ORD MANUSCRIPT COVER SHEET

**Title:** Collaborative Evaluation of *In silico* Predictions for High Throughput Toxicokinetics

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**Four bullet point summary:**

* Toxicokinetic (TK) information, such as elimination half-life (thalf, plotted below), is critical for understanding chemical risk
* Here we collected *in silico* (quantitative structure-property relationship, QSPR) predictions of key *in vitro* determinants of TK from several different models
* The models were evaluated for ability to reproduce *in vitro* and *In vivo* measurements of TK
* Overall, high throughput physiologically-based TK (PBTK) model performed similarly when using TK QSPRs as when the actual *in vitro* measured data were used

**One sentence description:** This collaborative trial demonstrates that multiple QSPRs exist that make reasonably accurate chemical structure-based predictions for *in vitro* TK parameters

**Chemicals Involved:** 87 ToxCast chemicals with diverse uses for which high-throughput toxicokinetic (HTTK) data are available

**Collaborative Evaluation of *In Silico* Predictions for High Throughput Toxicokinetics**

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# Abstract

To assess public health risks posed by chemicals we need to understand chemical absorption, distribution, metabolism, and elimination by the body (that is, toxicokinetics or TK). Unfortunately, chemical-specific TK data are unavailable for thousands of chemicals in commerce and the environment*. In silico* predictions along with high throughput toxicokinetic (HTTK) methods have the potential to address this gap for chemical risk evaluators. This collaborative trial uses *in vivo* measured toxicokinetic data to evaluate *in silico* approaches. Through comparison with measured data, predictive performance and bias can be empirically estimated. Six different sets of quantitative structure-property-relationship (QSPR) tools for predicting TK were evaluated. Four of the QSPR models made predictions for chemical-specific *in vitro* measurements. These four models were evaluated using *in vitro*-measured data for 68 chemicals. The *in vitro* parameter QSPRs were generally consistent; however, accuracy varied by chemical. QSPR predictions were further evaluated by using the predicted parameters within a physiologically based TK (PBTK) model to predict *in vivo* measured plasma concentrations for 83 chemicals mostly in rats with limited human data. This analysis used the generic PBTK model in R package "httk", which is designed to use the *in vitro* parameters predicted by the QSPRs. Both the models and in vitro data performed better than a y-randomized model and worse than empirical fits to the data, with the QSPR trained to the largest dataset (ADMET Predictor) performing best. In addition to the four QSPR-parameterized PBTK models, two additional models were evaluated for predicting chemical half-life. All six models performed adequately across the across 83 chemicals evaluation chemicals. Depending on the required accuracy, multiple QSPRs make reasonable predictions for chemical-specific TK. These models can provide key information for risk-based prioritization of many thousands of chemicals without either *in vivo* or *in vitro* TK data

# Introduction

Toxicokinetics (TK) describes the absorption, distribution, metabolism, and excretion (ADME) of a chemical compound in the body as a function of time [1]. Since TK allows the prediction of internal tissue concentrations as a function of chemical exposure it provides critical information for assessing risk posed by a chemical to public health [2]. TK allows interpretation of biomonitoring data [3], dosimetric anchoring of animal toxicity studies [4], and quantitative *in vitro*-*in vivo* extrapolation (or IVIVE) from high throughput bioactivity studies [5]. These *in vitro* bioactivity data are available for thousands of chemicals (for example, the ToxCast [6] and Tox21 [7] screening programs). IVIVE of bioactivity data relies upon TK to relate the concentrations found to be active *in vitro* with doses that could cause these concentrations in tissues [8-12]. Unfortunately, chemical-specific information on TK are often unavailable for thousands of chemicals in commerce and the environment [13]. New approach methodologies (NAMs) are being developed throughput the chemical risk assessment process [14] including new methods for assessing TK [15,16].

For non-therapeutic compounds TK data were traditionally developed using animal studies; for reasons of both ethics and resources these studies are no longer desirable nor practical for the thousands of remaining chemicals [17]. An alternative technology developed by the pharmaceutical industry relies on characterizing certain aspects of TK *in vitro* and then extrapolating to *in vivo* conditions to estimate TK parameters such as AUC, often within a three-fold error [18]. For the past decade, government chemical regulatory agencies working with their collaborators and contractors have been collecting chemical-specific *in vitro* data that allow prediction of TK [8-12,19]. These data currently span approximately one thousand chemicals used for industry, pesticides, diet, therapy, and consumer products. However, thousands more remain uncharacterized.

The U.S. National Academies of Science, Engineering, and Mathematics have recognized that *in vitro* TK data “enabled first-tier risk-based rankings of chemicals on the basis of margins of exposure—the ratio of exposures that cause effects (or bioactivity) to measured or estimated human exposures” [20]. To address the remaining chemicals for which even *in vitro* TK data are unavailable, multiple organizations have developed *in silico* quantitative structure-property relationship (QSPR) models for predicting these values [21-25]. Meanwhile, EPA and other organizations are continuing to accumulate chemical-specific TK data, both *in vivo* – that is, curation of data from the scientific literature as well as a targeted animal studies only when needed [26,27] – as well as *in vitro* [11,19,12].

Here we examine the TK predictions from six different modeling teams. Four modeling teams produced QSPR models for two key toxicokinetic parameters that can be measured *in vitro*: intrinsic hepatic clearance (Clint measured with hepatocyte incubations, [28]) and fraction unbound in plasma (fup, typically measured via rapid equilibrium dialysis [29]). The models are initially evaluated for their ability to reproduce *in vitro* measured values. However, we focus on analysis of the predictions for chemical concentration as a function of time (CvT) that can be made when a generic physiologically-based TK (PBTK) model [30] is used with the predictions from each QSPR. For this study *in vivo* plasma and blood concentration vs. time data for rat and human were available for 101 chemicals from the CvTdb [27]. Models are evaluated for ability to reproduce the full CvT curve as well as summary statistics (such as peak plasma concentration) and parameters (such as chemical half-life). Two additional models for chemical half-life are also evaluated. The six modeling teams were provided with chemical identities and some physico-chemical descriptors but were not provided with the actual *in vivo* evaluation data.

# Methods

As summarized in Table 1, three levels of evaluation were made. First (Level 1), QSPR predictions of parameters were compared against *in vitro*-measured values for each chemical where data were available. Then (Level 2) the ability of a PBTK model to predict chemical concentration vs. time behavior was evaluated against *in vivo* measurements for the full time-course observed for each combination of QSPR and chemical. Finally (Level 3) the ability of the QSPRs and PBTK model to predict summary statistics (for example Cmax, AUC, half-life) was evaluated. All analyses were performed in the free, open-source statistical analysis language R [31] v4.2.0. Analyses were performed on a Dell Latitude 7280 laptop personal computer. Scripts to perform all analyses are available in RMarkdown [32] format as supplemental material.

## HTTK

R package “httk” [30] v2.0.5 was used for this analysis. “httk” can parameterize a physiologically based toxicokinetic (PBTK) model based on chemical-specific values for fraction unbound in plasma (fup, unitless) and intrinsic hepatic clearance (Clint, µL/min/106 hepatocytes). These experimentally measured values are collected from peer-reviewed literature and provided by “httk”. All the QSPRs analyzed here were trained to human data.

The generic PBTK model used here (model “pbtk”) consists of well-mixed compartments for the gut, kidney, liver, and rest of body. The model is parameterized for a chemical using fup and Clint plus equilibrium tissue:plasma partition coefficients predicted with a modified Schmitt’s method [33,34]. The model simulates both oral and intravenous dosing. Oral dosing is subject to first-pass metabolism by the liver before the compound distributes systemically. Among other species, the model includes physiological information for parameterizing both humans and rats (primarily from [35,33,36]. The model was simulated using command “solve\_pbtk()” with option default.to.human=TRUE – that is, since no rat-specific values are predicted by the models under evaluation, comparisons to data from rats were done using rat physiology but human in vitro TK parameters.

The steady-state ratio of the concentration of chemical in blood and plasma (Rb:p) is an important parameter in the PBTK model and is used for converting between plasma predictions and observations in whole blood. The “httk” function get\_rblood2plasma either retrieves measured values of Rb:p from the literature or predicts the ratio by predicting the red blood cell:plasma equilibrium partition coefficient and then using the hematocrit fraction for the relevant species.

R is an interpreted language (primarily operated by a user from the command-line although scripts are common). The user can alter the values within an “httk” table which stores the fup and Clint values for all chemicals (chem.phys\_and\_invitro.data). After alteration, the httk function will proceed using the new values in the table. “httk” can be returned to its default chem.phys\_and\_invitro.data table via the command “reset\_httk()”. By default, no QSPR values are included in the table. However, predictions can be loaded with the commands “load\_sipes2017(overwrite=TRUE)”, “load\_pradeep2020(overwrite=TRUE)”, or “load\_dawson2021(overwrite=TRUE)” [23-25]. The argument “overwrite=TRUE” is needed so that *in vitro* measured data are overwritten whenever a chemical-specific prediction is available. To facilitate comparisons, a custom function “clear\_httk()” is included in the supplemental material which deletes all Human Clint and fup values. The OPERA predictions are available as Supplemental Table 1.

## QSPR Models

The QSPR models evaluated are summarized in Table 2. Four different modeling teams previously [23-25] produced quantitative structure-property relationship (QSPR) models for two key toxicokinetic parameters that can be measured *in vitro*: intrinsic hepatic clearance (Clint measured with hepatocyte incubations) and fraction unbound in plasma (fup). Two additional models for chemical half-life were also evaluated. Individual model predictions are available in Supplemental Table 1.

Due to the potential presence of the model evaluation data (that is, measured *in vitro* parameters) in the training sets for some or all the models, we attempted to remove predictions that seemed more like a direct retrieval of the chemical-specific values from a training set. Model predictions were removed for a particular model-chemical combination if both the predictions for Clint and fup were within 1% absolute fold error of the measured values, the predictions were omitted from the evaluations. Omitted predictions are listed in Supplemental Table 6. When a QSPR model prediction was missing for a particular chemical the mean prediction of the other models was used for evaluation purposes.

## *In vitro* Data

For 63 of the test chemicals, *in vitro* measurements were also available for comparison. These data are collected by the R package “httk” but are drawn from the peer reviewed scientific literature (including [9,5,12]). The *in vitro* measured values are available in Supplemental Table 1.

## *In vivo* Data

EPA has developed a public database of concentration vs. time data for building, calibrating, and evaluating TK models [27]. Curation and development of the database are ongoing, but when this study began there were 101 chemicals with either rat or human *in vivo* blood or plasma concentration vs. time data. The *in vivo* measured concentration vs. time values are available as Supplemental Table 2.

## Compartmental Model Fits

For each chemical with CvT data, parameters were estimated for empirical one- and two-compartment toxicokinetic models using R package “invivoPKfit” (<https://github.com/USEPA/CompTox-ExpoCast-invivoPKfit>). Between the one- and two-compartment models, the one with the lower Akaike Information Criterion (AIC) value – indicating model parsimony – was selected [37]. The empirical model fit was then used as a “best case” prediction scenario for comparison with PBTK parameterize by *in vitro* or QSPR predictions.

For both models a half-life was calculated from the terminal elimination rate as thalf = ln(2)/kelim. For the two-compartment model the volume of distribution at steady-state was used as Vd. For both models, clearance was calculated as Cltot = Vd \* kelim. The estimated TK parameters for both models are provided as Supplemental Table 3.

## Evaluation Metrics

Multiple statistics were used to evaluate predictions (*pred*) relative to observed values (*obs*) as appropriate. Relative Predictive Error (RPE) was calculated as RPE = , where if the observed value was 0 then the error was set to zero. We note that if the predicted value is 0 then RPE = -1. Absolute Average Fold Error (AAFE) was calculated as AAFE = , where if pred=0 and obs=0 we assigned = 0. Root Mean Squared Log Error (RMSLE) was calculated as RMSLE = .

Kolmogorov-Smirnov tests were performed using R function *ks.test*.

# Results

## Evaluation Chemicals and Predictions

There were 102 chemicals present in the CvTdb (Sayre, 2020) as of September 2019 that had plasma concentration data following either oral or intravenous doses given to rats or humans. These chemicals included: 57 from the Toxic Substances Control Act (TSCA) active inventory [38], 20 pharmaceuticals, 24 pesticides, 99 that are found in consumer products, 7 per- and poly-fluorinated substances (PFAS) [39], and 64 that are part of the ToxCast screening program. Note that a chemical could be in more than one of these categories.

Of the 102 chemicals, there were 10 chemicals that could only be predicted by OPERA and were omitted from the rest of the analysis (Supplemental Table 5). Two chemicals, Oxoacetic acid--water (1/1) and Nitrite have CvT data but do not have either measured or predicted values for both Clint and fup from any model to date.

The data for each chemical was fit using maximum likelihood estimation to one and two compartment empirical pharmacokinetic models, with separate fits for each combination of compound and species for which there were data. Maximum likelihood estimates could not be obtained for either model for 8 chemicals (listed in Supplemental Table 4). These chemicals were withheld from subsequent analysis. For each remaining chemical the better of the one or two compartment models was used on the basis of model parsimony.

Eliminating chemicals where the CvT data could not be described by an empirical model or for which there were only one QSPR that could make predictions left 83 chemicals with *in vivo* CvT data and 68 chemicals with *in vitro* measure fup and Clint.

For each QSPR we removed predictions where the predicted values for a given chemical were within 1% for both fup and Clint assuming these values reflected the chemical data present in the training set and the model method allowing for recall of the measurements. This only affected 21 chemicals as predicted by OPERA.

We summarize the chemical-specific properties and predictions in Figure 1. In Figure 1 similar chemicals (rows) and properties/predictions (columns) are clustered together based on Euclidean distance. All properties/predictions were centered (mean changed to zero) and scaled (divided by standard deviation) such that the value reflects the number of standard deviations from the mean. Interestingly, the first division between clusters in Figure 1 places all the Clint measurements and predictions on the one side and all the fup measurements and predictions on the other. The physico-chemical properties are divided between those two clusters, with Octanol:Water (partition coefficient, PC), Octanol:Air PC, Molecular Weight, Boiling Point, and Melting Point all clustering with Clint. Water solubility, vapor pressure, and the Henry’s law constant all clustered with fup.

## Level 1 Analysis

Our first level of evaluation directly compared the predictions of QSPR's with the *in vitro* measured values. We do not have predictions for all the chemicals across all QSPRs – when a prediction is missing, we assign the average prediction across those models that successfully made a prediction for that chemical. There were 63 chemicals with *in vitro* measured parameters. We evaluate model performance for Clint in Figure 2. The QSPR with the lowest mean RMSLE (root mean squared log10 error) is OPERA, which uses a nearest neighbors method to retrieve values from similar chemicals in the training set. On the basis of RMSLE the other three models – Dawson (2021), Pradeep (2020), and Simulations Plus ADMET predictor – perform similarly to each other despite the fact that the Dawson model is categorical (that is, predicting only three values: very slow, slow, and fast) while the other models are continuous. Figure 3 shows that all four models perform very well for predicting fup. Most models are within ten-fold and predictions are highly correlated with observed. Mean RMSLE ranged from 0.03 to 0.06 – all very small values. For chemicals where there is not a tight correlation it looks like most models over predict the fup value. OPERA has both over and under predictions in these “outliers". OPERA has the smallest variability in the relative error, ADMET has the most variability, and Dawson and Pradeep are rather on par with one another (between OPERA and SPlus).

We examined the distributions of fold errors between the predictions and the measured data using a Kolmogorov-Smirnov test. For both Clint and fup the only QSPR that differed from the others was OPERA, which had a significant (p-value < 0.05) difference between the distribution of predicted values and the distributions of the three other QSPRs. In Table 3 we summarize the fold errors for the four QSPRs. The median fold error for all for QSPRs is close to zero except for ADMET Predictor, which has a slight bias toward overestimating clearance (log10 fold error of 0.105 in Table 3 corresponding to median predictions being 27% higher than measured). We note that the median fold errors for OPERA are extremely low (effectively zero for fup), even with the obvious training set chemicals (Supplemental Table 6) removed.

Also shown in Table 3 is that the predictions of Clint range from 100x lower than experimental values (log10 folder error of -2) for all four QSPRs to 100x higher (log10 fold error of 2) for three of the four QSPRs. For fup the predictions range from 30x too low to more than a million times overestimated (log10 fold error of 6).

## Level 2 Analysis

For the second level of analysis, we compared predictions based on the QSPR predicted values with actual tissue concentration vs. time data. We evaluated based upon 83 chemicals as described above. All predicted values are used with the HTTK PBTK model to make predictions. We bracket model performance in three ways: First, we use the HTTK PBTK model with the actual in vitro measured values. In subsequent figures this is labeled as “HTTK-InVitro”. Next, for a best-case performance, we use empirical (one or two compartment toxicokinetic model) fits to the data themselves, labelled as “FitsToData”. The one and two compartment models are simpler than the high throughput PBTK model used for all other scenarios, but because they have been optimized to the evaluation data they are expected to outperform the other approaches here. Finally, for worst case performance we use y-randomization so that the measured values for the 83 chemicals are scrambled and assigned to the incorrect chemicals, labelled “HTTK-YRandom”. Figure 4 shows the full predicted time-courses for each set of model predictions as well as the actual *in vitro* data and empirical model fits. The PBTK model generally underpredicted the *in vivo* data, both when used with *in vitro*-measured parameters (mean RPE -0.71) and with the QSPR-predict values (MRPE for -0.66 for ADMET, -0.74 for Dawson, -0.80 for Pradeep, and -0.67 for OPERA).

In Panel A of Figure 5 we examine the distribution of RMSLE on a per chemical basis, aggregating over all doses, routes, and time points for each chemical. All observed time points are valued equally, without consideration of phase (absorption/distribution/metabolism) and measurement accuracy. First, we observe that the empirical fits to the data yield an average RMSLE of 0.54, corresponding on the arithmetic scale to predictions being on average within a factor of 3.5 times the observed values. The difference between the predictions made with *in vitro* measurements and y-randomized *in vitro* measurements is relatively small, with predictions based upon *in vitro* measurements being within 18x of observations on average and y-randomized values being with 22x of observations on average. All four QSPRs perform similarly to the *in vitro* data, with ADMET predictor being the best with an average prediction within 14x the observations.

Noting that the combination of ADMET predicted values and the generic PBTK model from “httk” produces values more accurate than either the actual measured *in vitro* values or the one compartment model fits, we looked to understand how the ADMET values differed from the rest. As discussed above, the clearance values predicted by ADMET were on average 27% higher than the experimentally measured values. In Panels B and C of Figure 5 we respectively break each time course into two phases, early (that is, time points less than the mean time for a given study) and late (the remaining points). We presume that the early phase will typically include the absorption and distribution phases, included the peak concentration. We note that the same absorption rate is used for all QSPR models (and indeed for all chemicals) based on the mean absorption observed in Wambaugh et al. [26]. All the models perform better for later time points than earlier time points, and the four QSPRs and *in vitro* measured data all perform effectively the same for later time points. The differences in performance of ADMET predictions seems to be in the early time points.

Early time points are dominated by the ability to correctly predict peak plasma concentration (Cmax). In Figure 6 we examine each methods accuracy in predicting Cmax as determined from the CvT data. As summarized in Table 4 optimal performance is given by the empirical fits to the data, with a coefficient of variation (R2) of 0.94 and a RMSLE of 0.32. Worst case performance is given by the y-randomized measured data with a R2 of 0.42 and RMSLE of .99. The QSPRs perform roughly as well, if not clearly better (ADMET being best) than the *in vitro* measured data, with R2 ranging from 0.48 to 0.59 and RMSLE ranging from 0.83 to 0.93. The superior ability of ADMET to predict Cmax likely is correlated with its better performance at early times.

In Figure 7 we examine the ability to predict time-integrated area under the plasma concentration time course (Area Under the Curve or AUC). Again, the empirical fits give a clear best-case scenario, but here the y-randomization more clearly gives a worst-case scenario. The other various methods are all roughly comparable, although again ADMET outperforms even the in vitro measured values.

In Figure 8 we plot each chemical-specific RMSLE by prediction method with each method as a row and each chemical as a column. These data are also provided in Supplemental Table 7. Chemicals and methods have been clustered based upon Euclidean distance. We see that the largest RMSLE is for the Tamoxifen measured data, potentially influencing the performance of in vitro measured data relative to the predictions of the QSPRs.

## Level 3 Analysis

We then proceed on to the third level of evaluation, in which we use the QSPR predictions to predict toxicokinetic summary parameters – volume of distribution (Vd), half-life for elimination from the body (thalf), and whole-body clearance (Cltot) – and compare the predictions to the values estimated from the empirical fits to the CvT data. The values predicted for each method are provided in Supplemental Table 8.

In Figure 9 we examine predicted vs. observed thalf. As summarized in Table 4, none of the models are very successful – the highest coefficient of variation is 0.15 for IFS-QSAR, while QSARINS-Chem and HTTK with ADMET both had an R2 of 0.11. The RMSLE for all models, including HTTK with y-randomized data, was just above 1 (a factor of 10x).

The models were distinctly better than the y-randomization for predicting Vd. As shown in Figure 10 and summarized in Table 4, the HTTK algorithm for predicting Vd [34] when used with y-randomized data had no skill. The models performed similarly to the measured *in vitro* data when used with the Vd algorithm – R2 ranged from 0.10 to 0.16 with the measured data being the worst. For all models the RMSLE again indicated a factor of 10x.

In Figure 11 we examine predictions for CLtot, which depends on both elimination rate (inverse of thalf) and Vd. As summarized in Table 4 the y-randomized predictions reassuringly have no skill at predicting *in vivo* clearance, while the combination of HTTK and ADMET predicted values had the most, with a R2 of 0.32 and a RMSLE indicating a factor of 17x. Both IFS-QSAR and QSARINS-Chem had comparable R2 of 0.25 and 0.2 (respectively) and an RMSLE indicating a factor of ~20x. The other QSPRs performed about as well as using the *in vitro* measured data.

# Discussion

* Here we have conducted a collaborative trial of four chemical structure-based predictors of *in vitro* TK parameters and two additional predictors of *in vivo* TK half-life
  + Evaluated with both *in vitro* data and, more importantly, evaluated impact of predictions on *in vivo* data
  + both in silico-in vitro extrapolation and in silico-in vivo extrapolation for toxicokinetics
  + Driven by 101 chemicals with rat or human data from CvTdb (Sayre et al. 2020)
* Two Goals:
  + Any remaining analyses to do?
  + After this meeting I'll turn the Discussion into a narrative – any additional points to add?
* TK information, especially elimination Vd, half-life (thalf), and whole-body clearance, is critical for understanding chemical risk.
  + The NAS has recognized [20] that high throughput (chemical-agnostic) TK models parameterized with chemical-specific *in vitro* data (that is, in vitro-in vivo extrapolation for toxicokinetics) are a powerful tool for interpreting high throughput screening data for chemical toxicity in terms of chemical risk [2] – in vitro-in vivo extrapolation for toxicity
  + This information allows evaluation of potential to accumulate and inference of exposure from biomonitoring data. – large data TK data gaps for analytes identified in biological matrices by both targeted (NHANES [40]) and non-targeted analyses (Woodruff [41])
* Governments and industry are continuing to accumulate chemical-specific TK data, both:
  + *In vivo* CvTdb, [27,26]
  + *In vitro* [8,9,11,12,10,19]
  + However, several thousand chemicals remain in need of TK info; the QSPRs evaluated here provide options to fill this gap
* Wambaugh et al. [26] found that in vitro-in vivo extrapolation for TK:
  + Vd: MSE 4.4
  + CLtot: MSE 2.4 for pharma, 2.93 for other, R2 0.19 for pharma, 0.5 for other
  + Cmax: MSE 5, R2 0.48
  + AUC: MSE 3.8, R2 0.62
  + Here we have used more chemicals, but many of the same as Wambaugh et al. [26]:
    - Vd: R2 0.04 RMSLE: 0.89
    - CLtot: R2: 0.03 RMSLE: 1.57
    - Cmax: R2: 0.57 RMSLE: 0.84
    - AUC: R2: 0.5 RMSLE: 1.11
    - Why the drop-off? Partially because we only have 63 chemicals with measured values and are including average predictions for the other 20. If we just look at the 63 AUC R2 is 0.63, Cmax, R2 is 0.6, Vd and CLtot still suck (0.05), and CLtot is 0.02 – Are some of these (like tamoxifen) especially challenging chemicals for HTTK? If so, how do we recognize this in advance?
* Here we have found the HTTK PBTK model performed similarly when using TK QSPRs for Clint and fup as when the actual *in vitro* measured data were used
* Model performance was closer to y-randomized predictions than to empirical fits to the data
* In some cases, QSPRs outperformed *in vitro* measurements, indicating value to intra-chemical averaging of data
  + Tamoxifen (Mike Devito): So here goes. Tamoxifen binds to a microsomal protein complex call the microsomal antiestrogen binding site (AEBS) that is involved in cholesterol-5,6-epoxide hydrolase (ChEH) activity. TAM is a non-competitive inhibitor of this enzyme and does not appear to be a substrate. Thus the binding of TAM to the AEBS/ChEH complex may be slowing the metabolism down by decreasing free TAM in the assays as well as in vivo. Because this binding is relatively restricted to a small structural class, this may be screwing up the in vitro assays. "5,6-Epoxy-cholesterols contribute to the anticancer pharmacology of tamoxifen in breast cancer cells." Segala G, de Medina P, Iuliano L, Zerbinati C, Paillasse MR, Noguer E, Dalenc F, Payré B, Jordan VC, Record M, Silvente-Poirot S, Poirot M. Biochem Pharmacol. 2013 Jul 1;86(1):175-89. <https://doi.org/10.1016/j.bcp.2013.02.031>
* Looking at Fig 4, the models tend to underpredict blood levels compared to the in vivo data. For IVIVE, we are trying to predict the oral dose that results in an equivalent blood level to the in vitro. If the HTTK models tend to underpredict blood levels compared to in vivo, then their oral equivalent dose would be higher than that obtained by in vivo studies.
  + Possible reasons: either clearing the chemical too quickly, or our oral absorption is not correct (too slow?)
* discussion on why they are all performing similarly in late time points vs differences when you consider overall trends and early trends might be useful.
* In Figure 8 it seems like there are chemicals where he RMSLE is greater than 1 for everything except the empirical fits. Even y-randomization doesn't change these much. What is it about those data sets that make the error so large?
* In Figure 1 describe distribution of chemicals quantitatively – means, upper, lower, distribution of high/medium/low Fup and Clint – this is why y-scrambling doesn't show a large range
* Could look at Arnot's larger data set of in vivo half-lives, y-randomization
* Point out that 80-100 chemicals is not "Big data" – need to expand CvT database
* Wrap up sentence: These QSPRs will enable public health risk-based prioritization of many more chemicals in commerce and the environment
* y randomization needs to be clarified
* more explanation of why in vitro data doesnt do better than the in silico
* we want to answer for a new chemical
* show CvT curve fits and in vitro curve
* "FitstoData" -> "FitstoInVivoData"
* add mention of versions used
* it's a constrained random set -- reflecting the correlation and distribuirton (frequency) in this set
* uniform draw would better show true random (no) information case -- call this "Randomized" or something
* it seems like fup and clint are correlated within the data set
* if it has low clearance doesnt matter how much it binds
* if it is highly bound doesant matter how fast it clears
* what error should we care about the most? ten-fold error in clearance or ten-fold or fup?
* fix clint zero point
* -- clarify that this is only for purposes of plotting
* just one point aove 10^3 for clint, 90% are within two fold of each other (y scramble doesn't do much)
* call out which compound had highest and lowest for clint and fup
* when chemical space is very narrow the overall statistics don't say anything about how good or bad are the statistics
* give some chemical-specific
* need to build new TK triage -- discuss the six worst chemicals, can we predict them
* ?
* note that CLtot and AUCinfinity are related
* level three -- terminal elimination from either one or two compartment model (selected by AIC)
* look at differences between rat and human?
* - everything is trained on human
* - only vary physiology
* - add to table
* add oral vs iv studies to table
* need supplemental table dose regimens (not per point)
* add to table 1 how many models compared
* Ester sent me in vivo predicted clearance (look August)
* -- model for in vitro intrinsic clearance
* mention taking in vitro or in vivo properties together is difficult
* mention phys-chem span (log p, etc)
* resend ten chemicals that only opera could predict
* add SMILES to that
* table of coverage percentages by model/chemical maybe
* section on imputations -- difference between regulatory data gaps and statistical perspective
* provide other figures (maybe for supplemental)
* need table to tell us how many were necessary
* could use consensu rmse
* how we handle missing observations
* could do both separate rmsle and consensus
* could fill in with average prediction for model
* note how many times consensus used per model
* overlap only evaluation
* admet is five cyps -- not an apples to apples comparison (figure 2)
* what are impacts of 3d effects in qsar? is that something we're missing?
* text:
* all the models are 2d right now because we don't have data to train qsar models to predict 3d differences (for example chiral pairs)
* like the cvt data , we can only evaluate and model things that vary across our dataset
* binning chemicals by what drives elimination route may be more important
* don't focus on the path that is not rate determining
* QSAR training set summary data
* number chemicals, max, min, average values
* will use the averages for missing values
* [10/26 1:11 PM]
* Wambaugh, John joined the conversation.
* [10/29 10:49 AM]
* Wambaugh, John named the meeting to Wrapping Up TK QSPR Collaborative Trial Manuscript.
* [10/29 10:49 AM]
* Sipes, Nisha and 9 others were invited to the meeting.
* [10/29 11:12 AM]
* Davidson, Sarah was invited to the meeting.
* [9:29 AM]
* [9:59 AM]
* 9:59 AM Meeting started
* [10:00 AM]
* Alessandro Sangion (Guest) has temporarily joined the chat.
* [10:01 AM]
* Ferguson, Stephen (NIH/NIEHS) [E] has temporarily joined the chat.
* [10:01 AM]
* John DiBella has temporarily joined the chat.
* [10:01 AM]
* Michael Lawless has temporarily joined the chat.
* [10:01 AM]
* Trevor Brown (Guest) has temporarily joined the chat.
* [10:01 AM]
* Mansouri, Kamel (NIH/NIEHS) [E] has temporarily joined the chat.
* [10:02 AM]
* Rocky Goldsmith has temporarily joined the chat.
* [10:02 AM]
* Jon Arnot (Guest) has temporarily joined the chat.
* [10:11 AM] Dawson, Daniel
* We had a very similar result in the Dawson et al. paper for Y-randomization.
* [10:11 AM] Devito, Michael
* the in vitro probably has a similar spread of values for technical reasons
* like 1
* [10:18 AM] Sayre, Risa
* to me, a takehome of this paper is that we could use to aggregate more in vivo and in vitro tk data to characterize the per-chemical (and across chemicals) spread. i'm pitching a version of this in strap4
* [10:18 AM]
* Papa Ester has temporarily joined the chat.
* [10:19 AM] Ferguson, Stephen (NIH/NIEHS) [E]
* agree with Mike...we need better in vitro models for metabolic and transport clearance for the liver
* [10:42 AM] Sayre, Risa
* we could compare all the one-comp and all the two-comp as two sets

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# Conflict of Interest

Please declare any COI here

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Please add your funding here

The views expressed in this publication are those of the authors and do not necessarily represent the views or policies of the U.S. EPA. Reference to commercial products or services does not constituteendorsement.

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# Figures

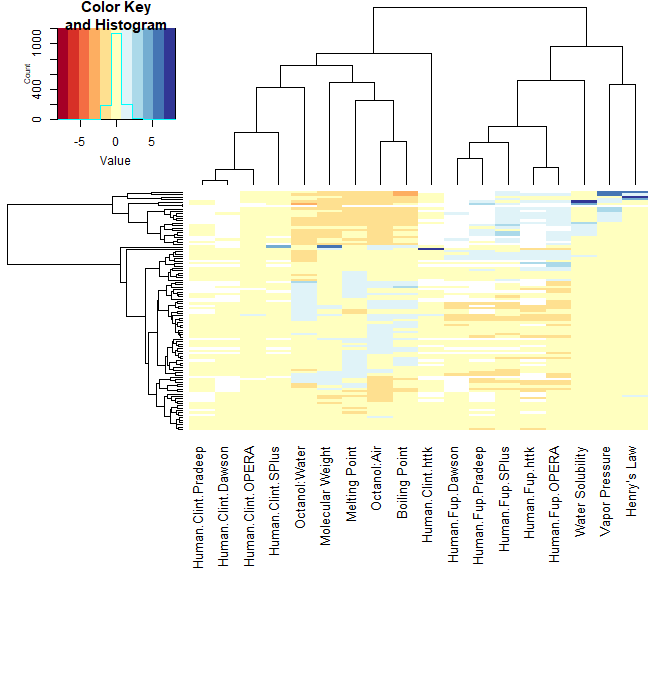
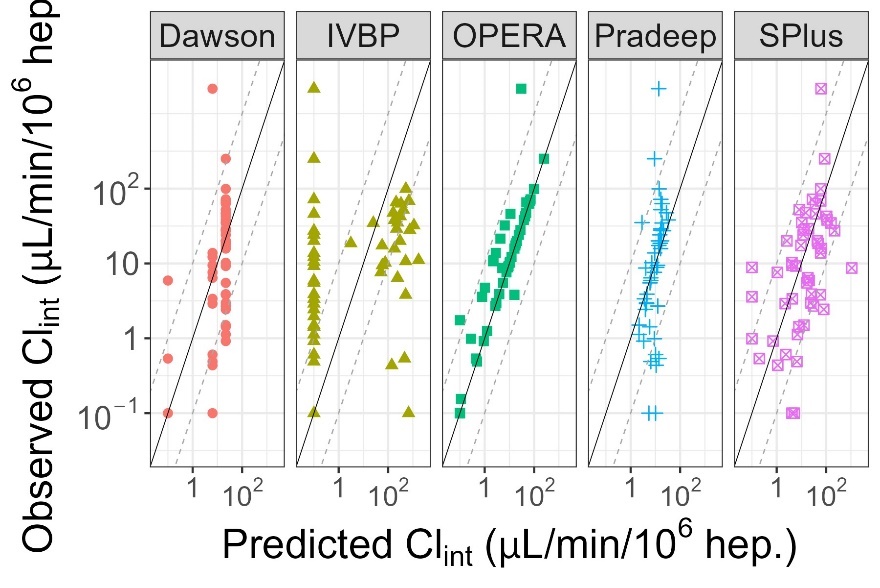


Figure 1:Columns in this heatmap indicate the physico chemical properties and measured/predicted values for in vitro TK (intrinsic hepatic clearance, Clint, and fraction unbound in plasma, fup). Each row corresponds to one of the 83 chemicals. The in vitro TK measurements (“Human.Clint.InVitro” and “Human.Fup.InvItro”) and predictions for these values from the various QSPRs (Table 2) are indicated by name. Data are normalized on a per column basis by centering (subtracting the mean) and scaling (by standard deviation). Thus, the "Value" of each entry in heatmap indicates the number of standard deviations from the mean. Blank values indicate no prediction.



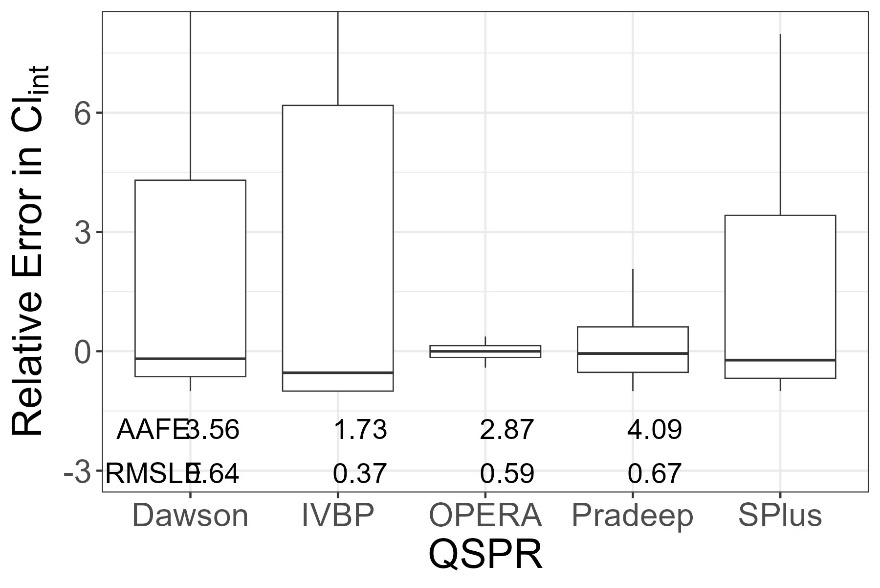
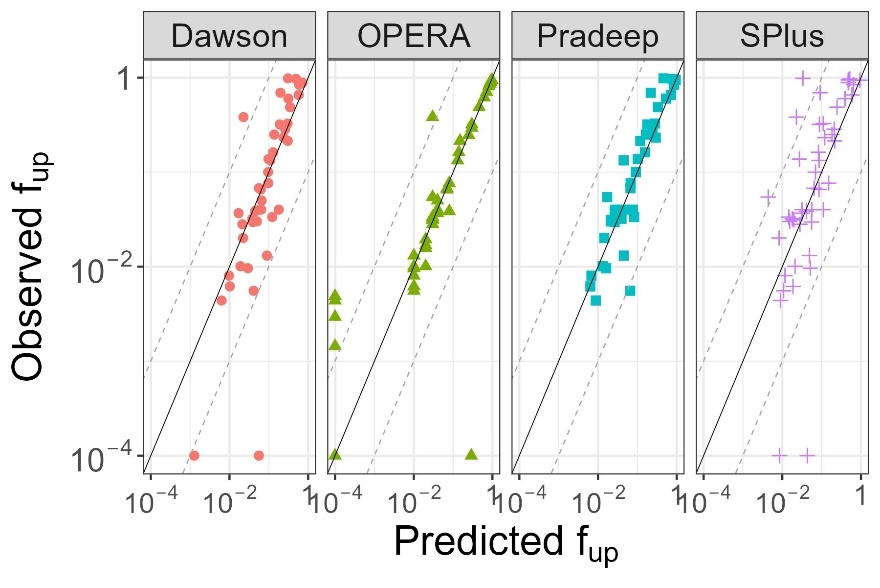


Figure 2: Evaluation of Predictions for Intrinsic Hepatic Clearance (Clint At top, zero values were plotted at 10-1, the solid line indicates identity (1:1) while the dashed lines indicate ten-fold difference. At bottom, the upper and lower extent of the box for each model indicates the 25th to 75th quantiles, the mid-line indicates the median (50th quantile) and vertical line indicates 1.5x the range of the box. The average absolute folder error (AAFE) and average root mean squared log10 error (RMSLE) are calculated for each model across all available predictions.



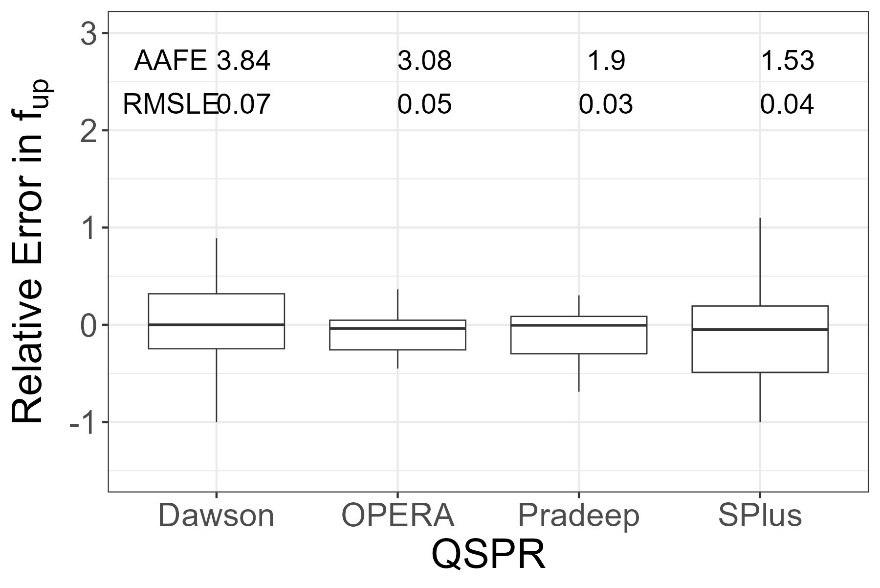


Figure 3: Evaluation of Predictions for Fraction Unbound in Plasma (fup). At top, zero values were plotted at 10-4, the solid line indicates identity (1:1) while the dashed lines indicate ten-fold difference. At bottom, the upper and lower extent of the box for each model indicates the 25th to 75th quantiles, the mid-line indicates the median (50th quantile) and vertical line indicates 1.5x the range of the box. The average absolute folder error (AAFE) and average root mean squared log10 error (RMSLE) are calculated for each model across all available predictions.

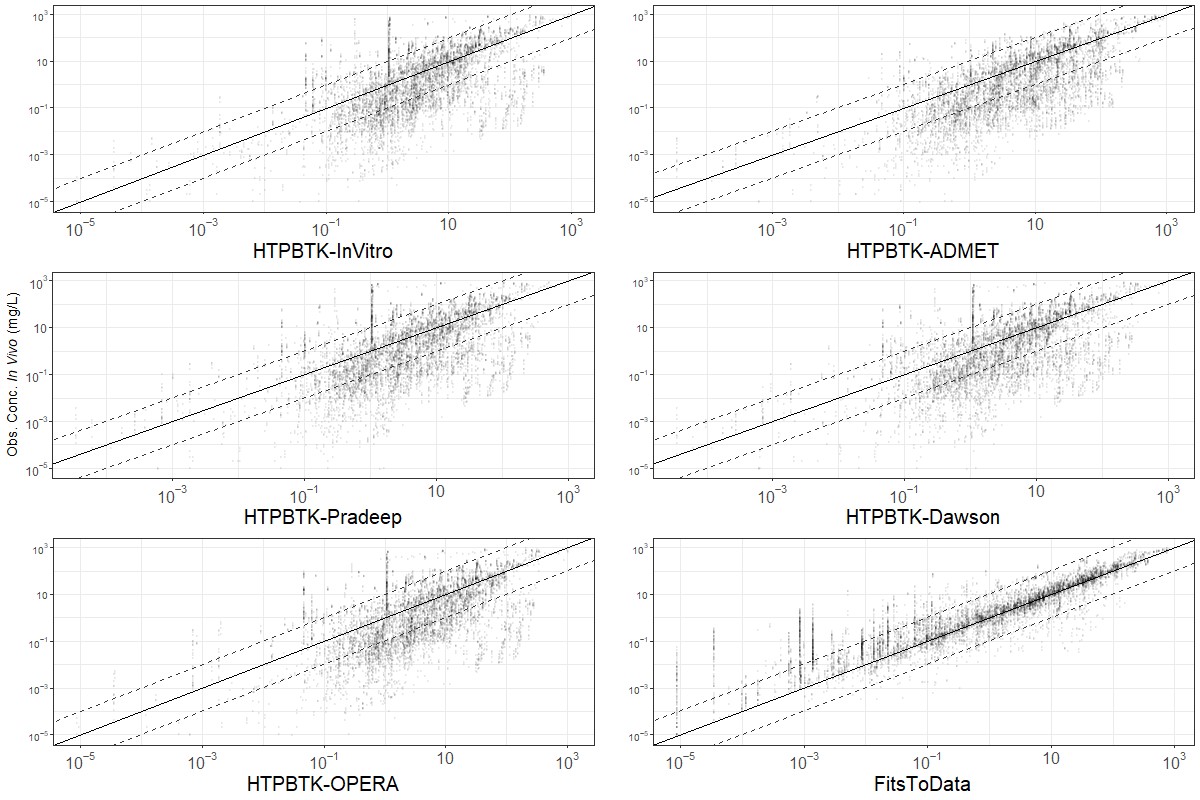


Figure 4: Comparison of in vivo measured chemical concentration vs. time (CvT) data [27] vs. predictions for empirical models fit to the data (“FitsToData”), and predictions for a PBTK model (“HTTK”) parameterized with chemical specific values either measured in vitro (“HTTK-InVitro”) or predicted with various QSPRs. In each sub-plot the y-axis shows the measured data while the x-axis shows the predictions made using chemical-specific parameters from the various sources. The solid line indicates identity (1:1) while the dashed lines indicate ten-fold difference.

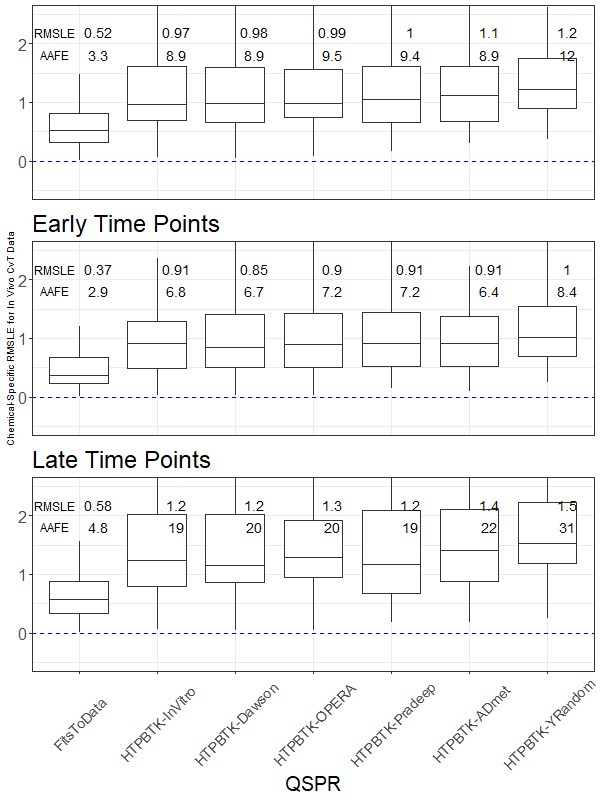


Figure 5: Chemical-Specific RMSLE for In vivo CvT Data. The upper and lower extent of the box for each model indicates the 25th to 75th quantiles, the mid-line indicates the median (50th quantile) and vertical line indicates 1.5x the range of the box.

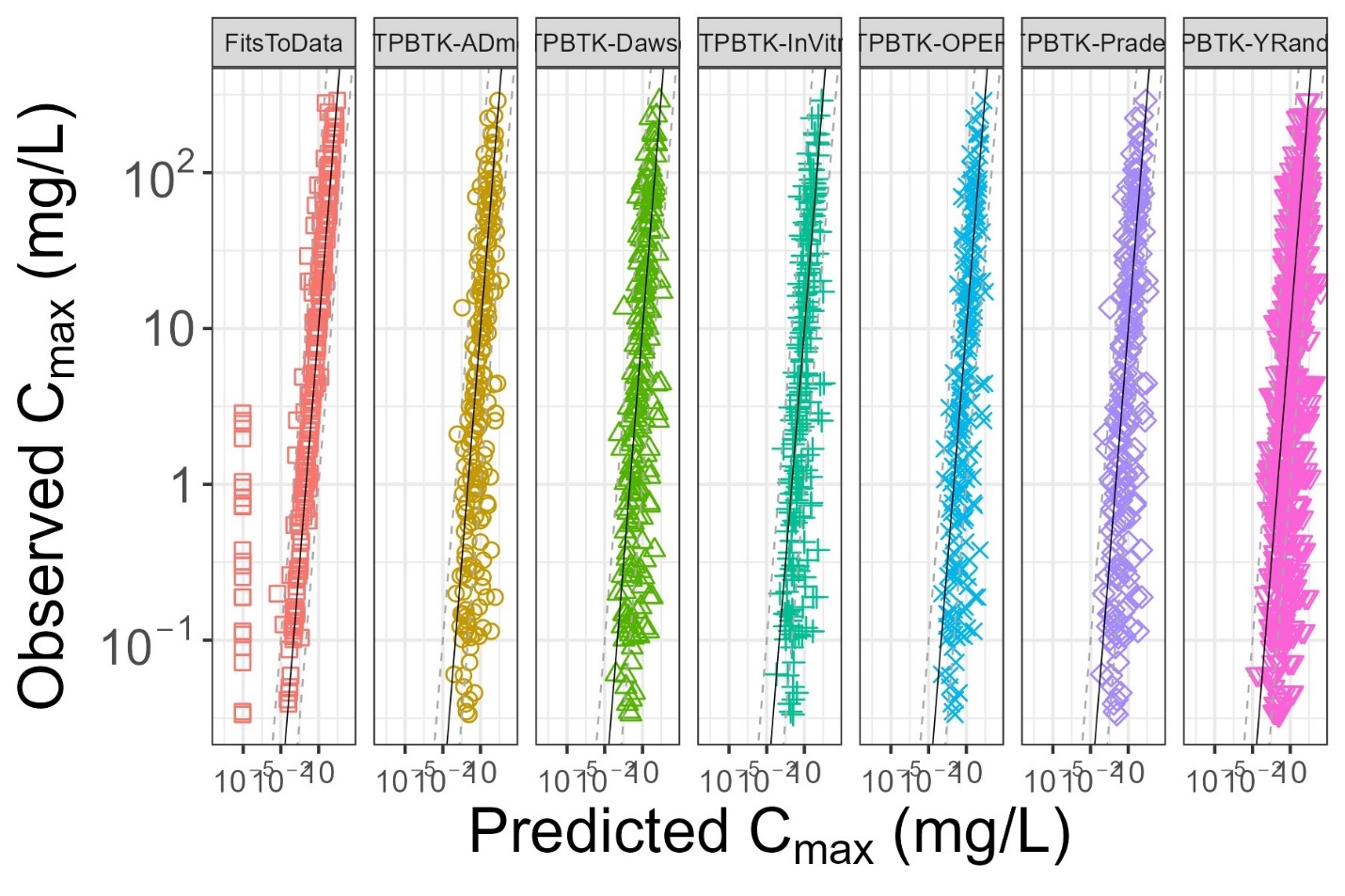


Figure 6: Evaluation of Predictions for Cmax based on empirical model fits (“1CompFits”), and a PBPK model (“HTTK”) parameterized with chemical specific values either measured in vitro (“HTTK-InVitro”) or predicted with various QSPRs.

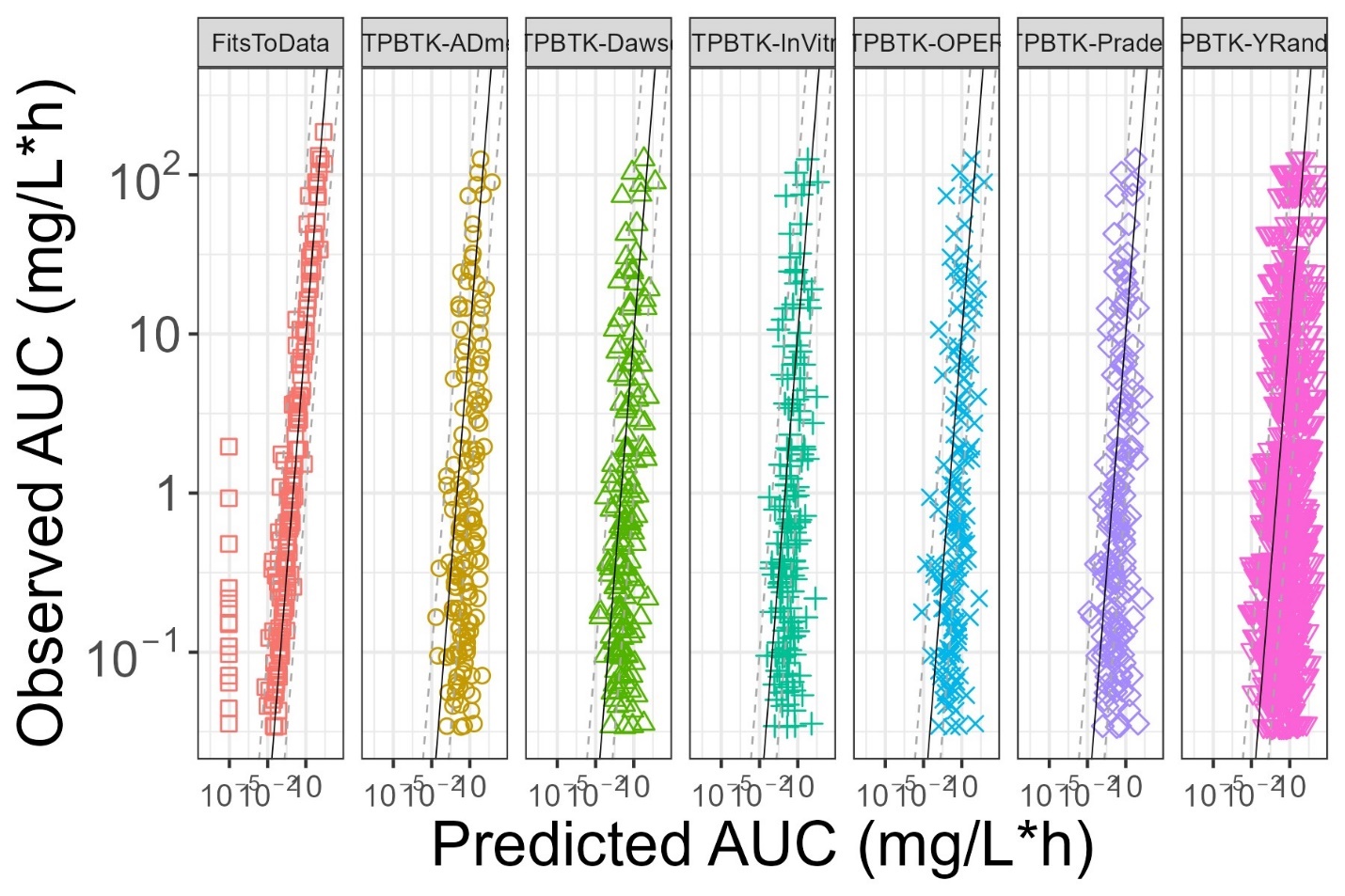


Figure 7: Evaluation of Predictions for AUC based on empirical model fits (“1CompFits”), and a PBPK model (“HTTK”) parameterized with chemical specific values either measured in vitro (“HTTK-InVitro”) or predicted with various QSPRs.

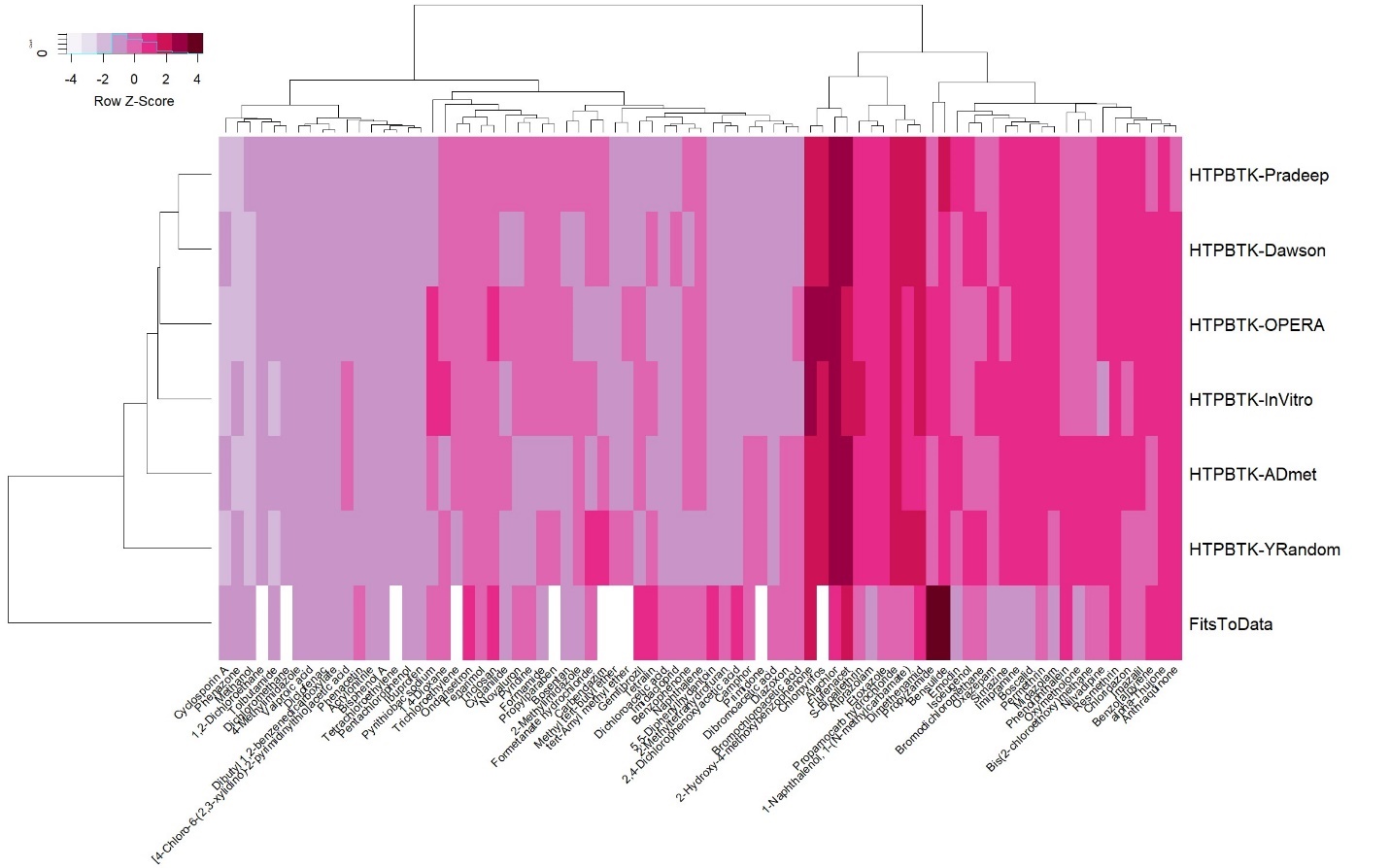
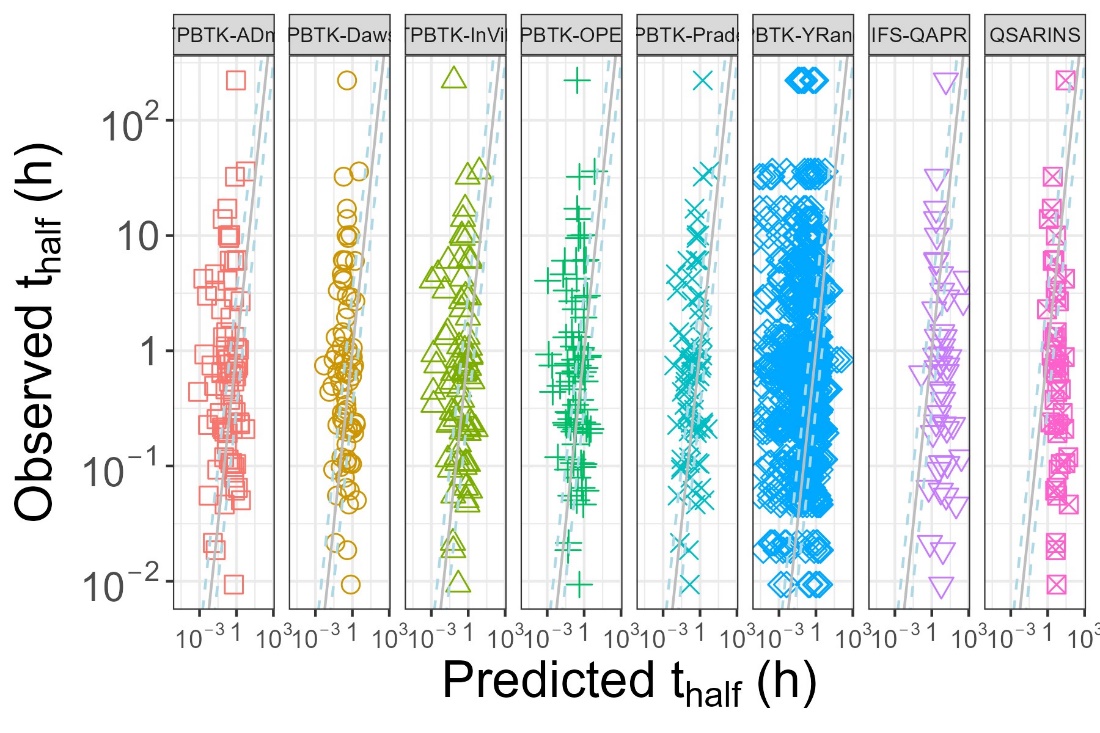


Figure 8: Values in this heatmap are the root mean squared log10 error (RMSLE) by chemical and predictor (that is, lighter indicates better predictive ability). The columns indicate different evaluation chemicals. The rows give the different prediction methods: The empirical fits to the data are given by “FitsToData”. All other values are calculated using the HTTK PBTK model and either measured values “In vitro”, y-randomized measured values (“Y-Random”) or the various QSPRS.



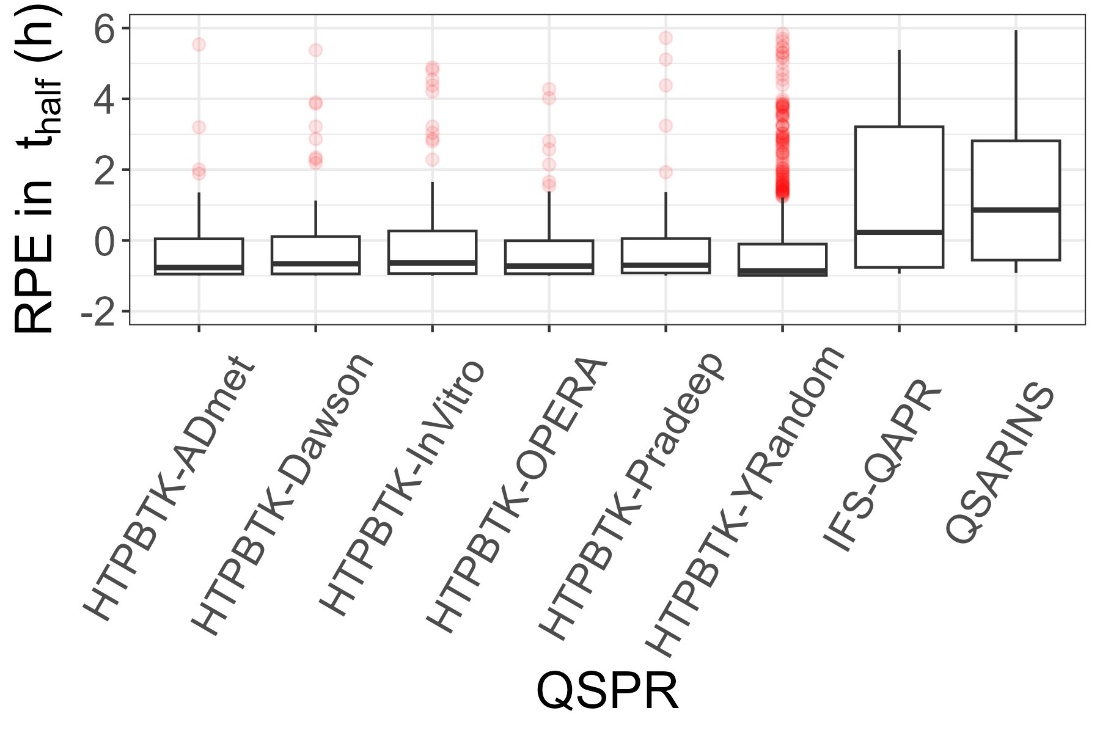
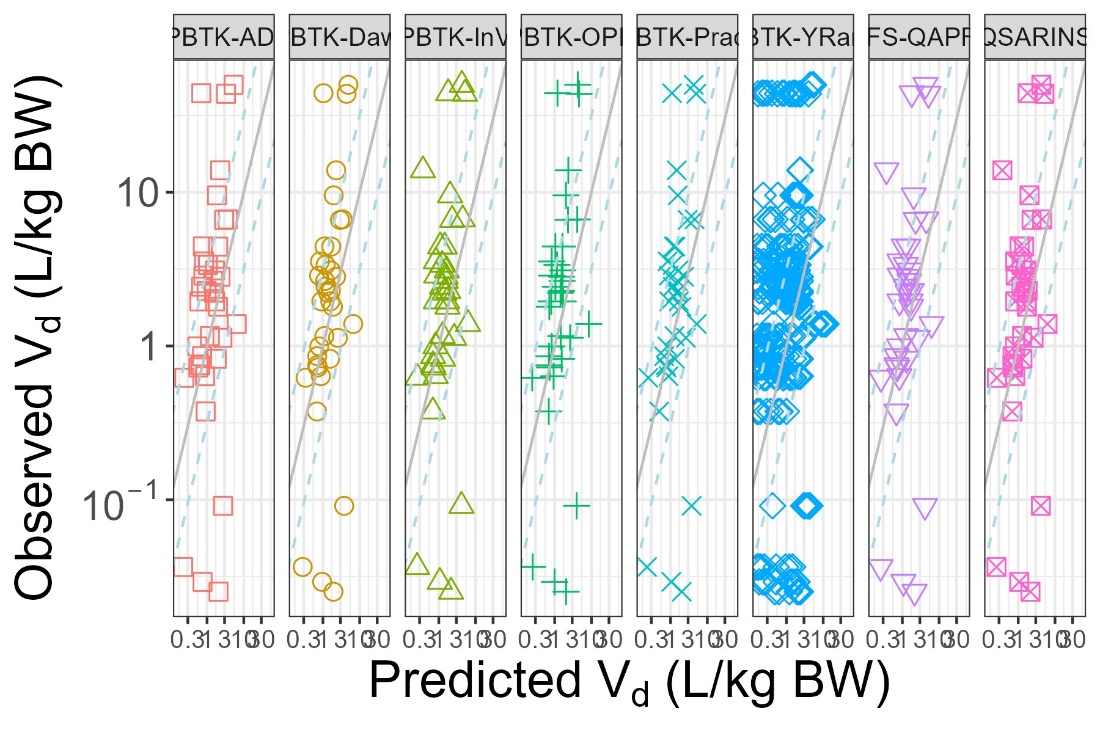


Figure 9: Comparison of “observed” chemical half-lives based empirical model fits and predictions for chemical half-life based on a PBPK model (“HTTK”) parameterized with chemical specific values either measured *in vitro* (“HTTK-InVitro”) or predicted with various QSPRs. The upper panel shows a scatter plot of predicted vs. observed values, while the lower panel shows the distribution of relative predictive error (RPE). The upper and lower extent of the box for each model indicates the 25th to 75th quantiles, the mid-line indicates the median (50th quantile) and vertical line indicates 1.5x the range of the box.



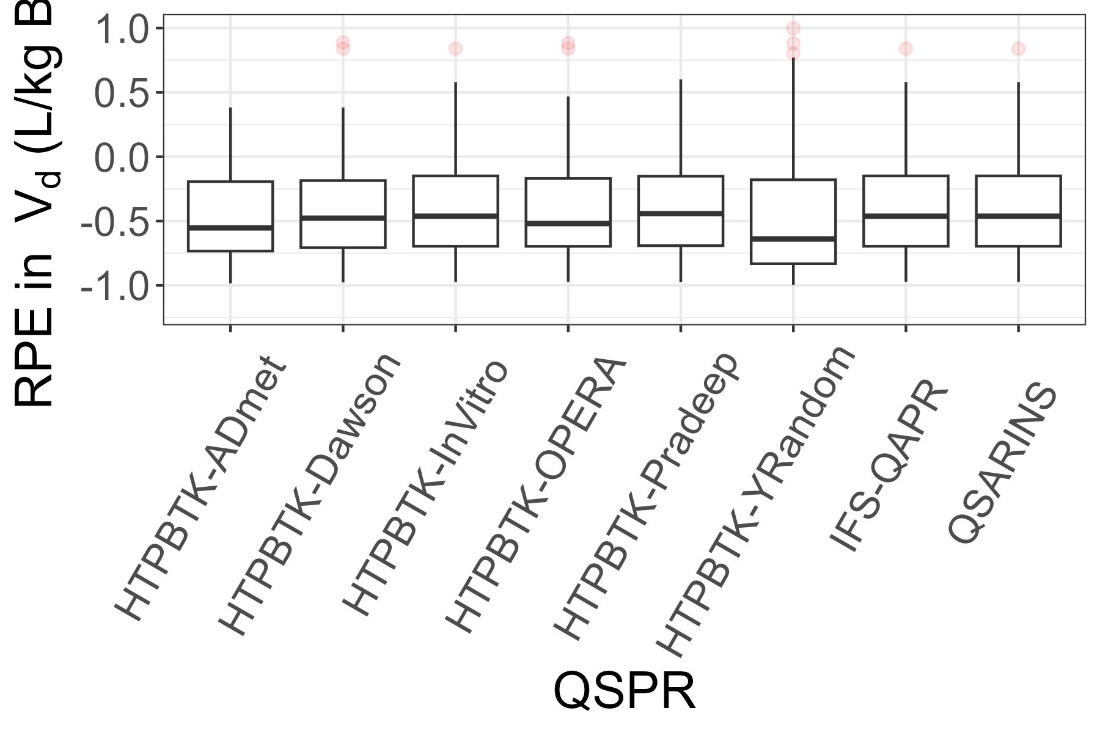
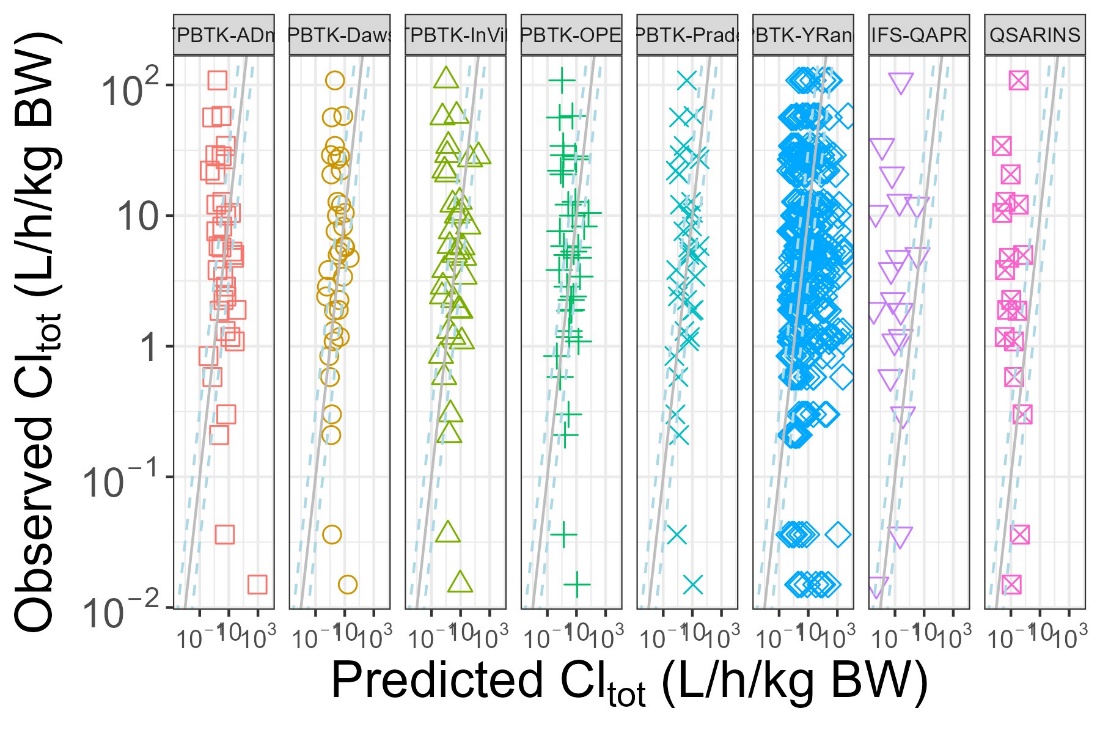


Figure 10: Comparison of “observed” chemical volumes of distribution based on empirical model fits and predictions for chemical half-life based on a PBPK model (“HTTK”) parameterized with chemical specific values either measured *in vitro* (“HTTK-InVitro”) or predicted with various QSPRs. The upper panel shows a scatter plot of predicted vs. observed values, while the lower panel shows the distribution of relative predictive error (RPE). The upper and lower extent of the box for each model indicates the 25th to 75th quantiles, the mid-line indicates the median (50th quantile) and vertical line indicates 1.5x the range of the box.



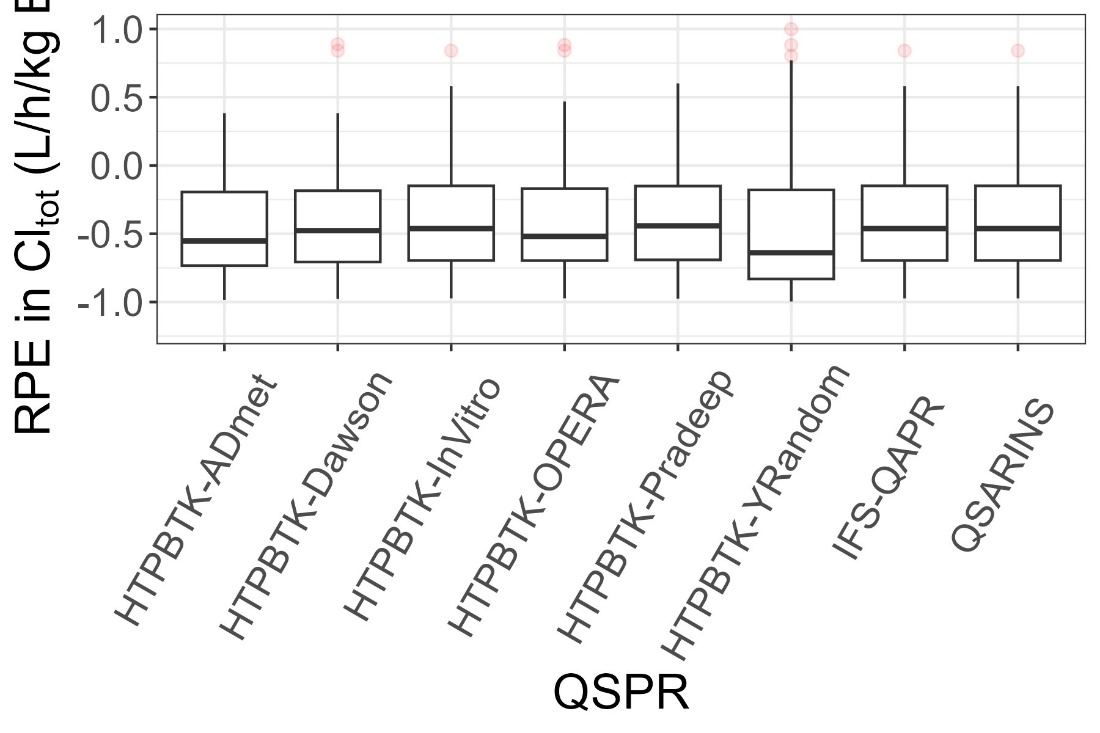


Figure 11: Comparison of “observed” chemical whole body clearance (Cltot) based on empirical model fits and predictions for chemical half-life based on a PBPK model (“HTTK”) parameterized with chemical specific values either measured *in vitro* (“HTTK-InVitro”) or predicted with various QSPRs. The upper panel shows a scatter plot of predicted vs. observed values, while the lower panel shows the distribution of relative predictive error (RPE). The upper and lower extent of the box for each model indicates the 25th to 75th quantiles, the mid-line indicates the median (50th quantile) and vertical line indicates 1.5x the range of the box.

# Tables

Table 1 Three Levels of Evaluation were performed

|  |  |  |  |
| --- | --- | --- | --- |
| **Evaluation** | **TK Quantities** | **Chemicals for Evaluation** | **Reference** |
| **Level 1** | *In vitro* TK Measurements  (fup, Clint) | 63 with Measured *In vitro* Data | [30] |
| **Level 2** | TK Concentration vs. Time  (all points, Cmax, time-integral/AUC) | 83 with predictions across multiple QSPRs and empirical model fits | [27] |
| **Level 3** | Summary Statistics  (Vd, thalf, Cltot) | 83 | [27] |

Table 2 QSPR Models Evaluated

|  |  |  |  |
| --- | --- | --- | --- |
| **Model** | **Predictions** | **Mechanism** | **Reference** |
| Simulations Plus ADMET Predictor® | Level 1  (*in vitro* parameters) | Sum of CYP-specific Artificial Neural Network (ANN) | [23] |
| Pradeep 2020 | Level 1 | Random forest and support vectors method | [24] |
| Dawson 2021 | Level 1 | Random forest, clearance organized by categories | [25] |
| OPERA | Level 1 | Nearest-neighbors | [42,43] |
| IFS-QSAR | Level 3  (Half-lives) | Fragment-based Multiple Linear Regressors (MRL) | [21] |
| QSARINS-Chem | Level 3 | Ordinary Least Squares MLR | [22] |

Table 3: Biases of the QSPRs for predicting in vitro measured values in terms of fold error (FE)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Median Clint AbsFE** | **Median Clint FE** | **Min Clint FE** | **Max Clint FE** | **Median fup AbsFE** | **Median fup FE** | **Min fup FE** | **Max fup FE** |
| SPlus | 0.503 | 0.105 | -2.34 | 2.1 | 0.274 | -0.0179 | -1.47 | 6.27 |
| Dawson | 0.348 | 0.0378 | -2.54 | 1.42 | 0.152 | -0.00384 | -1.23 | 6.38 |
| Pradeep | 0.232 | 0.0194 | -2.2 | 1.5 | 0.154 | -0.0191 | -0.506 | 1.92 |
| OPERA | 0.00141 | -0.00019 | -1.86 | 0.63 | 0.0281 | 0 | -1.11 | 7.1 |

Table 4 Analysis of predictions based on empirical model fits and a PBPK model (“HTTK”) parameterized with chemical specific values either measured in vitro (“HTTK-InVitro”) or predicted with various QSPRs. Both the coefficient of determination (R2) and root mean squared error (RMSLE) are calculated on a log10 scale. “HTTK-YRandom” indicates the HTTK PBPK model parameterized with a random permutation of the in vitro data.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Cmax** | | | **AUC** | | | | |
| **Predictor** | **R2** | **RMSLE** | **RPE** | **R2** | **RMSLE** | **RPE** | **RPE Low** | **RPE High** | |
| Empirical Fits | 0.95 | 0.95 | -0.09 | 0.96 | 0.31 | 0.32 | 0.41 | 0.06 | |
| HTTK-InVitro | 0.57 | 0.84 | -0.02 | 0.50 | 1.11 | 3.44 | 6.72 | -0.46 | |
| HTTK-ADmet | 0.62 | 0.78 | 0.15 | 0.62 | 0.97 | 5.88 | 10.50 | -0.44 | |
| HTTK-Dawson | 0.60 | 0.81 | 0.02 | 0.58 | 1.01 | 3.32 | 5.00 | -0.04 | |
| HTTK-Pradeep | 0.57 | 0.83 | -0.11 | 0.40 | 1.22 | 2.29 | 5.99 | -0.80 | |
| HTTK-OPERA | 0.58 | 0.83 | 0.11 | 0.56 | 1.04 | 4.67 | 7.93 | 0.39 | |
| HTTK-YRandom | 0.46 | 0.93 | 0.53 | 0.10 | 1.48 | 5.10 | 12.10 | -0.88 | |

Table 5 Analysis of predictions based on empirical model fits and a PBPK model (“HTTK”) parameterized with chemical specific values either measured in vitro (“HTTK-InVitro”) or predicted with various QSPRs. Both the coefficient of determination (R2) and root mean squared error (RMSLE) are calculated on a log10 scale. “HTTK-YRandom” indicates the HTTK PBPK model parameterized with a random permutation of the in vitro data.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Level 3** | | | | | |
|  | **thalf** | | **Vd** | | **Cltot** | |
| **Predictor** | **R2** | **RMSE** | **R22** | **R222** | **R23** | **RMSE3** |
| HTTK-InVitro | 0.04 | 1.43 | 0.04 | 0.89 | 0.03 | 1.57 |
| HTTK-ADmet | 0.02 | 1.45 | 0.13 | 0.85 | 0.20 | 1.42 |
| HTTK-Dawson | 0.09 | 1.39 | 0.11 | 0.86 | 0.20 | 1.42 |
| HTTK-Pradeep | 0.00 | 1.46 | 0.13 | 0.85 | 0.02 | 1.57 |
| HTTK-OPERA | 0.09 | 1.39 | 0.05 | 0.89 | 0.09 | 1.52 |
| HTTK-YRandom | 0.01 | 1.45 | 0.00 | 0.91 | 0.02 | 1.57 |
| QSARINS | 0.10 | 1.00 | 0.04 | 0.89 | 0.19 | 1.17 |
| IFS-QAPR | 0.15 | 0.97 | 0.04 | 0.89 | 0.25 | 1.12 |

# Supplemental Tables

Supplemental Table 1: List of 102 chemicals, physico-chemical descriptors, and *in vitro* measured values, and QSPR predictions

*SupTable-QSPRPredsandInVitroData.xlsx*

Supplemental Table 2: Concentration vs. time data (Sayre et al., 2020)

*SupTable-CvTData.xlsx*

Supplemental Table 3: Empirical (one and two compartment model) toxicokinetic parameter estimates

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Compound | CAS | Species | Reference | AIC.1comp | AIC.2comp | Model | Vdist | kelim | halflife |
| 1,2-dichloroethane | 107-06-2 | rat | 18 | 286.7 | 290.7 | 1Comp | 3.65 | 0.3431 | 2.02 |
| 1,4-dioxane | 123-91-1 | rat | 24, 6 | 1373 | 1373 | 2Comp | 0.4464 | 0.01508 | 45.97 |
| 1-chloro-2-propanol | 127-00-4 | rat | 176 | 5.553 | 10.39 | 1Comp | 2.835 | 1.344 | 0.5157 |
| 2,3,7,8-tetrachlorodibenzo-p-dioxin | 1746-01-6 | rat | 177 | -49.13 | -45.42 | 1Comp | 0.2341 | 0.001496 | 463.4 |
| 2,4-dichlorophenoxyacetic acid | 94-75-7 | rat | 164, 192 | 238.4 | 242.4 | 1Comp | 0.3759 | 0.2186 | 3.171 |
| 2-hydroxy-4-methoxybenzophenone | 131-57-7 | rat | 129 | -82.97 | -112 | 2Comp | 11.6 | 3.304 | 0.2098 |
| 2-methylimidazole | 693-98-1 | rat | 150 | 29.44 | 9.339 | 2Comp | 1.999 | 0.02467 | 28.1 |
| 2-methyltetrahydrofuran | 96-47-9 | rat | 175 | 420.5 | 417.6 | 2Comp | 1.072 | 0.9591 | 0.7227 |
| 4-methylimidazole | 822-36-6 | rat | 151 | 28.6 | 0.162 | 2Comp | 1.218 | 0.8444 | 0.8209 |
| acrylonitrile | 107-13-1 | rat | 43 | 195.4 | 170.3 | 2Comp | 0.7476 | 9.897 | 0.07003 |
| alachlor | 15972-60-8 | rat | 192 | -139 | -135.5 | 1Comp | 130.9 | 0.05883 | 11.78 |
| alpha-thujone | 546-80-5 | rat | 130 | -69.3 | -80.52 | 2Comp | 13.94 | 0.5856 | 1.184 |
| alprazolam | 28981-97-7 | rat | 192 | -87 | -116.9 | 2Comp | 9.42 | 3.702 | 0.1872 |
| anthraquinone | 84-65-1 | rat | 166 | 1.042 | NA | 1Comp | 4.74 | 0.09222 | 7.517 |
| antipyrine | 60-80-0 | rat | 192 | -4.438 | -2.125 | 1Comp | 1.133 | 0.5825 | 1.19 |
| benzophenone | 119-61-9 | rat | 171 | -27.96 | -52.25 | 2Comp | 12.76 | 0.2639 | 2.627 |
| bisphenol a | 80-05-7 | rat | 192 | -13.29 | -87.26 | 2Comp | 1.787 | 4.052 | 0.1711 |
| boscalid | 188425-85-6 | rat | 192 | -44.32 | -73.71 | 2Comp | 10.82 | 1.346 | 0.5151 |
| bosentan | 147536-97-8 | rat | 192 | -18.22 | -14.22 | 1Comp | 3.156 | 0.2439 | 2.841 |
| bromochloroacetic acid | 5589-96-8 | rat | 132 | 192.1 | NA | 1Comp | 0.3796 | 2.259 | 0.3069 |
| bromodichloromethane | 75-27-4 | rat | 154 | -7.809 | -45.51 | 2Comp | 22.72 | 3.576 | 0.1938 |
| carbaryl | 63-25-2 | rat | 192 | -55.78 | -24.19 | 1Comp | 34.44 | 6.131 | 0.1131 |
| carbendazim | 10605-21-7 | rat | 192 | 28.13 | 32.09 | 1Comp | 33.15 | 0.245 | 2.829 |
| chloridazon | 1698-60-8 | rat | 192 | -62.76 | NA | 1Comp | 9.044 | 0.07509 | 9.23 |
| chlorpyrifos | 2921-88-2 | rat | 192 | -88.14 | -84.14 | 1Comp | 100.3 | 0.1788 | 3.877 |
| cyclanilide | 113136-77-9 | rat | 192 | -30.3 | -66.03 | 2Comp | 0.3674 | 0.3297 | 2.102 |
| cyclosporin a | 59865-13-3 | rat | 192 | 113 | 76.75 | 2Comp | 1.593 | 0.3693 | 1.877 |
| di-n-butyl phthalate | 84-74-2 | rat | 135 | -3.349 | -35.07 | 2Comp | 1.587 | 8.08 | 0.08578 |
| diazinon-o-analog | 962-58-3 | rat | 192 | -58.93 | -68.18 | 2Comp | 177.3 | 0.000139 | 5001 |
| dibromoacetic acid | 631-64-1 | rat | 133 | 498.9 | 793 | 1Comp | 0.4029 | 1.692 | 0.4096 |
| dichloroacetic acid | 79-43-6 | rat | 134 | 230.2 | 240.1 | 1Comp | 0.3354 | 2.837 | 0.2443 |
| diltiazem | 34933-06-7 | rat | 192 | 12.39 | -9.811 | 2Comp | 2.747 | 3.384 | 0.2048 |
| dimethenamid | 87674-68-8 | rat | 192 | -120.1 | -142.7 | 2Comp | 246.7 | 2.019 | 0.3434 |
| dl-camphor | 76-22-2 | rat | 165 | 4.603 | -22.82 | 2Comp | 7.166 | 2.286 | 0.3032 |
| emodin | 518-82-1 | rat | 160 | 4.277 | 7.361 | 1Comp | 0.6335 | 2.886 | 0.2402 |
| etoxazole | 153233-91-1 | rat | 192 | -148.9 | -173 | 2Comp | 34.3 | 0.6847 | 1.012 |
| fenarimol | 60168-88-9 | rat | 192 | -68.23 | -91.56 | 2Comp | 12.17 | 0.3854 | 1.799 |
| flufenacet | 142459-58-3 | rat | 192 | -197.7 | -195.1 | 1Comp | 58.71 | 0.0321 | 21.59 |
| formamide | 75-12-7 | rat | 172 | 802.6 | NA | 1Comp | 0.5236 | 0.03136 | 22.1 |
| formetanate hydrochloride | 23422-53-9 | rat | 192 | -17.95 | -13.95 | 1Comp | 29.4 | 1.07E-08 | 64830000 |
| free carbon disulfide | 75-15-0 | rat | 167 | 52.7 | 33.58 | 2Comp | 3.469 | 6.702 | 0.1034 |
| gemfibrozil | 25812-30-0 | rat | 163 | 226.2 | NA | 1Comp | 0.7988 | 0.06215 | 11.15 |
| glyoxylic acid monohydrate | 563-96-2 | rat | 156 | 59.3 | 18.78 | 2Comp | 0.5298 | 19.34 | 0.03584 |
| hexachlorobenzene | 118-74-1 | rat | 174, 180 | NA | -242.2 | 2Comp | 7.088 | 0.002409 | 287.7 |
| hexobarbital | 15307-86-5 | rat | 192 | 222.2 | 492.2 | 1Comp | 0.4199 | 4.003 | 0.1732 |
| ibuprofen | 15687-27-1 | rat | 192 | 118.4 | 92.01 | 2Comp | 0.687 | 0.8539 | 0.8118 |
| imazalil | 35554-44-0 | rat | 192 | -54.53 | -91.25 | 2Comp | 10.4 | 5.226 | 0.1326 |
| imipramine | 50-49-7 | rat | 192 | -0.5092 | -20.95 | 2Comp | 45.44 | 0.2763 | 2.509 |
| isoeugenol | 97-54-1 | rat | 157 | 28.35 | -56.77 | 2Comp | 27.95 | 2.59 | 0.2676 |
| l-ephedrine | 299-42-3 | rat | 136 | -36.84 | -65.5 | 2Comp | 11.6 | 0.9385 | 0.7385 |
| methanol | 67-56-1 | rat | 17 | 46.39 | 47.83 | 1Comp | 0.992 | 0.3046 | 2.275 |
| methyl tert-butyl ether | 1634-04-4 | human | 51 | -134.9 | -141.9 | 2Comp | 0.8526 | 1.356 | 0.511 |
| methylene chloride | 75-09-2 | rat | 18 | 312.7 | 313.7 | 1Comp | 1.883 | 0.4908 | 1.412 |
| methyleugenol | 93-15-2 | rat | 158, 170 | NA | -5.438 | 2Comp | 8.303 | 3.048 | 0.2274 |
| midazolam | 59467-70-8 | rat | 192 | -36.58 | -49.41 | 2Comp | 3.487 | 3.998 | 0.1734 |
| nilvadipine | 75530-68-6 | rat | 192 | -93.6 | -113.4 | 2Comp | 10.74 | 1.03 | 0.673 |
| nitrite | 14797-65-0 | rat | 137 | 121.8 | 124.2 | 1Comp | 2.528 | 0.3789 | 1.829 |
| novaluron | 116714-46-6 | rat | 192 | -82.71 | -102.7 | 2Comp | 7.068 | 0.07823 | 8.861 |
| octylphenol | 140-66-9 | rat | 60 | -207.3 | -299.5 | 2Comp | 49.92 | 0.3106 | 2.231 |
| oxazepam | 604-75-1 | rat | 159 | 146 | 102.8 | 2Comp | 16.9 | 0.05402 | 12.83 |
| oxymetholone | 434-07-1 | rat | 168 | 67.48 | 30.16 | 2Comp | 8.674 | 3.082 | 0.2249 |
| pentachlorophenol, purified | 87-86-5 | rat | 161 | 519.8 | 522.2 | 1Comp | 0.0887 | 0.08031 | 8.631 |
| perfluorodecanoic acid | 335-76-2 | rat | 147 | 293.3 | NA | 1Comp | 0.2088 | 0.001265 | 548 |
| perfluorohexane-1-sulphonic acid – potassium salt | 3871-99-6 | rat | 143 | 464.4 | NA | 1Comp | 0.2362 | 0.001256 | 552 |
| perfluorooctane sulfonate | 45298-90-6 | rat | 148 | 173.9 | NA | 1Comp | 0.2185 | 0.000885 | 782.9 |
| perfluorooctanoic acid | 335-67-1 | rat | 146, 192 | 1075 | 1012 | 2Comp | 46.58 | 1.43E-07 | 4843000 |
| phenacetin | 62-44-2 | rat | 1, 192, 62, 63, 67 | 265.4 | 215.2 | 2Comp | 1.599 | 0.7362 | 0.9415 |
| phenytoin | 57-41-0 | rat | 192 | 228.4 | NA | 1Comp | 1.273 | 0.2112 | 3.282 |
| potassium perfluorobutane sulfonate | 29420-49-3 | rat | 144 | 330.5 | 318 | 2Comp | 0.2398 | 0.1955 | 3.545 |
| primidone | 125-33-7 | rat | 140 | 125.7 | 129.7 | 1Comp | 1.107 | 0.1883 | 3.68 |
| propamocarb hydrochloride | 25606-41-1 | rat | 192 | -68.01 | -95.77 | 2Comp | 8.131 | 3.207 | 0.2161 |
| propylparaben | 94-13-3 | rat | 64 | 139.9 | NA | 1Comp | 0.03969 | 333.2 | 0.00208 |
| propyzamide | 23950-58-5 | rat | 192 | -113.1 | NA | 1Comp | 11.09 | 0.09099 | 7.618 |
| pyridine | 110-86-1 | rat | 149, 162 | 516.2 | 516.4 | 1Comp | 1.241 | 0.07107 | 9.753 |
| pyrithiobac sodium | 123343-16-8 | rat | 192 | -6.596 | -2.142 | 1Comp | 1.09 | 0.08863 | 7.821 |
| resmethrin | 10453-86-8 | rat | 192 | -70.11 | NA | 1Comp | 59.95 | 0.2174 | 3.189 |
| s-bioallethrin | 28434-00-6 | rat | 192 | -77.36 | -83.84 | 2Comp | 47.54 | 0.831 | 0.8341 |
| simazine | 122-34-9 | rat | 192 | -64.68 | -62.89 | 1Comp | 3.532 | 1.992 | 0.348 |
| solvent red1 | 1229-55-6 | rat | 66 | -54.87 | -60 | 2Comp | 1.875 | 1.066 | 0.6502 |
| tamoxifen | 10540-29-1 | rat | 141 | -26.92 | -26.42 | 1Comp | 37050 | 0.07607 | 9.112 |
| tert-amyl methyl ether | 994-05-8 | human | 51 | -149.4 | -172.1 | 2Comp | 2.608 | 1.342 | 0.5167 |
| tetrachloroethylene | 127-18-4 | rat | 4 | 70.07 | 72.5 | 1Comp | 12.73 | 0.08628 | 8.034 |
| thiodiglycolic acid | 123-93-3 | rat | 155 | 43.13 | 20.29 | 2Comp | 0.7874 | 5.729 | 0.121 |
| trichloroethylene | 79-01-6 | rat | 18, 29 | 80.68 | 1508 | 1Comp | 2.565 | 0.8173 | 0.8481 |
| valproic acid | 99-66-1 | rat | 192 | 432.8 | 430.5 | 2Comp | 0.574 | 0.7342 | 0.9441 |
| wyeth-14643 | 50892-23-4 | rat | 173 | 113.1 | 102.8 | 2Comp | 0.4481 | 0.6937 | 0.9992 |
| bis 2-chloroethoxy methane | 111-91-1 | rat | 155 | NA | -0.4358 | 2Comp | 2.621 | 3.149 | 0.2202 |
| fluorotelomer alcohol 8+2 | 678-39-7 | rat | 131 | NA | -74.51 | 2Comp | 12.96 | 1.419 | 0.4884 |
| naphthalene | 91-20-3 | rat | 169 | NA | -29.5 | 2Comp | 5.786 | 2.63 | 0.2635 |
| perfluorohexanoic acid | 307-24-4 | rat | 145 | NA | 429.3 | 2Comp | 1.783 | 0.1387 | 4.996 |
| tetralin | 119-64-2 | rat | 152 | NA | 0.244 | 2Comp | 5.271 | 1.94 | 0.3573 |
| triclosan | 3380-34-5 | rat | 192 | NA | 27.86 | 2Comp | 0.3428 | 0.2387 | 2.904 |

Supplemental Table 4: Chemicals that could not be fit by either a one- or two-compartment model using R package invivoPKfit

|  |  |  |
| --- | --- | --- |
| **DTXSID** | **PREFERRED\_NAME** | **CASRN** |
| DTXSID0021125 | Phenolphthalein | 77-09-8 |
| DTXSID2020139 | Benzo(a)pyrene | 50-32-8 |
| DTXSID2021103 | Pentachloroanisole | 1825-21-4 |
| DTXSID5032442 | Imidacloprid | 138261-41-3 |
| DTXSID8021359 | Tolbutamide | 64-77-7 |
| DTXSID8022292 | Permethrin | 52645-53-1 |
| DTXSID8023393 | Ondansetron | 99614-02-5 |
| DTXSID9032329 | Bensulide | 741-58-2 |

Supplemental Table 5: Chemicals that could only be predicted by OPERA

|  |  |  |
| --- | --- | --- |
| DTXSID | PREFERRED\_NAME | CASRN |
| DTXSID0022985 | Ephedrine | 299-42-3 |
| DTXSID1051432 | Thiodiglycolic acid | 123-93-3 |
| DTXSID3032179 | 3,3',4,4',5-Pentachlorobiphenyl | 57465-28-8 |
| DTXSID30575892 | Oxoacetic acid--water (1/1) | 563-96-2 |
| DTXSID3061635 | 1-((2-Methoxyphenyl)azo)-2-naphthol | 1229-55-6 |
| DTXSID5020285 | 1-Chloro-2-propanol | 127-00-4 |
| DTXSID5024219 | Nitrite | 14797-65-0 |
| DTXSID50881104 | Diltiazem | 34933-06-7 |
| DTXSID7030066 | 2,3,4,7,8-Pentachlorodibenzofuran | 57117-31-4 |
| DTXSID80108992 | Perfluorooctanesulfonate | 45298-90-6 |

Supplemental Table 6: Chemicals whose measured values were potentially retrieved “as is” from model training sets and were therefore removed from the evaluation:

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| DTXSID | PREFERRED\_NAME | CASRN | Human.Clint.httk | Human.Fup.httk | Human.Clint.pred | Human.Fup.pred | QSPR | Human.Clint.AbsFE | Human.fup.AbsFE |
| DTXSID0020652 | Gemfibrozil | 25812-30-0 | 52.2 | 0.03 | 52 | 0.03 | OPERA | 0.00175 | 0 |
| DTXSID1021087 | Oxazepam | 604-75-1 | 3.27 | 0.04 | 3.27 | 0.04 | OPERA | 0.000133 | 0 |
| DTXSID1021116 | Phenacetin | 62-44-2 | 9.35 | 0.6 | 9.35 | 0.6 | OPERA | 0.000186 | 0 |
| DTXSID1022265 | Alachlor | 15972-60-8 | 62.9 | 0.133 | 62.9 | 0.13 | OPERA | 0.000276 | 0.00991 |
| DTXSID1043881 | Imipramine | 50-49-7 | 35 | 0.1 | 35 | 0.1 | OPERA | 0.00062 | 0 |
| DTXSID2032552 | Flufenacet | 142459-58-3 | 29 | 0.137 | 29 | 0.14 | OPERA | 0.00015 | 0.00941 |
| DTXSID3034872 | Chloridazon | 1698-60-8 | 1.87 | 0.427 | 1.87 | 0.43 | OPERA | 0 | 0.00304 |
| DTXSID4021268 | Simazine | 122-34-9 | 6.33 | 0.284 | 6.34 | 0.28 | OPERA | 0.000686 | 0.00616 |
| DTXSID4032376 | Dimethenamid | 87674-68-8 | 19.9 | 0.232 | 20 | 0.23 | OPERA | 0.000654 | 0.00376 |
| DTXSID4032405 | Formetanate hydrochloride | 23422-53-9 | 13.7 | 0.885 | 13.7 | 0.89 | OPERA | 0.000317 | 0.00245 |
| DTXSID5025607 | Methyleugenol | 93-15-2 | 26.6 | 0.142 | 26.5 | 0.14 | OPERA | 0.000818 | 0.00494 |
| DTXSID5032442 | Imidacloprid | 138261-41-3 | 2.81 | 0.656 | 2.81 | 0.66 | OPERA | 0 | 0.00264 |
| DTXSID5032600 | Cyclanilide | 113136-83-9 | 0.34 | 0.984 | 0.34 | 0.98 | OPERA | 0 | 0.00183 |
| DTXSID5037523 | Diazoxon | 962-58-3 | 34.6 | 0.327 | 34.6 | 0.32 | OPERA | 0.000125 | 0.0094 |
| DTXSID6021117 | Phenazone | 60-80-0 | 0.506 | 0.97 | 0.51 | 0.96 | OPERA | 0.00325 | 0.0045 |
| DTXSID6034849 | Propamocarb hydrochloride | 25606-41-1 | 5.81 | 0.843 | 5.81 | 0.84 | OPERA | 0 | 0.00155 |
| DTXSID7046627 | Bosentan | 147536-97-8 | 2.34 | 0.02 | 2.34 | 0.02 | OPERA | 0.000743 | 0 |
| DTXSID8021359 | Tolbutamide | 64-83-7 | 1.32 | 0.04 | 1.32 | 0.04 | OPERA | 0.00131 | 0 |
| DTXSID8023393 | Ondansetron | 99614-02-5 | 1.4 | 0.25 | 1.41 | 0.25 | OPERA | 0.00247 | 0 |
| DTXSID8024151 | Imazalil | 35554-44-0 | 1.02 | 0.03 | 1.02 | 0.03 | OPERA | 0 | 0 |
| DTXSID9020247 | Carbaryl | 63-25-2 | 27.3 | 0.692 | 27.3 | 0.69 | OPERA | 0 | 0.00126 |

Supplemental Table 7: Chemical-specific root mean square log10 errors for the full TK concentration time course data by QSPRs

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | FitsToData | ADMET | Dawson | InVitro | OPERA | Pradeep | Y-Random |
| 1,2-Dichloroethane | 0.406 | 0.576 | 1.05 | 1.11 | 0.957 | 1.06 | 0.65 |
| 1,4-Dioxane | 0.598 | 1.79 | 1.82 | 1.82 | 1.8 | 1.83 | 1.84 |
| 2-Hydroxy-4-methoxybenzophenone | 0.751 | 1.64 | 1.73 | 2 | 1.98 | 1.87 | 1.71 |
| 2-methylimidazole | 0.157 | 1.36 | 1.36 | 1.36 | 1.36 | 1.36 | 1.36 |
| 2-Methyltetrahydrofuran | 0.731 | 1.25 | 1.89 | 1.89 | 1.94 | 1.99 | 1.91 |
| 2,3,7,8-Tetrachlorodibenzo-p-dioxin | 0.417 | 0.417 | 0.417 | 0.417 | 0.417 | 0.417 | 0.417 |
| 2,4-D | 0.476 | 1.16 | 0.696 | 0.624 | 0.624 | 1.14 | 1.68 |
| 4-methylimidazole | 0.21 | 1.13 | 1.31 | 1.31 | 0.879 | 1.44 | 1.32 |
| acrylonitrile | 0.421 | 0.272 | 1.24 | 1.38 | 1.17 | 1.2 | 1.4 |
| Alachlor | 0.901 | 1.28 | 1.43 | 1.09 | 1.29 | 1.68 | 2.89 |
| alpha-Thujone | 0.584 | 1.08 | 1.12 | 1.11 | 1.12 | 1.11 | 1.17 |
| Alprazolam | 0.396 | 1.3 | 1.28 | 1.17 | 1.18 | 1.28 | 1.17 |
| Anthraquinone | 0.319 | 1.19 | 1.02 | 1.05 | 1.01 | 1.09 | 0.948 |
| Antipyrine | 0.0183 | 0.626 | 0.626 | 0.626 | 0.626 | 0.626 | 0.626 |
| Benzophenone | 0.383 | 0.637 | 0.673 | 0.663 | 0.659 | 0.655 | 0.622 |
| Bis 2-Chloroethoxy Methane | 0.483 | 1.44 | 1.68 | 1.68 | 1.74 | 1.7 | 1.72 |
| Bisphenol A | 0.127 | 0.614 | 0.401 | 0.419 | 0.348 | 0.469 | 0.36 |
| Boscalid | 0.247 | 0.902 | 0.671 | 0.695 | 0.7 | 0.694 | 0.652 |
| Bosentan | 0.396 | 0.766 | 0.666 | 0.432 | 0.669 | 0.445 | 0.746 |
| Bromochloroacetic acid | 0.632 | 1.08 | 1.24 | 1.24 | 1.2 | 1.33 | 1.3 |
| Bromodichloromethane | 0.634 | 0.643 | 0.768 | 0.768 | 0.797 | 0.917 | 1.02 |
| Carbaryl | 0.141 | 0.627 | 0.608 | 0.488 | 0.46 | 0.71 | 0.553 |
| Carbendazim | 0.303 | 2.06 | 2.12 | 2.15 | 2.15 | 2.07 | 1.49 |
| Chloridazon | 1.24 | 2.44 | 2.6 | 2.32 | 2.6 | 2.8 | 1.17 |
| Chlorpyrifos | 0.803 | 2.09 | 2.09 | 2.46 | 2.43 | 2.11 | 2.15 |
| Cyclanilide | 0.552 | 1.5 | 2.3 | 2.03 | 2.26 | 2.39 | 1.82 |
| Cyclosporin A | 0.154 | 0.894 | 0.879 | 0.688 | 0.596 | 0.655 | 0.811 |
| Di-n-butyl phthalate | 0.32 | 0.368 | 0.35 | 0.34 | 0.341 | 0.36 | 0.429 |
| Diazinon-o-analog | 0.737 | 3.19 | 2.98 | 3.02 | 3.07 | 2.93 | 0.933 |
| dibromoacetic acid | 0.57 | 1.02 | 1.43 | 1.43 | 1.39 | 1.48 | 1.54 |
| dichloroacetic acid | 0.604 | 0.929 | 0.939 | 0.939 | 0.924 | 0.953 | 0.947 |
| Dimethenamid | 0.756 | 0.983 | 0.994 | 0.994 | 0.994 | 0.996 | 1.2 |
| DL-Camphor | 0.396 | 0.56 | 1.24 | 1.34 | 1.39 | 1.19 | 0.998 |
| Emodin | 0.426 | 0.342 | 0.691 | 0.608 | 0.705 | 0.434 | 0.856 |
| Etoxazole | 0.525 | 1.12 | 0.891 | 0.874 | 0.874 | 0.849 | 0.837 |
| Fenarimol | 0.305 | 0.69 | 0.684 | 0.875 | 0.862 | 0.85 | 0.674 |
| Flufenacet | 1.27 | 1.49 | 1.34 | 1.29 | 1.35 | 1.34 | 1.23 |
| Formamide | 0.326 | 1.2 | 1.31 | 1.31 | 1.31 | 1.33 | 1.27 |
| Free Carbon disulfide | 0.213 | 0.247 | 0.325 | 0.325 | 0.227 | 0.446 | 1.15 |
| Gemfibrozil | 0.683 | 0.715 | 0.82 | 0.942 | 0.808 | 0.734 | 0.761 |
| Hexachlorobenzene | 0.315 | 0.333 | 0.647 | 0.643 | 0.643 | 0.7 | 0.743 |
| Ibuprofen | 0.272 | 0.722 | 1.18 | 0.858 | 0.993 | 0.801 | 1.44 |
| Imazalil | 0.245 | 0.961 | 0.659 | 1.44 | 1 | 1.4 | 1.23 |
| Imipramine | 0.307 | 0.649 | 0.638 | 0.626 | 0.642 | 0.753 | 0.627 |
| Isoeugenol | 0.419 | 0.837 | 0.924 | 0.905 | 0.9 | 0.844 | 1.29 |
| methanol | 0.0488 | 0.689 | 0.689 | 0.689 | 0.689 | 0.689 | 0.689 |
| methyl tert-butyl ether | 0.307 | 0.427 | 0.768 | 0.768 | 0.702 | 0.768 | 0.768 |
| methylene chloride | 0.274 | 0.564 | 1.04 | 1.1 | 0.935 | 1.07 | 1.13 |
| Midazolam | 0.373 | 1.01 | 1.01 | 1.01 | 1.01 | 1.01 | 2.42 |
| Nilvadipine | 0.633 | 1.13 | 0.842 | 1.33 | 0.91 | 0.857 | 0.899 |
| Novaluron | 0.281 | 1.12 | 1.12 | 0.807 | 0.807 | 0.855 | 1.25 |
| Oxazepam | 0.249 | 0.795 | 0.813 | 0.88 | 0.815 | 0.823 | 1.13 |
| Oxymetholone | 0.262 | 0.759 | 0.622 | 0.617 | 0.636 | 0.609 | 0.614 |
| Pentachlorophenol, purified | 0.332 | 0.565 | 0.825 | 0.885 | 0.868 | 0.892 | 0.867 |
| Perfluorodecanoic acid | 0.313 | 0.919 | 1.02 | 0.858 | 0.903 | 1.11 | 1.22 |
| perfluorohexane-1-sulphonic acid – potassium salt | 0.834 | 2.04 | 2.35 | 1.35 | 2.23 | 2.51 | 1.5 |
| Perfluorohexanoic Acid | 0.937 | 1.15 | 1.34 | 0.834 | 0.799 | 1.66 | 2.08 |
| Permethrin | 0.352 | 1.01 | 1.12 | 1.08 | 1 | 1.09 | 1.43 |
| phenacetin | 0.7 | 0.861 | 0.9 | 0.97 | 0.94 | 0.995 | 0.893 |
| Phenytoin | 0.576 | 0.395 | 0.841 | 0.481 | 0.462 | 0.67 | 0.494 |
| Potassium Perfluorobutane Sulfonate | 0.56 | 1.61 | 1.86 | 1.31 | 1.32 | 2.3 | 2.25 |
| Primidone | 0.318 | 1.96 | 1.94 | 1.96 | 1.66 | 1.94 | 1.61 |
| Propamocarb hydrochloride | 0.414 | 1.13 | 1.93 | 1.01 | 1.01 | 1.02 | 1.06 |
| propylparaben | 0.602 | 0.891 | 0.645 | 0.752 | 0.757 | 0.713 | 0.465 |
| Propyzamide | 0.62 | 1.42 | 1.52 | 1.05 | 1.06 | 1.08 | 2.09 |
| Pyridine | 0.445 | 1.28 | 1.35 | 1.35 | 1.37 | 1.33 | 0.958 |
| Pyrithiobac sodium | 0.937 | 1.25 | 2.27 | 0.75 | 0.647 | 2.58 | 2.66 |
| Resmethrin | 0.316 | 0.833 | 1.06 | 0.694 | 0.694 | 1.18 | 1.49 |
| S-Bioallethrin | 0.393 | 0.928 | 1.07 | 2.29 | 1.22 | 1.25 | 1.01 |
| Simazine | 0.455 | 0.836 | 0.636 | 0.639 | 0.717 | 0.637 | 0.717 |
| Tamoxifen | 0.347 | 0.938 | 1.83 | 4.24 | 3.99 | 2.15 | 2.68 |
| tert-amyl methyl ether | 0.0947 | 0.348 | 0.847 | 0.847 | 0.7 | 0.847 | 0.847 |
| tetrachloroethylene | 0.183 | 0.369 | 0.876 | 0.38 | 0.357 | 1.27 | 1.39 |
| Tetralin | 0.689 | 0.575 | 0.981 | 1.29 | 1.58 | 0.802 | 0.496 |
| trichloroethylene | 0.476 | 0.436 | 0.689 | 0.848 | 0.427 | 0.805 | 0.983 |
| Valproic acid | 0.434 | 0.881 | 0.971 | 0.969 | 0.843 | 1.37 | 1.6 |
| Wyeth-14643 | 0.458 | 0.503 | 0.764 | 0.555 | 0.628 | 0.875 | 0.95 |

Supplemental Table 8: Level 3 Predictions

*SupTable-Level3.xlsx*