated concentration over the time spanned to estimate C_{avg} . All PK/PD model simulations were conducted using MATLAB version 2015a (The MathWorks, Inc., Natick, MA). C_{avg} concentrations for the parent (P) and metabolite (M) were added into a PD model, along with two measures of in vitro dose–response data, the half-maximal effective concentration (EC_{50} ; mg/L), and the maximum inhibition activity (E_{max}). The E_{max} represents the maximum percent of inhibition/induction that a chemical might have on the normal activity of its molecular target. The PD model was used to calculate AChE inhibition activity for each chemical/grouping, assuming a baseline inhibition of 0, and according to the following equation:

$$A = \left[\frac{E_{max(P)} C_{avg(P)}}{EC_{50(P)} + C_{avg(P)}} \right] + \left[\frac{E_{max(M)} C_{avg(M)}}{EC_{50(M)} + C_{avg(M)}} \right] \quad (4)$$

Parameterizing the Reference Scenario

A literature search was conducted to obtain published data related to daily intake concentrations (assuming 100% absorption), $Stoich_M$, Cl_M for metabolites, and Cl_{int} for parent compounds. These values are provided in the Supporting Information, Table S1.

For the 13 active parent chemicals that do not have active metabolites and for the six active metabolites present in the environment (Figure 1), total clearance rates (L/h) account for the disappearance of these chemicals. Thus, for these chemicals, Cl_{int} in eq 1 was set to 0, and total clearance rates were used to replace the K_e term representing renal clearance. Although naled and thiodicarb are active parents with active metabolites (Figure 1), no hepatic clearance data were found in the literature, and thus, total clearance rates were also used for these two chemicals in lieu of separate K_e and Cl_{int} values. The majority of naled is metabolized to the active metabolite, dichlorvos,²⁴ and thiodicarb is metabolized rapidly to the active metabolite methomyl prior to further detoxification to acetonitrile and other volatiles.²⁵ Therefore, replacing the Cl_{int} term in eq 2 with total clearance and setting the K_e term to 0 for these two parent chemicals was a reasonable approach. Total clearance rate was estimated according to the following equation

$$Cl_{total} = \frac{\ln(2)V_d}{t_{1/2}} BW \quad (5)$$

where V_d is the volume of distribution of the chemical (L/kg), $t_{1/2}$ is the chemical plasma half-life (h), and BW assumes an average of 70 kg for an adult. For environmental chemicals, $t_{1/2}$ is often difficult to obtain, and V_d is rarely available. Because no measurements were found for the selected chemicals, V_d was predicted using ADMET Predictor (Simulations Plus, Inc., Lancaster, CA). Published $t_{1/2}$ values were available for 22 of the 25 chemicals. For malaoxon, chlorpyrifos oxon, and azamethiphos, $t_{1/2}$ was predicted using a published model:²⁶

$$\log t_{1/2} = 0.452 + 0.288(\log P) \quad (6)$$

The predictive ability of this model was examined using data for 670 intravenously administered drugs.²⁷ This equation was found to be most appropriate for chemicals with values $-4 < \log P < 4$ and optimal for values $-3 < \log P < 3$ (70% of predictions were within a factor of 5 of observations). The $\log P$ values for malaoxon, chlorpyrifos oxon, and azamethiphos predicted by ADMET Predictor were 1.38, 3.22, and 1.46, respectively.

Predicting Absorbed Doses

The HT Stochastic Human Exposure and Dose Simulation (SHEDS-HT) model¹⁴ was used to predict absorbed doses (mg/kg/d) of the 25 chemicals for a simulated population that contains 100000 adults weighing an average of 70 kg (predicted values in mg/h can be found in the SI, Table S2). Total intake (in mg/day) was estimated as the sum of the absorbed doses from all routes of exposure. SHEDS-HT was developed to predict distributions of absorbed doses for chemicals in consumer products based on reported weight fractions²⁸ and population use patterns, and details regarding model development can be found elsewhere.¹⁴ SHEDS-HT includes three routes of exposure—dermal, inhalation, and oral (both ingestion and transfer of chemical from the hands to the mouth).

Predicting Clearance Mechanisms

It has been suggested that use of separate models to predict clearance rates of chemicals undergoing different mechanisms of elimination (e.g., hepatic, renal) would result in a better performance than use of a single model to predict overall clearance.²⁹ A published model that predicts clearance mechanisms through either hepatic metabolism or renal elimination²⁹ was modified and retrained using measured clearance data for 437 pharmaceuticals.^{27,30} The retrained model used in the current study was developed using the Molecular Operating Environment (MOE) software (Chemical Computing Group, Montreal, Canada). The 17 most important physicochemical descriptors determined through a contingency analysis were subjected to a principal component analysis (PCA) to reduce dimensionality of the correlated variables. A binary classification tree³¹ was then created, and the PCA component calculations were used as the classifiers for clearance mechanism type. Additional details regarding model development and PCA analysis are provided in the SI (Figure S1).

Predicting Clearance Rates

Three predictive quantitative clearance models were developed for this study: (1) a hepatic metabolism model, (2) a renal clearance model, and (3) a total clearance model. A multiple

linear regression (MLR) using a genetic algorithm (GA) technique was applied to create each model in the custom MOE support vector language (SVL) program QuaSAR-Evolution (Ryoka Systems, Inc.). The known renal and hepatic clearance rate for each of the pharmaceutical compounds from the clearance mechanism data set developed using the binary classification tree was used as the dependent variable against all 202 physicochemical descriptors in MOE. Additional details regarding model development, along with predicted values, are provided in the SI (Table S2).

Prioritizing Chemicals

A published approach used to prioritize pharmaceuticals as potential environmental hazards³² was applied in the current study to place the 31 chemicals/groupings into five discrete priority bins. Briefly, chemicals/groupings were sorted from lowest to highest inhibition activity and converted into Weibull ranks. Activity values and Weibull ranks were then plotted on a log-probability scale, and a linear distribution was fit to determine the slope and intercept using the open-source statistical program RStudio (version 0.98.1103; R Core Team, 2013). The threshold activity values at the 20th, 40th, 60th and 80th percentiles were then used to place chemicals/groupings into five priority bins: (1) lowest, (2) low, (3) medium, (4) high, and (5) highest for each scenario. Classification results from the reference scenario were then compared against results from the 11 scenarios having various combinations of predicted absorbed doses, clearance rates, and stoich_M, in order to assess misassignments.

A confusion matrix was created for each predictive scenario based on which bins chemicals fell into compared to bin placement in the reference scenario (SI, Figure S2). The confusion matrix was used to calculate scores (higher is better) for the statistical measures of overall scenario accuracy (A), class-average accuracy (CAA), class-average precision (CAP), class-average recall (CAR), class-average specificity (CAS), and F score according to the following equations

$$A = \frac{1}{n_{tot}} \sum_{i=1}^c TP_c \quad (7)$$

$$CAA = \frac{1}{n_{class}} \sum_{i=1}^c \frac{TP_c + Tn_c}{Tp_c + Tn_c + Fp_c + Fn_c} \quad (8)$$

$$CAP = \frac{1}{n_{class}} \sum_{i=1}^c \frac{Tp_c}{Tp_c + Fp_c} \quad (9)$$

$$CAR = \frac{1}{n_{class}} \sum_{i=1}^c \frac{Tp_c}{Tp_c + Fn_c} \quad (10)$$

$$CAS = \frac{1}{n_{class}} \sum_{i=1}^c \frac{Tn_c}{Tn_c + Fp_c} \quad (11)$$

where n_{tot} is the total number of observations for the scenario, n_{class} is the number of classes, c is the class, Tp are the number of true positives for a class, Tn are the number of true negative for a class, Fp are the number of false positives for a class, and Fn are the number of false negatives for a class. The number of false negatives is equal to the sum of all values not contained in a row or column for that class. The F score is the harmonic mean of the CAP and CAR.

Evaluating Parameter Uncertainties

We examined the sensitivity of model parameters on activity over possible ranges for those parameters. This additional analysis, which examines how activity changes as an individual parameter changes (while holding all other parameters constant), allows evaluation of the influence that each model parameter exhibits on activity across a larger chemical space than that covered by the chemicals used in our case study. As such, the findings are applicable to other chemicals and biological end points. These parameter ranges included (1) doses for each chemical spanning from the fifth to the 99th population percentile in SHEDS-HT, and the literature-reported value if it was excluded from this predicted range; (2) physiological ranges for Q (60–83 L/h²²), V_b (4.7–5.5 L²¹), and K_e (60–130 mL/min GFR²³ and assuming an f_u of 0.001–1); (3) stoich and E_{max} from 0.1 to 1; (4) EC₅₀ along the minimum to maximum of the dose–response curve used for the AChE inhibition in vitro assay; and (5) Cl_{int} , Cl_M , and Cl_{tot} spanning from 1 to ~10 times the literature-reported clearance value for each chemical. In addition, for those parameters linearly related to activity, a sensitivity index (sens) was calculated according to the equation³³

$$Sens = \frac{O_{max} - O_{min}}{O_{max}} \quad (12)$$

where O_{max} is the model output value using the maximum for the parameter and O_{min} is the output value using the minimum for the parameter, which allows for an estimation of sensitivity of the activity to each of the modeled parameters.

RESULTS AND DISCUSSION

In the reference scenario, most chemicals having both high doses and high potencies were placed into the highest priority bin due to the resulting high activity values (Table 1). The absorbed dose was a major factor influencing placement of chemicals into the lowest priority

bin as well. All chemicals placed into this bin have low reported absorbed doses, except for malathion. The high potency and slower clearance of the oxon likely resulted in placement of the malathion/malaoxon grouping into the bin above that of the parent alone. Potency does not appear to be as influential for the two lowest priority bins; eight of the 12 chemicals in these two bins exhibit low to moderate potency, and the other four (bendiocarb, toluene 2,4-diisocyanate [2,4-TDI], oxamyl, and mevinphos) exhibit high potency (Figure 1). Despite the high potencies of bendiocarb and 2,4-TDI, and high absorbed doses of malathion and dichlorvos, their moderate to rapid clearance rates resulted in their placement into the lower two priority bins. Generally, the absorbed doses and clearance characteristics of chemicals/groupings in the medium and high bins were balanced or countered by their potencies. For example, the thiodicarb/methomyl grouping, profenofos, and didecyldimethylammonium chloride (DDAC) have high absorbed doses and slow to moderate clearance rates (Table S1) but have low potencies (Figure 1). In contrast, the high potencies of carbofuran and carbosulfan (Figure 1) are balanced by their low absorbed doses or rapid clearance (Table S1).

The SHEDS-HT exposure model was developed for HT exposure-based prioritization of chemicals,¹⁴ so it takes, as input, limited amounts of data to rapidly estimate absorbed doses with large margins of uncertainty. SHEDS-HT was designed to be conservative with respect to several model parameters, such as the number of product types in which chemicals are found, chemical concentrations in various formulations, and differential consumer product use patterns. Differences between the distributions for each of these inputs and parameters can significantly affect predicted absorbed doses. For example, predicted absorbed doses between the 50th and 95th population percentiles for naled and thiodicarb span several orders of magnitude (Table S2).

Scenarios using predicted absorbed doses and literature-reported values for clearance, as well as scenarios using predicted values for both, resulted in lower scores for all statistical measures, especially regarding accuracy, CAP, CAR, and F score (Table 2), compared to scenarios using predicted clearance rates and literature-reported absorbed dose values. Those scenarios having higher scores also had a higher predictive capability and a lower number of bin misassignments (SI, Tables S3–S6). In addition, bin misassignments using predicted absorbed doses and literature-reported clearance rates, along with use of predicted values for both parameters, often involved changes across multiple bins. For example, the large differences between literature-reported and predicted absorbed doses at the 95th percentile for carbaryl resulted in misassignment from the lowest to the highest priority bin (Tables S4 and S6). In contrast, misassignment of bins in scenarios using predicted clearance rates and literature-reported absorbed doses mostly involved changes across only one priority bin (Table S3).

Intake doses found in the literature are also likely to hold some degree of uncertainty due to the difficulty in measuring all possible sources of exposure (e.g., food, water, surface contact), along with detection limits of analytical instruments. Furthermore, more recent literature reporting intake doses was not available, and the older sources used in the current study may not account for chemical bans, limits, or discontinuations after publication.

Presently, in vivo clearance values extrapolated from in vitro studies are used to prioritize chemicals in risk assessment.³⁴ In addition to in vitro measurements, several approaches exist that attempt to predict clearance mechanisms or rates in an in silico environment using physicochemical descriptors.^{29,35,36} The present study utilizes a two-step in silico approach that first determines primary clearance mechanism (either hepatic metabolism or renal clearance), and then predicts clearance rate.

The 17 most important physicochemical descriptors influencing primary clearance mechanism type, as selected through contingency analysis, were equally divided among twodimensional and three-dimensional descriptors—nine and eight, respectively. Due to the distinctive nature of hepatic and renal clearance, the majority of descriptors were related to hydrophilic or hydrophobic properties and demonstrated very high loadings on PC1 (SI, Figure S1 and Table S7). The first principal component explained 77% of the variance within the data set, while the first two components explained 85%. The binary classification tree produced for hepatic clearance and renal clearance using the training set of 200 random compounds was nearly identical to the tree produced from the training set tested through cross-validation of compound subsets. Primarily renally cleared compounds were misclassified at a rate of 12% for both tree models, while hepatically cleared compounds had a 1.3% misclassification rate using the random training set and a 0.84% misclassification rate using compound subsets.

Using the binary classifier model, eight of the 25 chemicals were predicted to be cleared primarily through renal mechanisms, while the remaining 17 were predicted to be cleared via hepatic metabolism. Predicting primary clearance mechanisms for environmental chemicals is not as straightforward as predicting these mechanisms for pharmaceuticals³⁷ because exposure to environmental chemicals is, more often than not, incidental, and detoxification occurs through the most optimal mechanism based on a chemical's physicochemical properties. Findings within the literature indicated that the majority of the chemicals selected for the current study undergo primarily hepatic elimination or a mixture of both hepatic metabolism and renal clearance. For seven of the eight chemicals (naled, azamethiphos, methomyl, oxamyl, bronopol, 2,4-TDI, and malaoxon) predicted to be renally cleared, total clearance rate was used in the PK model for the reference scenario. Thus, the renal clearance rate model was not implemented to predict clearance for these chemicals, and the total clearance rate model was used instead. Naled, azamethiphos, and methomyl exhibit 20–40% elimination through exhalation,^{24,25,38} which was a mechanism not accounted for in our binary classifier model; the rest of these eight chemicals are cleared through a mixture of hepatic metabolism and renal mechanisms.³⁹⁻⁴² The eighth chemical, malathion, is the parent of the active metabolite malaoxon, so the hepatic clearance rate model was used instead of the renal clearance model.

Comparing the three MLR-GA clearance models, the renal model held the best predictability, with 71% and 90% of compounds falling within a 2-fold and 3-fold difference, respectively (SI, Table S8). Although the renal model was not used in this particular study, it can be applied to future studies examining alternative biological end points. The predictive ability of the hepatic model was only slightly lower than that of the total clearance model, with 74% and 77% of the compounds falling within a 3-fold

difference for each model, respectively (SI, Table S8). The predictive abilities of the renal and hepatic clearance models from this study were comparable to the models developed by Lombardo et al.,²⁹ 51% vs 53% below 2-fold difference for hepatic clearance; and 71% vs 77% below 2-fold difference for renal clearance.

The models generated by Lombardo et al.²⁹ contained 55 descriptors in the renal clearance model and 80 descriptors in the hepatic clearance model. In contrast, the models developed in the current study constrained descriptor numbers to 5–6% of the total number of individual chemicals in each data set to avoid overfitting. These descriptors, along with their descriptions and constants for each model, can be found in the SI (Tables S9–S11). The rates predicted by the hepatic model were used to represent the Cl_{int} term in eqs 1 and 2 in order to determine hepatic clearance for the 6 parent compounds generating active metabolites. For the remainder of the compounds, the rates predicted by the total clearance model were used (SI, Table S2).

Uncertainty and sensitivity estimations involved a number of chemical-specific parameters (e.g., dose, EC_{50} , etc.), and presenting these results in their entirety is beyond the scope of this paper. Rather, the relationships that exist among changes in activity and changes in parameter values for each of the chemical categories are summarized here, and plots of representative chemicals for each of the chemical categories are provided in the SI (Figures S3–S12). The relationship between activity and several parameters was linear or approximately linear across the ranges evaluated. These parameters included $dose_p$, $dose_M$, Q_l , $stoich$, K_e , V_b , and E_{max} , and sensitivities varied widely depending on absorbed dose or potency of chemicals when grouped with their active metabolites. For example, activity was more sensitive to $stoich$ and K_e when considering exposure to parents alone (sens of 0.725 for $stoich$ and -0.217 for K_e) than when grouped with their active metabolites (sens of 0.334 for $stoich$ and -0.03 for K_e). Activity was highly sensitive to both E_{max} and absorbed doses of parent and metabolites when exposed alone (sens of 0.89 to 0.95 for both), and when grouped with their respective metabolites, the moiety with the higher potency or larger absorbed dose had the higher sensitivity (e.g., sens for E_{max} of 0.72 for thiodicarb and 0.40 for methomyl). Activity was only weakly sensitive to V_b (sens of -0.17 for all chemicals) and was not sensitive to Q_l (sens < 0.01).

Cl_{tot} , CL_M , Cl_{int} , and EC_{50} were nonlinearly related to activity. As can be derived from eq 4, when C_{avg} for parents or metabolites $\gg EC_{50}$, the EC_{50} term in the denominator becomes negligible, and activity approaches the E_{max} for the target chemical but should never exceed 1. At higher values of EC_{50} , C_{avg} approaches 0. A similar constraint exists for the relationship between hepatic blood flow (Q_l) and intrinsic hepatic clearance (Cl_{int}). When $Q_l \gg Cl_{int}$, the intrinsic hepatic clearance rate in the denominator becomes negligible and M approaches Cl_{int} . Similarly, when $Q_l \ll Cl_{int}$, the liver flow rate in the denominator becomes negligible and M approaches Q_l . The magnitude of change in activity with changing Cl_{int} quickly saturates and is minimal at high values of this parameter. Both Cl_{tot} and CL_M showed a negative exponential relationship with activity. That is, small changes in these parameters initially have strong effects on the chemical activity at relatively low values (generally below ~ 10 L/h), but once the relation between the parameter and activity reaches its inflection point, the effect saturates.

Due to the nonlinear relationship between the clearance terms and activity, uncertainties in clearance rate at low values can contribute significantly to the uncertainty in activity. As the sensitivity of activity decreases with increasing clearance values, uncertainty in clearance rate matters less. In addition, the overall uncertainty in activity contributed by uncertainty in clearance rate is bound, due to physiological mechanisms and constraints. In contrast, changes in activity can be approximated as linear over a reasonable range of absorbed dose. However, the magnitude of uncertainty in clearance rates is far exceeded by uncertainty in absorbed doses, as is demonstrated by many of the literature-reported absorbed doses falling outside the predicted range (the fifth to the 99th population percentile). These differences are anywhere from 2× to 10 orders of magnitude. In contrast, the largest difference between predicted clearance rates and literature-reported values is only ~20×.

Since several chemicals in our case study have exposure guidance levels derived from in vivo dose–response studies, one may be interested in comparing those levels with our priority ranking results. However, such a comparison is inappropriate because prediction of our in vivo activity levels involved incorporating estimates of population exposure concentrations to predict blood concentrations with in vitro response data. In contrast, exposure guidance levels are hazardous concentrations of a toxicant above which there may be significant irreversible and adverse health effects and which are determined through in vivo testing, without consideration of population exposure directly; these guidance levels can then be compared to estimated population exposure values using a margin of exposure approach.

As chemical production and environmental dispersal continues to increase, so too will the need for rapidly evaluating health risks for the population exposed to these chemicals. Here, we have presented a framework that can apply to the evaluation of other chemicals and biological end points by incorporating limited data on intake doses and PK characteristics to prioritize chemicals identified as active in in vitro assays. It should be recognized that, even in the reference scenario using literature-reported data, uncertainties exist due to the inconsistencies between studies or paucity of necessary information. However, this study reveals how using even limited information on exposure and PK properties can aid in improving confidence during chemical prioritization.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Abt E, Rodricks JV, Levy JI, Zeise L, Burke TA. Science and Decisions: Advancing Risk Assessment. *Risk Anal.* 2010; 30(7):1028–1036. [PubMed: 20497395]

2. Krewski D, Andersen ME, Mantus E, Zeise L. Toxicity testing in the 21st century: Implications for human health risk assessment. *Risk Anal An Off Publ Soc Risk Anal*. 2009; 29(4):474–479.
3. Hau, J., Schapiro, SJ., Jr, GL, VH. *Handbook of Laboratory Animal Science: Animal Models*. 2. CRC Press; Boca Raton: 2002.
4. MacIntosh, DL., Spengler, JD., Gutschmidt, K., Lyle, K. *Human Exposure Assessment; Environmental Health Criteria Monograph 214*. International Programme on Chemical Safety; Geneva, Switzerland: 2000.
5. Risk assessment forum white paper: Probabilistic risk assessment methods and case studies; Highlights of EPA/100/R-14/004. United States Environmental Protection Agency Office of the Science Advisor; Washington, DC: 2014. <http://www.epa.gov/sites/production/files/2014-12/documents/raf-pra-white-paper-final.pdf> [15 Dec 2015]
6. Phillips RD, Bahadori T, Barry BE, Bus JS, Gant TW, Mostow J, Smith C, Willuhn M, Zimmer U. Twenty-first century approaches to toxicity testing, biomonitoring, and risk assessment: perspectives from the global chemical industry. *J Exposure Sci Environ Epidemiol*. 2009; 19(6): 536–543.
7. Zurlo, J., Rudacille, D., Goldberg, AM. *Animals and Alternatives in Testing: History, Science, and Ethics*. Mary Ann Liebert, Inc; Larchmont, NY: 2001.
8. Egeghy PP, Judson R, Gangwal S, Mosher S, Smith D, Vail J, Cohen Hubal EA. The exposure data landscape for manufactured chemicals. *Sci Total Environ*. 2012; 414:159–166. [PubMed: 22104386]
9. Binetti R, Costamagna FM, Marcello I. Exponential growth of new chemicals and evolution of information relevant to risk control. *Ann Ist Super Sanita*. 2008; 44(1):13–15. [PubMed: 18469371]
10. Sun H, Xia M, Austin CP, Huang R. Paradigm Shift in Toxicity Testing and Modeling. *AAPS J*. 2012; 14(3):473–480. [PubMed: 22528508]
11. Austin, C., Kavlock, RJ., Tice, R. AltTox.org. Alttox.org; 2008. Tox21: Putting a lens on the vision of toxicity testing in the 21st century. <http://alttox.org/tox21-putting-a-lens-on-the-vision-of-toxicity-testing-in-the-21st-century/> [22 Apr 2016]
12. Lioy PJ, Smith KR. A Discussion of Exposure Science in the 21st Century: A Vision and a Strategy. *Environ Health Perspect*. 2013; 121(4):405–409. [PubMed: 23380895]
13. Judson RS, Kavlock RJ, Setzer RW, Hubal EAC, Martin MT, Knudsen TB, Houck KA, Thomas RS, Wetmore BA, Dix DJ. Estimating toxicity-related biological pathway altering doses for high-throughput chemical risk assessment. *Chem Res Toxicol*. 2011; 24(4):451–462. [PubMed: 21384849]
14. Isaacs KK, Glen WG, Egeghy P, Goldsmith MR, Smith L, Vallero D, Brooks R, Grulke CM, Özkaynak H. SHEDS-HT: an integrated probabilistic exposure model for prioritizing exposures to chemicals with near-field and dietary sources. *Environ Sci Technol*. 2014; 48(21):12750–12759. [PubMed: 25222184]
15. Judson RS, Martin MT, Egeghy P, Gangwal S, Reif DM, Kothiya P, Wolf M, Cathey T, Transue T, Smith D, et al. Aggregating Data for Computational Toxicology Applications: The U.S. Environmental Protection Agency (EPA) Aggregated Computational Toxicology Resource (ACToR) System. *Int J Mol Sci*. 2012; 13(2):1805–1831. [PubMed: 22408426]
16. Groh KJ, Carvalho RN, Chipman JK, Denslow ND, Halder M, Murphy CA, Roelofs D, Rolaki A, Schirmer K, Watanabe KH. Development and application of the adverse outcome pathway framework for understanding and predicting chronic toxicity: I. Challenges and research needs in ecotoxicology. *Chemosphere*. 2015; 120:764–777. [PubMed: 25439131]
17. Phillips MB, Leonard JA, Grulke CM, Chang D, Edwards SW, Brooks R, Goldsmith MR, El-Masri HA, Tan YM. A Workflow to Investigate Exposure and Pharmacokinetics Influences on High-Throughput in vitro Chemical Screening Based on Adverse Outcome Pathways. *Environ Health Perspect*. 2016; 124:53–60. [PubMed: 25978103]
18. Lep datu C, Enache M, Walker JD. Toward a More Realistic QSAR Approach to Predicting Metal Toxicity. *QSAR Comb Sci*. 2009; 28(5):520–525.
19. Baell J, Walters MA. Chemistry: Chemical con artists foil drug discovery. *Nature*. 2014; 513(7519):481–483. [PubMed: 25254460]

20. Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, et al. Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets. *Toxicology*. 2011; 282(1-2):1–15. [PubMed: 21251949]
21. Brown RP, Delp MD, Lindstedt SL, Rhomberg LR, Beliles RP. Physiological parameter values for physiologically based pharmacokinetic models. *Toxicol Ind Health*. 1997; 13(4):407–484. [PubMed: 9249929]
22. Carlisle KM, Halliwell M, Read AE, Wells PN. Estimation of total hepatic blood flow by duplex ultrasound. *Gut*. 1992; 33(1):92–97. [PubMed: 1740284]
23. Delanaye P, Schaeffner E, Ebert N, Cavalier E, Mariat C, Krzesinski JM, Moranne O. Normal reference values for glomerular filtration rate: what do we really know? *Nephrol Dial Transplant*. 2012; 27(7):2664–2672. [PubMed: 22802582]
24. Cheng, HM. Metabolism of [ethyl-1-¹⁴C] naled in rats characterization of ¹⁴C in tissues; Highlights of 928924215-025. Department of Pesticide Regulation; Chevron Chemical Company; Sacramento, CA: 1981. <http://www.cdpr.ca.gov/docs/risk/rcd/naled.pdf> [15 Feb 2015]
25. Hawkins, DR., Mayo, BC., Pollard, AD., Haynes, LM. ¹⁴C-Thiodicarb The metabolism in monkeys; Highlights of HRC/RNP/398/92/1553. Rhone-Poulenc, Inc; Research Triangle Park, NC: 1993. Unpublished. <http://www.inchem.org/documents/jmpr/jmpmono/v00pr09.htm> [22 Apr 2016]
26. Sarver JG, White D, Erhardt P, Bachmann K. Estimating xenobiotic half-lives in humans from rat data: influence of log P. *Environ Health Perspect*. 1997; 105(11):1204–1209. [PubMed: 9370523]
27. Obach RS, Lombardo F, Waters NJ. Trend Analysis of a Database of Intravenous Pharmacokinetic Parameters in Humans for 670 Drug Compounds. *Drug Metab Dispos*. 2008; 36(7):1385–1405. [PubMed: 18426954]
28. Goldsmith M-R, Grulke CM, Brooks RD, Transue TR, Tan YM, Frame A, Egeghy PP, Edwards R, Chang DT, Tornero-Velez R, Isaacs K, Wang A, Johnson J, Holm K, Reich M, Mitchell J, Vallero DA, Phillips L, Phillips M, Wambaugh JF, Judson RS, Buckley TJ, Dary CC. Development of a consumer product ingredient database for chemical exposure screening and prioritization. *Food Chem Toxicol*. 2014; 65:269–279. [PubMed: 24374094]
29. Lombardo F, Obach RS, Varma MV, Stringer R, Berellini G. Clearance mechanism assignment and total clearance prediction in humans based upon in silico models. *J Med Chem*. 2014; 57(10):4397–4405. [PubMed: 24773013]
30. Berellini G, Waters NJ, Lombardo F. *In silico* prediction of total human plasma clearance. *J Chem Inf Model*. 2012; 52(8):2069–2078. [PubMed: 22715914]
31. Breiman, L., Friedman, J., Ohlsen, R., Stone, C. Classification and regression trees. Wadsworth International Group; 1984.
32. Berninger JP, LaLone CA, Villeneuve DL, Ankley GT. Prioritization of pharmaceuticals for potential environmental hazard through leveraging a large-scale mammalian pharmacological dataset. *Environ Toxicol Chem*. 2016; 35(4):1007–1020. [PubMed: 25772004]
33. Hoffman, FO., Gardner, R. Evaluation of uncertainties in environmental radiological assessment models. In: Till, JE., Meyer, HR., editors. Radiological Assessments: A Textbook on Environmental Dose Assessment. US Nuclear Regulatory Commission; Washington D.C: 1983. p. 11.1-11.55. report no. NUREG/CR-3332
34. Wetmore BA, Wambaugh JF, Ferguson SS, Sochaski MA, Rotroff DM, Freeman K, Clewell HJ, Dix DJ, Andersen ME, Houck KA, Allen B. Integration of dosimetry, exposure, and high-throughput screening data in chemical toxicity assessment. *Toxicol Sci*. 2012; 125(1):157–174. [PubMed: 21948869]
35. Chang, DT., Goldsmith, MR., Tornero-Velez, R., Tan, YM., Grulke, CM., Chen, LJ., Ulrich, EM., Lindstrom, AB., Pasquinelli, MA., Rabinowitz, JR., Dary, CC. Parameters for Pesticide QSAR and PBPK/PD Models for Human Risk Assessment. Vol. 1099. American Chemical Society; Washington, DC: 2012. *In silico* strategies for modeling stereoselective metabolism of pyrethroids; p. 245-269.
36. Peyret T, Krishnan K, Peyret T, Krishnan K. Quantitative Property-Property Relationship for Screening-Level Prediction of Intrinsic Clearance of Volatile Organic Chemicals in Rats and Its

Integration within PBPK Models to Predict Inhalation Pharmacokinetics in Humans, Quantitative Property-Property. *J Toxicol.* 2012; 2012:1–22.

37. Landis, W., Sofield, R., Yu, MH., Landis, WG., Sofield, RM. Introduction to Environmental Toxicology: Molecular Substructures to Ecological Landscapes. 4. CRC Press; Boca Raton: 2010.
38. Azamethiphos summary report 2; Highlights of EMEA/MRL/527/98-Final. European Agency for the Evaluation of Medicinal Products, Veterinary Medicines Evaluation Unit; London: 1999. http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500010779.pdf [6 Jan 2015]
39. Bouchard M, Gosselin NH, Brunet RC, Samuel O, Dumoulin MJ, Carrier G. A toxicokinetic model of malathion and its metabolites as a tool to assess human exposure and risk through measurements of urinary biomarkers. *Toxicol Sci.* 2003; 73(1):182–194. [PubMed: 12657741]
40. Interim reregistration eligibility decision: oxamyl; Highlights of EPA 738-R-00-015. United States Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances; Washington, DC: 2000. http://archive.epa.gov/pesticides/reregistration/web/pdf/oxamyl_red.pdf [11 Sep 2015]
41. Reregistration eligibility decision for bronopol; Highlights of EPA 738-R-95-034. United States Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances; Washington, DC: 1995. <http://archive.epa.gov/pesticides/reregistration/web/pdf/2770red.pdf> [11 Sep 2015]
42. Moore DH, Chasseaud LF, Bucke D, Risdall PC. The percutaneous absorption and disposition of the antibacterial agent bronopol in rats and rabbits. *Food Cosmet Toxicol.* 1976; 14(3):189–192. [PubMed: 950212]
43. Gunderson EL. FDA Total Diet Study, April 1982–April 1984, dietary intakes of pesticides, selected elements, and other chemicals. *J Assoc Off Anal Chem.* 1988; 71(6):1200–1209. [PubMed: 3240978]
44. Tracqui A, Flesch F, Sauder P, Raul JS, Géraut A, Ludes B, Jaeger A. Repeated measurements of aldicarb in blood and urine in a case of nonfatal poisoning. *Hum Exp Toxicol.* 2001; 20(12):657–660. [PubMed: 11936581]
45. Evaluation on: azamethiphos: use as a public hygiene insecticide; Highlights of 207. Department for Environment, Food, and Rural Affairs, Pesticides Safety Directorate; York, U.K: 2003. http://www.pesticides.gov.uk/Resources/CRD/ACP/207_azamethiphos.pdf [11 Sep 2015]
46. Whitmore RW, Immerman FW, Camann DE, Bond AE, Lewis RG, Schaum JL. Non-occupational exposures to pesticides for residents of two U.S. cities. *Arch Environ Contam Toxicol.* 1994; 26(1):47–59. [PubMed: 8110023]
47. Challis IR, Adcock JW. The metabolism of the carbamate insecticide bendiocarb in the rat and in man. *Pestic Sci.* 1981; 12(6):638–644.
48. Feldmann RJ, Maibach HI. Percutaneous penetration of some pesticides and herbicides in man. *Toxicol Appl Pharmacol.* 1974; 28(1):126–132. [PubMed: 4853576]
49. Revised preliminary risk assessment for chlorethoxyfos; Highlights of 129006. United States Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances; Washington, DC: 1999. http://www3.epa.gov/pesticides/chem_search/hhbp/R000222.pdf [11 Sep 2015]
50. Didecyldimethyl ammonium chloride risk assessment; Highlights of 069149. Office of Pesticide Programs Antimicrobials Division; Arlington, VA: 2006. <http://pi.ace.orst.edu/search/getDocketDocument.s?document=EPA-HQ-OPP-2006-0338-0019> [6 Jan 2015]
51. Reregistration eligibility decision for alkylbenzene sulfonates; Highlights of EPA 739-R-06-006. United States Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances; Washington, DC: 2006. http://archive.epa.gov/pesticides/reregistration/web/pdf/alkylbenzene_red.pdf [11 Sep 2015]
52. Cresswell DG, Baldock GA, Chasseaud LF, Hawkins DR. Toxicology studies of linear alkylbenzene sulphonate (LAS) in rhesus monkeys. II. The disposition of [¹⁴C] LAS after oral or subcutaneous administration. *Toxicology.* 1978; 11(1):5–17. [PubMed: 100903]

53. Cochran, R. Mevinphos risk characterization document. California Environmental Protection Agency, Department of Pesticide Regulation; Sacramento, CA: 1994. <http://www.cdpr.ca.gov/docs/risk/rcd/mevinphos.pdf> [6 Jan 2015]
54. Menzie, CM. Metabolism of pesticides: Update III; Highlights of Special Scientific Report Wildlife No 232. United States Department of the Interior Fish and Wildlife Service; Washington, DC: 1980. https://archive.org/stream/specialscientifi232unit/specialscientifi232unit_djvu.txt [6 Jan 2015]
55. Harvey J, Han JCY. Metabolism of oxamyl and selected metabolites in the rat. *J Agric Food Chem.* 1978; 26(4):902–910. [PubMed: 670572]
56. Reregistration eligibility decision for profenofos; Highlights of 2540. United States Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances; Washington, DC, USA: 1996. http://archive.epa.gov/pesticides/reregistration/web/pdf/profenofos_red.pdf [11 Sep 2015]
57. Summary of toxicology data: Profenophos; Highlights of T051005. California Environmental Protection Agency, Department of Pesticide Regulation Medical Toxicology Branch; Sacramento, CA: 1997. <http://www.cdpr.ca.gov/docs/risk/toxsums/pdfs/2042.pdf> [6 January 2015]
58. Sanborn, JR. Human exposure assessment for propoxur; Highlights of HS-1655. California Environmental Protection Agency, Department of Pesticide Regulation, Worker Health and Safety Branch; Sacramento, CA: 1990. <http://www.cdpr.ca.gov/docs/risk/rcd/propoxur.pdf> [11 Sep 2015]
59. Musk AW, Peters JM, DiBerardinis L, Murphy RL. Absence of respiratory effects in subjects exposed to low concentrations of TDI and MDI. *J Occup Med Off Publ Ind Med Assoc.* 1982; 24(10):746–750.
60. Environmental health criteria 75: Toluene diisocyanates. World Health Organization International Programme on Chemical Safety; Geneva, Switzerland: 1987. <http://www.inchem.org/documents/pims/chemical/pim534.htm> [22 Apr 2016]
61. Gartrell MJ, Craun JC, Podrebarac DS, Gunderson EL. Pesticides, selected elements, and other chemicals in adult total diet samples, October 1980–March 1982. *J Assoc Off Anal Chem.* 1986; 69(1):146–159. [PubMed: 3949687]
62. Buratti FM, D'Aniello A, Volpe MT, Meneguz A, Testai E. Malathion Bioactivation in the Human Liver: The Contribution of Different Cytochrome P450 Isoforms. *Drug Metab Dispos.* 2004; 33(3):295–302. [PubMed: 15557345]
63. Buratti FM, Testai E. Malathion detoxification by human hepatic carboxylesterases and its inhibition by isomalathion and other pesticides. *J Biochem Mol Toxicol.* 2006; 19(6):406–414.
64. Eaton DL, Daroff RB, Autrup H, Bridges J, Buffler P, Costa LG, Coyle J, McKhann G, Mobley WC, Nadel L, et al. Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. *Crit Rev Toxicol.* 2008; 38:1–125.
65. Dong, MH., Haskell, DE. Human pesticide exposure assessment: Naled; Highlights of HS-1739. California Environmental Protection Agency, Department of Pesticide Regulation; Sacramento, CA: 1997. <http://www.cdpr.ca.gov/docs/whs/pdf/hs1739.pdf> [22 Apr 2016]
66. Duprey Z, Rivers S, Lubner G, Becker A, Blackmore C, Barr D, Weerasekera G, Kieszak S, Flanders WD, Rubin C. Community Aerial Mosquito Control and Naled Exposure. *J Am Mosq Control Assoc.* 2008; 24(1):42–46. [PubMed: 18437813]
67. Pesticide residues in food - 2003. World Health Organization, Food and Agriculture Organization of the United Nations; Geneva, Switzerland: 2003. <ftp://ftp.fao.org/docrep/fao/006/y5221E/y5221E00.pdf> [22 Apr 2016]
68. Abass K, Reponen P, Mattila S, Pelkonen O. Metabolism of carbosulfan. I. Species differences in the in vitro biotransformation by mammalian hepatic microsomes including human. *Chem-Biol Interact.* 2009; 181(2):210–219. [PubMed: 19523935]
69. Abass K, Reponen P, Mattila S, Pelkonen O. Metabolism of carbosulfan II. Human interindividual variability in its in vitro hepatic biotransformation and the identification of the cytochrome P450 isoforms involved. *Chem-Biol Interact.* 2010; 185(3):163–173. [PubMed: 20307514]
70. Reregistration eligibility decision for thiodicarb; Highlights of EPA-738-F-98-020. United States Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances; Washington, DC: 1998. <http://www.epa.gov/pesticides/reregistration/REDs/factsheets/2675fact.pdf> [12 Sep 2015]

71. Hiles, RA. Pesticide Residues in Food- 2000: Evaluations Residues, Part 1. Food and Agriculture Organization; 2000. The metabolism of thiodicarb (acetyl-1-14C) in albino rats. [21 Apr 2016]
72. Marty MA, Dawson SV, Bradman MA, Harnly ME, Dibartolomeis MJ. Assessment of exposure to malathion and malaoxon due to aerial application over urban areas of southern California. J Exposure Anal Environ Epidemiol. 1994; 4(1):65–81.
73. Lyon J, Taylor H, Ackerman B. A case report of intravenous malathion injection with determination of serum half-life. J Toxicol Clin Toxicol. 1987; 25(3):243–249. [PubMed: 3612901]
74. Nolan RJ, Rick DL, Freshour NL, Saunders JH. Chlorpyrifos: pharmacokinetics in human volunteers. Toxicol Appl Pharmacol. 1984; 73(1):8–15. [PubMed: 6200956]
75. Dichlorvos toxicology assessment. Australian Pesticides and Veterinary Medicines Authority; Kingston, Australia: 2008. <http://apvma.gov.au/sites/default/files/publication/15066-dichlorvos-tox.pdf> [12 September 2015]
76. Gallo, MA., Lawryk, NJ. Handbook of Pesticide Toxicology. Vol. 2. Academic Press; San Diego: 1991. Organic phosphorus pesticides; p. 917-1123.
77. Rubin, AL., Evert, S. Risk characterization document: Carbofuran. California Environmental Protection Agency, Department of Pesticide Regulation Medical Toxicology Branch; Sacramento, CA: 2006. <http://www.cdpr.ca.gov/docs/risk/rcd/carbofuran.pdf> [22 April 2016]
78. Menzie, CM. Metabolism of pesticides: An update; Special Scientific Report Wildlife 184. United States Department of the Interior Fish and Wildlife Service; Washington, D.C: 1974. <http://babel.hathitrust.org/cgi/pt?id=mdp.39015077582008;view=1up;seq=10> [6 Jan 2015]
79. Hawkins, DR., Mayo, BC., Pollard, AD., Haynes, LM. Pesticide Residues in Food-2000: Evaluations Residues, Part 1. Food and Agriculture Organization; 2000. The metabolism of [1-14C] methomyl in rats. <http://www.inchem.org/documents/jmpr/jmpmono/v00pr09.htm> [22 Apr 2016]
80. Yoon M, Efremenko A, Blaauboer BJ, Clewell HJ. Evaluation of simple in vitro to in vivo extrapolation approaches for environmental compounds. Toxicol In Vitro. 2014; 28(2):164–170. [PubMed: 24216301]

Environmental Exposure	Internal Metabolism	Chemical/Mixture	EC ₅₀ (mg/L)	E _{max}
<div>Active Parent (13)</div>	No metabolites considered	Aldicarb	7.610	0.207
		Azamethiphos	2.143	0.866
		Bendiocarb	0.913	0.986
		Bronopol	4.660	0.520
		Carbaryl	4.829	0.441
		Chlorethoxyfos	6.048	0.305
		DDAC	4.381	0.875
		DBSA	6.301	1.000
		Mevinphos	1.145	1.000
		Oxamyl	1.623	0.658
		Profenofos	19.801	0.393
		Propoxur	2.657	0.978
		2,4-TDI	1.529	0.593
<div>Active Metabolite (6)</div>	No metabolites considered	Malaoxon	9.743	0.212
		Chlorpyrifos Oxon	0.050	0.937
		Chlorpyrifos Methyl Oxon	0.050	0.937
		Dichlorvos	6.850	0.499
		Carbofuran	0.092	1.000
		Methomyl	2.255	0.642
<div>Inactive Parent (3)</div>	<div>Active Metabolite</div>	Malathion to oxon	0.000	0.000
		Chlorpyrifos to oxon	0.000	0.000
		Chlorpyrifos Methyl to oxon	0.000	0.000
<div>Active Parent (3)</div>	<div>Active Metabolite</div>	Naled to dichlorvos	0.385	0.819
		Carbosulfan to carbofuran	0.460	0.955
		Thiodicarb to methomyl	17.723	0.432
<div>Active metabolite plus Inactive Parent (3)</div>	<div>Active Metabolite</div>	Malathion & oxon	Contribution from metabolite only	
		Chlorpyrifos & oxon	Contribution from metabolite only	
		Chlorpyrifos Methyl & oxon	Contribution from metabolite only	
<div>Active metabolite plus Active Parent (3)</div>	<div>Active Metabolite</div>	Naled & dichlorvos	Contribution from parent and metabolite	
		Carbosulfan & carbofuran	Contribution from parent and metabolite	
		Thiodicarb & methomyl	Contribution from parent and metabolite	

Figure 1.

Categories of acetylcholinesterase inhibiting chemicals used as a case study for prioritization, along with their in vitro potencies. Abbreviations are as follows: DDAC, didecyldimethylammonium chloride; 2,4-TDI, toluene 2,4-diisocyanate; DBSA, dodecylbenzene sulfonic acid.

Figure 2.

Decision tree for selecting category of chemical of interest (A) and the appropriate pharmacokinetic (PK) and pharmacodynamic (PD) model inputs and parameters for that chemical category (B). The open circle in (A) represents a diverging step. Shaded boxes in (B) represent a need for the model input or parameter for that chemical category, whereas empty boxes represent exclusion of that parameter from the model. See text for how parameters were used in the PK and PD model.

Log (10) Transformed Inhibition Activity Values for Each of the Chemicals^a and Chemical Groupings (Parents and Metabolites) Derived from Integration of the Pharmacokinetic and Pharmacodynamic Model for the Reference^b and Predictive Scenarios^b

chemical or chemical group	ref	ExpClear			ExpClear			50expclear			95expclear		
		50th	95th	5%	50%	90%	5%	50%	90%	5%	50%	90%	
1. malathion to oxon	-7.28	-6.90	-3.59	-7.98	-6.98	-6.73	-7.61	-6.61	-6.35	-4.29	-3.30	-3.04	
1. methomyl	-7.18	-5.70	-4.70	-7.98	-7.98	-7.98	-6.50	-6.50	-6.50	-5.50	-5.50	-5.50	
1. bendiocarb ^d	-6.82	N/A	-6.19	-7.17	-7.17	-7.17	N/A	N/A	N/A	-6.54	-6.54	-6.54	
1. carbaryl	-6.64	-5.49	-2.04	-7.29	-7.29	-7.29	-6.13	-6.13	-6.13	-2.67	-2.67	-2.67	
2. 2,4-TDI ^d	-5.57	N/A	-6.85	-4.57	-4.57	-4.57	N/A	N/A	N/A	-5.85	-5.85	-5.85	
2. malaoxon	-5.49	-7.53	-6.77	-5.98	-5.98	-5.98	-8.02	-8.02	-8.02	-7.26	-7.26	-7.26	
2. malathion and oxon	-5.48	-6.81	-3.59	-5.98	-5.94	-5.91	-7.46	-6.59	-6.34	-4.29	-3.30	-3.04	
2. dichlorvos	-5.44	-6.75	-4.59	-4.36	-4.36	-4.36	-5.68	-5.68	-5.68	-3.52	-3.52	-3.52	
2. aldicarb	-5.38	-6.93	-5.80	-6.49	-6.49	-6.49	-8.04	-8.04	-8.04	-6.91	-6.91	-6.91	
2. oxamyl	-5.28	-5.81	-4.76	-5.94	-5.94	-5.94	-6.47	-6.47	-6.47	-5.42	-5.42	-5.42	
2. mevinphos	-5.04	-5.97	-4.32	-6.09	-6.09	-6.09	-7.01	-7.01	-7.01	-5.36	-5.36	-5.36	
2. chlorethoxyfos	-5.01	-5.63	-2.62	-6.33	-6.33	-6.33	-6.95	-6.95	-6.95	-3.94	-3.94	-3.94	
3. propoxur	-4.27	-6.96	-3.99	-4.82	-4.82	-4.82	-7.51	-7.51	-7.51	-4.54	-4.54	-4.54	
3. thiodicarb to methomyl	-4.13	-11.86	-6.18	-5.14	-4.87	-4.72	-12.87	-12.60	-12.45	-7.19	-6.92	-6.77	
3. thiodicarb and methomyl	-4.13	-5.70	-4.68	-5.14	-4.87	-4.72	-6.50	-6.50	-6.50	-5.49	-5.48	-5.48	
3. carbosulfan to carbofuran	-4.13	-4.73	-3.88	-5.17	-4.88	-4.73	-5.77	-5.48	-5.33	-4.92	-4.63	-4.47	
3. profenofos	-3.94	-7.47	-6.38	-3.81	-3.81	-3.81	-7.33	-7.33	-7.33	-6.25	-6.25	-6.25	
4. DDAC	-3.75	-2.71	-1.69	-4.95	-4.95	-4.95	-3.91	-3.91	-3.91	-2.88	-2.88	-2.88	
4. chlorpyrifos to oxon	-3.70	-3.58	-0.68	-5.16	-4.17	-3.91	-5.05	-4.05	-3.79	-2.03	-1.07	-0.85	
4. chlorpyrifos-methyl to oxon	-3.55	-5.05	-3.47	-5.02	-4.02	-3.77	-6.52	-5.52	-5.26	-4.94	-3.94	-3.68	
4. carbofuran	-3.43	-4.49	-3.64	-4.15	-4.15	-4.15	-5.21	-5.21	-5.21	-4.36	-4.36	-4.36	
4. carbosulfan and carbofuran	-3.35	-4.29	-3.44	-4.11	-4.08	-4.05	-5.11	-5.03	-4.96	-4.25	-4.17	-4.11	
4. chlorpyrifos oxon	-3.06	-7.14	-4.18	-3.77	-3.77	-3.77	-7.86	-7.86	-7.86	-4.90	-4.90	-4.90	
4. chlorpyrifos and oxon	-2.97	-3.58	-0.68	-3.76	-3.63	-3.54	-5.05	-4.05	-3.79	-2.03	-1.07	-0.85	
4. chlorpyrifos-methyl oxon	-2.94	-7.14	-4.18	-3.65	-3.65	-3.65	-7.86	-7.86	-7.86	-4.90	-4.90	-4.90	
5. chlorpyrifos-methyl and oxon	-2.84	-5.04	-3.39	-3.63	-3.50	-3.40	-6.50	-5.51	-5.26	-4.61	-3.89	-3.66	

chemical or chemical group	ref	ExpClear			ExpClear			50expclear			95expclear		
		50th	95th	5%	50%	90%	5%	50%	90%	5%	50%	90%	5%
5. azamethiphos	-2.76	-4.36	-2.21	-3.50	-3.50	-3.50	-3.50	-5.10	-5.10	-5.10	-2.94	-2.94	-2.94
5. bronopol	-2.62	-5.11	-3.66	-3.31	-3.31	-3.31	-3.31	-5.81	-5.81	-5.81	-4.36	-4.36	-4.36
5. DBSA	-2.05	-1.98	-0.87	-1.72	-1.72	-1.72	-1.72	-1.65	-1.65	-1.65	-0.60	-0.60	-0.60
5. naled to dichlorvos	-1.99	-11.44	-5.14	-1.72	-1.72	-1.72	-1.72	-11.17	-11.17	-11.16	-4.87	-4.87	-4.87
5. naled and dichlorvos	-1.99	-6.75	-4.49	-1.72	-1.72	-1.72	-1.72	-5.68	-5.68	-5.68	-3.50	-3.50	-3.50

^aChemical abbreviations are as follows: DDAC, didecyltrimethylammonium chloride; DBSA, dodecylbenzene sulfonic acid; 2,4-TDI, toluene 2,4-diisocyanate.

^bThe reference scenario represents inhibition activities using literature-reported values for each parameter, as found in Table S1. Chemicals are listed in order of increasing activity for this reference scenario, and the numbers before each chemical represent which priority bin they fall into. These numbers are as follows: 1, Lowest; 2, Low; 3, Medium; 4, High; 5, Highest.

^cThe remaining scenarios represent inhibition activity values using combinations of predicted values for each parameter, as found in Table S2. Abbreviations for the 11 scenarios are as follows: Exp (mg/h), use of literature-reported doses or predicted absorbed doses at the 50th and 95th population percentiles; Clear (L/h), use of literature-reported clearance rates or predicted clearance rates assuming a 5%, 50%, or 90% stoichiometric yield for an active metabolite, when applicable. The parameters in bold were predicted within each scenario, and those in normal font are parameters for which literature-reported values were used.

^dThese two chemicals had a predicted absorbed dose of 0 mg/h at the 50th population percentile, and so calculation of inhibition activities at this percentile was not possible.

Table 2

Statistical Measures^a Calculated from the Confusion Matrix Associated with Misassignment of Priority Bins for Each of the 11 Predictive Scenarios,^b Compared to Bin Placement of Chemicals in the Reference Scenario

scenario	accuracy	CA-accuracy	CA-precision	CA-recall	CA-specificity	F-score
50ExpClear	0.323	0.729	0.299	0.298	0.828	0.299
95ExpClear	0.290	0.716	0.311	0.297	0.825	0.304
ExpClear5	0.710	0.884	0.771	0.743	0.927	0.757
ExpClear50	0.710	0.884	0.775	0.735	0.927	0.754
ExpClear90	0.710	0.884	0.775	0.735	0.927	0.754
50ExpClear5	0.323	0.729	0.339	0.298	0.829	0.317
50ExpClear50	0.290	0.716	0.286	0.273	0.822	0.279
50ExpClear90	0.323	0.729	0.304	0.298	0.829	0.301
95ExpClear5	0.258	0.703	0.260	0.272	0.818	0.266
95ExpClear50	0.258	0.703	0.248	0.263	0.813	0.256
95ExpClear90	0.290	0.716	0.282	0.288	0.820	0.285

^aMeasures are accuracy of the scenario as a whole, class-average accuracy, class-average precision, class-average recall, class-average specificity, and F-score.

^bThe parameters in bold were predicted within each scenario, and those in normal font are parameters for which literature-reported values were used. Abbreviations for the 11 scenarios are as follows: Exp (mg/h), use of literature-reported doses or predicted absorbed doses at the 50th and 95th population percentiles; Clear (L/h), use of literature-reported clearance rates or predicted clearance rates assuming a 5%, 50%, or 90% stoichiometric yield for an active metabolite, when applicable.