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IN VITRO TO IN VIVO EXTRAPOLATION OF BIOTRANSFORMATION RATES FOR ASSESSING BIOACCUMULATION OF HYDROPHOBIC ORGANIC CHEMICALS IN MAMMALS

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Abstract: Incorporating biotransformation in bioaccumulation assessments of hydrophobic chemicals in both aquatic and terrestrial organisms in a simple, rapid, and cost-effective manner is urgently needed to improve bioaccumulation assessments of potentially bioaccumulative substances. One approach to estimate whole-animal biotransformation rate constants is to combine in vitro measurements of hepatic biotransformation kinetics with in vitro to in vivo extrapolation (IVIVE) and bioaccumulation modeling. An established IVIVE modeling approach exists for pharmaceuticals (referred to in the present study as IVIVE-Ph) and has recently been adapted for chemical bioaccumulation assessments in fish. The present study proposes and tests an alternative IVIVE-B technique to support bioaccumulation assessment of hydrophobic chemicals with a log octanol–water partition coefficient (K_{OW}) ≥ 4 in mammals. The IVIVE-B approach requires fewer physiological and physiochemical parameters than the IVIVE-Ph approach and does not involve interconversions between clearance and rate constants in the extrapolation. Using in vitro depletion rates, the results show that the IVIVE-B and IVIVE-Ph models yield similar estimates of rat whole-organism biotransformation rate constants for hypothetical chemicals with log $K_{OW} \geq 4$. The IVIVE-B approach generated in vivo biotransformation rate constants and biomagnification factors (BMFs) for benzo[*a*]pyrene that are within the range of empirical observations. The proposed IVIVE-B technique may be a useful tool for assessing BMFs of hydrophobic organic chemicals in mammals. *Environ Toxicol Chem* 2017;36:1934–1946. © 2016 SETAC

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INTRODUCTION

International and national regulations controlling hazardous chemicals—including the United Nations Environment Programme Stockholm Convention on Persistent Organic Pollutants, the European Union Registration Evaluation Authorisation and Restriction of Chemicals, the Canadian Environmental Protection Act, and the United States Toxic Substances Control Act—specify criteria for categorizing the bioaccumulative behavior of commercial chemicals based on the chemical's bioconcentration factor (BCF), bioaccumulation factor (BAF), and logarithm of octanol–water partition coefficient ($\log K_{OW}$) [1]. However, these regulatory criteria for identifying potentially bioaccumulative substances are subject to significant limitations. First, the criteria developed for assessing bioaccumulation in aquatic (i.e., water-breathing) organisms are not always applicable to air-breathing organisms including humans [2,3]. Kitano [4] showed that 5 of 21 persistent organic pollutants recognized in the Stockholm Convention at that time do not meet BCF- and K_{OW}-based bioaccumulation criteria but are highly bioaccumulative in nonaquatic organisms. The failure of the BCF and K_{OW} to identify chemicals that have the potential to biomagnify in mammals and other air-breathing species is attributable to the fact that the BCF in fish has limited relevance to bioaccumulation in air-breathing organisms [2]. Therefore, there is a need to develop methods for assessing bioaccumulation of chemicals in air-breathing organisms, particularly mammalian wildlife and humans. Second, empirical BCF and BAF values that can be used in a bioaccumulation assessment do not exist for the great majority of commercial chemicals [1]. This has resulted in a reliance on the application of the K_{OW} and bioaccumulation models for bioaccumulation assessment. However, K_{OW} is an inherent property of the chemical and provides no direct information on the potential for biotransformation of that chemical. Bioaccumulation models that include quantitative structure–activity relationships for the estimation of whole-organism biotransformation rates have been developed for fish [5,6], but this approach has not yet been developed for mammalian species other than humans. Hence there is a need to develop methods for bioaccumulation assessment that include biotransformation. Accounting for biotransformation in bioaccumulation assessment is of particular importance for very hydrophobic ($\log K_{OW} \geq 5$) and poorly volatile (logarithm of octanol–air partition coefficient [$\log K_{OA}$] ≥ 5) chemicals because for such chemicals even low rates of biotransformation can dominate the overall depuration rate of the chemical. Third, for air-breathing organisms, the biomagnification factor (BMF) may be a more useful metric than the BCF because of the importance of dietary intake as a source of contaminants in the environment and the occurrence of biomagnification or trophic dilution in food webs [7]. Hence there is a need to develop models that can estimate BMFs of chemicals in mammals and other species.

Bioaccumulation models for mammals and other air-breathing organisms have been developed for the purpose of bioaccumulation assessment [8–10]. However, these models do not contain algorithms for estimating chemical biotransformation rates a priori. One approach that has been proposed to improve bioaccumulation assessment is the application of in vitro biotransformation assays followed by in vitro to in vivo extrapolation (IVIVE) [11]. This in vitro method is used widely

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in the pharmaceutical sciences to predict hepatic and total-body clearance of drugs for clinical applications [11–14] and is an alternative to in vivo testing that reduces costs, increases throughput, and reduces animal use [15–17]. In this technique, in vitro depletion rates of test chemicals are measured using isolated perfused fish livers [18,19] or fish liver preparations including S9 [20–24], microsomes [21,22], freshly isolated hepatocytes [20,21,25], and cryopreserved hepatocytes [26]. The measured in vitro biotransformation rate constant for a test chemical is converted into an in vitro intrinsic clearance rate and then extrapolated to obtain a hepatic intrinsic clearance rate. Subsequently, a well-stirred liver model is used to calculate the chemical's hepatic clearance by accounting for 3 biological factors: hepatic blood flow, enzyme activity, and nonspecific chemical binding in the blood [27]. This approach will be referred to in the present study as the in vitro to in vivo extrapolation–pharmaceuticals (IVIVE-Ph) approach and aims to assess the clearance of administered pharmaceuticals from blood. Nichols et al. [16,17] have adapted this approach for bioaccumulation assessment in fish and showed that model-calculated BCFs using this in vitro approach are closer to BCFs measured in whole fish than those that do not consider biotransformation [20,21,24–26].

The IVIVE-Ph approach uses the well-stirred liver model [27], involves interconversion between clearance and rate constants in the extrapolation process, and requires an estimate of the volume of distribution of chemicals to obtain the whole-organism biotransformation rate constants for bioaccumulation modeling. The clearance concept is useful for clinical applications to relate the administered dose to therapeutic concentrations of a drug in plasma, and the well-stirred liver model is useful for predicting the effects of alterations in hepatic blood flow and enzyme activity on the drug concentration-time profile caused by disease or drug interactions [27] but is not required for bioaccumulation assessments that aim to assess whole-organism concentrations. Also, the IVIVE-Ph approach requires information about hepatic blood flow, fraction of unbound chemical in the blood, and blood–water partition coefficients. These data are difficult to obtain for mammalian wildlife species and may not be needed to assess the bioaccumulation behavior of chemicals.

In the present study, we propose an alternative IVIVE application that is based on the extrapolation of in vitro biotransformation rate constants for hydrophobic organic chemicals ($\log K_{OW} \geq 4$) for the purpose of bioaccumulation assessment of chemicals. We will refer to this method as the IVIVE-B approach. This approach does not use the well-stirred liver model for reasons described below and there is no interconversion between rate constants and clearance rates in the extrapolations. Hepatic blood flow, blood composition, fraction of unbound chemicals in the blood, and blood–water partition coefficients are not required in the proposed IVIVE-B approach. The reason for developing and investigating this approach is to provide a practical bioaccumulation assessment methodology that can be applied to many species including water- and air-breathing organisms. By reducing the need for difficult-to-obtain data, bioaccumulation assessment may be extended to include a wider array of species than fish.

The objectives of the present study are 1) to develop an IVIVE-B approach for assessing whole-organism biotransformation rate constants for hydrophobic chemicals that have bioaccumulation potential, 2) to evaluate the proposed IVIVE-B model by comparing model-predicted, whole-organism biotransformation rates with those obtained from the IVIVE-Ph method, 3) to develop and test a mechanistic bioaccumulation model that accounts for biotransformation by using in vitro bioassay data to estimate BMFs, and 4) to demonstrate the application of the proposed IVIVE-B modeling approach as a tool for bioaccumulation assessments of hydrophobic chemicals. Rats were chosen to explore the behavior of the model because they are widely used in laboratory experiments and their physiological parameters are well established. The IVIVE-B approach is developed for the extrapolation of in vitro biotransformation rate data derived using liver S9 subcellular fractions. The liver S9 fraction was chosen because its preparation is simple and relatively quick compared with other commonly used in vitro systems such as liver microsomes or hepatocytes. Also, liver S9 fractions contain both microsomal and cytosolic enzymes, providing a more complete enzymatic profile than liver microsomes, and hence may be more useful for bioaccumulation screening assessments of large numbers of chemicals with unknown biotransformation pathways. Benzo-[*a*]pyrene and chrysene were chosen as model chemicals because both are hydrophobic substances with bioaccumulation potential ($\log K_{OW} \geq 5$) and biotransformation potential; their in vitro biotransformation rate constants and unbound fractions in the rat liver S9 incubation mixture have been measured previously [28], and in vivo data are available for model testing. The ultimate goal of our study is to improve bioaccumulation assessments for hydrophobic organic chemicals.

THEORY

The IVIVE-B approach for bioaccumulative substances in mammals

The framework of the proposed IVIVE-B approach for potentially bioaccumulative substances in mammals is illustrated in Figure 1. The major steps of the IVIVE-B approach are described in the following sections.

*Step 1: Experimental measurement of the apparent in vitro biotransformation rate constant (*kr*) using the substrate depletion method.* Rates of substrate depletion are measured instead of metabolite formation rates because the metabolites of most commercial chemicals are unknown and can be difficult to measure. The in vitro biotransformation rate constant can be measured in S9 liver preparations using a thin-film, sorbent-phase dosing procedure that delivers the test chemical from a sorbent phase to the incubation medium [28–30] or by introducing the test chemical to the incubation medium in a spiking solvent. In the latter method, the k_r (h^{-1}) can exhibit strong dependence on the initial substrate concentrations [30,31], that is

$$k_{r,C\rightarrow 0} = k_r / \left(1 - \frac{C_I}{C_I + K_M}\right) \quad (1)$$

where $k_{r,C\rightarrow 0}$ (h^{-1}) is the maximum in vitro biotransformation rate constant at infinitesimally low substrate concentration, C_I is the initial concentration of the test chemical (substrate) in the incubation medium (μM); and K_M is the pseudo Michaelis-Menten constant (μM) for substrate depletion assays [31,32]. If C_I is well below a known K_M or if it is acceptable to assume that C_I is well below K_M, then $k_{r,C\rightarrow 0}$ can be approximated by k_r. If K_M is unknown, it can be measured by conducting solvent delivery-based depletion experiments using a range of initial substrate concentrations [30,31]. If k_r is measured using the thin-film, sorbent-phase dosing approach, it may be possible to approximate $k_{r,C\rightarrow 0}$ from a

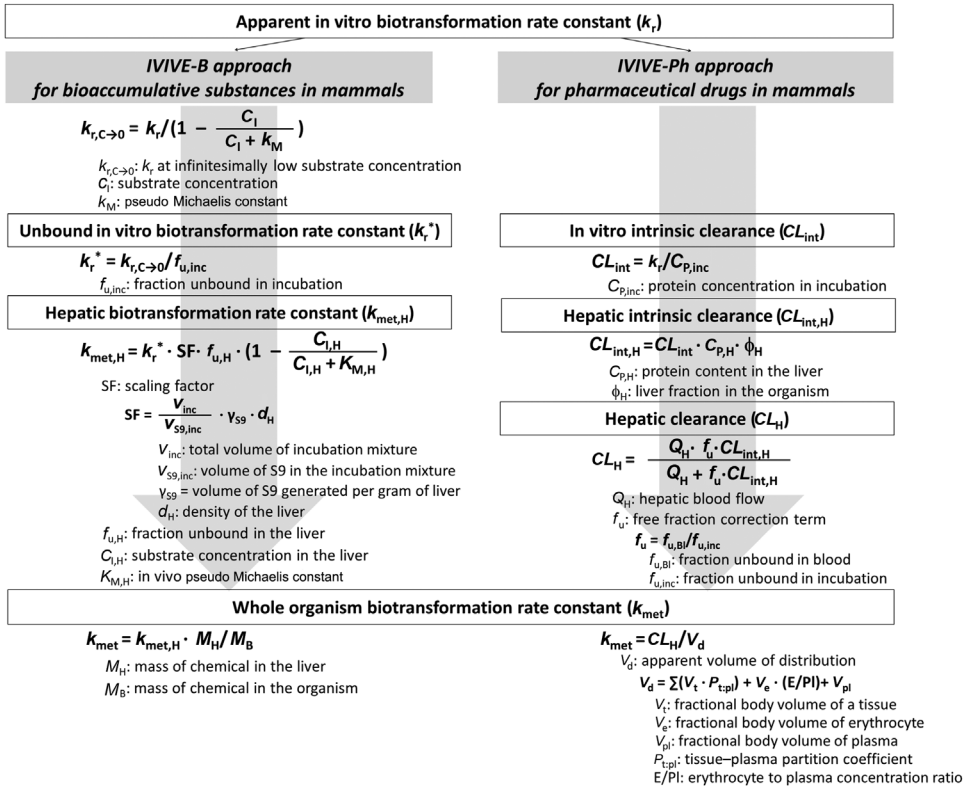

Figure 1. Framework of the IVIVE-B and IVIVE-Ph models for predicting the biotransformation rate constants of chemicals in mammals. IVIVE-B = in vitro to in vivo extrapolation–bioaccumulation; IVIVE-Ph = in vitro to in vivo extrapolation–pharmaceuticals.

single substrate concentration experiment because the substrate concentration in the incubation medium is initially 0 and may remain below K_M throughout the incubation period because of the slow release of the hydrophobic chemical from the sorbent phase to the incubation medium [30]. However, K_M cannot be verified within the constraints of a single substrate concentration experiment.

The in vitro biotransformation rate constant $k_{r,C\to0}$ can then be normalized to the fraction of substrate that is freely dissolved in the incubation medium, as shown in Equation 2

$$k_r^* = \frac{k_{r,C\to0}}{f_{u,inc}} \quad (2)$$

where k_r^* (h^{-1}) is the in vitro biotransformation rate constant of the unbound chemical in the incubation medium. The value of $f_{u,inc}$ can be measured in the depletion experiment [23,28], estimated from empirical relationships [17,22,33], or estimated by assuming the partitioning of the chemical in the incubation medium among 3 phases (i.e., lipids, proteins, and water)

$$f_{u,inc} = \frac{f_{W,inc}}{f_{L,inc} \times K_{LW} + f_{P,inc} \times K_{PW} + f_{W,inc}} \quad (3)$$

where $f_{L,inc}$, $f_{P,inc}$, and $f_{W,inc}$ are the fractions of lipid, protein, and water of the incubation medium (v/v; unitless), respectively; and K_{LW} and K_{PW} are lipid–water and protein–water partition coefficients, respectively. Lipid–water and protein–water partition coefficients (v/v; unitless) can be obtained from several sources, including empirical observations, surrogate partition coefficients (e.g., K_{OW}), and polyparameter linear free energy relationships [34]. For non-ionic hydrophobic organic chemicals with a log K_{OW} between approximately 1 and 6, K_{OW} is often a reasonable surrogate for K_{LW} (i.e., $K_{LW} \approx K_{OW}$) in both storage and membrane lipids [35]; and K_{PW} can be approximated as $0.05 \times K_{OW}$ [36].

*Step 2: Calculation of the hepatic biotransformation rate constant (*k*met,H) from the unbound in vitro biotransformation rate constant (k_r*).* This calculation is based on the simplifying assumptions that: 1) $k_{met,H}$ for bioaccumulative substances in mammals is determined by liver enzyme function and the fraction of unbound chemical in the liver, and is not significantly influenced by the hepatic blood flow, and 2) $k_{met,H}$ exhibits a substrate concentration-dependent relationship that can be described by Michaelis-Menten kinetics. The rationale for not considering hepatic blood flow is 2-fold. First, hydrophobic chemicals are often slowly metabolized, and their hepatic $k_{met,H}$ values are not limited by delivery of the chemical to the liver via the blood. In pharmaceutical terminology, such chemicals have low hepatic extraction ratios and their removal by the liver is dependent on enzyme activity and is not affected significantly by liver blood flow [27]. Second, in mammals, dietary uptake is the major route of exposure to hydrophobic organic chemicals of limited volatility. After oral exposure, chemicals first enter the liver from the gastrointestinal tract via the hepatic portal vein, and the extraction of unbound chemical in the liver depends on enzyme activity, not on blood flow to the liver [27,37]. The $k_{met,H}$ value (h^{-1}) is calculated as

$$k_{met,H} = k_r^* \times SF \times f_{u,H} \times \left(1 - \frac{C_{I,H}}{C_{I,H} + K_{M,H}}\right) \quad (4)$$

where SF is a scaling factor (unitless) that accounts for the dilution of enzymes that occurs during the preparation of the

liver fraction; $f_{u,H}$ is the unbound fraction (unitless) of the chemical in the liver; $C_{I,H}$ is the substrate concentration in the liver (μM); and $K_{M,H}$ is the in vivo hepatic pseudo Michaelis-Menten constant (μM) for substrate depletion measurements.

For liver S9 preparations, the scaling factor can be obtained through a series of volume ratios associated with the stepwise process of the preparations of liver S9 and expressed as

$$SF = \frac{V_{inc}}{V_{S9,inc}} \times \frac{V_{S9}}{V_H} = \frac{V_{inc}}{V_{S9,inc}} \times \frac{V_{S9}}{W_H} \times d_H = \frac{V_{inc}}{V_{S9,inc}} \times \gamma_{S9} \times d_H \quad (5)$$

where V_{inc} and $V_{S9,inc}$ are the total volume of incubation mixture (mL) and the volume of S9 (mL) used in the in vitro experiments, respectively; V_{S9} is the volume of liver S9 fraction (mL) collected after spinning the liver homogenate at $9000 \times g$; V_H is the volume of liver (mL) used for preparing the liver S9 fraction, and it can be obtained from the wet weight of the liver (W_H; g) and the density of the liver (d_H; g/mL); and γ_{S9} is the yield of S9 fraction generated per gram of liver (i.e., V_{S9}/W_H; mL/g liver).

The unbound fraction of the chemical in the liver ($f_{u,H}$) in Equation 4 can be estimated by assuming that the liver consists of 3 phases (i.e., lipids, proteins, and water) and that the chemical partitions as follows

$$f_{u,H} = \frac{f_{W,H}}{f_{L,H} \times K_{LW} + f_{P,H} \times K_{PW} + f_{W,H}} \quad (6)$$

where $f_{L,H}$, $f_{P,H}$, and $f_{W,H}$ are the fractions of lipid, protein, and water of the liver (v/v; unitless), respectively.

The concentration dependence of the in vivo hepatic biotransformation rate constant in Equation 4 is expressed by $K_{M,H}$, which is generally unknown and may be difficult to measure. However, in cases where exposure concentrations are low, it may be reasonable to assume that $K_{M,H}$ is approximately equal to the K_M measured in the in vitro assay. If the chemical concentration in the liver ($C_{I,H}$) is well below $K_{M,H}$, the last part in Equation 4 (i.e., $1–[C_{I,H}/(C_{I,H}+K_{M,H})]$) approximates 1. Thus, the in vivo hepatic biotransformation rate constant $k_{met,H}$ is substrate concentration independent. If $C_{I,H}$ appears to approach $K_{M,H}$, $k_{met,H}$ is substrate concentration dependent according to Equation 4.

Step 3: Derivation of the whole-organism kmet *from the* kmet,H. This calculation is based on the assumptions that the liver is the major organ of xenobiotic biotransformation, and chemical partitioning within the organism is fast and maintains a near-equilibrium between the chemical in the liver and in the rest of the organism. The whole-organism k_{met} (d^{-1}) can be calculated as

$$k_{met} = 24 \times k_{met,H} \times \frac{M_H}{M_B} \quad (7)$$

where M_H and M_B are the mass (g) of the chemical in the liver and in the whole organism (including the liver), respectively. A factor of 24 converts the unit of k_{met} from h^{-1} to d^{-1}. The ratio of M_H/M_B represents the fraction of the total chemical mass in the organism (M_B) that is in the liver (M_H). For many non-ionic hydrophobic substances, it can be estimated by assuming that the liver and the organism consist of 3 phases—lipids, proteins, and water—and that the chemical partitions, according to Equation 8

$$\frac{M_H}{M_B} = \phi_H \times \frac{f_{L,H} \times K_{LW} + f_{P,H} \times K_{PW} + f_{W,H}}{f_{L,B} \times K_{LW} + f_{P,B} \times K_{PW} + f_{W,B}} \quad (8)$$

where ϕ_H is the volumetric fraction of the liver in the organism (v/v; unitless), that is, V_H/V_B, where V_B is the volume of the organism (mL); and $f_{L,B}$, $f_{P,B}$, and $f_{W,B}$ are the fractions of lipid, protein, and water of the organism (v/v; unitless), respectively. It is interesting to note that in the extrapolation to k_{met} from in vitro data (Equations 1–8), the term $f_{L,H} \times K_{LW} + f_{P,H} \times K_{PW} + f_{W,H}$ that appears in Equations 6 and 8 tends to cancel out. This is because in the model, an increase in the lipid or protein content both reduces the fraction of unbound chemical available for biotransformation in the liver and increases the chemical mass present in the liver. This implies that for the estimation of the whole-organism biotransformation rate, the model is insensitive to the actual liver lipid and protein composition. The derivations of Equations 3, 6, and 8 are given in the Supplemental Data.

The IVIVE-Ph approach for pharmaceutical drugs in mammals

An IVIVE approach to estimate in vivo hepatic clearance of pharmaceuticals in mammals was recently applied to fish, and estimates of whole-fish biotransformation rate constants were incorporated into aquatic bioaccumulation assessments [16,17,20,22–26]. In the present study, we refer to this process as the IVIVE-Ph approach and discuss its application to mammals with the purpose of comparing it with the proposed IVIVE-B approach.

The major steps in the IVIVE-Ph method in mammals are illustrated in Figure 1 and summarized in the following sections.

Step 1. The k_r (h^{-1}) measured by substrate depletion in liver S9 is normalized to total S9 protein concentration in the incubation medium ($C_{P,inc}$; mg S9 protein/mL) to obtain the in vitro intrinsic clearance (CL_{int}; $mL\,h^{-1}\,mg$ S9 $protein^{-1}$), according to Equation 9

$$CL_{int} = \frac{k_r}{C_{P,inc}} \quad (9)$$

Step 2. The CL_{int} is scaled up to the whole liver to obtain the hepatic intrinsic clearance ($CL_{int,H}$; $mL\,h^{-1}\,g\ organism^{-1}$)

$$CL_{int,H} = CL_{int} \times C_{P,H} \times \phi_H \quad (10)$$

where $C_{P,H}$ is the protein content of the liver (mg S9 protein/g liver) and ϕ_H is the wet weight fraction of the liver in the organism (g liver/g organism).

The well-stirred liver model [27] is then used to calculate hepatic clearance (CL_H; $mL\,h^{-1}\,g\ organism^{-1}$), considering hepatic intrinsic clearance, hepatic blood flow, and nonspecific chemical binding, according to Equation 11

$$CL_H = \frac{Q_H \times f_u \times CL_{int,H}}{Q_H + f_u \times CL_{int,H}} \quad (11)$$

where Q_H is the hepatic blood flow ($mL\ blood\ h^{-1}\,g\ organism^{-1}$) obtained from the product of cardiac output ($mL\ blood\ h^{-1}\,g\ organism^{-1}$) and the fraction of blood flow that goes through the liver (unitless), and f_u is the free fraction correction term defined as

$$f_u = \frac{f_{u,Bl}}{f_{u,inc}} \quad (12)$$

where $f_{u,Bl}$ and $f_{u,inc}$ are the unbound fractions (unitless) of the chemical in the blood and in the incubation medium, respectively. Empirical equations for calculating $f_{u,Bl}$ have been reported previously using blood-binding data from mammals and fish (for neutral chemicals with log K_{OW} ranging from –0.78 to 6.19) [20,25], and specifically from rainbow trout (for neutral chemicals with log K_{OW} ranging from 1.5 to 8.2) [17]. This approach may be applicable to the IVIVE of potentially bioaccumulative substances in mammals. An alternative method for estimating $f_{u,Bl}$ is to consider the blood as consisting of 3 phases (i.e., lipids, proteins, and water) and assuming that the chemical partitions among these phases

$$f_{u,Bl} = \frac{f_{W,Bl}}{f_{L,Bl} \times K_{LW} + f_{P,Bl} \times K_{PW} + f_{W,Bl}} \quad (13)$$

where $f_{L,Bl}$, $f_{P,Bl}$, and $f_{W,Bl}$ are the fractions of lipid, protein, and water of the blood (v/v; unitless), respectively; K_{LW} is the lipid–water partition coefficient, which for non-ionic organic chemicals within a log K_{OW} range of approximately 1 to 6 can be approximated by K_{OW} [35]; and K_{PW} is the protein–water partition coefficient, which for some non-ionic organic compounds can be estimated as $0.05 \times K_{OW}$ [36]. The $f_{u,inc}$ can be measured experimentally [23,28], or estimated from empirical relationships [17,22], or from Equation 3.

Step 3. The whole-organism k_{met} (d^{-1}) is calculated by dividing the hepatic clearance (CL_H; mL h^{-1} g $organism^{-1}$) by the apparent volume of distribution of the chemical (V_d; mL/g organism)

$$k_{met} = \frac{24 \times CL_H}{V_d} \quad (14)$$

where a factor of 24 is obtained by converting the unit of k_{met} from h^{-1} to d^{-1}. In pharmacology, V_d is defined as the theoretical volume that the administered drug dose would have to occupy (if it were uniformly distributed) to provide the same concentration as that in blood plasma, or alternatively as the ratio of the total amount of drug in the organism and the drug plasma concentration [38]. In previous studies where this approach was applied to fish [16,17,20,22–26], V_d was viewed as the sorptive capacity of the fish relative to that of the blood and calculated as the ratio of a partitioning-based estimate of the BCF (in the absence of biotransformation or organism growth) and the blood–water partition coefficient [16]. The partitioning-based estimate of the BCF may not be meaningful and practical for mammals. Alternatively, the volume of distribution of chemicals in mammals can be calculated using a mechanism-based procedure that considers lipophilicity and plasma-protein binding as 2 main determinants of V_d, as proposed by Poulin and Theil [39]. The volume of distribution in mammals at steady-state is calculated as

$$V_d = \Sigma\left(V_t \times P_{t:pl}\right) + V_e \times (E/Pl) + V_{pl} \quad (15)$$

where V_t, V_e, and V_{pl} are the fractional body volume (mL g $organism^{-1}$) of a tissue, erythrocyte, and plasma, respectively; E/Pl is the erythrocyte to plasma concentration ratio (unitless), which is set equal to 1 for chemicals that distribute homogeneously into tissues [39]; and $P_{t:pl}$ is the tissue–plasma partition coefficient (unitless), including the non-adipose tissue–plasma partition coefficient ($P_{t:pl\_non\text{-}adipose}$) and the adipose tissue–plasma partition coefficient ($P_{t:pl\_adipose}$). The non-adipose tissues include the bone (plus marrow), brain, gut, heart, kidney, liver, lung, skeletal muscle, skin, and spleen; and the adipose tissue refers to subcutaneous white fat. The tissue–plasma partition coefficient for a non-adipose tissue ($P_{t:pl\_non\text{-}adipose}$; unitless) is calculated as

$$P_{t:pl\_non-adipose} = \frac{K_{OW} \times \left(f_{NL,t} + 0.3 \times f_{PhL,t}\right) + 1 \times \left(f_{W,t} + 0.7 \times f_{PhL,t}\right)}{K_{OW} \times \left(f_{NL,pl} + 0.3 \times f_{PhL,pl}\right) + 1 \times \left(f_{W,pl} + 0.7 \times f_{PhL,pl}\right)} \times \frac{f_{u,pl}}{f_{u,t}} \quad (16)$$

where $f_{NL,t}$, $f_{PhL,t}$, and $f_{W,t}$ are the fractions of neutral lipids, phospholipids, and water of the tissue (v/v; unitless), respectively; $f_{NL,pl}$, $f_{PhL,pl}$, and $f_{W,pl}$ are the fractions of neutral lipids, phospholipids, and water of the plasma (v/v; unitless), respectively; and $f_{u,pl}$ and $f_{u,t}$ are the unbound fractions (unitless) in the plasma and tissue, respectively. The value of $f_{u,pl}$ can be estimated by assuming that the chemicals partitions in the plasma, consisting of 3 phases (i.e., lipids, proteins, and water)

$$f_{u,pl} = \frac{f_{W,pl}}{f_{L,pl} \times K_{LW} + f_{P,pl} \times K_{PW} + f_{W,pl}} \quad (17)$$

where $f_{L,pl}$, $f_{P,pl}$, and $f_{W,pl}$ are the fractions of lipids (including neutral lipids and phospholipids), proteins, and water of the plasma (v/v; unitless), respectively. The value of $f_{u,t}$ can be estimated from $f_{u,pl}$ based on an empirical equation for mammals [39]

$$f_{u,t} = \frac{1}{1 + \left[\left(1 - f_{u,pl}\right)/f_{u,pl}\right] \times 0.5} \quad (18)$$

The tissue–plasma partition coefficient for the adipose tissue ($P_{t:pl\_adipose}$; unitless) is calculated as

$$P_{t:pl\_adipose} = \frac{K_{VO:W} \times \left(f_{NL,t} + 0.3 \times f_{PhL,t}\right) + 1 \times \left(f_{W,t} + 0.7 \times f_{PhL,t}\right)}{K_{VO:W} \times \left(f_{NL,pl} + 0.3 \times f_{PhL,pl}\right) + 1 \times \left(f_{W,pl} + 0.7 \times f_{PhL,pl}\right)} \times \frac{f_{u,pl}}{1} \quad (19)$$

where $K_{VO:W}$ is the olive oil–water partition coefficient (unitless). It was reported that olive oil is a better surrogate of the adipose tissue lipids than octanol [40]. The logarithm of $K_{VO:W}$ can be calculated based on an empirical equation for neutral compounds [39]

$$\log K_{VO:W} = 1.115 \times \log K_{OW} - 1.35 \quad (20)$$

The derivations of Equations 13 and 17 are given in the Supplemental Data.

Bioaccumulation model

For the purpose of bioaccumulation assessment, a whole-organism toxicokinetic bioaccumulation model that describes

the major uptake and elimination pathways of chemicals in the organism can be used to obtain the BMF. The major uptake processes of chemicals in mammals include respiratory and dietary uptake; and the major elimination processes include respiratory elimination, fecal elimination, urinary elimination, biliary elimination, biotransformation, lactation, and growth dilution. The organism is described as a single compartment in which the chemical is distributed homogeneously based on the assumption of rapid internal partitioning of the chemical. The change of chemical concentrations in the organism over time is described as

$$\frac{dC_B}{dt} = k_{AU} \times C_A + k_D \times C_D - (k_{AE} + k_F + k_U + k_{Bi} + k_{met} + k_L + k_G) \times C_B \quad (21)$$

where C_B, C_A, and C_D are the concentrations (mol/m^3) of the chemical in the organism, air, and diet, respectively; k_{AU} and k_D are the rate constants (d^{-1}) describing the first-order kinetics of chemical uptake from air and diet, respectively; and k_{AE}, k_F, k_U, k_{Bi}, k_{met}, k_L, and k_G are the rate constants (d^{-1}) describing the first-order kinetics of chemical elimination via respiratory elimination, fecal elimination, urinary elimination, biliary elimination, biotransformation, lactation, and growth dilution, respectively. The k_{met} can be obtained from the IVIVE-B or IVIVE-Ph approach. The equations for deriving k_{AU}, k_D, k_{AE}, k_F, k_U, k_{Bi}, and k_L are described in the Supplemental Data. This model can be used to derive an expression for the steady-state BMF if dietary uptake is the major route of exposure and chemical uptake from air is negligible.

$$\mathrm{BMF} = \frac{C_B}{C_D} = \frac{k_D}{(k_{AE} + k_F + k_U + k_{Bi} + k_{met} + k_L + k_G)} \quad (22)$$

This model can often be further simplified. For example, when applied to adult male mammals, lactation does not need to be considered and growth dilution may be negligible (i.e., $k_L = 0$ and $k_G = 0$). The lipid-normalized biomagnification (BMF_L; kg lipid/kg lipid) can be obtained by multiplying the unitless steady-state BMF (Equation 22) by a factor of $(d_D \times f_{L,D})/(d_B \times f_{L,B})$, where d_D and d_B are the densities of diet and organism, respectively; and $f_{L,D}$ and $f_{L,B}$ are the lipid fractions (w/w; unitless) of diet and organism, respectively.

METHODS

Model evaluation

The proposed IVIVE-B model for potentially bioaccumulative substances was evaluated using 3 approaches. First, we compared the model-calculated k_{met} values in rats for hypothetical chemicals ($\log K_{OW}$ ranging from 0 to 10) with those calculated by the IVIVE-Ph model. The input in vitro biotransformation rate constants were set at 0.1 h^{-1}, 0.2 h^{-1}, 0.5 h^{-1}, 1 h^{-1}, 2 h^{-1}, 5 h^{-1}, and 10 h^{-1} (corresponding to in vitro half-lives of 6.9 h, 3.5 h, 1.4 h, 42 min, 21 min, 8.3 min, and 4.2 min, respectively), considering typical experimental conditions of in vitro experiments. The $f_{u,inc}$ value was calculated according to Equation 3.

To examine the assumption of the perfusion-independent hepatic biotransformation rate in the proposed IVIVE-B model for potentially bioaccumulative substances in mammals, the biotransformation rate constant in the IVIVE-B model was compared with that obtained from the IVIVE-Ph model. The well-stirred liver model (Equation 11) was used to independently assess the relative contribution of hepatic blood flow and unbound hepatic intrinsic clearance to hepatic clearance for hypothetical chemicals ($\log K_{OW}$ ranging from 0 to 10) with in vitro biotransformation rate constants set at 0.1 h^{-1}, 0.2 h^{-1}, 0.5 h^{-1}, 1 h^{-1}, 2 h^{-1}, 5 h^{-1}, and 10 h^{-1}. The well-stirred liver model (Equation 11) was rearranged in an additive format as

$$\frac{1}{CL_H} = \frac{1}{Q_H} + \frac{1}{f_u \times CL_{int,H}} \quad (23)$$

The percentage contribution of hepatic blood flow to hepatic clearance ($CL_{H-Q\%}$; %) can then be calculated as

$$CL_{H-Q\%} = \left(\frac{1}{Q_H}\right) / \left(\frac{1}{CL_H}\right) \times 100\% \quad (24)$$

Similarly, the percentage contribution of unbound hepatic intrinsic clearance to hepatic clearance ($CL_{H-int\%}$; %) can be calculated as

$$CL_{H-int\%} = \left(\frac{1}{f_u \times CL_{int,H}}\right) / \left(\frac{1}{CL_H}\right) \times 100\% \quad (25)$$

Second, we evaluated the proposed IVIVE-B model by comparing the IVIVE-B model-calculated, whole-body k_{met} for benzo[*a*]pyrene and chrysene in rats with those calculated using the IVIVE-Ph model. Actual in vitro measurements of the depletion rate constant and unbound fraction in incubation mixture ($f_{u,inc}$) [28] were used in both models. We also compared the BMF values for benzo[*a*]pyrene and chrysene in rats calculated by the rat bioaccumulation model using input k_{met} values obtained from the IVIVE-B model with those calculated using k_{met} from the IVIVE-Ph model.

Third, the hepatic and whole-body biotransformation rate constant for benzo[*a*]pyrene in rats calculated by the IVIVE-B model was compared with the measured depuration rate constants for benzo[*a*]pyrene in rats from in vivo and ex vivo (e.g., isolated perfused liver) studies reported in the literature. The whole-body biotransformation rate constant for benzo[*a*]pyrene in rats calculated from the IVIVE-B model was used as an input parameter in the rat bioaccumulation model to obtain the BMF. The calculated BMF for benzo[*a*]pyrene in rats was then compared with empirical BMF data obtained from the literature.

Model parameterization

The IVIVE-B and IVIVE-Ph models were parameterized for rats. The input parameters for hypothetical chemicals and the 2 model chemicals (benzo[*a*]pyrene and chrysene) in both IVIVE models are summarized in the Supplemental Data, Table S1. For hypothetical chemicals, the log K_{OW} value was set ranging from 0 to 10, and the unbound fraction in incubation mixture ($f_{u,inc}$) was calculated according to Equation 3. For benzo[*a*]pyrene and chrysene, the log K_{OW} values were obtained from Mackay et al. [41] and adjusted for temperature to 37 °C, according to Beyer et al. [42]. The in vitro biotransformation rate constants and unbound fractions in the incubation mixture ($f_{u,inc}$) were obtained from previous measurements that used a thin-film, sorbent-phase dosing approach with liver S9 from male Sprague-Dawley rats [28].

In the IVIVE-B model, the volume of liver S9 in the incubation mixture ($V_{S9,inc}$) and the total volume of the incubation mixture (V_{inc}) were obtained from the experimental

conditions of the sorbent-phase dosing experiments using rat liver S9 fractions [28]. The yield of liver S9 fraction (γ_{S9}) was measured during the preparation of rat liver S9 fractions. The density of the liver (d_H) was measured previously for male Sprague-Dawley rats [43]. The lipid and water fractions in the liver ($f_{L,H}$ and $f_{W,H}$) were reported previously [44]. The protein fraction in the liver ($f_{P,H}$) was calculated by assuming that the sum of all fractions equaled unity. The lipid and protein fractions in the rat ($f_{L,B}$ and $f_{P,B}$) were reported previously [45]. The water fraction in the rat ($f_{W,B}$) was calculated by assuming that the sum of all fractions equaled unity. To mimic bioaccumulation in environmental situations where exposure concentrations are often low, it was assumed that the substrate concentration in the liver ($C_{I,H}$) in Equation 4 is well below the in vivo hepatic pseudo Michaelis-Menten constant ($K_{M,H}$), so that the term $1–[C_{I,H}/(C_{I,H}+K_{M,H})]$ approximates 1.

In the IVIVE-Ph model, the S9 protein concentrations in the incubation mixture and in the liver were obtained from the sorbent-phase dosing experiments using rat liver S9 fractions [28]. The volumetric fraction of the liver in the organism (ϕ_H) was estimated as the measured wet weight fraction (i.e., g liver/g animal) because a mass-to-volume conversion can be ignored for tissues with densities approximating 1 g/mL [46]. The cardiac output and fraction of blood flow through the liver in the rat were obtained from reported values [46]. The lipid and water fractions in the blood ($f_{L,Bl}$ and $f_{W,Bl}$) were reported previously for the rat [44]. The protein fraction in the blood ($f_{P,Bl}$) was calculated by assuming that the sum of all fractions equaled unity. The fraction of unbound chemicals in the blood was calculated using Equation 13. The volume of distribution (V_d) of chemicals was calculated using a mechanism-based approach developed by Poulin and Theil [39] using Equations 15 through 20. The fractional body volume of tissues (V_t), erythrocyte (V_e), and plasma (V_{pl}); fractions of neutral lipids of tissues ($f_{NL,t}$) and plasma ($f_{NL,pl}$); fractions of phospholipids of tissues ($f_{PhL,t}$) and plasma ($f_{PhL,pl}$); fractions of water of tissues ($f_{W,t}$) and plasma ($f_{W,pl}$); and erythrocyte to plasma concentration ratio (E/Pl) were obtained from Poulin and Theil [39]. The only modification of the Poulin and Thiel method for estimating V_d involved maintaining the contribution of adipose tissue to the volume of distribution constant at a value of approximately 1 mL g organism^{-1}, for chemicals with a log $K_{OW} > 3$ instead of declining with increasing log K_{OW}. As detailed in the Supplemental Data, this modification is likely more realistic and has only a small effect on the estimation of k_{met} for chemicals with a log $K_{OW} > 3$ (Supplemental Data, Figure S1) because adipose tissue contributes only up to 24% of the whole organism's volume of distribution. This modification does not affect the IVIVE-B model, which does not require the estimation of V_d.

The input parameters for the rat bioaccumulation model are summarized in the Supplemental Data, Table S2. The log K_{OA} values for benzo[*a*]pyrene and chrysene were obtained from Mackay et al. [41] and adjusted for temperature to 37 °C according to Beyer et al. [42]. There are 2 sets of dietary absorption efficiency used in the present study. First, to investigate the role of hepatic biotransformation on the BMF for hypothetical chemicals, the dietary absorption efficiencies of hypothetical chemicals in rats were calculated using a K_{OW}-dependent relationship for nonmetabolizable reference chemicals of polychlorinated biphenyls reported by Armitage and Gobas [10]

$$\frac{1}{E_{D,N}} = 6.87 \times 10^{-9} K_{OW} + 1.12 \quad (26)$$

where $E_{D,N}$ is the dietary absorption efficiency (unitless), which does not consider intestinal biotransformation. Second, to compare model-calculated BMFs of benzo[*a*]pyrene and chrysene with empirical BMFs, we used empirically derived dietary absorption efficiencies ($E_{D,IM}$; unitless) obtained from measured fecal excretion rates of benzo[*a*]pyrene and chrysene in rat dietary exposure experiments [47]. The measured $E_{D,IM}$ values, which reflect any intestinal biotransformation that occurred, were used for deriving the dietary uptake rate constants (k_D; Supplemental Data, Equation S4). The $E_{D,N}$ values that does not consider intestinal biotransformation (obtained from Equation 26) were used for deriving the fecal elimination rate constants (k_F; Supplemental Data, Equation S7) for benzo[*a*]pyrene and chrysene.

The $f_{u,inc}$ for benzo[*a*]pyrene (i.e., 4.44×10^{-4}) was obtained from sorbent-phase dosing experiments [28] and was almost identical to the value calculated from Equation 3 (4.63×10^{-4}). The $f_{u,inc}$ for chrysene (i.e., 4.55×10^{-4}) was also obtained from sorbent-phase dosing experiments but was 2.7-fold lower than that calculated from Equation 3.

Model application

The relationship between in vitro biotransformation rate constants (k_r) and calculated BMF_L values in rats using the proposed IVIVE-B model in combination with the rat BMF model was investigated for adult male rats for a set of hypothetical chemicals with log K_{OW} values ranging from 1 to 10 and log K_{OA} values ranging from 4 to 10, at selected in vitro biotransformation rate constants of 0 h^{-1}, 0.1 h^{-1}, and 0.3 h^{-1}. The fraction of unbound chemical in the incubation mixture ($f_{u,inc}$) was calculated according to Equation 3. The dietary absorption efficiencies ($E_{D,N}$) for the hypothetical chemicals were calculated according to Equation 26, hence assuming no intestinal biotransformation. To estimate at what value of $E_{D,N}$ chemicals can be expected to lack biomagnification potential (i.e., $BMF_L < 1$) despite their hepatic biotransformation rate, we also conducted model calculations where we varied values of $E_{D,N}$ for the calculation of the BMF_L while keeping k_{met} at 0 d^{-1}. This model application attempts to assess the upper dietary uptake efficiency below which biomagnification is not expected to occur. The values of other input parameters are listed in the Supplemental Data, Tables S1 and S2 for the IVIVE-B and rat BMF models, respectively.

RESULTS AND DISCUSSION

Model evaluation

Figure 2 illustrates the comparison of the whole-body k_{met} in rats calculated by the IVIVE-B and IVIVE-Ph models for hypothetical chemicals with a wide range of log K_{OW} values given a fixed in vitro biotransformation rate constant. Figure 2 shows that k_{met} values calculated by the IVIVE-B and IVIVE-Ph models both decreased sigmoidally with increasing log K_{OW} and that at log $K_{OW} \geq 4$, k_{met} values remained approximately constant. The sigmoidal relationship of k_{met} with log K_{OW} is a result of the higher lipid and protein contents of the liver (in the IVIVE-B model) and the blood (in the IVIVE-Ph model) compared with those in the incubation medium (Supplemental Data, Table S1). This causes $f_{u,H}/f_{u,inc}$ and $f_{u,Bl}/f_{u,inc}$ to fall with increasing log K_{OW} for chemicals with a log $K_{OW} < 4$ in the

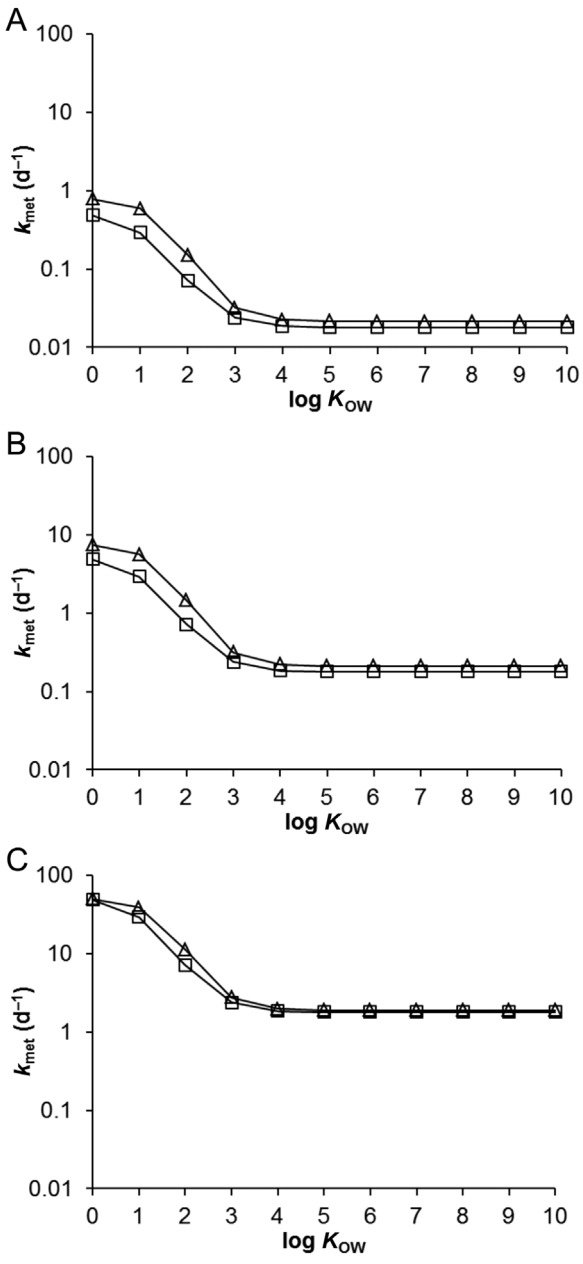

Figure 2. Relationship between calculated whole-body biotransformation rate constants (k_{met}; d^{-1}) and octanol–water partition coefficients (log K_{OW}) for hypothetical chemicals in rats using the IVIVE-B model (open squares) or the IVIVE-Ph model (open triangles) at input in vitro biotransformation rate constants of 0.1 h^{-1} (**A**), 1 h^{-1} (**B**), and 10 h^{-1} (**C**). IVIVE-B = in vitro to in vivo extrapolation-bioaccumulation; IVIVE-Ph = in vitro to in vivo extrapolation-pharmaceuticals.

IVIVE-B and IVIVE-Ph models, respectively. At higher log K_{OW} ($\log K_{OW} \geq 4$), the ratios $f_{u,H}/f_{u,inc}$ and $f_{u,Bl}/f_{u,inc}$ reach constant values because the chemical is predominantly bound in the liver, the blood, and the incubation medium, causing the unbound fractions in liver, blood, and incubation medium to fall with increasing log K_{OW} at essentially the same rate.

For chemicals with log $K_{OW} < 4$, the k_{met} values calculated by the IVIVE-B model were on average 1.77-fold, 1.71-fold, and 1.27-fold lower than those calculated by the IVIVE-Ph model when the in vitro biotransformation rate constant was set to 0.1 h^{-1}, 1 h^{-1}, and 10 h^{-1}, respectively (Figure 2A–C). For chemicals with log $K_{OW} \geq 4$, differences in k_{met} between the 2 models were very small. The IVIVE-B model yielded values of

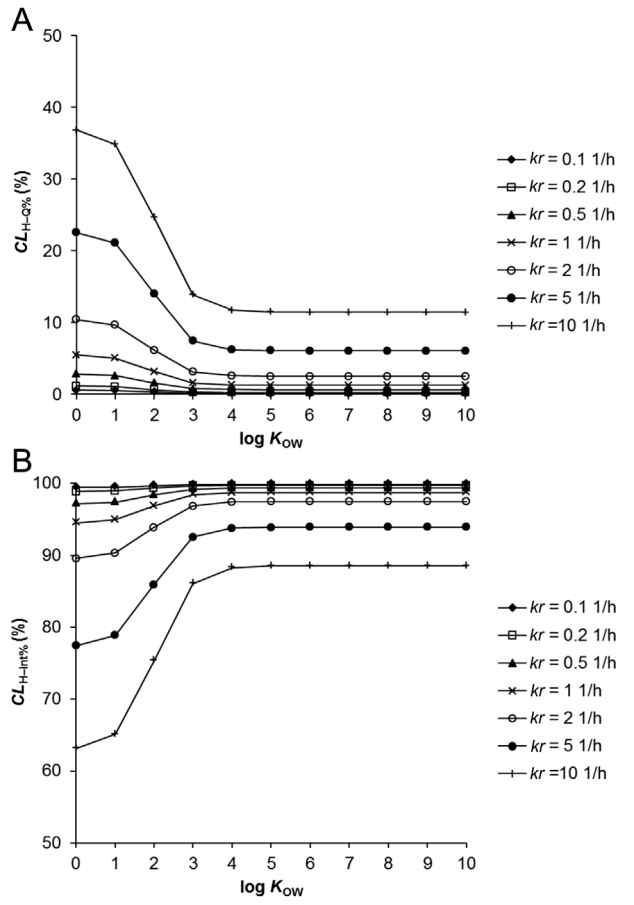

Figure 3. The contribution (%) of hepatic blood flow to rat hepatic clearance ($CL_{H-Q\%}$; calculated using Equation 24) as a function of the octanol–water partition coefficients (log K_{OW}; **A**), and the contribution (%) of unbound hepatic intrinsic clearance to rat hepatic clearance ($CL_{H-int\%}$; calculated using Equation 25) as a function of log K_{OW} (**B**) derived from the well-stirred liver model (Equations 11 and 23) at input in vitro biotransformation rate constant (k_r) ranging from 0.1 h^{-1} to 10 h^{-1}.

k_{met} on average 1.19-fold, 1.18-fold, and 1.06-fold lower than those of the IVIVE-Ph model when the in vitro biotransformation rate constant was set to 0.1 h^{-1}, 1 h^{-1}, and 10 h^{-1}, respectively. This indicates that the k_{met} values produced by the IVIVE-B and IVIVE-Ph models are in good agreement for chemicals with log $K_{OW} \geq 4$ for in vitro biotransformation rate constants ranging from 0.1 h^{-1} to 10 h^{-1}, corresponding to in vitro half-lives ranging from 6.9 h to 4.2 min.

Figure 3 details the relative contribution of hepatic blood flow and unbound hepatic intrinsic clearance to hepatic clearance in the well-stirred liver model for a set of hypothetical chemicals. Figure 3A shows that the relative contribution of hepatic blood flow to hepatic clearance ($CL_{H-Q\%}$) decreased with increasing log K_{OW} (with log $K_{OW} < 4$) and achieved a constant value (with log $K_{OW} \geq 4$). The $CL_{H-Q\%}$ values increased with increasing in vitro biotransformation rates. Figure 3B illustrates an opposite trend: the relative contribution of unbound hepatic intrinsic clearance to hepatic clearance ($CL_{H-int\%}$) increased with increasing log K_{OW} (with log $K_{OW} < 4$) and remained approximately constant (with log $K_{OW} \geq 4$). The $CL_{H-int\%}$ values increased with decreasing in vitro biotransformation rates. Figure 3B displays that more than 85% of the hepatic clearance is a result of the unbound hepatic intrinsic clearance for hydrophobic chemicals (log $K_{OW} \geq 4$) if in vitro depletion half-lives exceed 4.2 min. This supports the

assumption in the IVIVE-B modeling approach that for hydrophobic chemicals with bioaccumulative potential ($\log K_{OW} \geq 5$), the hepatic biotransformation rate is predominantly controlled by nonspecific chemical binding and inherent metabolic activity in the liver rather than the liver perfusion rate.

Supplemental Data Figure S2A and B further illustrates that at in vitro biotransformation rate constants of $0.1\ h^{-1}$ and $1\ h^{-1}$, the calculated hepatic clearance is identical to the unbound hepatic intrinsic clearance over the entire range of the log K_{OW} values, suggesting that nonspecific chemical binding and inherent metabolic activity in the liver are the major determinants of hepatic clearance for slowly metabolized chemicals and that hepatic blood flow does not affect the hepatic clearance under such conditions. When the in vitro biotransformation rate constant was set to a high value of $10\ h^{-1}$, hepatic clearance was controlled by unbound hepatic intrinsic clearance and hepatic blood flow for chemicals with log $K_{OW} < 4$, but was essentially independent of hepatic blood flow and fully controlled by unbound hepatic intrinsic clearance for the more hydrophobic chemicals with a log $K_{OW} \geq 4$ (Supplemental Data, Figure S2C). The results in Figure 3 and Supplemental Data, Figure S2 support the use of the IVIVE-B model for evaluating hydrophobic chemicals with a log K_{OW} greater than approximately 4. Even for chemicals with a log $K_{OW} < 4$, the blood flow often has a minor effect on the hepatic clearance and hence k_{met}. Because hepatic clearance data are not required, the IVIVE-B method simplifies the assessment of bioaccumulation potential in rats and possibly other mammalian species.

Figure 4 shows the comparison of the calculated whole-body k_{met} and lipid-normalized BMF_L values in rats for 2 hydrophobic model chemicals, benzo[*a*]pyrene and chrysene (log K_{OW} of 6.04 and 5.60 at 25 °C, respectively), with those obtained using the IVIVE-Ph model. In vitro biotransformation rate constants and unbound fractions in the incubation mixture for benzo[*a*]pyrene and chrysene in both models were taken from the same source [28]. For benzo[*a*]pyrene, the calculated k_{met} values in rats were $0.40 \pm 0.06\ d^{-1}$ and $0.46 \pm 0.07\ d^{-1}$ (mean ± standard error of the mean; $n = 3$) for the IVIVE-B and IVIVE-Ph models, respectively. The calculated lipid-normalized BMF_L values in adult male rats were 0.098 ± 0.013 kg lipid/kg lipid and 0.085 ± 0.011 kg lipid/kg lipid (mean ± standard error of the mean; $n = 3$) using k_{met} calculated from the IVIVE-B and IVIVE-Ph models, respectively. For chrysene, the calculated k_{met} values in rats were $1.22 \pm 0.08\ d^{-1}$ and $1.33 \pm 0.08\ d^{-1}$ (mean ± standard error of the mean; $n = 3$) for the IVIVE-B and IVIVE-Ph models, respectively. The calculated lipid-normalized BMF_L values in adult male rats were 0.012 ± 0.001 kg lipid/kg lipid and 0.011 ± 0.001 kg lipid/kg lipid (mean ± standard error of the mean; $n = 3$) using k_{met} calculated from the IVIVE-B and IVIVE-Ph models, respectively. For both chemicals, the calculated whole-body biotransformation rate constants and BMF_L values using the proposed IVIVE-B model were not statistically different from those calculated using the IVIVE-Ph model (Figure 4). The agreement between the models further supports our contention that the IVIVE-B model is a good alternative for estimating whole-body biotransformation rate constants and BMFs for hydrophobic chemicals in rats.

Figure 5A and the Supplemental Data, Table S3 show that the calculated $k_{met,H}$ for benzo[*a*]pyrene in rats from the IVIVE-B model ($0.38\ h^{-1} \pm 0.06\ h^{-1}$; mean ± standard error of the mean; $n = 3$) is within the range of the previously reported hepatic elimination rate constants for benzo[*a*]pyrene ($0.05-5\ h^{-1}$) in measured in vivo rat studies [48–50] and an

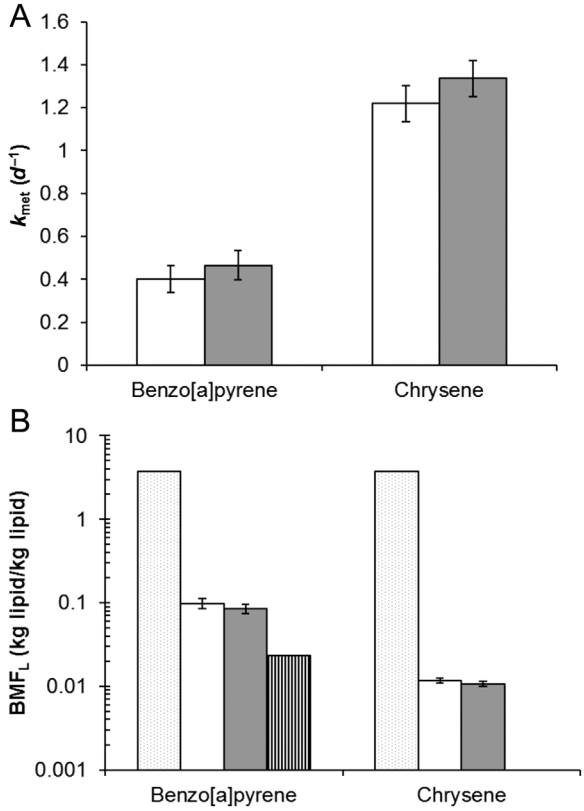

Figure 4. The whole-body biotransformation rate constants (k_{met}; **A**) and lipid-normalized biomagnification factors (BMF_L; **B**) for benzo[*a*]pyrene and chrysene in rats calculated from the IVIVE-B (open bars) and IVIVE-Ph (filled bars) models with measured in vitro biotransformation rate constants from Lee et al. [28]; a BMF_L derived from an in vivo study by Kang et al. [56] (striped bar), and calculated BMF_L by setting $k_{met} = 0$ using the IVIVE-B model (dotted bars). Error bars are standard errors of the mean. IVIVE-B = in vitro to in vivo extrapolation–bioaccumulation; IVIVE-Ph = in vitro to in vivo extrapolation–pharmaceuticals.

ex vivo study using isolated perfused rat liver [51]. Figure 5B and Supplemental Data, Table S3 show that the calculated k_{met} for benzo[*a*]pyrene in rats from the IVIVE-B model ($0.40 \pm 0.06\ d^{-1}$; mean ± standard error of the mean; $n = 3$) is in reasonable agreement with the measured whole-body elimination rate constants for benzo[*a*]pyrene administered via oral gavage and intravenous injection ($0.41\ d^{-1}$ and $0.53\ d^{-1}$, respectively) from a recent in vivo rat study [52] and with the median value ($1.06\ d^{-1}$) of measured whole-body elimination rate constants [48,50,52–55]. The wide range of observed elimination rate constants for benzo[*a*]pyrene in rats reported in the literature may be associated with different experimental designs and conditions such as different dose levels and routes of administration (e.g., oral gavage, intravenous injection, and intratracheal injection) used in the in vivo experiments. The concentration dependence of in vivo biotransformation rate constants (Equation 4), extrahepatic biotransformation, and technical difficulties in conducting in vivo and ex vivo experiments for very hydrophobic chemicals (e.g., incomplete dissolution in aqueous solution) may also have contributed to the variability in the observed values.

Figure 4B shows that the calculated lipid-normalized BMF_L value for benzo[*a*]pyrene in adult male rats (0.098 ± 0.013 kg lipid/kg lipid; mean ± standard error of the mean; $n = 3$) is approximately 4.3-fold greater than the BMF_L estimate of 0.023 kg lipid/kg lipid derived as the ratio of the highest concentration of benzo[*a*]pyrene observed in rat muscle

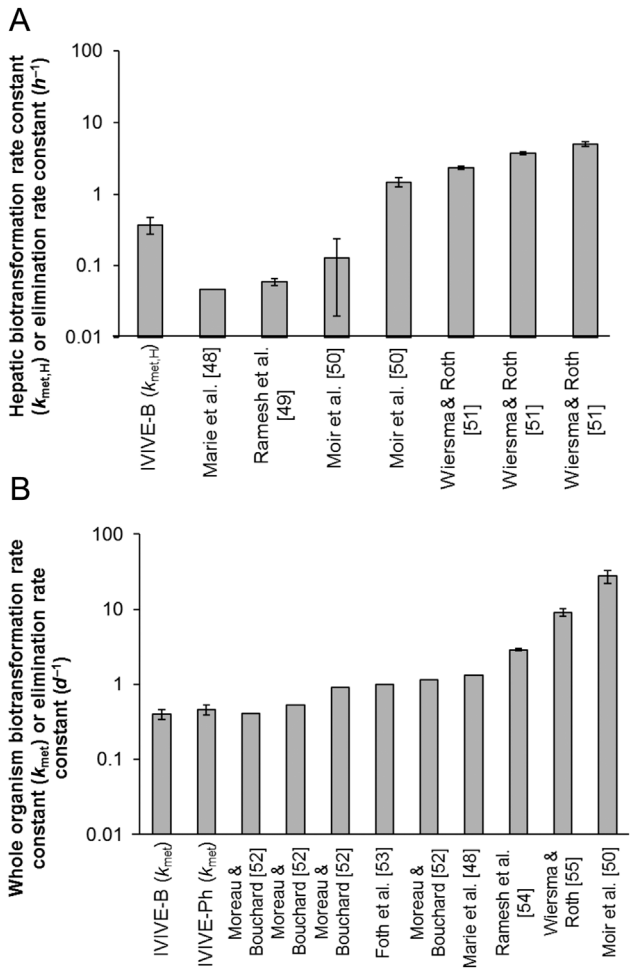

Figure 5. The hepatic biotransformation rate constant ($k_{met,\ H}$) calculated from the IVIVE-B model or measured hepatic elimination rate constants obtained from previous studies (**A**), and the whole-organism biotransformation rate constants (k_{met}) calculated from the IVIVE-B, and IVIVE-Ph models or measured whole-organism elimination rate constants obtained from previous studies (**B**) for benzo[*a*]pyrene in rats. Details of each study can be found in the Supplemental Data, Table S3. Error bars are standard errors of the mean. IVIVE-B = in vitro to in vivo extrapolation–bioaccumulation; IVIVE-Ph = in vitro to in vivo extrapolation–pharmaceuticals.

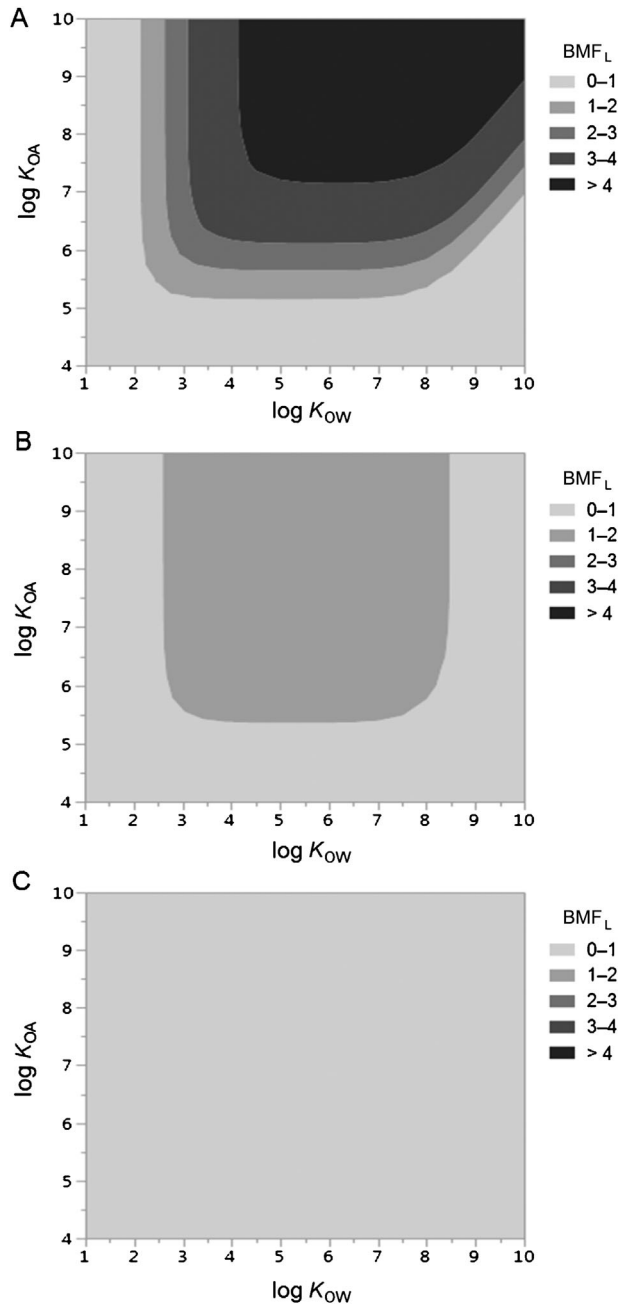

Figure 6. Calculated lipid-normalized biomagnification factor (BMF_L) values in adult male rats for hypothetical chemicals as a function of log K_{OW} and log K_{OA} using the IVIVE-B model in combination with a rat BMF model at input in vitro biotransformation rate constant of 0 h^{-1} (**A**), 0.1 h^{-1} (**B**), and 0.3 h^{-1}, or a dietary absorption efficiency less than 18% (**C**). K_{OW} = octanol–water partition coefficient; K_{OA} = octanol–air partition coefficient; IVIVE-B = in vitro to in vivo extrapolation–bioaccumulation.

(34.5 ng/g, assuming 2% lipid) and the concentration of benzo[*a*]pyrene in corn oil (75 μg/mL) administered daily by oral gavage for 30 d from the results of an in vivo study by Kang et al. [56]. Figure 4B also shows that both the model-calculated and empirical BMF_L values are well below the calculated BMF_L that does not account for biotransformation (i.e., $k_{met} = 0$). Hence, the proposed IVIVE-B and BMF modeling approach improves predictions of the BMF_L over models that do not account for biotransformation but tend to overestimate the BMF_L. Unfortunately, data required for a more thorough evaluation of the IVIVE-B approach are, as far as we know, not available at this time. Additional investigations are needed to further evaluate the proposed IVIVE-B modeling method.

Model application`

The IVIVE-B modeling approach for relating measured in vitro biotransformation rate constants to the BMF may be useful for bioaccumulation screening. Figure 6A illustrates that when no biotransformation is measured in an in vitro liver assay, chemicals with a log $K_{OW} > 2$ and a log $K_{OA} > 5$ have a biomagnification potential in rats ($BMF_L > 1$). These results are in line with previous studies showing that nonmetabolized chemicals with a log $K_{OW} > 2$ and a log $K_{OA} > 5$ have the potential to biomagnify in mammals and terrestrial food chains [2,8,9]. In contrast, Figure 6B shows that when chemicals are metabolized slowly (at an in vitro rate constant of 0.1 h^{-1}), BMF_L values are lower than those for nonmetabolized chemicals, and only chemicals with a log K_{OW} between 2.5 and 8.5 and a log $K_{OA} > 5.5$ have the potential to biomagnify in rats. Figure 6C shows that at the higher in vitro biotransformation rate constant of 0.3 h^{-1}, the estimated BMF_L values of hydrophobic chemicals in rats are all <1. This in vitro

biotransformation rate constant of $0.3\,h^{-1}$ may be useful as a preliminary guideline (cut-off value) for identifying chemicals that are not expected to biomagnify in rats. This cut-off value may be useful for chemical screening because it does not require the full execution of the IVIVE-B modeling approach. However, caution should be exercised because of the limited testing of the IVIVE-B modeling method to date and the lack of standardized protocols for measuring in vitro biotransformation rate constants in rats for the purpose of bioaccumulation screening of very hydrophobic chemicals. It should also be emphasized that chemicals can lose their ability to biomagnify not only as a result of hepatic biotransformation but also because of biotransformation in the gastrointestinal tract. Intestinal biotransformation reduces the dietary uptake efficiency of chemicals [57]. Figure 6C illustrates that the calculated BMF_L values of chemicals in rats are all <1, not only with an in vitro biotransformation rate constant of $0.3\,h^{-1}$ but also when in vivo dietary absorption efficiencies are <0.18 in absence of in vitro hepatic biotransformation rates ($k_r = 0$). The model results suggest that an in vivo dietary absorption efficiency < 0.18 (or 18%) may also be a useful screening tool for chemicals that can be expected not to biomagnify in rats and possibly other mammals. The model calculations illustrate the value of developing in vitro bioassays to assess the intestinal biotransformation rate of chemicals. A high degree of intestinal biotransformation can negate the ability of chemicals to biomagnify.

Advantages and limitations of the proposed IVIVE-B approach

The proposed IVIVE-B approach can be used for estimating in vivo biotransformation rate constants and BMF values for hydrophobic chemicals ($\log K_{OW} \geq 4$) in mammals and possibly other terrestrial animals. This approach has several advantages. First, the extrapolation from in vitro to in vivo is relatively straightforward because it involves the extrapolation of rate constants. Clearance and volume of distribution values are useful properties for describing the therapeutic dosages of pharmaceuticals but are, in most cases, not required for bioaccumulation assessment of high log K_{OW} chemicals. Second, the well-stirred liver model is not incorporated into the proposed IVIVE-B because it is not needed for bioaccumulation assessment. Consequently, information such as cardiac output, fraction of blood flow through the liver, and fraction unbound in blood, as well as estimates of the volume of distribution are not required in the IVIVE-B approach. This is advantageous because several of these parameters may not be available or may be hard to measure for most wildlife species and may introduce uncertainty in predictions for bioaccumulation assessment. Third, the scaling factor involved in the IVIVE-B process is comprised of several volume ratios that are easy to measure in the preparation of liver S9 fractions and in the in vitro substrate depletion experiments.

The proposed IVIVE-B approach is also subject to limitations. First, the prediction of whole-body biotransformation rate constants from the IVIVE-B model is sensitive to the unbound fractions of chemicals in the incubation mixture ($f_{u,inc}$) and in the liver ($f_{u,H}$). The unbound fractions can be measured [23,28], calculated from empirical relationships (e.g., Han et al. [22] and Nichols et al. [17] using binding data for drugs with log K_{OW} ranging from 1.54 to 6.34 from Austin et al. [33]), or estimated (e.g., using Equations 3 and 6). The sorbent-phase dosing approach is a useful method for hydrophobic chemicals because it is solvent-free; the concentrations of very hydrophobic chemicals in the incubation medium often remain very low (hence avoiding saturation effects) because of slow release of the chemical from the sorbent phase [28,29]; and the in vitro biotransformation rate constant and the unbound fraction in the incubation mixture can be determined in the same experiment. In the present study, the estimation of $f_{u,inc}$ and $f_{u,H}$ using Equations 3 and 6 is based on the assumption that K_{OW} is an adequate surrogate for lipid–water partition coefficients (K_{LW}) of non-ionic organic chemicals. This assumption is valid for neutral storage and membrane lipids [35], and this method is useful for illustrating model estimates for hypothetical chemicals with respect to K_{OW} for model comparison (IVIVE-B vs IVIVE-Ph). However, this assumption may not be appropriate for highly polar or ionic chemicals. Alternatively, K_{LW} may be determined from measured data or estimated using the polyparameter linear free energy relationships [34].

Second, the BMF estimations by the IVIVE-B approach are sensitive to the value used for dietary absorption efficiency, which is not derived in the in vitro bioassay. Dietary absorption efficiency of chemicals can be affected by the composition of diet [58] and intestinal biotransformation [57]. Values chosen for the assimilation efficiencies for lipid, protein, and carbohydrate can therefore have a large effect on the BMF estimations. The BMF model is insensitive to assimilation efficiency for water as well as the increase in solubility of chemical in bile compared with that in water as reported previously [10].

Other potential limitations of the IVIVE-B approach result from key assumptions of the model, such as the assumption that no extrahepatic metabolism occurs. The small intestines contribute to the first-pass metabolism of ingested and absorbed chemicals [58], and intestinal biotransformation has been reported to contribute substantially to biotransformation of hydrophobic organic chemicals in fish [57]. This is a limitation of both the IVIVE-B and IVIVE-Ph approaches. Although there is a clear need for further testing of the IVIVE-B approach for bioaccumulation assessment, we submit that the proposed IVIVE-B modeling approach presented and evaluated in the present study can be a useful tool for screening the bioaccumulative potential of hydrophobic chemicals that undergo biotransformation in mammals and possibly other terrestrial and nonaquatic animals.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3718.

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Data Availability—All data used for the model are included in the present paper. A spreadsheet version of the model will be made available on our website (http://www.sfu.ca/rem/toxicology.html).

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