

Prediction of Time-Dependent PAH Toxicity in *Hyalella azteca* Using a Damage Assessment Model

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A damage assessment model (DAM) was developed to describe and predict the toxicity time course for PAH in *Hyalella azteca*. The DAM assumes that death occurs when the cumulative damage reaches a critical point and was described by a combination of both first-order toxicokinetic and toxicodynamic models. In aqueous exposures, body residues increase in proportion to the water concentration. Damage is assumed to accumulate in proportion to the accumulated residue and damage recovery in proportion to the cumulative damage when damage is reversible. As a result, the toxicity time course, $LC_{50}(t)$, is determined by both a damage recovery rate and an elimination rate. The constant critical body residue (CBR) and the critical area under the curve (CAUC) models can be derived as two extreme cases from the DAM, and all three models were reanalyzed using a hazard modeling approach. As a result, the critical cumulative damage (D_L) is the determinant of the concentration–time response relationship and not simply the CBR or the CAUC. Finally, from the DAM, two parameters, a damage recovery rate constant k_r and the killing rate k_t , were estimated and found to be relatively constant for selected PAH.

Introduction

The time- and concentration-dependent toxicity curve that yields 50% mortality is a graphic summary of mortality data acquired in a toxicity experiment (1, 2). To fit time series data of $LC_{50}(t)$ or $LT_{50}(c)$, the empirically selected models for $LC_{50}(t)$ are generally given by a hyperbolic function of time (3–5), e.g., Ostwald's equation (6). The toxicity level should be linearly related to the product dose times duration, or " ct " (concentration \times time). Therefore, at the fixed toxicity level, the product of dose and duration should be constant.

In the field of aquatic toxicology, a critical body residue (CBR) model was used as a first attempt to describe and predict the time course of toxicity based on the assumption that CBR, the body residue for 50% mortality, is constant for exposure conditions such as exposure concentration and

time (2). For nonpolar narcotic (anesthetic) compounds such as PAH, this model assumed a first-order toxicokinetic model to predict the time-dependent LC_{50} and the incipient median lethal concentration ($LC_{50}(\infty)$) at steady state (1, 7–10). From the constant CBR model, both the $LC_{50}(t)$ and the CBR should be constant at steady state.

In the companion paper (11), the bioaccumulation process of PAH in *H. azteca* was described and predicted by a one-compartment first-order toxicokinetic model. However, both the $LC_{50}(t)$ and the CBR values decreased with increasing exposure time after *H. azteca* attained steady state (11). Thus, it is necessary to assume a time-limiting step other than toxicokinetics to model the toxicodynamic process.

To investigate the toxicity time course for reactive and receptor-mediated compounds, the critical area under the curve (CAUC) model was recently derived on the basis of integrals of time and concentration as a dose metric (12, 13). The CAUC model is compared with the constant CBR model, which is based on the peak concentration as a dose metric. The CAUC model was considered as the first theoretical explanation for the hyperbolic relationship between exposure concentration and time shown in Ostwald's equation (12).

The simplest case for a toxicodynamic model would be for recovery from an acute injury to follow a one-compartment model (14). Recently, Ankley et al. (15) suggested a conceptual model for prediction of the phototoxicity of PAH using a one-compartment first-order toxicodynamic model adapted to the condition of constant body residue. However, Ankley et al. (15) failed to describe and predict the time course of toxicity for PAH.

For this study, a one-compartment first-order toxicokinetic–toxicodynamic model for minimally metabolized nonpolar organic compounds was derived to describe and predict the time course of toxicity and compared with the CBR and CAUC models (see Table 1 for significant abbreviations used in the text). Furthermore, the concentration–time response relationship based on the toxicokinetic–toxicodynamic model was compared with the empirically derived Ostwald's equation.

Theory

Empirically Derived Toxicity Model: Ostwald's Equation.

Usually, there is a balance between dose and time, with a low dose and a long exposure time producing a similar effect to a high dose and a short exposure time. A formalization of this is provided by Ostwald's equation (6): if a given level of response (k_p), e.g., percent mortality ($p\%$) is produced by concentration c and time-to-response t , then Ostwald's equation predicts the relationship

$$ct^\lambda = k_p \quad (1)$$

for constant k_p and λ .

Variability in the toxic response results from the variability in the time course of accumulation and the amount of compound delivered to the site(s) of toxic action (toxicokinetics) and/or the variability in the time course of the response to the target dose (toxicodynamics) (14). Various toxicodynamic models have been suggested (see ref 16) with the common objective to describe and predict the concentration–time response relationship of exposure concentration to time-to-death or LC_{50} or LD_{50} at fixed exposure times. These models are not based on toxic mechanism but rather on empirical relationships, e.g., a hyperbolic relationship between time and concentration, and/or statistical assump-

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TABLE 1. List of Symbols and Their Dimension for Variables and Parameters

symbol	unit	interpretation
c	mmol L ⁻¹	water concentration
t	h	exposure time
$R(t; c)$	mmol kg ⁻¹	body residue of the compound
k_u	L kg ⁻¹ h ⁻¹	uptake clearance rate (eq 13)
k_e	h ⁻¹	elimination rate constant (eq 13)
$D(t; c)$		cumulative damage (eq 15)
k_a	mmol ⁻¹ ·kg·h ⁻¹	damage accrual rate (eq 14)
k_r	h ⁻¹	damage recovery rate constant (eq 14)
$LC_{50}(t)$	mmol L ⁻¹	median lethal concentration (eq 16)
$LC_{50}(\infty)$	mmol L ⁻¹	incipient LC_{50} at the infinite time
$CBR(t)$	mmol kg ⁻¹	critical body residue level corresponding to 50% mortality (eq 17)
$CBR(\infty)$	mmol kg ⁻¹	incipient CBR at infinite time
LT_{50}	h	the median lethal time
MLR	mmol kg ⁻¹	mean lethal residues for dead animals within treatment
D_L		critical cumulative damage level corresponding to 50% mortality (eq 16)
R_{ss}	mmol kg ⁻¹	body residue at steady state
$AUC(t; c)$	mmol kg ⁻¹ h	area under the curve ($\int R(t, c) dt$)
$CAUC$	mmol kg ⁻¹ h	critical area under curve corresponding to 50% mortality (eq 7)
$h(t; c)$	h ⁻¹	hazard rate function (eq 28)
$H(t; c)$		cumulative hazard function (eq 29)
$S(t; c)$		survival probability function (eq 30)
k_1	mmol ⁻¹ kg	killing rate constant in hazard model (eq 31)
k_2	mmol ⁻¹ kg h ⁻¹	killing rate based on body residue corresponding to k_a in DAM (eq 33)
k_3		coefficient between H and D (eq 36)
k_t	mmol ⁻¹ kg h ⁻¹	killing rate based on body residue corresponding to $(k_3 k_a)$ (eq 46)
x		% of mortality
D_x		damage level for $x\%$ of mortality
LT_x	h	time-to-death for $x\%$ of mortality
LC_x	mmol L ⁻¹	lethal concentration corresponding to $x\%$ of mortality
$P(t)$ or $P(LT_{50})$	h	time scale function in toxicokinetic and toxicodynamic processes (eqs 40 and 41)

tions such as log-normal distribution of individual tolerance in probit analysis:

$$\text{Green's model (3): } LD_{50} = a_1 + b_1(1/t) \quad (2)$$

$$\text{Finney's model (17): } P_p = a_2 + b_2 \ln(D) + c_2 \ln t \quad (3)$$

$$\text{Newman's model (18): } \ln LT_{50} = a_3 + b_3 \ln c \quad (4)$$

where LD_{50} is the median lethal dose yielding 50% mortality; t is exposure time; P_p is the probit of $p\%$ mortality; D is the dose yielding $p\%$ mortality; LT_{50} is the median lethal time for half of the test animals to die in each treatment; c is the external exposure concentration; $a_1, a_2, a_3, b_1, b_2, b_3,$ and c_2 are constants.

According to these models, the toxicity time course is a hyperbolic time function independent of the bioconcentration kinetics. However, the constants in these empirically derived models are not defined by toxicological mechanism but rather on statistical significance.

Mechanism-Based Toxicity Model: The Constant CBR Model and the CAUC Model. *Constant CBR Model.* According to the narcosis hypothesis, CBR is constant for exposure concentration and time within the compound groups with the same mode of toxic action (19). The time-dependent nature of CBR was actually expressed as the difference between the CBR for acute and chronic tests (19). In the case of narcotic compounds, the ratio of acute to chronic CBR for lethal effect was estimated to be about 10 from the experimental data (20). The difference between acute and chronic CBR values was usually explained as differences in the mode of toxic action between acute and chronic toxicity.

CAUC Model. Recently, for reactive or receptor-mediated toxicants, theoretically derived toxicology models having a hyperbolic time function were suggested (12, 13). The model for these irreversibly bound toxicants was well-explained by

two models that considered exposure time and body residue, the CAUC model (12) and the critical target occupation (CTO) model (13). One of the major differences between the toxicity of narcotics and reactive chemicals is that reactive chemicals interact essentially irreversibly with the target, while narcotics interact reversibly. With irreversible receptor binding, it is not simply the target site concentration but the total amount of target affected or occupied that is the relevant dose parameter (12, 13, 21). Thus, the concentration of affected target ($C_{\text{affected target}}$) can be modeled as follows (12):

$$\frac{dC_{\text{affected target}}}{dt} = k_a C_{\text{target}} C_{\text{toxicant}} - k_d C_{\text{affected target}} \quad (5)$$

where C_{target} is a concentration of target, C_{toxicant} is a concentration of toxicant, and k_a and k_d are constants. Assuming absolutely irreversible interaction ($k_d = 0$), the model leads to

$$LC_{50}(t) = \frac{CAUC}{BCF} \frac{1}{t - (1 - e^{-k_e t})/k_e} \quad (6)$$

In the case that target site can be renewed by biosynthesis, the $LC_{50}(t)$ is eventually expected to reach an incipient value ($LC_{50}(\infty)$):

$$LC_{50}(t) = \frac{CAUC}{BCF} \frac{1}{t - (1 - e^{-k_e t})/k_e} + LC_{50}(\infty) \quad (7)$$

Thus, the CBR at the target site depends on the exposure time:

$$CBR(t) = \frac{CAUC}{\frac{t}{(1 - e^{-k_e t})} - \frac{1}{k_e}} + CBR(\infty) \quad (8)$$

If the exposure time is sufficiently long to reach steady state, the $LC_{50}(t)$ is a hyperbolic function of the exposure time t :

$$LC_{50}(t) = \frac{CAUC}{BCF} \frac{1}{t} + LC_{50}(\infty) \quad (9)$$

and the concentration–time response relationship under the steady state is given by

$$\log(LC_{50}(t) - LC_{50}(\infty)) + \log t = \log(CAUC/BCF) \quad (10)$$

This relationship is essentially the same as Green's empirically derived model (eq 2).

While the CAUC model and other empirical models such as Green's model (eq 2) assume an irreversible interaction between toxicant and target site, typical narcotics such as PAH are assumed to interact reversibly with the target site, membrane lipid. If the toxicity is rapidly and absolutely reversible, the exposure history does not matter, and the peak concentration at the target tissue is more important.

Derivation of DAM. To create a general model, we must not assume a priori whether the toxicity of a compound is reversible, but rather we must investigate the extent of reversibility. The DAM is based on three assumptions. First, the compound accumulates by the simple first-order kinetics:

$$\frac{dR}{dt} = k_u c - k_e R \quad (11)$$

where c is the aqueous exposure concentration (mmol L^{-1}), R is the tissue residue (mmol kg^{-1}), k_u is the uptake clearance rate ($\text{L kg}^{-1} \text{h}^{-1}$), k_e is the elimination rate constant (h^{-1}), and t is exposure time (h). For actively metabolized organic compounds such as PAH in fish and naphthalene in *H. azteca*, the model would need to be modified.

Second, organism damage accumulates in proportion to the tissue residue, and the damage recovery is proportional to the cumulative damage (reversible damage):

$$\frac{dD}{dt} = k_a R - k_r D \quad (12)$$

where D is the cumulative damage (dimensionless), k_a is a rate for accrual of damage ($\text{kg mmol}^{-1} \text{h}^{-1}$), and k_r is a first-order rate constant for damage recovery (h^{-1}). This model can be applied to compounds with rapid reversible binding to the target site ($k_r \approx \infty$) as well as to reactive and receptor-mediated compounds with irreversible binding ($k_r \approx 0$) (12, 13). However, for this model, it is simply assumed that in addition to the bioconcentration kinetics there is a second rate-limiting step that is critical for modeling the time-dependent toxic response.

The third assumption is that death occurs when damage accrues to a certain critical lethal level, D_L . If c is constant and $D(0)$ is zero, the cumulative damage function $D(t)$ is given by

$$D(t) = k_a \frac{k_u}{k_e} c \left(\frac{e^{-k_r t} - e^{-k_e t}}{k_r - k_e} + \frac{1 - e^{-k_r t}}{k_r} \right) \quad (13)$$

If $D(t)$ can be denoted by D_L for the extent of damage that produces 50% mortality, the time-dependent median lethal concentration $LC_{50}(t)$ and the time-dependent critical body residue (CBR(t)) are as follows:

$$LC_{50}(t) = \frac{D_L/k_a}{\frac{k_u}{k_e} \left(\frac{e^{-k_r t} - e^{-k_e t}}{k_r - k_e} + \frac{1 - e^{-k_r t}}{k_r} \right)} \quad (14)$$

$$CBR(t) = \frac{D_L/k_a}{\frac{1}{(1 - e^{-k_e t})} \left(\frac{e^{-k_r t} - e^{-k_e t}}{k_r - k_e} + \frac{1 - e^{-k_r t}}{k_r} \right)} \quad (15)$$

with D_L/k_a in $\text{mmol kg}^{-1} \text{h}$. The coefficient D_L/k_a is equivalent to the product of tissue residue and exposure time. Therefore, the coefficient D_L/k_a can be viewed as the compound equivalent toxic damage level required for 50% mortality.

Relationship of the DAM to the CBR and CAUC Models.

Both the constant CBR model and the CAUC model are special cases of the damage assessment model.

According to the narcosis hypothesis, the damage is reversible (not accumulated), for any given exposure duration, and eq 12 should always equal zero:

$$\frac{dD}{dt} = k_a R - k_r D = 0 \quad (16)$$

Thus, the tissue residue corresponding to D_L should be a constant value equivalent to the CBR:

$$D_L/k_a = \frac{CBR}{k_r} \quad (17)$$

with CBR in mmol kg^{-1} . Under these conditions, $D(t)$ would also be zero, if $k_a = 0$ (no damage accumulation) or $k_r = \infty$ (extremely rapid reversibility). If k_a was zero, then the biological response would not be caused by the internal dose. If k_r is infinite, then the damage is not accumulated. In this case, eq 14 is modified as follows:

$$LC_{50}(t) = \lim_{k_r \rightarrow \infty} \frac{D_L/k_a}{\frac{k_u}{k_e} \left(\frac{e^{-k_r t} - e^{-k_e t}}{k_r - k_e} + \frac{1 - e^{-k_r t}}{k_r} \right)} \quad (18)$$

$$= \frac{CBR}{\frac{k_u}{k_e} (1 - e^{-k_e t})} \quad (19)$$

Equation 19 is the same as the constant CBR model (7, 22).

In the case of reactive or receptor-mediated compounds, the interaction of these compounds with target is assumed to be irreversible (12). Therefore, the dose metric is not the instantaneous peak concentration such as the CBR but is the time-integrated concentration such as the CAUC. This situation is similar to the condition where k_r equals zero. If k_r equals zero, then eq 12 is simplified as follows:

$$\frac{dD}{dt} = k_a R = k_a \frac{k_u}{k_e} c (1 - e^{-k_e t}) \quad (20)$$

Thus, $D(t)$ is then given by

$$D(t) = k_a \int_0^t R dt = k_a \times AUC(t) \quad (21)$$

where $AUC(t)$ stand for "area under the curve" from time 0 to time t and has units of $\text{mmol kg}^{-1} \text{h}$. Under conditions of 50% mortality, $D(t)$ can be denoted D_L and $AUC(t)$ would be a time-independent constant, "critical area under the curve (CAUC)". Therefore, in the case of $k_r = 0$, a relationship between D_L and CAUC is as follows:

$$D_L/k_a = CAUC \quad (22)$$

with both D_L/k_a and CAUC in units of $\text{mmol kg}^{-1} \text{h}$. Therefore, if k_r is zero, eq 14 is simplified as follows:

$$LC_{50}(t) = \frac{D_L/k_a}{\frac{k_u}{k_e} \left(t + \frac{e^{-k_e t}}{k_e} - \frac{1}{k_e} \right)} \quad (23)$$

which reduces to

$$LC_{50}(t) = \frac{\text{CAUC}}{\frac{k_u}{k_e} \left(t - \frac{1 - e^{-k_e t}}{k_e} \right)} \quad (24)$$

Therefore, the CAUC model (12, 13) is derived from the DAM under the assumption that k_r equals zero.

Hazard Modeling Using the DAM. To investigate the relationship among body residues, cumulative damage, and survival rate, a hazard model can be used (23, 24). The simple hazard model without control mortality is given by

$$h(t) = -\frac{1}{S(t)} \frac{dS(t)}{dt} \quad (25)$$

$$H(t) = \int_0^t h(t) dt = -\ln S(t) \quad (26)$$

$$S(t) = e^{-H(t)} \quad (27)$$

where $h(t)$ is the hazard rate function in h^{-1} , $H(t)$ is the cumulative hazard (dimensionless), and $S(t)$ is the survival probability (dimensionless). To simplify the simulation, it is assumed that there is no toxicity threshold. There can be three types of hazard models relating the survival probability and lethal residue or cumulative damage, which corresponds to the CBR and CAUC models and the DAM.

For the constant CBR model, the cumulative hazard function ($H(t)$) is proportional to the body residue:

$$H_1(t) = k_1 R(t) \quad (28)$$

$$= k_1 \frac{k_u}{k_e} c (1 - e^{-k_e t}) \quad (29)$$

where the unit of k_1 is kg mol^{-1} and k_1 can be called a killing rate constant. In eq 29, the time-dependent toxicity is controlled only by kinetic processes. Thus, the lethal effects of a toxicant should be constant as tissue residue approaches steady state. This equation yields the same toxicity time course as the constant CBR model. It also indicates that the cumulative damage function results from the assumption of very large k_r .

For the CAUC model, the hazard function ($h(t)$) is proportional to the body residue:

$$h_2(t) = k_2 R(t) \quad (30)$$

where the units of k_2 are $\text{kg mol}^{-1} \text{h}^{-1}$ and k_2 is a killing rate. This equation is the same as the Kooijman's hazard model (24) except for the assumption that the no effect concentration is zero. From eqs 30 and 26, the cumulative hazard function $H(t)$ is given by

$$H_2(t) = \int_0^t h_2(t) dt = k_2 \frac{k_u}{k_e} c \left(t - \frac{1 - e^{-k_e t}}{k_e} \right) \quad (31)$$

Equation 31 has the same form as the DAM under the assumption that k_r is zero. Thus, k_2 corresponds to k_a in the DAM. According to this model, if there is no toxicity threshold, the survival rate for any treatment level at infinite time should be zero.

Finally, for the DAM, the cumulative hazard is proportional to the cumulative damage level:

$$H_3(t) = k_3 D(t) \quad (32)$$

where k_3 is a dimensionless coefficient. This equation is based on a process of damage accumulation that can be limited by both k_e and k_r :

$$H_3(t) = k_3 k_a \frac{k_u}{k_e} c \left(\frac{e^{-k_r t} - e^{-k_e t}}{k_r - k_e} + \frac{1 - e^{-k_r t}}{k_r} \right) \quad (33)$$

According to eq 33, the survival rate ($\exp(-k_3 H_3(\infty))$) for any treatment level at the infinite time is zero or a positive constant value.

Table 2 summarizes the characteristics of the hazard models corresponding to the CBR and CAUC models and the DAM, respectively. On the basis of the above analysis, cumulative hazard ($H(t)$) corresponds to the cumulative damage ($D(t)$) in the DAM. Therefore, using eqs 27 and 33, the survival probability can be related to the body residue and the cumulative damage function based on the DAM.

Finally, from eq 33, the survival probability, $S(t; c)$, for a given treatment level c at the exposure time t is given by

$$S(t; c) = \exp(-k_3 D(t; c)) \quad (34)$$

where $S(t; c)$ is the survival probability for a given treatment level c at exposure time t , and $D(t; c)$ is the cumulative damage. Thus, for a given percent of mortality (x) in a given treatment c , $S(t; c) = (100 - x)/100$, the relationship between a given treatment level c and time-to-death LT_x at that treatment level for a given $x\%$ mortality is

$$D_x(LT_x; c) = -1/k_3 \ln((100 - x)/100) \quad (35)$$

where $D_x(LT_x; c)$ is a constant value for a given mortality level $x\%$. This leads to two combinations with (LT_x, c) or (t, LC_x) as follows:

$$\ln((100 - x)/100) = -k_3 k_a (k_u/k_e) c P(LT_x) \quad (36)$$

$$P(LT_x) = \left(\frac{e^{-k_r LT_x} - e^{-k_e LT_x}}{k_r - k_e} + \frac{1 - e^{-k_r LT_x}}{k_r} \right) \quad (37)$$

or

$$\ln((100 - x)/100) = -k_3 k_a (k_u/k_e) LC_x(t) P(t) \quad (38)$$

$$P(t) = \left(\frac{e^{-k_r t} - e^{-k_e t}}{k_r - k_e} + \frac{1 - e^{-k_r t}}{k_r} \right) \quad (39)$$

where $P(LT_x)$ and $P(t)$ are a time scale function for both the toxicokinetic and toxicodynamic processes. In eq 36, for a given $x\%$ mortality, LT_x is a dependent variable and c is an independent variable. Thus, eq 36 is the inverse function of LT_x . In contrast, in eq 37 for a given $x\%$ mortality, t is an independent variable and $LC_x(t)$ is a dependent variable. Therefore, eqs 36 and 38 are essentially the same.

For 50% mortality, the survival function $S(t)$ would be 1/2, and the exposure time t would be the median lethal

TABLE 2. Comparison among Hazard Models Based on Constant CBR Model, CAUC Model without Toxicity Threshold, and DAM^a

	constant CBR model	CAUC model without toxicity threshold	DAM
hazard model	$H_1(t, c) = k_1 R(t, c)$	$h_2(t, c) = k_2 R(t, c)$	$H_3(t, c) = k_3 D(t, c)$
critical assumption	CBR \equiv constant	CAUC \equiv constant	$D_L/k_a \equiv$ constant
implication	$k_r \equiv \infty$	$k_r \equiv 0$	$0 \leq k_r \leq \infty$
limiting parameter	k_r	k_e ($k_r = 0$)	k_e and k_r
$D(t; c)$	$R(t; c)(k_a/k_r)$	$k_a \times \text{AUC}(t; c)$	$k_a(k_u/k_e)cP(t)$
$\text{LC}_{50}(t)$	$\text{CBR}/((k_u/k_e)(1 - e^{-k_e t}))$	$\text{CAUC}/((k_u/k_e)c(t - (1 - e^{-k_e t})/k_e))$	$(D_L/k_a)/((k_u/k_e)P(t))$
$\text{LT}_x(c)$	$-(1/k_e) \ln(1 - \text{LC}_x(\infty)/c)$	$\text{CAUC}(\text{LT}_x; c) \equiv \text{constant}$	$D(\text{LT}_x; c) \equiv \text{constant}$
$\text{CBR}(t)$	$(k_u/k_e)\text{LC}_{50}(1 - e^{-k_e t})$	$\text{CAUC}/(t/(1 - e^{-k_e t}) - 1/k_e)$	$(D_L/k_a)/((1 - e^{-k_e t})P(t))$
$S(t; c)$	$\exp(-k_1 R(t; c)(k_a/k_r))$	$\exp(-k_2 k_a \times \text{AUC}(t; c))$	$\exp(-k_3 k_a(k_u/k_e)cP(t))$
$D(t; c)_{t \rightarrow \infty}$	$c(k_u/k_e)(k_a/k_r)$	∞	$(k_a/k_r)(k_u/k_e)c$
$\text{LC}_{50}(t)_{t \rightarrow \infty}$	$\text{CBR}/(k_u/k_e) > 0$	0	$(D_L/k_a)k_r/(k_u/k_e) > 0$
$\text{LT}_x(c)_{c \rightarrow 0}$	∞	∞	∞
$\text{CBR}(t)_{t \rightarrow \infty}$	$(k_u/k_e)\text{LC}_{50}$	0	$(D_L/k_a)k_r \geq 0$
$S(t; c)_{t \rightarrow \infty}$	constant ($0 < S \leq 1$)	0	constant ($0 \leq S \leq 1$)
target compds	narcotic compds	reactive and receptor-mediated compds	all org compds (without active metabolites)
reference	McCarty et al. (7)	Verhaar et al. (12)	this study

^a $R(t, c) = (k_u/k_e)c(1 - e^{-k_e t})$; $\text{AUC}(t, c) = \int R(t, c) dt$; $P(t)$ is a time scale function in DAM (see eq 42).

TABLE 3. Input Parameters and Estimated Parameters from Constant CBR Model, CAUC Models with and without Toxicity Threshold ($\text{LC}_{50}(\infty)$), and DAM (See Figure 1)^a

compd	input parameters ^b		estimated parameters					
	k_u ($\text{L g}^{-1} \text{h}^{-1}$)	k_e (h^{-1})	CBR ^c (mmol kg^{-1})	CAUC ^d ($\text{mmol kg}^{-1} \text{h}^{-1}$)	CAUC ^e ($\text{mmol kg}^{-1} \text{h}^{-1}$)	D_L/k_a ^f ($\text{mmol kg}^{-1} \text{h}^{-1}$)	k_r ^f ($\text{mmol kg}^{-1} \text{h}^{-1}$)	k_r ^f (h^{-1})
fluorene	0.27 (± 0.07)	0.97 (± 0.25)	1.44 (± 0.22)	111 (± 9)	79 (± 19)	86 (± 3)	0.008 (± 0.0003)	0.008 (± 0.001)
phenanthrene	0.15 (± 0.01)	0.32 (± 0.03)	1.47 (± 0.21)	80 (± 7)	56 (± 9)	57 (± 13)	0.012 (± 0.003)	0.014 (± 0.009)
pyrene	0.52 (± 0.04)	0.15 (± 0.02)	1.91 (± 0.21)	257 (± 20)	165 (± 30)	170 (± 23)	0.004 (± 0.001)	0.007 (± 0.002)

^a Values of the estimated parameters are presented \pm standard deviation (SD). ^b Uptake clearance rate (k_u) and elimination rate constant (k_e) are from ref 11. ^c CBR values are estimated using constant CBR model. ^d CAUC values are estimated using CAUC model without toxicity threshold ($\text{LC}_{50}(\infty)$). ^e CAUC values are estimated using CAUC model with toxicity threshold ($\text{LC}_{50}(\infty)$). ^f Parameter D_L/k_a , where D_L is cumulative damage level corresponding to 50% mortality and k_a is the damage accrual rate. The killing rate (k_r) and the damage recovery rate constant (k_r) are estimated using DAM. k_r values and SD are calculated by $(\ln 2/D_L/k_a)$.

time (LT_{50}). Then from eqs 36 and 38, $\text{LC}_{50}(t)$ and $\text{CBR}(t)$ are given by

$$\text{LC}_{50}(t) = (\ln 2) / \{k_3 k_a (k_u/k_e) P(t)\} \quad (40)$$

$$\text{CBR}(t) = \{(\ln 2)(1 - \exp(-k_e t))\} / \{(k_3 k_a) P(t)\} \quad (41)$$

Equations 40 and 41 can be comparable to eqs 14 and 15. Thus, D_L/k_a is given by

$$D_L/k_a = (\ln 2) / (k_3 k_a) \quad (42)$$

$$= (\ln 2) / k_r \quad (43)$$

where k_3 is a dimensionless coefficient and k_a is the damage accrual rate ($\text{mmol kg}^{-1} \text{h}$). Then $(k_3 k_a)$ stand for a killing rate (k_r) based on molar concentration of body residue ($\text{mmol}^{-1} \text{kg h}^{-1}$). The killing rate (k_r) can be interpreted as an integrated parameter of chemical potency and exposure time. In addition, from the assumption of a critical damage level (D_L), the toxicity time course can be predicted in terms of the time scale function $P(t)$. Both the killing rate (k_r) and the damage recovery rate constant k_r can be used to classify chemicals into groups with the same mode of toxic action.

Methods

Data Analysis. Data for modeling the time variable mortality and toxicokinetics were taken from Lee et al. (11). Toxicity curve for PAHs in *Hyalella azteca* was fit by the constant CBR model, the CAUC model with and without toxicity threshold, and the DAM. Data sets, which were used for the above modeling, are the measured $\text{LT}_{50}(c_i)$ and $\text{MLR}(\text{LT}_{50}(c_i))$ values.

These data sets included data from different batches of toxicity experiments (Exp I, II, and III) and, thus, actually reflect the variation among different batches of toxicity experiments as well as the variation among different treatments within each experiment. Unfortunately, functions of $\text{LT}_{50}(c_i)$ and $\text{MLR}(\text{LT}_{50}(c_i))$ cannot be analytically resolved. However, $\text{LC}_{50}(t)$ and $\text{LT}_{50}(c_i)$ are theoretically the same, and so are $\text{CBR}(t)$ and $\text{MLR}(\text{LT}_{50}(c_i))$. Equations of $\text{LC}_{50}(t)$ and $\text{CBR}(t)$ in Table 2 were used for modeling by the CBR model, the CAUC model without toxicity threshold, and the DAM. The CAUC model with a toxicity threshold $\text{LC}_{50}(\infty)$ is calculated using eqs 7 and 9. Measured toxicokinetic parameters (k_u and k_e) were used for estimating CBR, CAUC, D_L/k_a or k_r , and k_r (Table 3). $\text{LC}_{50}(\infty)$ and $\text{CBR}(\infty)$ values were also estimated from each model and compared with each other. The data were fit by an iterative least-squares fit to the equations using the fourth-order Runge-Kutta approach with a time step 0.01 using Scientist, Version 2.01 (MicroMath, Salt Lake City, UT).

Results

Curve Fitting of the DAM to $\text{LT}_{50}(c)$ Data and Parameter Estimation. In Figure 1, the measured $\text{LT}_{50}(c)$ values for fluorene, phenanthrene, and pyrene in *H. azteca* were plotted. These measured $\text{LT}_{50}(c)$ values increase after *H. azteca* attains steady state (~ 48 h) and showed a similar trend to the $\text{LC}_{50}(t)$ (Figure 2 in ref 11). Results of fitting the four models are presented in Table 3, and statistics associated with the fits are given in Table 4. Estimated $\text{LC}_{50}(\infty)$ and $\text{CBR}(\infty)$ values are also presented in Table 5.

The constant CBR model and CAUC model without toxicity threshold failed to describe the time-dependent

TABLE 4. Coefficients of Determination (r^2) and Sum of Squares of Deviations (SSD) of Fits of Constant CBR Model, CAUC Models with and without Toxicity Threshold ($LC_{50}(\infty)$), and DAM to the $LT_{50}(c)$ Data for *H. azteca* (See Figure 1)

compd	n	constant CBR model		CAUC model without $LC_{50}(\infty)$		CAUC model with $LC_{50}(\infty)$		DAM	
		r^2	SSD	r^2	SSD	r^2	SSD	r^2	SSD
fluorene	7		23.87	0.76	5.67	0.99	0.34	0.98	0.30
phenanthrene	5		3.94	0.62	1.49	0.93	0.28	0.94	0.24
pyrene	6		0.09	0.37	0.06	0.89	0.01	0.86	0.01

TABLE 5. Calculated Incipient LC_{50} and CBR Values from Constant CBR Model, CAUC Model, and DAM (See Figure 1)^a

compd	constant CBR model		CAUC model		DAM	
	$LC_{50}(\infty)$ (μ M)	$CBR(\infty)$ (mmol kg ⁻¹)	$LC_{50}(\infty)$ (μ M)	$CBR(\infty)$ (mmol kg ⁻¹)	$LC_{50}(\infty)^b$ (μ M)	$CBR(\infty)^b$ (mmol kg ⁻¹)
fluorene	4.91 (\pm 0.75)	1.44 (\pm 0.22)	1.79 (\pm 0.20)	0.51 (\pm 0.06)	2.48 (\pm 0.40)	0.69 (\pm 0.11)
phenanthrene	3.21 (\pm 0.46)	1.47 (\pm 0.21)	1.17 (\pm 0.34)	0.55 (\pm 0.16)	1.71 (\pm 0.49)	0.80 (\pm 0.70)
pyrene	0.51 (\pm 0.06)	1.91 (\pm 0.21)	0.22 (\pm 0.06)	0.83 (\pm 0.23)	0.34 (\pm 0.14)	1.19 (\pm 0.50)

^a Values of the estimated parameters are presented \pm standard deviation (SD). ^b SD for $CBR(\infty)$ are calculated by the rule of the propagation of errors, and SD for $LC_{50}(\infty)$ are calculated using $CBR(\infty) \pm$ SD and fixed mean BCF values (fluorene, 278; phenanthrene, 469; pyrene, 3467).

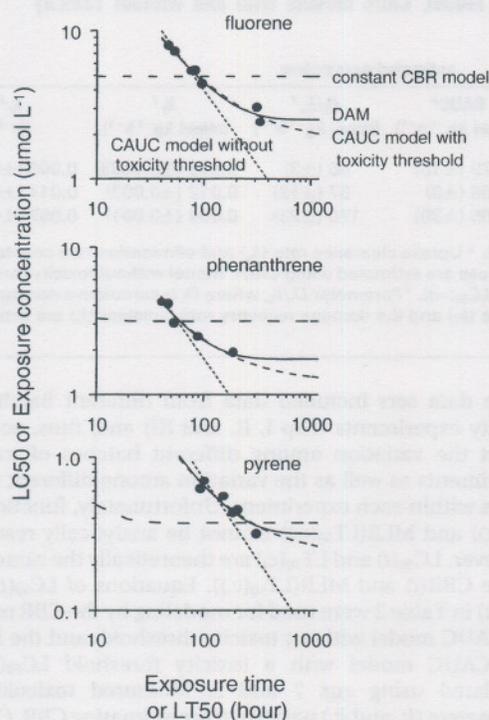


FIGURE 1. Fits of the constant CBR model, CAUC models with $LC_{50}(\infty)$ and without $LC_{50}(\infty)$, and damage assessment model (DAM) to water exposure concentration—the median lethal time ($LT_{50}(c)$) for fluorene, phenanthrene, and pyrene in *H. azteca*. Note: The solid symbols represent $LT_{50}(c)$ and conversely the $LC_{50}(t)$ at the respective LT_{50} .

toxicity data (Figure 1 and Table 4). The constant CBR model underestimates the LC_{50} value at short exposure times and overestimates the LC_{50} as time increases. The CAUC model without a toxicity threshold accurately estimates toxicity at short exposure times but substantially underestimates the LC_{50} values as time increases. In contrast, the CAUC model with a toxicity threshold and the DAM accurately estimate the LC_{50} values over the whole exposure period. Estimated $LC_{50}(\infty)$ and $CBR(\infty)$ values for the DAM were slightly higher than those for the CAUC model (Table 5).

As shown in Figure 1, the results of fitting the $LT_{50}(c)$ to the DAM and the CAUC model with toxicity threshold were

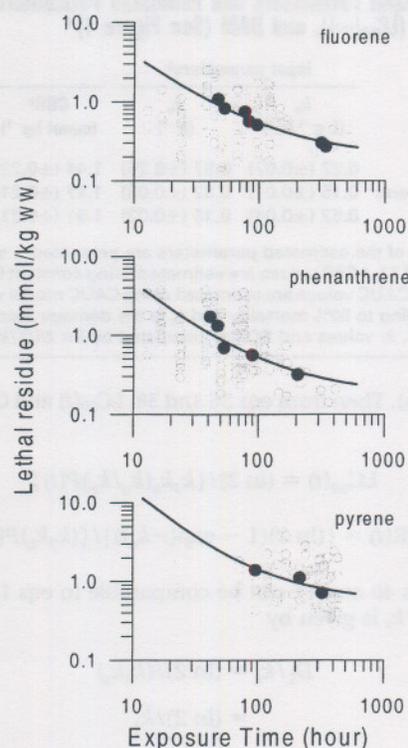


FIGURE 2. Measured lethal residues of fluorene, phenanthrene, and pyrene in *H. azteca* (open symbols) for the different times of death. Solid lines were fitted from the mean lethal residue (MLR- (LT_{50})) using the damage assessment model (DAM, eq 17).

similar. However, the assumptions on which the two models were based are very different: the CAUC model assumes that the interaction of toxicant and receptor or target site is irreversible, but the DAM assumed that the interaction is essentially reversible and tried to estimate the actual damage recovery rate constant k_r . As a result, the nonzero k_r values and D_1/k_a or k_f values were relatively constant for PAH within a factor of 2 (Table 3).

Curve Fitting of the DAM to Measured Lethal Body Residue Data. Individual lethal body residues for fluorene, phenanthrene, and pyrene ranged from 0.1 to 3.0 mmol kg⁻¹ wet wt, from 0.08 to 4.5 mmol kg⁻¹ wet wt, and from 0.2 to 4 mmol kg⁻¹ wet wt, respectively (11). Figure 2 shows the

TABLE 6. Estimated Parameters, Coefficients of Determination (r^2), and Sum of Squares of Deviation (SSD) of Fits from MLR Data Using DAM (See Figure 2)^a

comps	D_0/k_a (mmol kg ⁻¹ h ⁻¹)	k_f (mmol kg ⁻¹ h ⁻¹)	k_r (μ M)	CBR(∞) ^b (mmol kg ⁻¹ h ⁻¹)	LC ₅₀ (∞) ^b (μ M)	SSD	r^2
fluorene	41 (\pm 3)	0.017 (\pm 0.001)	0.006 (\pm 0.002)	0.24 (\pm 0.10)	0.86 (\pm 0.36)	0.026	0.95
phenanthrene	27 (\pm 5)	0.025 (\pm 0.005)	0.012 (\pm 0.005)	0.33 (\pm 0.20)	0.70 (\pm 0.43)	0.005	0.93
pyrene	88 (\pm 16)	0.008 (\pm 0.001)	0.010 (\pm 0.003)	0.88 (\pm 0.42)	0.25 (\pm 0.12)	0.093	0.73

^a Values of the estimated parameters are presented \pm standard deviation (SD). ^b SD for CBR(∞) are calculated by the rule of the propagation of errors, and SD for LC₅₀(∞) are calculated using CBR(∞) \pm SD and fixed mean BCF values (fluorene, 278; phenanthrene, 469; pyrene, 3467).

fitted result of the measured MLR($LT_{50}(c)$) values by eq 15. Estimated parameters are summarized in Table 6. D_0/k_a or k_f and the incipient LC₅₀ and CBR values estimated using the measured MLR values were about one-half or one-third of those estimated using the measured LC₅₀ values (Tables 3, 4, and 6). In contrast, considering the standard deviation of estimated k_r values, there is no significant difference between the estimated k_r values (Tables 3 and 6).

Discussion

The DAM as a New Type of Concentration–Time Response Relationship. The previous work (11) demonstrated that there was a second time-limiting step independent of the bioconcentration process that regulated the toxic response. Here, the time-limiting step was described by a damage accumulation process. The damage accumulation process is largely governed by k_r , which is related to the mode of toxic action. The two limiting cases for the DAM are $k_r = 0$ for irreversibly bound receptor-mediated compounds and $k_r = \infty$ for very rapidly reversible compounds such as some nonpolar narcotics. This study estimated the k_r value from time-to-death data without any assumption of the mode of action or the reversibility of receptor binding. The critical cumulative damage level was assumed to be constant. The modeling produced relatively constant k_r values (Table 3). In addition, the killing rates (k_f) were also relatively similar, within a factor of 2 or 3 (Table 3).

The first-order kinetic model described and predicted the bioconcentration process but failed to relate the bioconcentration process to the time course of toxicity of PAH in *H. azteca* (11). Thus, CBR is not constant among exposure concentrations for the exposure period. The kinetics, specifically k_e , which has been estimated for some organisms from toxicity data (6), cannot be estimated from the LC₅₀(t) data for *H. azteca*. This is consistent with other studies that exhibited time variable toxicity (e.g., ref 16) where k_e could not be estimated and the CAUC and LC₅₀(∞) could only be estimated by using a fixed k_e value. Theoretically, damage recovery cannot be distinguished from elimination of the PAH based strictly on the relationship of toxicity to tissue residue. As shown for the parameter estimates, it is apparent that the toxicodynamic process is independent of toxicokinetic process. This requires some direct measurement or independent estimate of the time course of toxicant elimination if the extent of damage recovery is to be inferred from toxicity test (13).

Finally, we derived a new type of concentration–time response relationship (eqs 36 and 38), which can be applied to time–mortality data, and estimated new toxicological parameters from the results in the traditional bioassay. The constant CBR model and the CAUC model are derived from the DAM as specific cases in the DAM. The empirically derived toxicity models represented by Ostwald's equation are also derived from the DAM.

Limitations of the DAM. It is apparent that the DAM has some limitations based on its assumptions including simple first-order toxicokinetics and toxicodynamics, the need to compare time-dependent toxicity among treatments as a

fixed effect level, e.g., 50% mortality, and ignorance of the toxicity threshold.

The DAM treated only the simplest situation of non-polar narcotic compounds under water-only exposure. The toxicokinetic model in the DAM would need to be modified to incorporate other factors such as growth dilution and biotransformation. The DAM also assumes a simple first-order toxicodynamic process reflecting only the reversibility of the toxic response. The damage recovery process likely includes several physiological and genetic processes, thus selection of such a simple model should be examined for use with other chemicals of differing mechanisms of action.

In this study, the concentration–time response relationship was analyzed at the fixed mortality level, 50%. The analysis method needs to be extended to multiple effect levels, LC_x(t) or LT_x(c). In addition, the toxicodynamic parameters k_r and k_f were also estimated at 50% mortality under the assumption that the two parameters were constant among treatments. This basic assumption should be tested.

Finally, the DAM assumed the first-order toxicodynamic process without consideration of a toxicity threshold. According to the DAM, the toxicity threshold cannot be defined as a body residue but rather as a time-integrated internal exposure reflecting accumulated damage. The no effect concentration is not constant over time but is a function of the potential toxicity of a toxicant (k_a), the recovery time (k_r), and the accumulated damage (D_0) corresponding to no effect. At $t = \infty$, the incipient no effect body residue would be given by $(D_0/k_a)/k_r$.

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Literature Cited

- Chew, R. D.; Hamilton, M. A. *Trans. Am. Fish. Soc.* **1985**, *114*, 403–412.
- Rand, G. M.; Wells, P. G.; McCarty, L. S. Introduction to Aquatic Toxicology. In *Fundamentals of Aquatic Toxicology*, 2nd ed.; Rand, G. M., Ed.; Taylor & Francis: Washington, DC, 1995; pp 3–67.
- Green, R. H. *Ecology* **1965**, *46*, 887.
- Sprague, J. B. *Water Res.* **1969**, *3*, 793–821.
- Mayer, F. L.; Krause, G. F.; Buckler, D. R.; Ellersieck, M. R.; Lee, G. *Environ. Toxicol. Chem.* **1994**, *13*, 671–678.
- Morgan, B. J. T. *Analysis of Quantal Response Data*; Chapman & Hall: London, 1992.
- McCarty, L. S.; Ozburn, G. W.; Smith, A. D.; Bharath, A.; Orr, D.; Dixon, D. G. *Hydrobiologia* **1989**, *188/189*, 533–542.
- Mackay, D.; Puig, H.; McCarty, L. S. *Environ. Toxicol. Chem.* **1992**, *11*, 941–951.
- Van den Heuvel, M. R.; McCarty, L. S.; Lanno, R. P.; Hickie, B. E.; Dixon, D. G. *Aquat. Toxicol.* **1991**, *20*, 235–252.
- Hickie, B. E.; McCarty, L. S.; Dixon, D. G. *Environ. Toxicol. Chem.* **1995**, *14*, 2187–2197.
- Lee, J. H.; Landrum, P. F.; Koh, C. H. *Environ. Sci. Technol.* **2002**, *36*, 3124–3130.

- (12) Verhaar, H. J. M.; de Wolf, W.; Dyer, S.; Legierse, K. C. H. M.; Seinen, W.; Hermens, J. L. M. *Environ. Sci. Technol.* **1999**, *33*, 758–763.
- (13) Legierse, K. C. H. M.; Verhaar, H. J. M.; Vaes, W. H. J.; Wouter, H. J.; De Bruijn, J. H. M.; Hermens, J. L. M. *Environ. Sci. Technol.* **1999**, *33*, 917–925.
- (14) Rozman, K. K.; Doull, J. *Toxicology* **2000**, *144*, 169–178.
- (15) Ankley, G. T.; Erickson, R. J.; Phipps, G. L.; Mattson, V. R.; Kosian, P. A.; Sheedy, B. R.; Cox, J. S. *Environ. Sci. Technol.* **1995**, *29*, 2828–2833.
- (16) Suter, G. W., II. *Ecological Risk Assessment*; Lewis Publishers: Boca Raton, FL, 1993.
- (17) Finney, D. J. *Probit Analysis*, 3rd ed.; Cambridge University Press: Cambridge, 1971.
- (18) Newman, M. C. *Quantitative Methods in Aquatic Ecotoxicology*; Lewis Publishers: Boca Raton, FL, 1995.
- (19) McCarty, L. S.; Mackay, D. *Environ. Sci. Technol.* **1993**, *27*, 1719–1728.
- (20) McCarty, L. S. *Environ. Toxicol. Chem.* **1986**, *5*, 1071–1080.
- (21) Freidig, A. P.; Verhaar, H. J. M.; Hermens, J. L. M. *Environ. Sci. Technol.* **1999**, *33*, 3038–3043.
- (22) Sijm, D. T. H. M.; Schipper, M.; Opperhuizen, A. *Environ. Toxicol. Chem.* **1993**, *12*, 1117–1127.
- (23) Dixon, P. M.; Newman, M. C. Analyzing Toxicity Data Using Statistical Models for Time-to-Death: An Introduction. In *Metal Ecotoxicology: Concepts and Application*; Newman, M. C., McIntosh, A. W., Eds.; Lewis Publishers: Chelsea, MI, 1991; pp 207–242.
- (24) Kooijman, S. A. L. M.; Bedaux, J. J. M. *The Analysis of Aquatic Toxicity Data*; VU University Press: Amsterdam, 1996.

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