Development of PK- and PBPK-based modeling tools for derivation of biomonitoring guidance values


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ABSTRACT

There are numerous programs ongoing to analyze environmental exposure of humans to xenobiotic chemicals via biomonitoring measurements (e.g.: EU ESBO, COPHES; US CDC NHANES; Canadian Health Measures Survey). The goal of these projects is to determine relative trends in exposure to chemicals, across time and subpopulations. Due to the lack of data, there is often little information correlating biomarker concentrations with exposure levels and durations. As a result, it can be difficult to utilize biomonitoring data to evaluate if exposures adhere to or exceed hazard/exposure criteria such as the Derived No-Effect Level values under the EU REACH program, or Reference Dose/Concentration values of the US EPA. A tiered approach of simple, arithmetic pharmacokinetic (PK) models, as well as more standardized mean-value, physiologically-based (PBPK) models, have therefore been developed to estimate exposures from biomonitoring results. Both model types utilize a user-friendly Excel spreadsheet interface. QSAR estimations of chemical-specific parameters have been included, as well as accommodation of variations in urine production. Validation of each model's structure by simulations of published datasets and the impact of assumptions of major model parameters will be presented.

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1. Introduction

The time-course of drug concentrations in blood or tissues is often predicted using pharmacokinetic modeling calculations. These calculations are generally based on assumptions that the body can be represented simply as a single, homogeneous compartment (pharmacokinetic (PK) model) [1], or as a more complex system of separate tissues with differing pharmacokinetic properties and incorporating physiological parameters such as blood flow and tissue size (physiologically based (PBPK) model) [2]. Measured or estimated rates of absorption, distribution, metabolism and excretion (ADME) are utilized in these pharmacokinetic models, which can be validated with data from numerous clinical studies.

These models can also be used to interpret biomonitoring data, which consists of blood or tissue concentrations of xenobiotic chemicals or their metabolites in workers or non-occupationally exposed humans. There are numerous programs ongoing to analyze environmental exposure of humans to xenobiotic chemicals, in which single time-point samples are analyzed for concentrations of a variety of compounds in a subset of the population (e.g.: EU ESBO, COPHES, NewGeneris; US CDC NHANES; Canadian Health...
Measures Survey) [3–7]. While these single-sample assays may result in some error of exposure predictions, due to the known diurnal variability in metabolite levels [8,9], these large biomonitoring programs have an overall goal to determine relative trends in exposure to chemicals, across time and subpopulations. However, these programs do not attempt to calculate actual exposures from the biomarker measurements, or compare if biomonitoring-derived exposure estimates are below or exceed acceptable limit values, such as the REACH Derived No-Effect Level or the US EPA RfC/RfD values. As a result, it can be difficult to utilize biomonitoring data to evaluate acceptable levels of exposure to chemicals.

Some methods, such as the Biomonitoring Equivalents approach [10] have been developed to define biomarker levels, or biomonitoring guidance values (BGVs) [11], that are equivalent to reference exposure values. This approach requires the use of PK or PBPK models to correlate animal or human points of departure (POD) with biomarker levels at the human equivalent POD. A tiered approach utilizing simple PK models, as well as more standardized mean-value PBPK models, would aid researchers in their derivation of BGVs.

There are numerous examples of how chemical-specific PK or PBPK models have been used to derive biomonitoring guidance values [12–14]. Several PBPK models have also been developed directly in the Microsoft™ Excel application, therefore requiring no user programming [15,16]. However, these models, while well-validated, can require a substantial amount of empirical ADMET data and model optimization for accurate prediction of biomarker levels. There is a paucity of this type of data for most synthetic chemicals. As a result, for a large percentage of biomonitoring data, only limited interpretation has been conducted. The recent Excel-based PBPK model of Jongeneelen et al., is a substantial improvement in this area, in that it also provides model-derived predictions of tissue distribution, dermal absorption and renal clearance and accommodates modeling of metabolites [17].

The purpose of the work described in this manuscript is to develop several user-friendly computer applications that support exposure estimates from biomonitoring data. A multi-tissue compartment PBPK model, as well as an arithmetic, one-compartment PK model requiring minimal ADMET data, have been developed. Both applications have been written for the Microsoft Excel spreadsheet interface and include Quantitative Structure–Property Relationship (QSPR) estimations of chemical-specific parameters, such as blood:tissue partition coefficients, as in the recent model of Jongeneelen and Berge [17]. The PBPK model includes accommodation of variations in urine micturition, to allow for direct comparison of urine biomarker concentrations with timed samples collected from biomonitoring studies. This model utilizes an Excel interface to a compiled acslX model, allowing for full utilization of a classic modeling tool in a user-friendly manner. Both models also support automatic back-calculation (“reverse dosimetry”) of exposure levels from biomonitoring data. Verification of each model’s structure by simulations of published datasets and the impact of assumptions of major model parameters will be presented.

![Diagram of one-compartment PK model for reverse dosimetry.](Image)

### 2. Computational methods

An overview of the design of the PK and PBPK models for reverse dosimetry is presented in the following sections. In addition, empirical data used for the derivation of the oil:water partition QSPR required for volume of distribution (Vd) or tissue:plasma partition (Ptp) coefficients is discussed.

#### 2.1. PK model for reverse dosimetry

As shown in Text Fig. 1, a one-compartment PK model is employed to calculate single- and steady-state exposure levels from plasma (Cp) or urine (Cu) biomarker concentrations. Classic PK equations for single-dose and steady state exposures [1] were incorporated into this model and are fully defined in an appendix section of the Microsoft Excel™-compatible workbook file. A first-order uptake of the chemical is employed (kabs), which is most relevant for oral exposures, but is also assumed adequate for short-term dermal or inhalation exposures. The apparent first-order elimination rate (k) defined by Gibaldi and Perrier is set equal to the urinary excretion rate (kexcr), as it is assumed that any metabolism involved in production of the relevant biomarker is primarily a first-pass phenomenon, and as such does not need to be separately included in the model. The assumption of linear kinetics is felt appropriate for non-occupational exposure estimates from biomonitoring data. Non-linearities in PK parameters may occur at higher worker exposure scenarios.

Equations used for the PK model are shown below and also fully described in the PK model, available as supplemental material to this manuscript:

**Single Exposure Amount (from plasma conc)**

\[
\text{Cpt} \times \text{Vd} \times \frac{(\text{kabs} - \text{kexcr})}{(\text{kabs} \times \text{FrXAbs}) \times (e^{-\text{kexcr}T} - e^{-\text{kabs}T})} \quad (1)
\]

**Single Exposure Amount (from urine conc)**

\[
\frac{\text{Cur}T \times \text{Vur} \times (\text{kabs} - \text{kexcr})}{(\text{kexcr} \times \text{kabs} \times \text{FrXExcr}) \times (e^{-\text{kexcr}T} - e^{-\text{kabs}T})} \quad (2)
\]

**Steady-state Exposure Amount (from plasma conc)**

\[
\frac{\text{CpSS} \times \text{Vd} \times \text{kexcr} \times \text{Tau}}{\text{FrXAbs}} \quad (3)
\]
Steady-state Exposure Amount (from urine conc.)

\[ \text{CurSS} \times \text{Vur24hr} \]

where \( C_p \) is plasma concentration of biomarker (Cu) at time \( T \), \( k_{abs} \) and \( k_{exc} \) are absorption and excretion rates, respectively. \( T \) is time since exposure, \( F_{rxAbs} \) is fraction of dose absorbed, \( V_d \) is the calculated or measured volume of distribution, \( C_{pu} \) is “instantaneous” concentration of biomarker in urine (Cu) at time \( T \), \( F_{rxExc} \) is fraction of dose excreted in urine as biomarker, \( C_{pSS} \) is plasma biomarker concentration at steady state, \( T_{au} \) is interval between steady state exposures (assumed 24 h), \( C_{uSS} \) is urine biomarker concentration at steady state and \( V_{ur24hr} \) is the daily urine volume.

2.2. PBPK model for reverse dosimetry

The PBPK model for reverse dosimetry describes the time-course of ADME for a chemical and up to one biomarker metabolite following a single exposure. Input routes include oral, dermal and inhalation (Text Fig. 2). Metabolism of the parent compound, if relevant, occurs in the liver and the metabolite formed is input into the venous blood compartment of the parallel metabolite PBPK model. A first-order elimination from the venous blood is used for both parent chemical and metabolite to account for loss via urinary excretion (micturition).

This PBPK model is run from a custom Microsoft Excel workbook file. User-input parameters are transferred from the Excel file automatically to a compiled, acs19 model (AEGis Technologies, Huntsville, Alabama, USA). Once the model completes calculations of time-course blood and tissue concentrations, this model output is written back to the Excel file. After 11 iterations of model runs across an array of dose levels, an estimate of exposure is calculated from the various dose time-course datasets.

Concentrations of the test chemical and metabolite in representative physiological compartments are calculated across time via a series of differential equations, in which partitioning between blood and tissues is instantaneous (“well-stirred” approach [18]). The general approach for this model design has been well described previously [2]. A copy of the model code is included in the Excel interface workbook, available as supplementary material to this manuscript.

2.3. QSAR for oil:water partition coefficient

The QSAR approaches for predicting \( V_d \) or \( P_{TP} \) values for the PK and PBPK models, respectively, rely on a fundamental relationship for partitioning of the test chemical between octanol:water (Koc:water) or vegetable oil:water (Koi:water) [19-22]. While Koc:water values are generally empirically derived for chemicals, the Koi:water parameter is often not available. Previous researchers have utilized linear relationships between these two parameters to estimate Koi:water from Koc:water [23]. However, this linear relationship may not be appropriate for fairly lipophilic compounds (logKoc:water > 4) and may result in an overestimate of the Koi:water parameter [24].

To evaluate the relationship between Koi:water and Koc:water, published Koi:water data for 104 compounds with log Koc:water values of −1.9 to 6.0 [19,23,25] were combined with new Koi:water data for eight lipophilic chemicals (log Koc:water 4.3-8.3) as well as the positive control p-ethylphenol (log Koc:water 2.5), described in the following section. This combined data was then fit to a sigmoidal dose response curve (with variable slope), using the Prism application (v5.0; GraphPad Software Inc, La Jolla, CA, USA).

Equations used for the PK model are shown below and also fully described in the PK model, available as supplementary material to this manuscript:

2.3.1. Koi:water determinations

\[ ^{3}H(G)-benzo(a) pyrene, \; 7-^{3}H-cholesterol, \; ^{3}H-retinol, \; ^{14}C-p- \]

ethylphenol, and \( ^{14}C-1\)-octodecanol were purchased from American Radiolabeled Chemicals (St. Louis, MO). \( ^{14}C(u)-\)

octachlorostyrene was purchased from Dupont, NEN Products (Boston, MA). Chlorpyrifos (ring-4-3H) was obtained from Amersham Biosciences (now GE Healthcare, Piscataway, NJ). \( ^{14}C\)-hexachlorobenzene, \( ^{14}C\)-XDE-175J, and \( ^{14}C\)-XDE-007 were obtained and used with permission from Dow AgroSciences LLC (Indianapolis, IN). A bottle of 100% pure all natural Filipo Berio Olive Oil (Hackensack, NJ) was purchased from a local grocery store. Each chemical was dissolved in 10 ml of olive oil in a 24-ml glass vial. An equal volume of Milli-Q water was added to each vial. The concentration of each chemical fortified into the oil was intended to produce a final water concentration of the chemical at approximately 1/10 the water solubility of the chemical if partitioning were to occur according to the published (or QSAR-derived) Koi:water values. Each vial contained a glass magnetic stir bar, the two phases were gently stirred for 7 days on a magnetic stir plate at 25 °C. An aliquot of oil and an aliquot of water were taken on the seventh day and assayed by radiochemical detection with liquid scintillation counting. Each chemical treatment was performed in duplicate. Koi:water was calculated as the ratio of total counts (dpm) in the oil phase vs. the total counts (dpm) in the water phase.

3. Program descriptions

3.1. PK model for reverse dosimetry

Use of the PK model for reverse dosimetry is fairly straightforward. User input is minimal, requiring only basic test chemical information and selected pharmacokinetic (PK) parameters, on the User Entry page of the Excel workbook (Fig. 1). The test chemical and biomarker names (if metabolite is biomarker), and molecular weights are needed for molar conversions of biomarker concentration to exposure amounts within the PK model. An estimated time between exposure and biomarker sampling is needed to back-calculate exposure levels. The biomarker concentration in plasma (\( C_p \)) and/or urine (\( C_u \) for a given individual/time point is entered, in units of mg/L (or possibly mg/g creatinine for urine). Note that whole blood concentrations can be used instead of plasma levels, if the blood/plasma ratio for the biomarker is ~1.0. The PK parameters required for this model are absorption and
elimination rates ($k_{abs}$, $k_{exr}$), fractions absorbed and eliminated as biomarker, and volume of distribution ($V_d$), which is needed for plasma biomarkers only. Urine hourly production rate is set at the default for a 70 kg adult male [26], which can be adjusted by the user. Hourly creatinine production rate is based on a population average creatinine production rate (mg/L) urine [27] for individuals ages 20–59, multiplied by the average hourly urine production rate (L/h). Note that correction of urine biomarker levels for creatinine concentration should be done on a compound-specific basis, and is dependent on the renal clearance mechanism and water solubility of the compound [28]. Required PK values for reverse dosimetry calculations are flagged with a red asterisk, once a biomarker value is entered (plasma, urine, urine/Cr). A more complete description on the use of this model can be found in the user’s manual, available as supplementary material to this manuscript.

These PK parameters are generally obtained from controlled metabolism/exposure studies in animals or human volunteers. Some parameters, such as $k_{abs}$, fraction absorbed and $V_d$ can be estimated separately with QSAR programs such as ADME Suite (Advanced Chemistry Development, Inc. (ACD/Labs), Toronto, On, Canada) or ADMET Predictor (Simulations Plus, Lancaster, CA, USA). Since $V_d$ is a summation of tissue:plasma partition coefficients multiplied by tissue volumes (see text Eq. (5)), a QSAR to derive this parameter from a few physical–chemical properties is included within this spreadsheet-based model. This $V_d$ QSAR is based on the compilation of published tissue:plasma partition coefficient algorithms of Rodgers and Rowland [29], which utilizes a compound’s solubility in octanol or vegetable oil as a surrogate for distribution into the lipid fraction of tissues. Note that these partitioning algorithms have been developed and validated primarily for pharmaceutical compounds ($\log K_{oct}$:water $< 4$), which are often less lipophilic than industrial chemicals in the environment.

$$V_d = \frac{V_p}{f_u} + \sum (V_t \times P_{tp})$$ (5)

where $V_p$ is the volume of plasma in the body, $f_u$ is the fraction of the chemical or metabolite not bound to protein, $V_t$ is the volume of individual tissues in the body and $P_{tp}$ is the tissue:plasma partition coefficient for individual tissues.
Since the exposure amount calculations are direct rearrangements of PK equations for Cp or Cu at time T post-exposure, a predicted exposure amount is immediately calculated from any biomarker concentration, applying Eqs. (1) and (2), above. The model output is given in several formats. Exposure amounts, in total mg chemical for a nominal human of 70 kg body weight, are given for single exposure or steady-state assumptions, based on entered plasma or urine biomarker concentrations (Fig. 1). For visualization purposes, time-course graphs of biomarker levels in plasma, urine and cumulative in urine are also shown for the exposure amount calculated from the inputted biomarker value. Since the accuracy in exposure amount determinations falls off over time, due to variability in analytical measurements, no exposure amounts are calculated for single-exposure scenarios when the time between exposure and sampling is greater than 5 times the sum of the absorption and elimination half-lives. This summation of half-lives will also accommodate “flip-flop” model scenarios, where absorption rates are slower than elimination rates. This phenomenon is seen following dermal uptake of a chemical or from sustained release drug formulations, where the initial positive slope of the plasma time–course curve represents the elimination rate and the subsequent negative slope represents the slow absorption rate [30].

3.2. PBPK model for reverse dosimetry

The data required for the PBPK model is similar to that of the PK model. Chemical-specific and PK data are required for both the chemical and metabolite (entered in the User Entry page of the Excel workbook) (Fig. 2). First-order uptake rates are assumed for oral exposures. Dermal exposures are assumed to occur from infinite-volume solutions (mg/L) of the chemical, on a nominal skin surface area of 100 cm² (15.5 in²). First-order Kp rates for dermal uptake can be user-entered or derived via a published QSAR, used in the public-access Dermwin Application (v1.43, US EPA) [31]. For inhalation exposures, a continuous air concentration is assumed, for a user-defined period of time in hours. Biomarker concentrations are inputted for either parent chemical or metabolite, from blood, plasma, urine or urine corrected for creatinine concentrations. Metabolism rates for loss of both chemical and metabolite are assumed to be saturable, therefore Vmax and Km terms are required for both parent and metabolite. Plasma elimination rates are required for both chemical and
metabolite. Urine micturition times (up to 20 values) can be inputted to match concentrations that would be measured from timed urine collections, otherwise a default micturition interval of 4 h is assumed. Tissue-specific Ptp values can be user entered. These Ptp values can also be derived within the model from published QSPR methods that derive partitioning based on water/lipid compositions in tissues [19,20], or offline with a more unified algorithm, incorporating distribution between cells, interstitial fluid, erythrocytes and plasma [32]. Note that the Koil:water parameter, derived from the updated correlation with Koct:water, as discussed in Section 2.3 (above), can be used as an input in these Ptp QSPR methods. Note that Ptp values are calculated by default for tissue:plasma, unless biomarker values
are entered for whole blood, in which case these values are calculated for tissue:blood, as per Poulin and Krishnan [33].

Tissue : blood partition coefficient (Ptp)

\[
\frac{[\text{Kox} : \text{water}] \times ([\text{Vnl} + 0.3 \times \text{Vpht}] + (1 \times \text{Vut} + 0.7 \times \text{Vpht}))}{[\text{Kox} : \text{water}] \times ([\text{Vnl} + 0.3 \times \text{Vpht}] + (1 \times \text{Vut} + 0.7 \times \text{Vpht}))} \times \text{fut}
\]

(6)

where Kox:water is Koct:water or Koil:water, Vnl, Vpht,Vnlp and Vphtp are the volume of neutral lipids (nl) and phospholipids (ph) in the tissues and plasma, respectively, Vut and Vup are the volume of water in the tissue and plasma, respectively, and fut and fup are the fractions of the chemical or metabolite not bound to protein in the plasma or tissue, respectively.

Although this modeling tool is designed to interpret human biomonitoring data, the algorithm for predicting Ptp values has been expanded to facilitate potential modeling in species besides human. Calculated Ptp values for mouse, rat, rabbit, or dog can be found on the Ptp derivation page of the modeling workbook. In addition to Ptp values, the required, analogous blood:air partition coefficients (Pba) for the PBPK model were derived from calculated Kwater:air and Koil:air partition coefficients (as defined by Kelly et al. [34]), in addition to the derived Koil:water values from the QSPR derived in this project.

Reverse dosimetry calculations are then made from this Excel workbook. The program first runs the PBPK model iteratively, at 11 different exposure concentrations, logarithmically distributed across the user-defined range of low and high exposure values. Simulations are conducted for 24 h or up to the last micturition time, whichever is longer. Once model runs are complete, a combined set of four graphs are created, showing the time-courses of parent chemical and metabolite levels in blood and excreted urine (Fig. 3). A reverse dosimetry calculation [10] of exposure from the input biomarker value is determined from a “Lookup” of biomarker time-course values from each model run. Exact exposure values are linearly interpolated from the two closest predicted biomarker levels that bracket the user-input biomarker value. If the exposure amount is outside of the user-defined exposure range, the model will need to be re-run with new exposure values. As with the PK model, exposure amounts are outputted in units of mg/total body weight in kg. Note that unlike the PK model’s default body weight of 70 kg, the user can input specific body weights for each model run, to better match empirical study data. A more complete description on the use of this model can be found in the User’s Manual, available as supplementary material to this manuscript.

4. Sample program output

4.1. PK model for reverse dosimetry

4.1.1. Biomarker predictions

The accuracy of the model to predict biomarker concentrations over time (i.e.: forward predictions) was evaluated. Simulations of predicted biomarker concentrations for several published datasets were made and the results shown in Fig. 4. The model does a reasonably good job of fitting urinary or blood biomarker concentrations for datasets of chlorpyrifos, diazinon and/or butoxyethanol following oral, dermal or inhalation exposures. Assuming that single-exposure evaluations via biomonitoring are generally done during or shortly after a workshift, refit values at timepoints of 0.5-, 1.0- and 2.0-fold the elimination rate value were calculated and are shown in Table 1.

For oral or dermal administration of chlorpyrifos, model simulations were conducted with the published kabs, kexcr, FreqAbs and FreqExc values reported by Nolan et al. [35]. The predicted blood and urine biomarker concentrations of the 3,5,6-trichloropyridinol (TCP) biomarker in simulations of oral exposures, were within 25% of actual values [35]. Lower, but still acceptable predictions were obtained for the dermal data, with all predictions within 70% of actual levels.

Diazinon biomarker concentrations in urine (sum of total dialkylphosphates (DAPs)), following oral administration, were predicted using an estimated absorption half-life of 0.5 h, as no data was derived by the authors [36]. This short absorption half-life was chosen, based on the rapid elimination half-life (2.13 h) and known rapid absorption of the related organophosphate, chlorpyrifos [35]. Predicted biomarker levels were all within 30% of actual dose levels. Simulation of dermal absorption was done using the same elimination t1/2 as oral uptake (2.13 h), and utilizing the reported 9.12 h elimination t1/2 as the actual dermal uptake rate, assuming flip-flop kinetics for this route. The overprediction of DAP levels at 0.5 × t1/2 of 4.2-fold measured concentration (Table 1) appears to be due to a delay in actual micturition, since predicted levels at 1- and 2-fold the elimination t1/2 were within 10% of actual values (Fig. 4).

Butoxyethanol (BE) biomarker concentrations (total butoxyacetic acid (BAA)) following inhalation exposure were calculated with an absorption t1/2 estimated at 1 h, which was equivalent to the t1/2 calculated for dermal uptake (below) since steady-state levels were not achieved via inhalation for 0.5 h [37]. The elimination t1/2 reported by Kezic et al. [37] of 3.4 h was used. Biomarker concentrations following dermal exposure were calculated with an absorption t1/2 estimated at 1 h, based on steady blood BE levels achieved within 4 h [37], while the reported elimination t1/2 of 5.8 h was used. The PK model predictions at 0.5 – through 2.0-fold the elimination t1/2 were all within 2.2-fold of actual levels for both routes. Note that the data from Kezic et al. was obtained by digitizing mean biomarker concentrations from Fig. 2A and B of their manuscript, with the application DigitizeIt v1.5.5 (Digital River, Köln, Germany). Accuracy of data digitization was less than 0.5% error, as determined by comparison of calculated and reported sample collection interval time-points. The absorption half-life of 1.0 h was selected for both the inhalation and dermal datasets, based on the reported time to steady-state plasma butoxyethanol levels of 2–4 h (Fig. 1B of Kezic et al.).

4.1.2. Model assumptions

The needed pharmacokinetic parameters may not always be available for the determination of exposure levels via the Biomonitoring PK model. However, exposure predictions could
still be made within this simple model if values for unknown parameters are estimated. The model that would require the fewest estimates for unknown parameters would be calculation of exposure from a urine biomarker concentration at steady-state exposure (Eq. (4)). In this case, urine production rates would be fairly constant, leaving exposure levels dependent only on FrxExcr (fraction of dose excreted in urine as biomarker). This value could be estimated from ADME data in animals. Alternately, it could be set at a default value of 10%, which would give at most a one-order of magnitude error in exposure levels (assuming FrxExcr is at least 1%). This maximum 10-fold error in exposure estimate, when using a default FrxExcr of 10%, is due to the fact that the calculated exposure amount is inversely proportional to FrxExcr, as defined in Eqs. (2) and (4) (Section 2.1, above). Note that for the three validation compounds discussed above, FrxExcr ranged from 86% to 70%.

The remaining three scenarios for the Biomonitoring PK model (single exposure blood, steady-state blood or urine level) require at least three PK parameters that are specific to a given chemical (kabs, kexcr, Vd, FrxAbs and/or FrxExcr). The Vd term can be estimated from QSPR applications, if not known. The FrxAbs and FrxExcr terms have only a linear impact on exposure calculations, so again assuming a value of 10% for either of these will only afford at most a 10-fold error in exposure calculations. As shown in the published data for the three validation compounds, biomarker measurements in urine matrix are generally more common than blood measurements for non-pharmaceutical compounds. As a result, the determination of kexcr from human volunteer exposure studies is generally reported more often than kabs, as urine collection frequency is insufficient for calculation of most absorption rates. So for the single exposure scenarios or steady-state exposure calculations from blood levels, there may be a need to estimate kabs for use in this Biomonitoring PK model.

An evaluation of the error in exposure calculations that could arise from inaccurate estimation of compound’s kabs (ln(2)/absorption t1/2) is shown in Fig. 6. Three of this study’s test data sets (diazinon-oral urine, chlorpyrifos-dermal urine and butoxyethanol-inhalation urine) were utilized to predict biomarker concentrations at 0.1, 0.2, 0.5, 1.0, 2.0, 3.0 and 5.0 times the reported absorption half-life. As expected, the largest errors in biomarker concentrations when varying the absorption t1/2 parameter were found in the dermal absorption dataset. The highest overall error was found in the early time-range of all datasets. Maximal errors in predicted biomarker values, at timepoints out to five times the absorption t1/2, ranged from 4 to 10 times higher or lower than values from the simulation with the default absorption half-life.

4.2. **PBPK model for reverse dosimetry**

4.2.1. Biomarker predictions

As with the PK model, above, the accuracy of the PBPK model to predict biomarker concentrations was also evaluated with
Fig. 4 – Comparison of PK and PBPK model fits to empirical biomonitoring data (red line (closed circles) = empirical data, green line = PK model prediction of biomarker time-course, blue line (open triangles) = PBPK model prediction of biomarker time-course). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
the test datasets for chlorpyrifos, diazinon and butoxyethanol. As shown in Fig. 4, the biomarker fits from the PBPK model matched the experimental data generally well. Note that the PK and PBPK model fits are also comparable to each other. This is most probably due to the chemical similarity of the three biomarkers (TCP, DAPs, BAA), all fairly hydrophilic, with Ptp values for all tissue compartments that ranged between 0.5 and 1.5, and the fact that both models utilize first-order uptake and elimination rates.

For the oral and dermal absorption routes for chlorpyrifos, the same kα and kexcr parameters were utilized as in the simpler PK model. Note that the plasma kexcr for the parent compound was set equal to that of the trichloropyridinol metabolite. Alteration of this rate did not impact the biomarker levels substantially, as the rate of metabolic loss of chlorpyrifos is quite high, with ~99% of total blood metabolites present as TCP [38]. The oral dose level was adjusted for percent absorption value of 72% reported by Nolan et al. [35]. The PBPK model assumes an infinite dose volume for dermal exposures. To account for the finite amount of test material solution used by Nolan et al. (8 μL of a 50% chlorpyrifos solution/cm² skin), the limited volume of applied dose concentration of 50% (w/v) was multiplied by 10%, to afford a more realistic, real-world potential infinite dose concentration of 50 g/L (5%) solution. As described below, this dose concentration used in the PBPK model afforded good fits with actual biomarker concentrations following dermal exposure, in both time-course and magnitude of TCP concentrations. The dermal penetration Kp value was taken from Timchalk et al. [38]. In the original PBPK model published for this chemical by Timchalk et al. [38], the authors define numerous rates for the various metabolic conversions. The Vmax and Km terms for the primary route of metabolism (chlorpyrifos → TCP) were utilized in the Biomonitoring PBPK model for chemical conversion to metabolite. Also, since TCP is not further metabolized [39], the metabolic rates for conversion of this metabolite were set to zero. Tissue-plasma partition coefficients for the parent chemical were derived from the QSRR within the PBPK model. The Ptp value for TCP was set to 0.5 for all tissues, consistent with the Vd value of 35 L/total body weight used by Timchalk et al. in their PBPK model of this biomarker [38].
The simulations of blood TCP levels with the PBPK model, from both exposure routes, were within 30% of actual values, while urine concentration predictions were generally within 50% (Table 1). These relatively good fits for TCP with the PBPK model show that the use of the enzymatic rate constants for the primary metabolic pathway of chlorpyrifos → TCP afforded an accurate representation of biomarker formation in human.

Diazinon DAP biomarker levels following oral absorption were estimated with the PBPK model using the same kabs and kexcr values as described in Section 4.1. The plasma kexcr value for dermal exposure was set equal to the urinary elimination rate observed by Garfitt et al. [36] and the dermal Kp value from Poet et al. [40]. The oral dose level was adjusted for the percent absorption of 80%, calculated by Poet et al. As
discussed for chlorpyrifos above, the finite dose volume of 20 µL of a 25% diazinon solution/cm² skin used by Garfitt et al. [36], was adjusted to a lower concentration of the infinite dose volume required by the PBPK model. The concentration of 0.25% (w/v) was found to afford good predictions of urinary DAP concentrations, and was therefore assumed appropriate for this limited dose to infinite dose volume extrapolation. As was the case for chlorpyrifos, the $V_{max}$ and $K_m$ terms for the primary route of metabolism, hepatic CYP-based dearylation, affording IMHP and DAP metabolites, were used to describe metabolic loss of parent compound. The $V_{max}$ for metabolism of the DAP metabolites was set to zero, as these biomarkers represent a high percentage of administered oral dose (66%) [36] and Timchalk et al. showed that these metabolites are quite metabolically stable in the rat [39].

Blood:tissue partition coefficients for the parent compound and DAP metabolites were derived from the QSRR within the PBPK model.

The predictions of DAP metabolite levels of diazinon by the PBPK model were quite acceptable (Fig. 4). Refits at 0.5 and 1.0 times the elimination $t_{1/2}$, were within 30%, while fits at 2.0× the elimination $t_{1/2}$ were within 85% of actual biomarker levels (Table 1).

The predictions of total BAA metabolite following inhalation or dermal exposure to BE are again shown in Fig. 4 (G and H). The plasma elimination $t_{1/2}$, for both parent compound and BAA metabolite was set equal to the reported BAA urinary elimination rates for both routes. The dermal dose was set equal to 100% solution (near BE), as an infinite dose volume, which is the default dose volume assumed by the model. The $V_{max}$ and $K_m$ terms for the primary route of metabolism (BE → BAA) were obtained from Corley et al. [41]. The $V_{max}$ for subsequent metabolism of BAA was set to zero, as BAA is primarily conjugated with glutamine, once formed [42], and this conjugation step is not relevant, as total BAA levels (free + conjugated) were used in the PK and PBPK model simulations. [39]. Blood:tissue partition coefficients for the parent compound and BAA metabolite were derived from the QSRR within the PBPK model.

Predictions of total BAA levels with the Biomonitoring PBPK model were all within 2-fold of measured concentrations (Fig. 4 and Table 1). No values were calculated at 0.5 × $t_{1/2}$, as the first urine samples collected were at 4 h for both exposure routes.

4.2.2. Model assumptions

The PBPK model was designed to require a minimal number of PK parameters for prediction of exposure from blood or urine biomarker levels. Chemical-specific parameters required for the simpler PK model were $k_{abs}$, $k_{exc}$, $V_d$, $FrXAbs$ and/or $FrXExc$. The PBPK model requires rates of absorption and elimination, as well as metabolic rates for at least the conversion of the parent chemical. Also, tissue:plasma partition coefficients are required for several tissue compartments, even when interpreting urine biomarker data only.

As discussed in Section 4.1, the parameters needed to predict biomarker concentrations with the PBPK model may not always be available for a given chemical. In that case, estimates can still be made if assumptions are made for key model parameters. The impact of these assumptions are shown in Fig. 7.

In predicting the effect of an absorption rate for a chemical up to 0.2–5-fold different than the actual value, a comparative analysis shows only a modest impact on predicted biomarker levels, with the maximum error in diazinon DAP metabolite values less than 4-fold of actual concentrations, when simulated out to 5 times the elimination half-life (Fig. 7A). These results are quite comparable to the assumptions for this parameter described for the PK model (above). In contrast, the errors in predicted biomarker concentrations with variations in elimination rates are more substantial. This error ranged up to ~4000-fold when evaluations were carried out to 5 times the elimination half-life of 2.1 h (Fig. 7B). This error was most pronounced when elimination half-lives were underestimated. However, as discussed above, the elimination rate for a biomarker can often be approximated from occupational exposure studies, in which workers are isolated from chemical exposure to measure decay in blood or urine levels that occurred prior to the isolation.

The impact of estimating the Michaelis–Menton metabolic terms on predicted biomarker levels is shown in Fig. 7C and D. One-fifth to five-fold variations in either of these parameters afforded only ~4-fold error in biomarker predictions, across the time range of 0–5 elimination half-lives. This is probably due to the fact that the low exposure amount (0.011 mg/kg body weight) is in the linear (first-order) range of CYP-based metabolism of diazinon (<$K_m$). With most environmental exposures quite low, it is likely that first-order chemical metabolism could be assumed. Venkataramanan et al. [43] have discussed this approach as the “model-independent in vitro half-life approach”, and have mentioned that, for substrate concentrations below 1 µM, numerous groups have calculated metabolic clearance in the liver as a linear process equal to $V_{max}/K_m$.

These model assumption results are consistent with a localized sensitivity analysis, conducted in acsIX for the chemical-specific model parameters of the diazinon (oral), chlorpyrifos (dermal) and butoxyethanol (inhalation) PBPK models. These sensitivity analysis results were obtained by varying individual parameter values and determining the impact on predicted maximal concentration of metabolite in urine. As shown in Table 2, the models were most sensitive to the elimination rate of the respective metabolites. Other rates (absorption, metabolism of parent compound) were found to have lower sensitivity coefficients, but still above 0.1 (or 10% of total parameter sensitivity).

4.3. QSRR for oil:water partition coefficient

The use of vegetable oil as a surrogate for tissue lipids has been recommended by researchers ever since early studies were conducted on anesthetic tissue distributions over 75 years ago [44]. The results of the current Koil:water analyses conducted with olive oil were found to be comparable to prior studies, with the measured log Koil:water value for p-ethylphenol of 1.69 quite similar to the previously reported value of 1.79 [23]. The new values for eight lipophilic compounds, derived with olive oil (Fig. 5), should also be comparable to the data from other studies employing vegetable or fish oils [19,23,25], as
Fig. 7 – Impact of model parameter assumptions on diazinon urinary DAP metabolite data following oral administration at 0.2, 0.5, 1.0, 2.0 and 5-fold the estimated or published PK values (actual values in green/thick line) across the time interval of 5 elimination half-lives (0–11 h): (A) absorption half-life; (B) elimination half-life; (C) $V_{\text{max}}$ Metabolic rate; (D) $K_{\text{m}}$ metabolic rate. Note: Stepped output represents incorporation of micturition intervals into model.

Table 2 – Sensitivity of the PBPK model parameters to prediction of maximal urine concentration of metabolite.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Diazinon oral</th>
<th>Chlorpyrifos dermal</th>
<th>Butoxyethanol inhalation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absorption</td>
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<tr>
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<tr>
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<td>0.1</td>
</tr>
<tr>
<td>$V_{\text{max}}$ for metabolite</td>
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<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>$V_{\text{max}}$ for parent compound</td>
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<tr>
<td>$K_{\text{m}}$ for metabolite</td>
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<td>0.0</td>
</tr>
<tr>
<td>$K_{\text{m}}$ for parent compound</td>
<td>0.2</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Partition coefficients for parent compound</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood:air</td>
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<td>0.0</td>
</tr>
<tr>
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<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>GI:blood</td>
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<tr>
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<td>0.0</td>
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<td>0.0</td>
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<tr>
<td>Muscle:blood</td>
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<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Richly perfused:blood</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Skin:blood</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Slowly perfused:blood</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Partition coefficients for metabolite</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood:air</td>
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<tr>
<td>Fat:blood</td>
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<tr>
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<tr>
<td>Muscle:blood</td>
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<tr>
<td>Richly perfused:blood</td>
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<tr>
<td>Skin:blood</td>
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<tr>
<td>Slowly perfused:blood</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>
concentrations.

Chiou [45] also conducted similar Koil-water derivations for a variety of lipophilic compounds. However, the synthetic triglyceride triolein (triglyceride of oleic acid) used as the oil in that study afforded log Koil:water values for several compounds substantially higher than those obtained with natural oils (hexachlorobenzene: 4.39 in new data vs. 5.5 in Chiou; pp-DDT: 2.4 in Kanazawa vs. 5.9 in Chiou). These differences may be due to triolein’s lack of lower molecular weight, unsaturated and phospholipids that can be present in vegetable or fish oils. As a result, the data of Chiou were not included in the new Koil:water regression analysis.

As shown in Fig. 5, the majority of the compounds evaluated by Leo and Hansch show a fairly linear correlation between log Koct:water and log Koil:water. However, the lipophilicity for these compounds ranged from log Koct:water of ~−2 to 4. Since there is a great deal of research ongoing to evaluate the PK fate for fairly lipophilic compounds (log Koct:water 3–8), a sigmoidal regression that included additional lipophilic test chemicals from Kanazawa, Poulin and the current data was derived. This sigmoidal regression gave a good fit to the data, where substantial nonlinearity in Koil:water values were observed above log Koct:water values of 5. A similar nonlinear trend was observed by Connell [46] in the data of Chiou [45], which Connell attributed to differential solubilities of compounds due to physical properties of the octanol or trioiln solvents (i.e.: solvent size). The overall correlation for the new regression model, based on the data presented in Fig. 5, was \( r^2 = 0.90 \), with an average error of 0.38 between predicted and actual log Koil:water values. Predicted log Koil:water values, obtained from this QSAR equation, are used for calculations of Vd, individual tissue:plasma Ptp as well as Pba values in the PK and PBPK models of this project.

5. Conclusions

A user-friendly set of computer-based modeling programs have been developed to aid in the estimation of chemical exposure levels from biomonitoring datasets. The Excel-based PK model can be used to solve for single- or steady-state exposures. The simple interface of this model, and the requirement for minimal chemical-specific parameters, provides the user a convenient tool for exposure calculations. The PBPK model that has been developed also utilized an Excel program interface interacting with acslX coded models. This physiologically-based model is similar to other generic PBPK models [47-49], but includes QSPrs for Kp, Ptp, and incorporates micturition intervals and repeat model runs with reverse dosimetry calculations of exposure levels. Both models were verified using published data for two pesticides and one industrial chemical. Additional comparisons to datasets from other compounds would extend the validation of these models to broader chemical domains.

These models should be applicable for estimation of chemical exposure levels, from blood or urine biomarker concentrations. These programs will also provide a good platform for training, to understand the impact of various chemical-specific or physiological parameters on chemical biomarker levels in humans.

6. Program access

The PK and PBPK biomonitoring programs described in this manuscript are available as supplemental material online (LINK). The PK model consists of a Microsoft Excel 2003 or higher-compatible workbook file. The PBPK model also consists of an Excel 2007 or higher-compatible workbook file, with the associated acslX generic PBPK model, in a compiled .dll file format. A detailed User’s Manual for these programs is also included as supplemental material.

Conflict of interest

The authors at The Dow Chemical acknowledge that two of the chemicals used to validate the Biomonitoring PK and PBPK models (chlorpyrifos and butoxyethanol) are produced by this company.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cmpb.2012.04.014.

References


