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Development of a Multi-Component Damage Assessment Model (MDAM) for Time-Dependent Mixture Toxicity with Toxicokinetic Interactions

JONG-HYEON LEE† AND PETER F. LANDRUM*,‡

Cooperative Institute for Limnology and Ecosystems Research, University of Michigan, Ann Arbor, Michigan 48105-1593, and Great Lakes Environmental Research Laboratory, National Oceanic and Atmospheric Administration, Ann Arbor, Michigan 48105-1593

A new mixture toxicity model was developed to predict the time-dependent toxicity of a mixture with toxicokinetic interactions directed specifically toward addressing biotransformation. The Damage Assessment Model (DAM), a toxicokinetic−toxicodynamic model that describes and predicts the time-dependent toxicity of a single compound, was extended to a multicomponent model for mixture toxicity. The model assumes that cumulative damage from the parent compound, metabolites, and/or a biotransformation inhibitor are additive, and the sum of the cumulative damage determines mixture toxicity. Since incorporation of the damage addition hypothesis into the DAM was equivalent to an independent action model for mixture toxicity, it was applied to describe the combined effect of mixture components with potentially dissimilar modes of action. From the multicomponent DAM, a time-dependent toxic unit model was derived and applied to determine the toxic units of mixture components. This model suggests a series of experimental designs required to assess the role of biotransformation in the toxicity of metabolized organic compounds and a data analysis method to separately estimate toxicodynamic parameters for the parent compound and metabolites.

Introduction

Biotransformation is a challenge for ecotoxicologists because no current method predicts the capability of a species to biotransform organic compounds from the physicochemical characteristics of the compound and species-specific properties of aquatic organisms. For the body residue approach to predict toxic effects, improvement is needed in predicting biotransformation and interpreting the metabolites' contribution to the observed toxicity (*1*−*2*). An additional complication in predicting the bioconcentration factor (BCF) and half-life for the parent compound and metabolites results from potential dose-dependent toxicokinetics (*3*).

The biotransformation of an organic compound may modify the response to the contaminant by altering the mechanism of action through detoxification or the formation of toxic metabolites. Therefore, the toxic effects of metabolized PAH should be assessed as a mixture of the parent compound and metabolites with different toxicokinetics and toxicodynamics (*1*, *2*).

In most bioassays, the contributions of parent compound and metabolites are not distinguished. To assess the relative toxicity of metabolites, separate toxicity assessments of the parent compound and metabolites are required. The toxicity of individual metabolites cannot generally be correctly measured because of additional biotransformation. However, when biotransformation of a metabolized organic compound is blocked by a biotransformation inhibitor, comparison of the toxicity of the parent compound and metabolites becomes possible if the direct contribution of the inhibitor to the toxic response is known. Then, the time-dependent toxicity of a mixture of parent compound and metabolites can be determined and analyzed with toxicokinetic and toxicodynamic models that include metabolic interaction.

Traditionally, mixture toxicity has been classified as additive, synergistic, or antagonistic based on mixture toxicity compared to that expected from Concentration Addition (CA) or Independence Action (IA) models. The CA and IA models were defined as follows.

CA model (Loewe additivity; *4*, *5*):

$$C_A/LC_{x,A} + LC_{x,B|A}/LC_{x,B} = LC_{x,A|B}/LC_{x,A} + C_B/LC_{x,B} = 1 \quad (1)$$

IA model (Bliss independence; *4*, *5*):

$$\begin{aligned} E(C_A, C_B) &= 1 - (1 - E(C_A))(1 - E(C_B)) \\ &= E(C_A) + E(C_B) - E(C_A)E(C_B) \end{aligned} \quad (2)$$

where $LC_{x,A}$ and $LC_{x,B}$ are the lethal concentrations of compound A and B for x% mortality in a single exposure to A and B, respectively; C_A and C_B are the concentrations for A and B in a mixture, and $LC_{x,A|B}$ and $LC_{x,B|A}$ are the lethal concentration of A and B for x% mortality in a mixture exposure; $E(C_A, C_B)$ is the joint response probability in a mixture exposure to A and B; $E(C_A)$ and $E(C_B)$ are the response probability for a given concentration of A or B acting alone (*4*, *5*).

The CA model compares relative toxicities, whereas the IA model compares relative probabilities of response. Thus, the CA and IA models have been considered to be mutually exclusive (*6*). However, the CA and IA models are related to each other quantitatively, and the relationship depends on the functional form (e.g., logistic, Weibull, and probit model), the shape parameter of the concentration−response relationship for individual components, the exposure concentration level (*4*, *7*), and the mode of toxic action (*8*).

The CA model is based on the assumption that mixture components have the same mode of toxic action, whereas the IA model is based on the assumption that all mixture components contribute to a given effect, but may do so by different modes of toxic action. Therefore, when using a mixture of compounds with potentially different modes of toxic action such as nonpolar narcosis by PAH and a specific mode of action by metabolites, the IA model is applied.

The CA and IA models use two different methods to compare the actual toxicity of the mixture to a reference, i.e, a noninteraction situation. Synergistic and antagonistic effects were classified as a deviation from the CA model as follows:

$$\text{Loewe synergism: } LC_{x,A|B}/LC_{x,A} + LC_{x,B|A}/LC_{x,B} < 1 \quad (3)$$

$$\text{Loewe antagonism: } LC_{x,A|B}/LC_{x,A} + LC_{x,B|A}/LC_{x,B} > 1 \quad (4)$$

* Corresponding author phone: 734-741-2276; fax: 734-741-2055; e-mail: peter.landrum@noaa.gov.
† University of Michigan.
‡ National Oceanic and Atmospheric Administration.

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TABLE 1. List of Symbols and Their Units for Variables and Parameters[a]

symbol	unit	definition
C_w	μmol L^{-1}	water concentration
$C_j(t)$	μmol g^{-1}	body residue, $C_j(t) = C_w BCF_j K_j(t)$
$D_j(t)$	$-$[b]	cumulative damage, $D_j(t) = k_{aj} C_j P_j(t) = k_{aj} C_w BCF_j K_j(t) P_j(t)$
$S_j(t)$	$-$	survival probability, $S_j(t) = \exp(-D_j(t))$
$M_j(t)$	$-$	mortality probability, $M_j(t) = 1 - S_j(t)$
$LC_{50,j}(t)$	μmol L^{-1}	median lethal concentration, $LC_{50,j}(t) = (D_j/k_{aj})/BCF_j K_j(t) P_j(t)$
$LBR_{50,j}(t)$	μmol g^{-1}	median lethal body residue, $LBR_{50,j}(t) = (D_j/k_{aj})/P_j(t)$
$LBR_{50,m0}(t)$	μmol g^{-1}	median lethal body residue for metabolites, $LBR_{50,m0}(t) \equiv (D_{tox}/(k_{am}/k_{rm}))/P_m(t)$, see eq 43 for details.
$K_j(t)$	$-$	toxicokinetic time-scale function
BCF_j	mL g^{-1}	bioconcentration factor
$P_j(t)$	h	toxicodynamic time-scale function
k_{uj}	mL g^{-1} h^{-1}	uptake rate coefficient
V_{max}	μmol g^{-1} h^{-1}	maximum reaction rate
K_M	μmol g^{-1}	half-saturation concentration
k_m	h^{-1}	biotransformation rate constant
k_{ej}	h^{-1}	elimination rate constant
k_{aj}	μmol^{-1} g h^{-1}	damage accrual rate coefficient
k_{rj}	h^{-1}	damage recovery rate constant
p	$-$	scaled $P_p(t)$, $p \equiv P_p(C_w,t)/P_{p0}(t)$
q	$-$	scaled $K_p(t)$, $q \equiv K_p(C_w,t)/K_{p0}(t)$
r	$-$	scaled BCF_p, $r \equiv BCF_p(C_w)/BCF_{p0}$
$\hat{p}$	$-$	scaled $P_m(t)$, $\hat{p} \equiv P_m(C_w,t)/P_{m0}(t)$
$\hat{q}$	$-$	scaled $K_m(t)$, $\hat{q} \equiv K_m(C_w,t)/K_{m0}(t)$
$\hat{r}$	$-$	scaled BCF_m, $\hat{r} \equiv BCF_m(C_w)/BCF_{m0}$
$TU_j(t)$	$-$	toxic unit, $TU_j(t) \equiv LBR_{50,j}(t)/LBR_{50,j0}(t)$ and
$^cTU_j(t)$	$-$	$^cTU_j(t) \equiv LC_{50,j}(t)/LC_{50,j0}(t)$
$f_m(t)$	$-$	percent of metabolites in total body residue
$\gamma(t)$	$-$	relative toxicity for metabolites compared to the parent compound, $LBR_{50,p0}(t)/LBR_{50,m0}(t)$
$\bar{\gamma}$	$-$	relative toxic potency for metabolites compared to the parent compound, $\bar{\gamma} \equiv \lim_{t\to\infty} \gamma(t)$

[a] j represents situations exposed to parent compound (p) or metabolites (m) in the absence of PBO, parent compound (p|I) and metabolites (m|I) in the presence of PBO, biotransformation inhibitor (I), only parent compound in the absence of biotransformation (p0), only metabolites (m0), and nonmetabolized organic compound (A|B, B|A, A, and B). [b] $-$ means dimensionless.

With regard to Bliss synergism and antagonism, there is no consensus on the concept and its application (*8*, *9*). These empirical approaches suggest criteria for the diagnosis of synergism and antagonism from mixture toxicity, but do not explain why the mixture toxicity is more or less toxic than expected.

In addition to the above empirical approaches, deviation from the CA or IA models can result from toxicokinetic and toxicodynamic interactions among components in a mixture. Toxicokinetic interactions involve the alteration of metabolism and disposition of one compound by another. These interactions can be mediated by the induction or inhibition of the activation or detoxicification of a compound. The toxicodynamic interactions include those processes that do not directly affect the metabolism or disposition of a xenobiotic, but affect a tissue's response or susceptibility to toxic injury. These interactions include depletion or induction of cytoprotective factors such as the depletion of glutathione (*10*) and alterations in tissue repair (*11*). Until now, relating the models for component interaction using the toxicokinetics and toxicodynamics has not been done, and combining information from both approaches is recognized as one of the challenges in toxicology (*5*).

The main objective was to develop a toxicokinetic and toxicodynamic model to predict time-dependent mixture toxicity with toxicokinetic interactions. The Damage Assessment Model (DAM, *12*), a toxicokinetic−toxicodynamic model developed to predict the time-dependent toxicity of a single compound, was extended to a multicomponent model for mixture toxicity. The DAM defines the determinant of toxicity in terms of cumulative damage rather than body residue or external concentration. This is the first application of a damage addition (DA) hypothesis applied to describe the combined effect of mixture components, which could includes dissimilar modes of action. The influence of a toxicokinetic interaction, such as the inhibition of biotransformation, can be easily incorporated into the DAM by changing the toxicokinetic model for biotransformation (*3*). From the multicomponent DAM (MDAM), a time-dependent toxic unit ($TU(t)$) model for mixtures with and without toxicokinetic interaction was derived.

A second objective was to suggest an experimental design to assess the role of biotransformation in the toxicity of metabolized organic compounds and evaluate the relative toxicity of metabolites. See Table 1 for significant abbreviations used in the text.

Theory

Toxicokinetics for a Metabolized Organic Compound. The toxicokinetics of an organic chemical accumulated from water with biotransformation can be described by coupled equations with first-order elimination of parent compound (PC) and metabolites and Michaelis−Menten (MM) type biotransformation

$$\frac{dC_p}{dt} = k_u C_w - \frac{V_{max} C_p}{K_M + C_p} - k_{ep} C_p \quad (5)$$

$$\frac{dC_m}{dt} = \frac{V_{max} C_p}{K_M + C_p} - k_{em} C_m \quad (6)$$

where C_w, C_p, and C_m are the concentrations in the exposure water (μmol L^{-1}), and organism concentrations of PC (μmol g^{-1}) and metabolites (μmol g^{-1}), respectively. The k_u is the uptake rate coefficient (mL g^{-1} h^{-1}). The elimination processes

for the PC include first-order kinetics (k_{ep}) for the diffusion out of the organism and Michaelis−Menten saturation kinetics for biotransformation (V_{max} and K_M are the maximum reaction rate (μmol g^{-1} h^{-1}) and the half-saturation concentration (μmol g^{-1}), respectively). For simplicity, the elimination process for the metabolites was described with a pseudo first-order kinetic constant (k_{em}, h^{-1}).

If the body residue of a compound (C_p^1) is smaller than K_M, eq 5 simplifies to first-order kinetics:

$$\frac{dC_p^{\,1}}{dt} = k_u C_w - (k_m + k_{ep}) C_p^{\,1} \quad (7)$$

If the body residue of a compound (C_p^0) is greater than K_M, eq 5 simplifies to zero-order kinetics:

$$\frac{dC_p^{\,0}}{dt} = k_u C_w - V_{max} - k_{ep} C_p^{\,0} \quad (8)$$

Therefore, the body residues of a compound showing different toxicokinetics (i) such as first-, zero-order, and MM-type kinetics are given by

$$C_p^{\,i}(C_w,t) = C_w \mathrm{BCF}_p^{\,i}(C_w) K_p^{\,i}(C_w,t) \quad (9)$$

$$\begin{aligned} C_m^{\,i}(C_w,t) &= (k_m/k_{em}) C_p^{\,i}(C_w,t) K_m^{\,i}(C_w,t) \\ &= C_w BCF_m^{\,i}(C_w) K_p^{\,i}(C_w,t) K_m^{\,i}(C_w,t) \end{aligned} \quad (10)$$

where $C_p^i(C_w,t)$ and $C_m^i(C_w,t)$ are the body residue, $\mathrm{BCF}_p^i(C_w)$ and $\mathrm{BCF}_m^i(C_w)$ are the bioconcentration factors, $K_p^i(t)$ and $K_m^i(C_w,t)$ are the toxicokinetic time-scale function for PC (p) and metabolites (m), respectively, the superscript i represents the 1st-order ($i = 1$), zero-order kinetics ($i = 0$), and the MM-type kinetics (i = M) (see Supporting Information part I for details). In particular, $K_p^M(C_w,t)$ and $K_m^M(C_w,t)$ are explicitly unknown functions of C_w and t. However, for a given C_w, $K_p^M(C_w,t)$ and $K_m^M(C_w,t)$ range as follows

$$K_p^{\,1}(t) \le K_p^{\,M}(t) \le K_p^{\,0}(t) \text{ and } K_m^{\,1}(t) \le K_m^{\,M}(C_w,t) \le K_m^{\,0}(t)$$

(see Supporting Information part II for details).

The bioconcentration factor for PC (BCF_p) in the toxicokinetic model for biotransformation (eq 5) is given as a function of C_w as follows

$$\mathrm{BCF}_p = -\left(a + b\frac{1}{C_w}\right) + \sqrt{\left(a + b\frac{1}{C_w}\right)^2 + c\frac{1}{C_w}} \quad (11)$$

where

$$a = -\frac{k_u}{2k_{ep}},\ b = \frac{K_M}{2} + \frac{V_{max}}{2k_{ep}},\ c = \frac{k_u K_M}{k_{ep}}$$

The bioconcentration factor for metabolites (BCF_m) and total body residue including PC and metabolites (BCF_{total}) are given by

$$\mathrm{BCF}_m = \frac{k_u}{k_{ep} + k_m(C_p)} \frac{k_m(C_p)}{k_{em}} \quad (12)$$

$$\begin{aligned} \mathrm{BCF}_{total} &= \frac{k_u}{k_{ep} + k_m(C_p)}\left(1 + \frac{k_m(C_p)}{k_{em}}\right) \\ &= \left(\frac{k_u}{k_{ep}}\right)\left(\frac{k_{ep}}{k_{em}}\right)\left(\frac{k_{em} + k_m(C_p)}{k_{ep} + k_m(C_p)}\right) \end{aligned} \quad (13)$$

where

$$k_m(C_p) \equiv \frac{V_{max}}{K_M + C_p} = \frac{V_{max}/C_w}{K_M/C_w + \mathrm{BCF}_p}$$

The BCF_p is a monotonically increasing function of C_w, but BCF_m is a monotonically decreasing function of C_w. It is notable that BCF_{total} increases with increasing C_w when k_{em} is greater than k_{ep}, and decreases with increasing C_w when k_{em} is smaller than k_{ep} (Figure S-1 in Supporting Information I). However, for a given C_w, BCF_p^M and BCF_m^M also range as follows

$$\mathrm{BCF}_p^{\,0} \le \mathrm{BCF}_p^{\,M} \le \mathrm{BCF}_p^{\,1}$$
$$\text{and } \mathrm{BCF}_m^{\,1} \le \mathrm{BCF}_m^{\,M}(C_w) \le \mathrm{BCF}_m^{\,0}(C_w)$$

Damage Assessment Model for Metabolized Organic Compounds. If the DAM (*12*) is applied to metabolized organic compounds, the cumulative damage from the PC and metabolites with different kinetics (i), i.e., first- and zero-order, and MM-type kinetics is:

$$\begin{cases} \dfrac{dD_p^{\,i}}{dt} = k_{ap} C_p^{\,i} - k_{rp} D_p^{\,i} \\ \dfrac{dD_m^{\,i}}{dt} = k_{am} C_m^{\,i} - k_{rm} D_m^{\,i} \end{cases} \quad (14)$$

Therefore, cumulative damage with different toxicokinetics is given by

$$\begin{aligned} D_p^{\,i}(C_w,t) &= \frac{k_{ap}}{k_{rp}} C_p^{\,i}(C_w,t) P_p^{\,i}(C_w,t) \\ &= \frac{k_{ap}}{k_{rp}} C_w \mathrm{BCF}_p^{\,i}(C_w) K_p^{\,i}(C_w,t) P_p^{\,i}(C_w,t) \end{aligned} \quad (15)$$

$$\begin{aligned} D_m^{\,i}(C_w,t) &= \frac{k_{am}}{k_{rm}} C_m^{\,i}(C_w,t) P_m^{\,i}(C_w,t) \\ &= \frac{k_{am}}{k_{rm}} C_w \mathrm{BCF}_m^{\,i}(C_w) K_p^{\,i}(C_w,t) K_m^{\,i}(C_w,t) P_m^{\,i}(C_w,t) \end{aligned} \quad (16)$$

where $D_p^i(C_w,t)$ and $D_m^i(C_w,t)$ are the cumulative damage (dimensionless) and $P_p^i(C_w,t)$ and $P_m^i(C_w,t)$ are the time-scale functions for toxicodynamics for the PC and metabolites, respectively (see Supporting Information I for details). Note that $P_p^M(C_w,t)$ and $P_m^M(C_w,t)$ are explicitly unknown functions of C_w and t. However, for a given C_w, $P_p^M(C_w,t)$ and $P_m^M(C_w,t)$ range as follows

$$P_p^{\,1}(t) \le P_p^{\,M}(C_w,t) \le P_p^{\,0}(t)$$
$$\text{and } P_m^{\,1}(t) \le P_m^{\,M}(C_w,t) \le P_m^{\,0}(t)$$

(see Supporting Information II for details).

According to the DAM (*12*), a toxic effect occurs for 50% mortality at a critical level of cumulative damage (D_L). Thus, if the metabolites toxicity is negligible ($D_L = D_p$), the time-dependent toxicity based on body residue ($\mathrm{LBR}_{50,p}(t)$) and water concentration ($\mathrm{LC}_{50,p}(t)$) are given by

$$\mathrm{LBR}_{50,p}^{\,i}(t) = \frac{D_p^{\,i}/(k_{ap}/k_{rp})}{P_p^{\,i}(t)} \quad (17)$$

$$\mathrm{LC}_{50,p}^{\,i}(t) = \frac{D_p^{\,i}/(k_{ap}/k_{rp})}{\mathrm{BCF}_p^{\,i} K_p^{\,i}(t) P_p^{\,i}(t)} \quad (18)$$

Therefore, the relationships of $\mathrm{LBR}_{50,p}(t)$ and $\mathrm{LC}_{50,p}(t)$ among different toxicokinetics are as follows

$$LBR_{50,p}{}^{0}(t) \le LBR_{50,p}{}^{M}(t) \le LBR_{50,p}{}^{1}(t) \le LBR_{50,p0}(t)$$

$$LC_{50,p0}(t) \le LC_{50,p}{}^{1}(t) \le LC_{50,p}{}^{M}(t) \le LC_{50,p}{}^{0}(t)$$

where $LBR_{50,p0}(t)$ and $LC_{50,p0}(t)$ are the median lethal body residue and the median lethal concentration in a separate exposure of the parent compound in the absence of biotransformation and metabolites (see Supporting Information II for details).

Damage Addition (DA) Hypothesis. Mortality in any toxicity experiment includes both death by the toxicant and background mortality. Total survival probability is given as a product of survival probability from the toxicant (S_{tox}) and from background (S_0) in control as follows

$$S_{total} = S_{tox}S_0 \tag{19}$$

(*13*). Since $S_{total} = 1 - M_{total}$, $S_{tox} = 1 - M_{tox}$, and $S_0 = 1 - M_0$, the above equation leads to

$$M_{total} = M_{tox} + M_0 - M_{tox}M_0 \tag{20}$$

where M_{total} is the total mortality probability, and M_{tox} and M_0 are the mortality probabilities for the toxicant and the background mortality probability, respectively. This is equivalent to stating that mortality from the toxicant and background are independent.

According to the DAM (*12*) the relationship between cumulative damage ($D(t)$) and survival probability ($S(t)$) is given by

$$S(t) = \exp(-kD(t))$$

Total cumulative damage (D_{total}) is the sum of cumulative damage by toxicant (D_{tox}) and from background (D_0) as follows:

$$D_{total} = D_{tox} + D_0$$

Similarly, if the toxicities of two compounds A and B are independent, the cumulative damage (D_{tox}) from compound A and B are additive as follows:

$$D_{tox} = D_A + D_B \tag{21}$$

then,

$$S_{tox} = S_AS_B \tag{22}$$

$$M_{tox} = M_A + M_B - M_AM_B \tag{23}$$

where D_A and D_B are cumulative damage from compound A and B; S_A, S_B and M_A, M_B are the survival (S) and mortality (M) probability from compound A and B; and D_{tox} is the cumulative damage that leads to the survival probability and mortality probability from a mixture of compound A and B. Therefore, the Damage Addition (DA) hypothesis (eq 21) is equivalent to the Independent Action (IA) model (eq 2). Thus, the DAM can be applied to a mixture of PC and metabolites, even where the mode of toxic action of the metabolites is different from that of the PC providing that the damage caused by metabolites contributes to the overall effect. Meanwhile, a mixture of compounds with similar modes of toxic action are explained by the Concentration Addition (CA) model (eq 1). According to the DAM and DA hypothesis, CA and IA models (eqs 1 and 2) are essentially the same when there is no toxicokinetic interaction among components within a mixture (see below).

Multicomponent Damage Assessment Model (DAM). The "damage addition hypothesis" (eq 21) was applied to three different situations: a simple binary mixture, and a metabolized compound showing first-, zero-order, and MM-type kinetics with and without co-exposure to a biotransformation inhibitor. Table 2 summarizes the toxicokinetic and toxicodynamic models and damage addition model for the three different situations.

Simple Binary Mixture of Nonmetabolized Compounds. For a binary mixture without toxicokinetic and toxicodynamic interactions between mixture components A and B, if the cumulative damage for two compounds in a mixture is additive, total cumulative damage (D_{tox}) equals the sum of the cumulative damage from A and B ($D_{A|B} + D_{B|A}$), and the cumulative damage by compound A or B from a separate exposure (D_A or D_B) for a given level of response as follows:

$$D_{tox} = D_{A|B} + D_{B|A} = D_A = D_B \tag{24}$$

with

$$D_{A|B} = (k_{aA}/k_{rA})C_{A|B}(t)P_A(t) = (k_{aA}/k_{rA})C_{wA|B}BCF_AK_A(t)P_A(t)$$

$$D_{B|A} = (k_{aB}/k_{rB})C_{B|A}(t)P_B(t) = (k_{aB}/k_{rB})C_{wB|A}BCF_BK_B(t)P_B(t)$$

$$D_A = (k_{aA}/k_{rA})C_A(t)P_A(t) = (k_{aA}/k_{rA})C_{wA}BCF_AK_A(t)P_A(t)$$

$$D_B = (k_{aB}/k_{rB})C_B(t)P_B(t) = (k_{aB}/k_{rB})C_{wB}BCF_BK_B(t)P_B(t)$$

Dividing both sides of eq 24 by $k_{aA}P_{r,A}(t)$ and $K_A(t)BCF_Ak_{aA}P_{r,A}(t)$, respectively, leads to

$$LBR_{50,A}(t) = LBR_{50,A|B}(t) + LBR_{50,B|A}(t)\frac{k_{aB}}{k_{aA}}\frac{P_{r,B}(t)}{P_{r,A}(t)} = LBR_{50,A}(t) + LBR_{50,B}(t)\frac{LBR_{50,A}(t)}{LBR_{50,B}(t)}$$

where

$$LBR_{50,A}(t) = \frac{D_{tox}/k_{aA}}{P_{r,A}(t)},\ LBR_{50,A|B}(t) = \frac{D_A/k_{aA}}{P_{r,A}(t)},$$
$$LBR_{50,B}(t) = \frac{D_{tox}/k_{aB}}{P_{r,B}(t)} \text{ and } LBR_{50,B|A}(t) = \frac{D_B/k_{aB}}{P_{r,B}(t)}$$

$$LC_{50,A}(t) = LC_{50,A|B}(t) + LC_{50,B|A}(t)\frac{BCF_B}{BCF_A}\frac{K_B(t)}{K_A(t)}\frac{k_{aB}P_{r,B}(t)}{k_{aA}P_{r,A}(t)} = LC_{50,A|B}(t) + LC_{50,B|A}(t)\frac{LC_{50,A}(t)}{LC_{50,B}(t)}$$

where

$$LC_{50,A}(t) = \frac{D_{tox}/k_{aA}}{BCF_AK_A(t)P_{r,A}(t)},\ LC_{50,A|B}(t) = \frac{D_A/k_{aA}}{BCF_AK_A(t)P_{r,A}(t)},$$
$$LC_{50,B}(t) = \frac{D_{tox}/k_{aB}}{BCF_BK_B(t)P_{r,B}(t)}, \text{ and } LC_{50,B|A}(t) = \frac{D_B/k_{aB}}{BCF_BK_B(t)P_{r,B}(t)}$$

$LBR_{50,A}(t)$, $LBR_{50,B}(t)$, $LC_{50,A}(t)$, and $LC_{50,B}(t)$ are the lethal body residues and the median lethal concentration of A or B for 50% mortality in separate exposures to A and B, respectively, and $LBR_{50,A|B}(t)$, $LBR_{50,B|A}(t)$, $LC_{50,A|B}(t)$, and $LC_{50,B|A}(t)$ are the lethal body residues and the median lethal concentration of A or B for 50% mortality in mixture exposures to A and B, respectively.

TABLE 2. Multicomponent Damage Assessment Model With and Without Toxicokinetic Interaction[a]

	toxicokinetics: body residue (C_i)	toxicodynamics: cumulative damage (D_i)	Damage Addition Model/Time-Dependent Toxic Unit Model ($TU_i(t)$)
a simple binary mixture of onmetabolized compound	$\left\{\begin{array}{l}\frac{dC_{A\|B}}{dt} = k_{uA}C_{wA} - k_{eA}C_{A\|B} \\ \frac{dC_{B\|A}}{dt} = k_{uB}C_{wB} - k_{eB}C_{B\|A}\end{array}\right.$	$\left\{\begin{array}{l}\frac{dD_{A\|B}}{dt} = k_{aA}C_{A\|B} - k_{rA}D_{A\|B} \\ \frac{dD_{B\|A}}{dt} = k_{aB}C_{B\|A} - k_{rB}D_{B\|A}\end{array}\right.$	$D_{tox} = D_{A\|B} + D_{B\|A} = D_A = D_B$ $TU_{A\|B}(t) + TU_{B\|A}(t) = 1$ ${}^cTU_{A\|B}(t) + {}^cTU_{B\|A}(t) = 1$
metabolized organic compound	$\left\{\begin{array}{l}\frac{dC_p}{dt} = k_u C_w - \frac{V_{max}C_p}{K_M + C_p} - k_{ep}C_p \\ \frac{dC_m}{dt} = \frac{V_{max}C_p}{K_M + C_p} - k_{em}C_m\end{array}\right.$	$\left\{\begin{array}{l}\frac{dD_p}{dt} = k_{ap}C_p - k_{rp}D_p \\ \frac{dD_m}{dt} = k_{am}C_m - k_{rm}D_m\end{array}\right.$	$D_{tox} = D_p + D_m = D_{p0} = D_{m0}$ $pTU_p(t) + \hat{p}TU_m(t) = 1$ $pqr\,{}^cTU_p(t) + \hat{p}\hat{q}\hat{r}\,{}^cTU_m(t) = 1$
biotransformation inhibition	$\left\{\begin{array}{l}\frac{dC_{p\|I}}{dt} = k_u C_w - \frac{V_{max}C_{p\|I}}{K_M + C_{p\|I}} - k_{ep}C_{p\|I} \\ \frac{C_{m\|I}}{dt} = \frac{V_{max}C_{p\|I}}{K_M + C_{p\|I}} - k_{em}C_{m\|I} \\ \frac{dC_I}{dt} = k_{uI}C_{wI} - k_{eI}C_I\end{array}\right.$	$\left\{\begin{array}{l}\frac{dD_{p\|I}}{dt} = k_{ap}C_{p\|I} - k_{rp}D_{p\|I} \\ \frac{dD_{m\|I}}{dt} = k_{am}C_{m\|I} - k_{rm}D_{m\|I} \\ \frac{dD_I}{dt} = k_{aI}C_I - k_{rI}D_I\end{array}\right.$	$D_{tox} = D_{p\|I} + D_{m\|I} + D_I = D_{p0} = D_{m0}$ $pTU_{p\|I}(t) + \hat{p}TU_{m\|I}(t) + TU_I(t) = 1$ $pqsr\,{}^cTU_p(t) + \hat{p}\hat{q}\hat{s}\hat{r}\,{}^cTU_m(t) + {}^cTU_I(t) = 1$
parent compound in the absence of biotransformation	$dC_{p0}/dt = k_u C_w - k_{ep}C_{p0}$	$dD_{p0}/dt = k_{ap}C_{p0} - k_{rp}D_{p0}$	$D_{tox} = D_{p0}$ $TU_{p0}(t) = {}^cTU_{p0}(t) = 1$

[a] See Table 1 for the definition of symbols.

Therefore, the above equations are converted to

$$LBR_{50,A}(t) = LBR_{50,A|B}(t) + LBR_{50,B|A}(t)\left(\frac{k_{aB}\,P_{r,B}(t)}{k_{aA}\,P_{r,A}(t)}\right)$$

$$\text{and } LC_{50,A}(t) = LC_{50,A|B}(t) + LC_{50,B|A}(t)\frac{LC_{50,A}(t)}{LC_{50,B}(t)}$$

The above equations can be rearranged to yield toxic unit models:

$$\frac{LBR_{50,A|B}(t)}{LBR_{50,A}(t)} + \frac{C_{B|A}(t)}{C_{A|B}(t)} = \frac{C_{A|B}(t)}{LBR_{50,A}(t)} + \frac{LBR_{50,B|A}(t)}{LBR_{50,B}(t)} = 1 \quad (25)$$

$$\frac{LC_{50,A|B}(t)}{LC_{50,A}(t)} + \frac{C_{w,B}}{LC_{50,B}(t)} = \frac{C_{w,A}}{LC_{50,A}(t)} + \frac{LC_{50,B|A}(t)}{LC_{50,B}(t)} = 1 \quad (26)$$

$$TU_{A|B}(t) + TU_{B|A}(t) = {}^cTU_{A|B}(t) + {}^cTU_{B|A}(t) = 1 \quad (27)$$

with

$$TU_{A|B}(t) \equiv C_{A|B}(t)/LBR_{50,A}(t) \equiv LBR_{50,A|B}(t)/LBR_{50,A}(t)$$

$$TU_{B|A}(t) \equiv C_{B|A}(t)/LBR_{50,B}(t) \equiv LBR_{50,B|A}(t)/LBR_{50,B}(t)$$

$${}^cTU_{A|B}(t) \equiv C_{w,A|B}/LC_{50,A}(t) \equiv LC_{50,A|B}/LC_{50,A}(t)$$

$${}^cTU_{B|A}(t) \equiv C_{w,B|A}/LC_{50,B}(t) \equiv LC_{50,B|A}/LC_{50,B}(t)$$

The eq 27 is a time-dependent Toxic Unit (TU) model for a simple binary mixture showing the characteristics of a concentration addition (CA) model for mixture toxicity. The assumption of damage addition (DA) in the DAM means independent action (IA) of mixture components, however, when there are no toxicokinetic and toxicodynamic interactions among mixture components, the DA hypothesis in the DAM is equivalent to the concentration addition (CA) model.

Metabolized Organic Compound. If the cumulative damage of the PC and metabolites is additive, the cumulative damage for the total body residue (D_{tox}) equals the sum of cumulative damage from the PC and metabolites ($D_p{}^i + D_m{}^i$), and the cumulative damage by the PC in the absence of biotransformation (D_{p0}) or by the metabolites in the absence of PC ($D^i{}_{m0}$) for a given level of response as follows

$$D_{tox} = D_p{}^i + D_m{}^i = D_{p0} = D_{m0}{}^i \quad (28)$$

where D_{p0} and $D_{m0}{}^i$ are the cumulative damage from separate exposures to the PC in the absence of biotransformation and metabolites. Even though it is actually impossible to directly measure C_{p0}, D_{p0}, $C_{m0}{}^i$, and $D_{m0}{}^i$ using a wild-type strain of test animal, it is acceptable to use the conceptual quantities such as C_{p0}, D_{p0}, $C_{m0}{}^i$, and $D_{m0}{}^i$ for the description of the intrinsic toxicity of the PC and metabolites, respectively (see Supporting Information II).

Dividing eq 28 by $(k_{ap}/k_{rp})P_{r,p0}(t)$ leads to

$$LBR_{50,p0}(t) = LBR_{50,p}{}^i(t)\frac{P_p{}^i(C_w,t)}{P_{p0}(t)} + LBR_{50,m}{}^i(t)\frac{LBR_{50,p0}(t)}{LBR_{50,m0}{}^i(t)}\frac{P_p{}^i(C_w,t)}{P_{50,m0}{}^i(t)} \quad (29)$$

where $LBR_{50,p0}(t)$ and $LBR_{50,m0}{}^i(t)$, and $P_{p0}(t)$ and $P_{m0}{}^i(t)$ are the median lethal body residues and the time-scale functions for toxicodynamics for PC in the absence of biotransformation

and metabolites from separate exposures to the PC and metabolites, respectively (see Supporting Information II), $LBR_{50,p}{}^{i}(t)$ and $LBR_{50,m}{}^{i}(t)$ are the lethal body residue of PC and metabolites in a mixture exposure.

Dividing eq 28 by $(BCF_{p0}K_{p0}(t)(k_{ap}/k_{rp})P_{p0}(t))$ leads to

$$LC_{50,p0}(t) = LC_{50,p}{}^{i}(t)\frac{P_p{}^{i}(C_w,t)}{P_{p0}(t)}\frac{K_p{}^{i}(C_w,t)}{K_{p0}(t)}\frac{BCF_p{}^{i}(C_w)}{BCF_{p0}} + LC_{50,m}{}^{i}(t)\frac{LC_{50,p0}(t)}{LC_{50,m0}{}^{i}(t)}\frac{P_m{}^{i}(C_w,t)}{P_{m0}{}^{i}(t)}\frac{K_m{}^{i}(C_w,t)}{K_{m0}{}^{i}(t)}\frac{BCF_m{}^{i}(C_w)}{BCF_{m0}{}^{i}} \quad (30)$$

where $LC_{50,p}{}^{i}(t)$, $LC_{50,m}{}^{i}(t)$, $LC_{50,p0}(t)$, and $LC_{50,m0}(t)$ are the median lethal concentration of PC and metabolites for 50% mortality from a mixture exposure (p and m), from separate exposures to the PC and metabolites assuming no biotransformation (p0 and m0), which are expressed as the water concentration of PC, but have different sources of toxicity, i.e, PC ($LC_{50,p0}(t)$) and metabolites ($LC_{50,m0}(t)$), $BCF_{m0}{}^{i}$ and $K_{m0}{}^{i}(t)$are the bioconcentration factor and the time-scale functions for toxicokinetics for PC in the absence of biotransformation and metabolites from separate exposures to the PC and metabolites, respectively (see Supporting Information II).

Thus, eqs 29 and 30 can be rearranged as follows:

$$p^{i}\frac{LBR_{50,p}{}^{i}(t)}{LBR_{50,p0}(t)} + \hat{p}^{i}\frac{LBR_{50,m}{}^{i}(t)}{LBR_{50,m0}{}^{i}(t)} = 1 \quad (31)$$

$$p^{i}q^{i}r^{i}\frac{LC_{50,p}{}^{i}(t)}{LC_{50,p0}(t)} + \hat{p}^{i}\hat{q}^{i}\hat{r}^{i}\frac{LC_{50,m}{}^{i}(t)}{LC_{50,m0}{}^{i}(t)} = 1 \quad (32)$$

with

$$p^{i} \equiv \frac{P_p{}^{i}(C_w,t)}{P_{p0}(t)},\ q^{i} \equiv \frac{K_p{}^{i}(C_w,t)}{K_{p0}(t)},\ r^{i} \equiv \frac{BCF_p{}^{i}(C_w)}{BCF_{p0}},$$
$$\hat{p}^{i} \equiv \frac{P_m{}^{i}(C_w,t)}{P_{m0}{}^{i}(t)},\ \hat{q}^{i} \equiv \frac{K_m{}^{i}(C_w,t)}{K_{m0}{}^{i}(t)},\ \text{and}\ \hat{r}^{i} \equiv \frac{BCF_m{}^{i}(C_w)}{BCF_{m0}{}^{i}}$$

The p^i, q^i, and r^i are scaled values for the toxicodynamic and toxicokinetic time-scale functions, and bioconcentration factors, respectively. In eqs 31 and 32, p^i is a function of time with the toxicokinetic parameters (k_{ep}, V_{max}, and K_m) and toxicodynamic parameter k_{rp}. The p^i is the interaction term for the toxicokinetics because the damage recovery rate constant for the PC is the same with or without biotransformation. Therefore, the extent to which p^i deviates from 1 represents the intensity of toxicokinetic interaction. From a simulation, the range of p^i and $p^iq^ir^i$ are given by

$$1 \le p^1 \le p^M \le p^0 \text{ and } p^1q^1r^1 \le p^Mq^Mr^M \le p^0q^0r^0 \le 1$$

$$1 \le \hat{p}^1 \le \hat{p}^M \le \hat{p}^0, \text{ and } \hat{p}^1\hat{q}^1\hat{r}^1 \le \hat{p}^M\hat{q}^M\hat{r}^M \le \hat{p}^0\hat{q}^0\hat{r}^0 \le 1$$

(see Supporting Information II).

As a result, the time-dependent TU model based on body residue (eq 31) can show a synergistic effect as follows:

$$TU_p(t) + TU_m(t) \le 1$$

where $TU_p(t) \equiv LBR_{50,p}(t)/LBR_{50,p0}(t)$, and $TU_m(t) \equiv LBR_{50,m}(t)/LBR_{50,m0}(t)$, because $1 \le p^i$ and $1 \le \hat{p}^i$. However, the time-dependent TU model based on water concentration (eq 32) would show an antagonistic effect for the same experiment as follows:

$${}^{c}TU_p(t) + {}^{c}TU_m(t) \ge 1$$

where ${}^{c}TU_p(t) \equiv LC_{50,p}(t)/LC_{50,p0}(t)$ and ${}^{c}TU_m(t) \equiv LC_{50,m}(t)/LC_{50,m0}(t)$, because $p^iq^ir^i \le 1$ and $\hat{p}^i\hat{q}^i\hat{r}^i \le 1$. These conflicting results are due to the metabolic interaction, which is a special case of toxicokinetic interactions.

Biotransformation Inhibition of a Metabolized Organic Compound. If the biotransformation process for an organic compound is inhibited, and cumulative damage (D_{tox}) equals the sum of cumulative damage from the PC, metabolites, and inhibitor ($D_{p|I}{}^{i} + D_{m|I}{}^{i} + D_I$), and cumulative damage from the PC in the absence of biotransformation (D_{p0}) or by the metabolites in the absence of PC ($D_{m0}{}^{i}$) for a given level of response as follows

$$D_{tox} = D_{p|I}{}^{i} + D_{m|I}{}^{i} + D_I = D_{p0} = D_{m0}{}^{i} \quad (33)$$

Thus, eq 33 leads to

$$p_I{}^{i}\frac{LBR_{50,p|I}{}^{i}(t)}{LBR_{50,p0}(t)} + \hat{p}_I{}^{i}\frac{LBR_{50,m|I}{}^{i}(t)}{LBR_{50,m0}{}^{i}(t)} + \frac{LBR_{50,I}(t)}{LBR_{50,I0}(t)} = 1 \quad (34)$$

$$p_I{}^{i}q_I{}^{i}r_I{}^{i}\frac{LC_{50,p|I}{}^{i}(t)}{LC_{50,p0}(t)} + \hat{p}_I{}^{i}\hat{q}_I{}^{i}\hat{r}_I{}^{i}\frac{LC_{50,m|I}{}^{i}(t)}{LC_{50,m0}{}^{i}(t)} + \frac{C_{w,I}}{LC_{50,I0}(t)} = 1 \quad (35)$$

where

$$p_I{}^{i} \equiv \frac{P_{p|I}{}^{i}(t)}{P_{p0}(t)},\ q_I{}^{i} \equiv \frac{K_{p|I}{}^{i}(t)}{K_{p0}(t)},\ r_I{}^{i} \equiv \frac{BCF_{p|I}{}^{i}}{BCF_{p0}},\ \hat{p}_I{}^{i} \equiv \frac{P_{m|I}{}^{i}(t)}{P_{m0}{}^{i}(t)},$$
$$\hat{q}_I{}^{i} \equiv \frac{K_{m|I}{}^{i}(t)}{K_{m0}{}^{i}(t)},\ \text{and}\ \hat{r}_I{}^{i} \equiv \frac{BCF_{m|I}{}^{i}}{BCF_{m0}{}^{i}}$$

$LBR_{50,p0}(t)$, $LBR_{50,m0}{}^{i}(t)$, and $LBR_{50,I0}(t)$ are the lethal body residues in separate exposures to the PC, metabolites, and inhibitor, respectively; $LBR_{50,p|I}{}^{i}(t)$, $LBR_{50,m|I}{}^{i}(t)$, and $LBR_{50,I}(t)$ are the lethal body residues in a mixture exposure of the PC or metabolites and inhibitor, respectively; $LC_{50,p0}(t)$, $LC_{50,m0}{}^{i}(t)$, and $LC_{50,I0}(t)$ are the median lethal concentrations in separate exposures to the PC, metabolites, and inhibitor, respectively; and $LC_{50,p|I}{}^{i}(t)$ and $LC_{50,m|I}{}^{i}(t)$ are the median lethal concentrations in a mixture exposure of the PC or metabolites and inhibitor, respectively (see Supporting Information I). The $LC_{50,m0}{}^{i}(t)$ and $LC_{50,m|I}{}^{i}(t)$ are expressed as the water concentration of PC, but their toxicity source is metabolites. The ranges of $p_I{}^{i}$,$q_I{}^{i}$,$r_I{}^{i}$, $\hat{p}_I{}^{i}$, and $\hat{p}_I{}^{i}\hat{q}_I{}^{i}\hat{r}_I{}^{i}$ are given by

$$1 \le p_I{}^{i} \le p^i, \text{ and } p^iq^ir^i \le p_I{}^{i}q_I{}^{i}r_I{}^{i} \le 1$$

$$1 \le p_I{}^{i} \le \hat{p}^i, \text{ and } \hat{p}^i\hat{q}^i\hat{r}^i \le \hat{p}_I{}^{i}\hat{q}_I{}^{i}\hat{r}_I{}^{i} \le 1$$

(see Supporting Information II for details).

In an inhibition experiment, if the inhibitor entirely blocks the metabolism of the PC, ($k_{m|I} = 0$), then $C_{m|I}$ is negligible, and all of p_I, q_I, and r_I equal 1. Therefore, when the water concentration of inhibitor ($C_{w,I}$) is constant, eqs 34 and 35 can be simplified as follows

$$\frac{LBR_{50,p|I}{}^{i}(t)}{LBR_{50,p0}(t)} + \frac{C_I(t)}{LBR_{50,I0}(t)} = 1 \quad (36)$$

$$\frac{LC_{50,p|I}{}^{i}(t)}{LC_{50,p0}(t)} + \frac{C_{w,I}}{LC_{50,I0}(t)} = 1 \quad (37)$$

Time-Dependent Toxic Unit (TU) Model. The MDAM with and without toxicokinetic interactions can be summarized by the time-dependent toxic unit model as follows:

$$p^i\mathrm{TU}^i{}_\mathrm{p}(t) + \hat{p}^i\mathrm{TU}^i{}_\mathrm{m}(t) = p^i{}_\mathrm{I}\mathrm{TU}^i{}_{\mathrm{p|I}}(t) + \hat{p}^i{}_\mathrm{I}\mathrm{TU}^i{}_{\mathrm{m|I}}(t) + \mathrm{TU}_\mathrm{I}(t) = \mathrm{TU}_{\mathrm{p0}}(t) = 1 \quad (38)$$

where $\mathrm{TU}^i{}_\mathrm{p}(t) \equiv \mathrm{LBR}^i{}_{50,\mathrm{p}}(t)/\mathrm{LBR}_{50,\mathrm{p0}}(t)$, $\mathrm{TU}^i{}_\mathrm{m}(t) \equiv \mathrm{LBR}^i{}_{50,\mathrm{m}}(t)/\mathrm{LBR}^i{}_{50,\mathrm{m0}}(t)$, $\mathrm{TU}^i{}_{\mathrm{p|I}}(t) \equiv \mathrm{LBR}^i{}_{50,\mathrm{p|I}}(t)/\mathrm{LBR}_{50,\mathrm{p0}}(t)$, $\mathrm{TU}^i{}_{\mathrm{m|I}}(t) \equiv \mathrm{LBR}^i{}_{50,\mathrm{m|I}}(t)/\mathrm{LBR}^i{}_{50,\mathrm{m0}}(t)$, and $\mathrm{TU}_\mathrm{I}(t) \equiv C_\mathrm{I}(t)/\mathrm{LBR}_{50,\mathrm{I0}}(t)$. From eq 38 it is, therefore, possible to compare the relative contribution on a toxic unit basis of the PC or metabolites in total toxicity in the presence and absence of the inhibitor at different exposure times t.

If the toxicity of metabolites is negligible, i.e., $\mathrm{TU}^i{}_\mathrm{m}$ and $\mathrm{TU}^i{}_{\mathrm{m|I}}$ are zero, eq 38 is simplified as follows:

$$p^i\mathrm{TU}^i{}_\mathrm{p}(t) = p^i{}_\mathrm{I}\mathrm{TU}^i{}_{\mathrm{p|I}}(t) + \mathrm{TU}_\mathrm{I}(t) = 1$$

If the inhibitor entirely blocks metabolism of the PC, i.e., $C^i{}_{\mathrm{m|I}}$ is negligible, eq 38 is changed as follows:

$$p^i\mathrm{TU}^i{}_\mathrm{p}(t) + \hat{p}^i{}_\mathrm{I}\mathrm{TU}^i{}_\mathrm{m}(t) = \mathrm{TU}^i{}_{\mathrm{p|I}}(t) + \mathrm{TU}_\mathrm{I}(t) = 1$$

Discussion

A Toxicokinetic Model for Biotransformation. In this study, two routes for the loss flux of PC (the satiable biotransformation process and the first-order elimination process) are assumed to be independent and the biotransformation process is neither inhibited nor induced by newly produced metabolites. Also both the uptake and elimination processes are assumed to be unaffected by the body residue level of PC or metabolites. In reality, a toxic compound can change the physiological conditions of test animals. Thus, the uptake and elimination rate may decrease by attenuating bioactivity (e.g., narcotics, *14*), or increase the uptake rate by boosting the respiration rate as a result of increasing oxygen consumption (e.g., pentachlorophenol, *15*). In the case of a PAH mixture, individual PAH congeners will be common substrates for the same biotransformation enzymes. So biotransformation of PAH mixture may be inhibited depending upon its composition, while some special components of a PAH mixture such as benzo[*a*]pyrene can induce the monooxygenase cytochrome P450 increasing biotransformation (*16*, *17*).

Comparison of Multicomponent Damage Assessment Model to Other Mixture Toxicity Models. To predict the time-dependent toxicity of a metabolized organic compound, the MDAM with toxicokinetic interactions was developed as an extended version of the DAM based on a damage addition hypothesis for the time-dependent toxicity of mixtures. Thus, total damage for toxicity is the sum of cumulative damage from PC and metabolites. The MDAM can be applied to a mixture containing compounds with different modes of toxic action such as a mixture of narcotic PC and apparently reactive toxic metabolites based on the damage addition (DA) hypothesis. If there is no toxicokinetic interaction, the DA model is equivalent to the CA model (eqs 27). In this case, the DA model represents the situation where the CA model equals the IA model. The equivalence of the CA and IA models was theoretically demonstrated for the condition when the individual tolerance distribution is a Weibull distribution (*4*, *7*, *18*). Experimentally the same situation can occur when the concentration−response relationship of each mixture component is described by the Weibull model, where the dose−response curves are strictly parallel, and the slope parameter takes a value of 1 based on the natural logarithm (*19*).

From the MDAM, a 'time-dependent toxic unit' model was derived (eq 38). Using an iso-effective approach for concentration−time-response relationship, for a given effect level, e.g., 50%, the time-dependent toxicity in the presence and absence of an biotransformation inhibitor can be compared with a time-dependent toxic unit model. This is the first derivation and development of a "time-dependent" toxic unit model for a chemical mixture with toxicokinetic interactions, and was made possible because a toxicokinetic and toxicodynamic model such as DAM could be successfully applied to the time-dependent mixture toxicity. It was particularly important, for the mixture of different chemicals with toxicokinetic interactions, to differentiate the definitions of "toxic unit" for PC based on water concentration ($^\mathrm{c}\mathrm{TU}(t) \equiv \mathrm{LC}_{50,\mathrm{p}}(t)/\mathrm{LC}_{50,\mathrm{p0}}(t)$) and body residue ($\mathrm{TU}(t) \equiv \mathrm{LBR}_{50,\mathrm{p}}(t)/\mathrm{LBR}_{50,\mathrm{p0}}(t)$), because $\mathrm{TU}(t)$ is not equal to $^\mathrm{c}\mathrm{TU}(t)$, but rather to $qr\,^\mathrm{c}\mathrm{TU}(t)$, where $q = K_\mathrm{p}(t)/K_{\mathrm{p0}}(t)$ and $r = \mathrm{BCF}_\mathrm{p}/\mathrm{BCF}_{\mathrm{p0}}$. Since qr is equal to $C_\mathrm{p}/C_{\mathrm{p0}}$, qr ranges from r to 1. Therefore, $\mathrm{TU}_\mathrm{p}(t)$ is always smaller than $^\mathrm{c}\mathrm{TU}_\mathrm{p}(t)$.

Role of Biotransformation and the Inhibition of Biotransformation. Metabolism of an organic compound in the field can be inhibited or induced during exposure to a complex mixture. Therefore, the role of inhibition and induction of biotransformation needs to be assessed as the combined toxicity of mixture components with toxicokinetic interaction (competitive inhibition, induction by inducers, or noncompetitive inhibition by other inhibitors or inducers). However, it is not easy to assess the role of biotransformation in toxicity, because criteria for diagnosing deviation from the reference condition of zero-interaction such as synergism and antagonism are not simple. Based on body residue, the role of biotransformation on the toxicity of metabolized compounds can be Loewe synergism (eq 3), but based on water concentration, it is always Loewe antagonism (eq 4). Thus, it is a more practical and important issue to assess the relative toxicity of metabolites compared with the PC using body residues for understanding the toxicity of a metabolized organic compound.

The role of biotransformation is usually classified as detoxification or intoxication based on whether metabolites are more toxic than the PC. However, it is not simple to measure the body residue of individual metabolites and to directly determine the toxicity of individual metabolites. So the fraction of metabolites in total body residue ($f_\mathrm{m}(t)$) is usually measured (*20*), and toxic unit of metabolites ($\mathrm{TU}_\mathrm{m}(t)$) corresponding to the relative contribution of metabolites to total toxicity are determined. From the $f_\mathrm{m}(t)$ and $\mathrm{TU}_\mathrm{m}(t)$, the relative toxicity of metabolites ($\gamma(t)$) can be calculated

$$\gamma(t) \equiv \frac{\mathrm{TU}_\mathrm{m}(t)/C_\mathrm{m}(t)}{\mathrm{TU}_\mathrm{p}(t)/C_\mathrm{p}(t)} = \frac{(1 - p\mathrm{TU}_\mathrm{p}(t))/f_\mathrm{m}(t)}{\hat{p}\mathrm{TU}_\mathrm{p}(t)/(1 - f_\mathrm{m}(t))}$$

where $f_\mathrm{m}(t)$ is the percent of metabolites in total body residue. Note that the $\mathrm{TU}_\mathrm{m}(t)$ and $\gamma(t)$ are not for an individual metabolite, but for a mixture of metabolites. The estimated $\mathrm{TU}_\mathrm{m}(t)$ and $\gamma(t)$, therefore, represent the total body residue of metabolites, including different metabolites whose toxicity is similar to, less than, or more toxic than the PC. Since the composition of individual metabolites as well as total amount of metabolites can change during the biotransformation process, the $\mathrm{TU}_\mathrm{m}(t)$ and $\gamma(t)$ are not constant, but a function of time and body residue of toxicant.

Meanwhile, if the individual metabolites are investigated, the $\gamma(t)$ can be given by

$$\gamma(t) \equiv \frac{\mathrm{LBR}_{50,\mathrm{p0}}(t)}{\mathrm{LBR}_{50,\mathrm{m0}}(t)} = \frac{(k_\mathrm{am}/k_\mathrm{rm})P_\mathrm{m0}(t)}{(k_\mathrm{ap}/k_\mathrm{rp})P_{\mathrm{p0}}(t)} \quad (39)$$

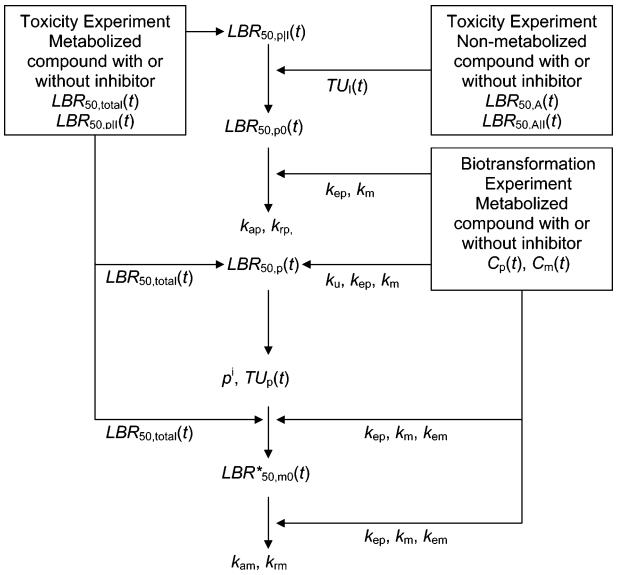

FIGURE 1. Scheme for the estimation of toxicodynamic parameters for parent compound and metabolites using time-dependent toxicity data for mixture of parent compound and metabolites. See Table 1 for the definition of symbols.

where the ratio of $P_{m0}(t)/P_{p0}(t)$ reflects the impact of the toxicokinetic interaction on the time-dependent toxicity. Therefore, $\gamma(t)$ does not give any concrete information about metabolite toxicity, so determination of a time-independent and intrinsic parameter, i.e., the relative toxic potency ($\bar{\gamma}$) of metabolites compared to that of PC provides more insight. The $\bar{\gamma}$ can be defined as

$$\bar{\gamma} \equiv \lim_{t\to\infty}\gamma(t) \equiv \lim_{t\to\infty}\frac{\mathrm{LBR}_{50,p0}(t)}{\mathrm{LBR}_{50,m0}(t)} = \frac{\mathrm{LBR}_{50,p0}(t=\infty)}{\mathrm{LBR}_{50,m0}(t=\infty)} = \frac{(k_{am}/k_{rm})}{(k_{ap}/k_{rp})} \quad (40)$$

Experimental Design and Data Analysis Method for the Assessment of the Relative Toxicity of Metabolites. According to MDAM, the time-dependent toxicity of a metabolized organic compound can be analyzed as mixture toxicity of PC and metabolites. To estimate the toxicodynamic parameters, follow the schematic given in Figure 1.

First, the toxicity of PC and metabolites are separately assessed by blocking the biotransformation using a biotransformation inhibitor. Body residues of PC and metabolites need to be separately measured and then are described by a toxicokinetic model. The metabolic interaction between test compound and inhibitor can be analyzed by comparing toxicokinetics in the presence and absence of the inhibitor. Since metabolites are not supposed to occur in the presence of the inhibitor, the toxicity in the presence of the inhibitor can be considered to be due to PC and the inhibitor. The toxicity in the absence of the inhibitor is due to the PC and metabolites.

The time-dependent toxicity of the PC ($\mathrm{LBR}_{50,p0}(t)$ or $\mathrm{LC}_{50,p0}(t)$) would be determined directly by blocking biotransformation if the relative contribution of the inhibitor to the total toxicity is negligible. However, the relative contribution of the inhibitor may be not negligible, because sufficient concentration of the inhibitor to block biotransformation can exert toxicity. Thus, it is necessary to determine the time-dependent toxicity of the inhibitor or at least the relative contribution of the inhibitor to the total toxicity ($\mathrm{TU}_I(t)$). However, it is difficult to determine the time-dependent toxicity of the inhibitor because it is not possible with reasonable effort to measure the inhibitor body residue in small test animals such as *H. azteca*. However, the time-dependent toxic unit of the inhibitor ($\mathrm{TU}_I(t)$) can be indirectly determined in toxicity experiments using a nonmetabolized compound in the presence and absence of the inhibitor. Since there is no metabolic interaction, the toxicokinetic parameters of nonmetabolized compound in the presence and absence of the inhibitor are statistically the same. Thus, it is reasonable to assume that the toxicity of the nonmetabolized compound (A) and inhibitor are additive ($\mathrm{TU}_I(t) + \mathrm{TU}_{A|I}(t) = 1$), and then $\mathrm{TU}_I(t)$ can be calculated as

$$\mathrm{TU}_I(t) = 1 - \mathrm{LBR}_{50,A|I}(t)/\mathrm{LBR}_{50,A}(t)$$

The $\mathrm{TU}_I(t)$ can be used for determination of $\mathrm{LBR}_{50,p0}(t)$ as follows:

$$\mathrm{LBR}_{50,p0}(t) = \frac{\mathrm{LBR}_{50,p|I}(t)}{1 - \mathrm{TU}_I(t)}$$

The $\mathrm{LBR}_{50,p0}(t)$ can be used to calculate the toxicodynamic parameters for PC (k_{ap} and k_{rp}) using the following equation:

$$\mathrm{LBR}_{50,p0}(t) = \frac{D_L/(k_{ap}/k_{rp})}{P_{p0}(t)} \quad (41)$$

(see Supporting Information I).

Since $\mathrm{TU}_p(t)$ can be calculated by $\mathrm{LBR}_{50,p}(t)/\mathrm{LBR}_{50,p0}(t)$, the time-dependent toxicity of metabolites ($\mathrm{TU}_m(t)$) can be also estimated by $(1 - p^i\mathrm{TU}_p(t))/\hat{p}^i$, where p^i is a function of time with k_{ep}, k_m, and k_{rp}, and $\hat{p}^i$ is a function of time with k_{ep}, k_m, k_{em}, and k_{rm}. Since k_{ep} and k_m are estimated by measuring body residues of PC and metabolites in toxicokinetic experiment, k_{rm} can be also estimated (see below).

From eq 31, $\mathrm{LBR}_{50,m0}{}^i(t)$ is given by

$$\mathrm{LBR}_{50,m0}{}^i(t) = \frac{P_m{}^i(C_w,t)}{P_{m0}{}^i(t)} \frac{\mathrm{LBR}_{50,m}{}^i(t)}{1 - p^i(\mathrm{LBR}_{50,p}{}^i(t)/\mathrm{LBR}_{50,p0}{}^i(t))} \quad (42)$$

Since $\mathrm{LBR}_{50,m0}{}^i(t)$ is also defined by DAM as

$$\mathrm{LBR}_{50,m0}{}^i(t) \equiv \frac{D_{tox}/(k_{am}/k_{rm})}{P_{m0}{}^i(t)}$$

eq 42 leads to

$$\mathrm{LBR}_{50,m0}{}^{i,*}(t) \equiv \frac{D_{tox}/(k_{am}/k_{rm})}{P_m{}^i(t)} = \frac{\mathrm{LBR}_{50,m}{}^i(t)}{1 - p^i(\mathrm{LBR}_{50,p}{}^i(t)/\mathrm{LBR}_{50,p0}{}^i(t))} \quad (43)$$

Meanwhile, $\mathrm{LBR}_{50,m}(t)$ and $\mathrm{LBR}_{50,p}(t)$ can be measured or calculated from $\mathrm{LBR}_{50,total}(t)$ and a fraction of metabolites as

$$\mathrm{LBR}_{50,m}{}^i(t) = \mathrm{LBR}_{50,total}{}^i(t) f_m{}^i(t) \quad \text{and} \quad \mathrm{LBR}_{50,p}{}^i(t) = \mathrm{LBR}_{50,total}{}^i(t)(1 - f_m{}^i(t))$$

Therefore, eq 43 is converted into

$$\mathrm{LBR}_{50,m0}{}^{i,*}(t) \equiv \frac{D_{tox}/(k_{am}/k_{rm})}{P_m{}^i(t)} = \frac{f_m{}^i(t)}{\dfrac{1}{\mathrm{LBR}_{50,total}{}^i(t)} - p^i(t)\dfrac{(1 - f_m{}^i(t))}{\mathrm{LBR}_{50,p0}(t)}} \quad (44)$$

where

$$f_m{}^i(t) \equiv \frac{C_m{}^i(t)}{C_m{}^i(t) + C_p{}^i(t)} = \frac{K_m{}^i(t)}{K_m{}^i(t) + (\mathrm{BCF_p}{}^i/\mathrm{BCF_m}{}^i)K_p{}^i(t)},$$

$$\mathrm{LBR}_{50,p0}(t) = \frac{D_{tox}/(k_{ap}/k_{rp})}{P_{p0}(t)},\ p^i(t) \equiv \frac{P_p{}^i(t)}{P_{p0}(t)}$$

and $\mathrm{LBR}_{50,total}{}^i(t)$ can be experimentally estimated or measured. The left side of eqs 43 and 44 is a function of both toxicodynamic parameters such as k_{am} and k_{rm}, and toxicokinetic parameters, which can be estimated from the independent experiments. Therefore, from eqs 43 and 44, k_{am} and k_{rm} can be estimated, but when the biotransformation process shows MM-type kinetics, a new method for parameter estimation needs to be developed, because $P_m{}^M(C_w,t)$ is not an explicitly known function. In addition, the relative toxic potency of metabolites ($\bar{\gamma}$) can be calculated (eq 40). Finally, the estimated toxicokinetic and toxicodynamic parameters can be used to assess the influence of toxicokinetic interaction such as biotransformation inhibition or induction.

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Supporting Information Available

Biocooncentration factor, toxicokinetic and toxicodynamic time-scale functions, median lethal body residue and median lethal concentration for parent compound and metabolites, and toxicokinetic interaction by biotransformation and time-dependent toxicity. This material is available free of charge via the Internet at http://pubs.acs.org.

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