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# Toxicokinetic and Toxicodynamic Models for Ecological Risk Assessment

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# 생태위해성 평가를 위한 독성동태학 및 독성역학 모델

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## 요 약

오염물질에 대한 생태위해성평가(ecological risk assessment)를 위해서는 노출평가(exposure assessment)와 함께 생물영향에 대한 평가(effect assessment)를 수행해야 한다. 노출평가의 경우는 지화학적 과정에 대한 이해를 바탕으로 환경농도를 예측하기 위한 화학평형모델이나 다매체환경거동모델 등 다양한 평가 및 예측모델을 활용해 왔다. 이와 달리 생물영향평가는 실험실 조건에서 제한된 독성자료를 대상으로 외부노출농도에 기반한 농도-반응관계를 통계적 방법을 통해서 추정하는 '경험적 모델(empirical model)'에 주로 의존해 왔다. 최근에 와서 생체 내 잔류량을 기반으로 농도-시간-반응관계를 기술하고 예측하는 독성동태학 및 독성역학 모델(toxicokinetic-toxicodynamic model)과 같은 독성작용에 기반한 모델(process-based model)들이 개발되어 활용되고 있다. 본 논문에서는 여러 종류의 독성동태학 및 독성역학 모델을 소개하고, 이를 통계적 추론에 기반한 전통적인 독성학 모델과 비교하였다. 서로 다른 종류의 독성동태학 및 독성역학 모델로부터 도출된 노출농도-시간-반응관계식을 비교하고, 동일 독성기작을 보이는 오염물질 그룹 내에서 미측정 오염물질의 독성을 예측할 수 있게 해주는 구조-활성관계(Quantitative Structure-Activity Relationship, QSAR) 모델을 여러 독성동태 및 독성역학모델로부터 유도하였다. 마지막으로 독성동태학 및 독성역학 파라미터를 추정하기 위한 실험계획을 제안하였고, 앞으로 독성동태학 및 독성역학 모델을 생태계 위해성평가에 활용하기 위해서 해결해야 될 연구과제를 검토하였다.

**Key words** : toxicokinetic model, toxicodynamic model, quantitative structure-activity relationship model, ecological risk assessment

## INTRODUCTION

Ecological risk assessment is based on the exposure assessment to estimate the predicted environmental concentration (PEC) and the effect assessment to estimate the predicted no-effect concentration (PNEC), which are determined by different kinds of environmental fate and effect models as shown in Fig. 1. Environmental fate models used to estimate the PEC are based on fate, transport, and reaction processes, whereas most of toxicity models used to determine the PNEC are empirically derived statistical models

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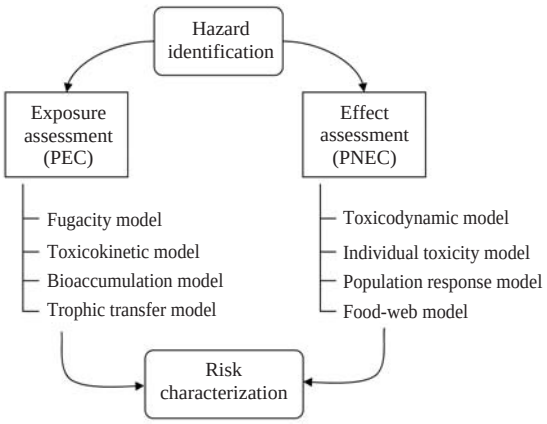


**Fig. 1.** Ecological risk assessment scheme and prediction model for the exposure assessment and effect assessment. PEC: predicted environmental concentration, PNEC: predicted no effect concentration.

such as probit or logistic model. Recently, different types of toxicity models were developed for the effect assessment. These models are toxicokinetic-toxicodynamic (TK-TD) models based on a one-compartment first-order kinetic (1CFOK) model, but include different toxicodynamic assumptions based on empirical inspection and toxicological process.

Here, different types of process-based toxicity models are introduced (see Table 1 for significant abbreviations used in the text). Importantly, TK-TD models such as constant Critical Body Residue (CBR) model (McCarty *et al.*, 1989), Critical Area Under the Curve (CAUC) model (Verhaar *et al.*, 1999), Damage Assessment Model (DAM) (Lee *et al.*, 2002b), and Dynamic Energy Budget toxicity (DEB-tox) model (Kooijman and Bedaux, 1996) are introduced and compared with

**Table 1.** List of symbols and their unit for variables and parameters used in toxicokinetic and toxicodynamic models

| Symbols | Unit | Definition |
|---|---|---|
| R, R(t) | mol $kg^{-1}$ | Body residue of the compound (eq 1 and 2) |
| c, $C_w$, c(t) | mol $L^{-1}$ | Water concentration of the compound (eq 1) |
| $k_u$ | L $kg^{-1}$ $h^{-1}$ | Uptake rate coefficient (eq 1) |
| $k_e$ | $h^{-1}$ | Elimination rate constant (eq 1) |
| $R_{ss}$ | mol $kg^{-1}$ | Body residue at steady state (eq 5) |
| BCF | L $kg^{-1}$ | Bioconcentration factor (eq 5) |
| CBR, CBR(t) | mol $kg^{-1}$ | Critical body residue (eq 13, 20) |
| $LC_{50}(t)$ | mol $L^{-1}$ | Median lethal concentration (eq 13) |
| $LC_{50,\infty}$ | mol $L^{-1}$ | The incipient median lethal concentration (eq 15) |
| CAUC | mol $kg^{-1}$ $h^{-1}$ | The critical area under the curve (eq 18) |
| D, D(t) | – | Cumulative damage (eq 24) |
| $k_a$ | $mol^{-1}$ kg $h^{-1}$ | Damage accrual rate coefficient (eq 24) |
| $k_r$ | $h^{-1}$ | Damage recovery rate constant (eq 24) |
| $CBR_{50}(t)$, $LR_{50}(t)$ | mol $kg^{-1}$ | Median lethal body residue (eq 27 and 45) |
| S(t) | – | Survival probability (eq 28) |
| h(t) | $h^{-1}$ | Hazard function (eq 28) |
| $k_\dagger$ | $mol^{-1}$ kg $h^{-1}$ | Killing rate based on body residue (eq 28) |
| $R_0$ | mol $kg^{-1}$ | A toxicological threshold based on body residue (eq 28) |
| $C_0$ | mol $L^{-1}$ | A toxicological threshold based on external water concentration (eq 29) |
| $h_0$ | $h^{-1}$ | A hazard rate in control (eq 29) |
| $K_{OW}$ | – | The *n*-octanol and water partition coefficient (eq 30) |
| $K_{LW}$ | – | The partition coefficient between water phase and lipid phase in membranes (eq 35) |
| $C_L$ | mol kg $lipid^{-1}$ | Target lipid concentration (eq 35) |
| K(t) | – | A toxicokinetic time-scale function (eq 40) |
| l | – | The scaled body length defined by $L/L_m$ with L=body length and $L_m$=the maximum body length |

empirically derived toxicity models. These TK-TD models are used to describe and predict the concentration-time-response relationship. The quantitative structure-activity relationship (QSAR) models are derived from different types of TK-TD model. The QSAR models are used to extrapolate the toxicity parameters such as $LC_{50}$ among chemicals.

The use of models in aquatic toxicology is an integral part of both toxicity testing and interpretation (Rand *et al.*, 1995). The models make it possible to derive experimental designs for toxicity test, where toxicokinetic and toxicodynamic parameters are estimated. The estimated parameters are interpreted based on the model. Here, a general form of experimental designs to investigate toxicokinetic and toxicodynamic processes for a compound is discussed. Finally, further study needs for the effect assessment based on the body residue approach are addressed.

## 1. Toxicokinetic and toxicodynamic models for the prediction of the concentration-time-response relationship

Three different types of models used in aquatic toxicology are introduced; toxicokinetic (TK) model, toxicodynamic (TD) model, and quantitative structure-activity relationship (QSAR) model. These models are process-based models, which deal bioaccumulation, intoxication, and modes of toxic action, respectively.

### 1) Toxicokinetic model

#### (1) One-compartment first-order toxicokinetic model

Most of the practical application of aquatic toxicology testing and interpretation is based on application of a one-compartment first-order kinetic (1CFOK) model (Spacie and Hamelink, 1982; Mancini, 1983; Hawker and Connell, 1985). This model assumes that organism is a single homogeneous, well-mixed compartment and all uptake, transformation, and elimination processes associated with toxicant accumulation are first order; that is rates are proportional to difference in toxicant concentration between phases.

If the distribution of a chemical between external water phase and internal tissue phase is not at thermodynamic equilibrium, there will be mass transfer down a gradient in the direction of that equilibrium state. For accumulation in an aquatic organism, a non-polar chemical dissolved in water diffuses into the organism until no more net change in either direction occurs. The rate of change in chemical concentration in an organism as it approached equilibrium is described by simple first-order kinetics:

$$\frac{dR}{dt} = k_u c(t) - k_e R \quad (1)$$

where R is body residue (mol $kg^{-1}$), c(t) is water concentration (mol $L^{-1}$), $k_u$ is uptake rate coefficient, and $k_e$ is elimination rate constant. The uptake rate coefficient is the amount of environmental medium (e.g., water or sediment) cleared of chemical by uptake into the organism per unit mass of organism per unit time, expressed as flow rate (L $kg^{-1}$ $h^{-1}$). Elimination is the removal of a compound from the body by diffusion, desorption or excretion. The first-order elimination rate constant is the ratio of body residue to remove and expressed as a unit of the reciprocal time ($h^{-1}$). Finally body residue is given by

$$R(t) = R_{t=0}\, e^{-k_e t} + k_u \int_0^t \exp\{-(t-t_1)k_e\}\, c(t_1)\, dt_1 \quad (2)$$

where $R_{t=0}$ is the initial body residue when the exposure starts.

If c(t) is constant and $R_{t=0}$ is zero, in uptake phase, the above equation is reduced as follows:

$$R(t) = \frac{k_u}{k_e} c(1 - e^{-k_e t}) \quad (3)$$

In depuration phase, the integrated form is given by

$$R(t) = R_{t=0}\, e^{-k_e t} \quad (4).$$

At steady state ($dR/dt = 0$), body residue ($R_{ss}$) is given by

$$R_{ss} = \frac{k_u}{k_e} c = BCF \cdot c \quad (5)$$

where BCF is bioconcentration factor defined ($R_{ss}/c$), which can be also given by $k_u/k_e$ (L $kg^{-1}$).

Although viewing organism as a 1CFOK model is clearly a dramatic oversimplification, it continues to be of great utility (Rand *et al.*, 1995). Modifications of the 1CFOK model may incorporate multiple sources (e.g., food or sediment), multiple elimination components (e.g., biotransformation), and growth, etc. However, enhanced realism with such models cannot be independent of the assumption of the simple model: constant uptake rate, instantaneous mixing within the compartment(s). Under field conditions, the rate of contaminant uptake may not be constant due to changes in behavior, food type, food availability, or other environmental factors.

2) Toxicodynamic model

Variability of results in toxicity experiment can result from the variability in the time course of accumulation and amount delivered to the site(s) of toxic action (toxicokinetics) and/or the variability in the time course of the response of individual organisms to the target dose (toxicodynamics). Various types of toxicodynamic models have been suggested (see Suter (1993)). Common objective in these models is to describe and predict the relationship of concentration-time-response.

(1) Experience-based toxicodynamic models

First group in toxicodynamic model is not based on toxic mechanism, but only on the experiential inspection such as hyperbolic relationship between log (time) and effect concentration, and/or statistical assumptions such as log-normal distribution of individual tolerance in probit analysis:

$$P_p = a_1 + b_1 \ln(D) + c_1 \ln t \quad \text{(Finney, 1978)} \quad (6)$$

$$LD_{50} = a_2 + b_2 (1/t) \quad \text{(Green, 1965)} \quad (7)$$

$$\ln LT_{50} = a_3 + b_3 \ln c \quad \text{(Newman, 1992)} \quad (8)$$

where $P_p$ is the probit of percent mortality p, D is the dose to produce of p% mortality, $LD_{50}$ is the median lethal dose to produce a 50% mortality, t is exposure time, $LT_{50}$ is the median lethal time when a half of test animals die in each treatment, c is the external concentration, $a_1$, $a_2$, $a_3$, $b_1$, $b_2$, $b_3$, and $c_2$ are constant. Recently, the probit transformation of proportion of response ($P_p$) has been replaced by logit ($\ln(p/(1-p))$) or Weibull transformation ($\ln(-\ln p)$) because of their computational simplicity (Morgan, 1992).

The above toxicodynamic models are based on empirical inspection. All of these models are given by a power function form as follows

$$c^n t^\lambda = K \quad \text{or} \quad n \ln c + \lambda \ln t = \ln K \quad (9)$$

$$\text{Finney's model:} \quad b_1 \ln D + c_1 \ln t = (P_p - a_1) \quad (10)$$

$$\text{Green's model:} \quad \ln(LD_{50} - a_2) + \ln t = \ln b_2 \quad (11)$$

$$\text{Newman's model:} \quad -b_3 \ln c + \ln LT_{50} = a_3 \quad (12)$$

where n and $\lambda$ are exponent, K are constant proportional to percent mortality, $a_1$ is a probit of percent mortality in control, $a_2$ corresponds to a toxicity threshold concentration, $b_2$ and $a_3$ are constant, and $P_p$, is given by a function of % mortality. Newman's model does not include the toxicity threshold term.

(2) Process-based toxicodynamic model

The empirically derived toxicological models do not rely on a specification of a mechanism, but simply aims to summarize the data. In contrary, a process-based model may provide much more, since the parameters of the model might correspond to definite aspects of the supposed mechanism and useful for prediction such as interpolation and extrapolation (Morgan, 1992).

① Narcosis hypothesis and constant CBR model

According to the 'narcosis hypothesis', in the case of poorly metabolized non-polar narcotic chemicals, there is a constant threshold for exposure concentration and time (van Hoogen and Opperhuizen, 1988). The constant threshold for 50% mortality is called as 'Critical Body Residue (CBR)', which is relatively constant (from 2 to 8 mmol/kg wet wt.) and is also independent of narcotic chemical and species (McCarty and Mackay, 1993).

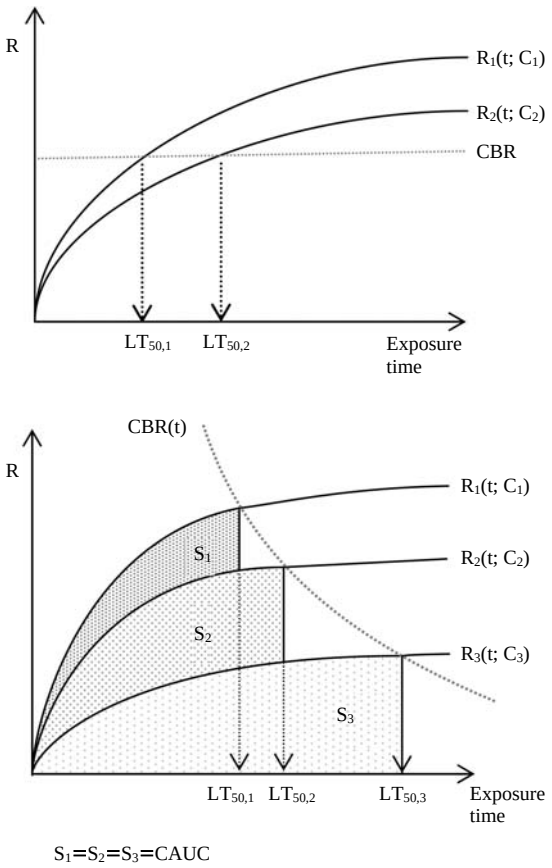


**Fig. 2.** Scheme of the critical body residue (CBR) model and the critical area under the curve (CAUC) model. $R_i$ (t, $c_i$)=body residue in test animals exposed to $C_i$ at time t, CBR=critical body residue, $LT_{50_i}$=the median lethal time for each exposure concentration i, CAUC=critical area under the curve.

Under the assumption of constant threshold (CBR), the one-compartment first-order kinetic (1CFOK) model was used for prediction of time-dependence of $LC_{50}$, especially the incipient median lethal concentration ($LC_{50,\infty}$) (McCarty *et al.*, 1989; Rand *et al.*, 1995). If the exposure concentration c is the median lethal concentration ($LC_{50}$) to produce 50% mortality in test animals, the body residue is corresponding to critical body residue (CBR) as follows:

$$CBR = LC_{50}\,(k_u/k_e)\,(1-e^{-k_e t}) \quad (13)$$

where $LC_{50}(t)$ is the median lethal concentration at exposure time t (mol $L^{-1}$), CBR is a constant body residue corresponding to 50% mortality (mol $kg^{-1}$) (Fig. 2). If the threshold body residue (CBR) is constant, $LC_{50}(t)$ is given by

$$LC_{50}(t) = (1-e^{-k_e t})\,CBR/(k_u/k_e) \quad (14)$$

In addition, the median lethal time ($LT_{50}(c)$) for a given exposure concentration is given by

$$LT_{50}(c) = -(1/k_e)\ln(1-LC_{50,\infty}/c) \quad (15)$$

where $LC_{50,\infty}$ is the incipient $LC_{50}$ at the infinitive time (mol $L^{-1}$), c is a water exposure concentration (mol $L^{-1}$). The $LC_{50,\infty}$ stands for CBR/($k_u/k_e$), where $k_u/k_e$ is equal to the bioconcentration factor (BCF) at steady state (L $kg^{-1}$). Thus, there is a temporal correlation between the attainment of bioconcentration steady state and attainment of a stationary $LC_{50}$ value. The time course of toxicity is regulated by only the bioconcentration process.

② Critical Area Under the Curve (CAUC) model

In contrast to non-polar narcotic compound, for reactive and receptor-mediated toxicants there is no constant CBR, but constant Critical Area Under the Curve (CAUC) for each species-compound combination (Verhaar *et al.*, 1999). One of the major differences between narcosis and toxicity of reactive chemicals is the fact that the interaction of reactive chemicals with the target is irreversible, while narcosis is due to a reversible interaction. In an irreversible interaction, it is not just the concentration at the target site (relevant for narcosis), but the amount of target that is occupied or depleted, which is the relevant parameter (Freidig *et al.*, 1999; Legierse *et al.*, 1999; Verhaar *et al.*, 1999). According to Verhaar *et al.* (1999) the concentration of affected target can be modeled as follows:

$$\frac{dC_{\text{affected target}}}{dt} = k_a C_{\text{target}} C_{\text{toxicant}} - k_d C_{\text{affected target}} \quad (16)$$

Thus, assuming irreversible interaction ($k_d$=0), the concentration of affected target is given by

$$C_{affected\ target}=k_a C_{target}\int_0^t C_{toxicant} \quad (17)$$

If it is assumed that there is a certain $C_{affected\ target}$ at which a certain effect manifests itself, it is not the internal concentration ($C_{toxicant}$) that is constant, but the time integral of $C_{toxicant}$, or Critical Area Under the Curve (CAUC).

$$CAUC=\int_0^t BCF \cdot C_w(1-e^{-k_e t})dt \equiv constant \quad (18)$$

(Fig. 2). In this case, the $LC_{50}(t)$ is given by

$$LC_{50}(t)=\frac{CAUC}{BCF}\times\frac{1}{t-(1-e^{-k_e t})/k_e} \quad (19)$$

Thus, the CBR at the target site is dependent on the exposure time.

$$CBR(t)=\frac{CAUC}{(1-e^{-k_e t})}\times\frac{1}{t-(1-e^{-k_e t})/k_e} \quad (20)$$

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

$$LC_{50}(t)=\frac{CAUC}{BCF}\times\frac{1}{t-(1-e^{-k_e t})/k_e}+LC_{50,\infty} \quad (21)$$

If the exposure time is sufficiently longer than ($1/k_e$), $LC_{50}(t)$ is a hyperbolic function of the exposure time t:

$$LC_{50}(t)=\frac{CAUC}{BCF}\times\frac{1}{t}+LC_{50,\infty} \quad (22)$$

Therefore, the concentration-time-response relationship is given by

$$\ln(LC_{50}(t)-LC_{50,\infty})+\ln t=\ln(CAUC/BCF). \quad (23)$$

This relationship is similar to that of the Green's hyperbolic model.

The CAUC model and other empirical models such as Green's model assumed the irreversible interaction between toxicant and target site. However, for a typical narcotics polycyclic aromatic hydrocarbons (PAH), it is unreasonable to assume the irreversible interaction between PAH and its target site such as membrane lipids. It has been reported, however, for chlorobenzenes known as typical non-polar narcotic compound groups, critical body residue (CBR) is not constant, but decrease with increasing exposure time and concentration (Chaisuksant *et al.*, 1997; de Maagd *et al.*, 1997; Yu *et al.*, 1999). Thus, it is inevitable to assume another time-limiting step in the toxicodynamic process to illustrate the recovery of response.

③ Damage Assessment Model (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 (Lee *et al.*, 2002). First, the compound accumulates by the simple first-order kinetics as follows:

$$R(t)=\frac{k_u}{k_e}c(1-e^{-k_e t}) \quad (24).$$

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

$$\frac{dD}{dt}=k_a R-k_r D \quad (25)$$

where D is the cumulative damage (dimensionless), $k_a$ is a rate for accrual of damage (kg $mol^{-1}$ $h^{-1}$), 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 ($k_r=\infty$) as well as to reactive and receptor-mediated compounds with irreversible binding ($k_r=0$) (Verharr *et al.*, 1999; Legierse *et al.*, 1999). 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(t=0) is zero, the cumulative damage function D(t) is given by

$$D(t)=k_a\frac{k_u}{k_e}C_w\left(\frac{e^{-k_r t}-e^{-k_e t}}{k_r-k_e}+\frac{1-e^{-k_r t}}{k_r}\right) \quad (26)$$

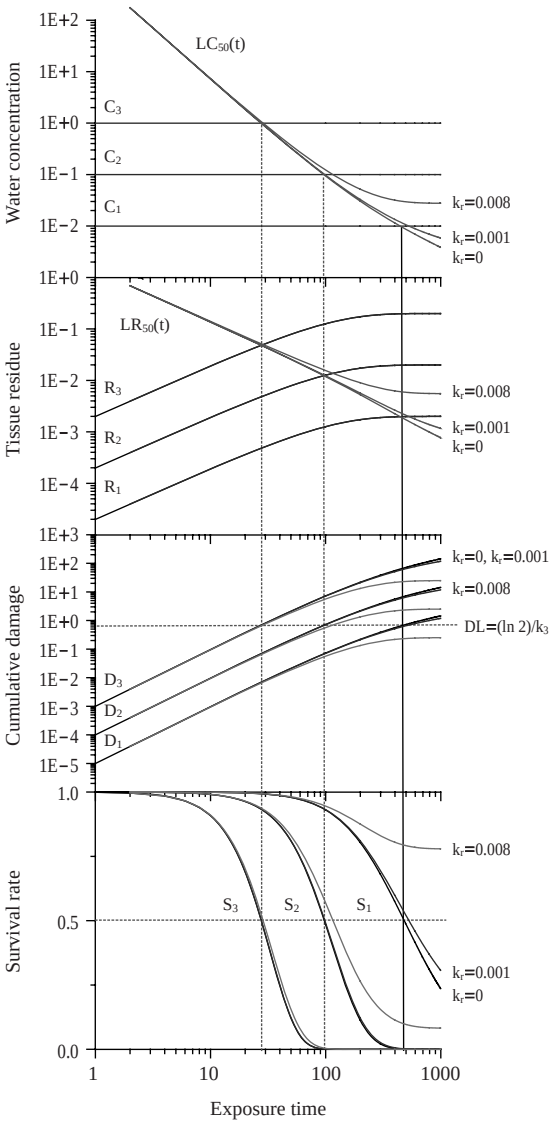


**Fig. 3.** Simulation results for the basic principle of the damage assessment model. Fixed parameter values were $k_u$=2 L $kg^{-1}$ $h^{-1}$, $k_e$=0.01 $h^{-1}$, $k_3$=1, $k_a$=1. For given $k_r$ values such as 0, 0.001, and 0.008 $h^{-1}$, R (c; t), D (c; t), were calculated using eqs 24, 26 and 27. S (c; t)=exp (−D (c,t)).

(Fig. 3). 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:

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

$$CBR_{50}(t)=\frac{D_L/k_a}{\frac{1}{(1-e^{-k_e t})}\left(\frac{e^{-k_r t}-e^{-k_e t}}{k_r-k_e}+\frac{1-e^{-k_r t}}{k_r}\right)} \quad (27)$$

with $D_L/k_a$ in mol $kg^{-1}$ h (Fig. 3). 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.

The constant CBR and CAUC models can be derived as two extreme cases from the DAM with the first-order damage recovery rate constant $k_r=\infty$ and $k_r=0$, respectively. Rozman *et al.* (2002) just theoretically suggested the applicability of a first- and zero-order damage recovery processes in toxicodynamic model. The DAM is corresponding to the first-order toxicodynamic model. Meanwhile, a toxicodynamic model including a zero-order toxicodynamic model called as 'Dynamic Energy Budget toxicology (DEB-tox) model' was suggested by Kooijman and Beaudaux (1996) and Kooijman (2000).

④ Dynamic Energy Budget toxicology (DEB-tox) model

The DEB-tox model assumes that the hazard rate, defined as a relative mortality rate, h(t), is proportional to the difference between body residue and toxicity threshold as follows:

$$h(t)\equiv-\frac{1}{S}\frac{dS}{dt}=k_†(R-R_0)_+ \quad (28)$$

where S is survival probability (dimensionless), h(t) is hazard rate ($h^{-1}$), $k_†$ is killing rate coefficient based on the internal concentration ($mol^{-1}$ kg $h^{-1}$), R is body residue (mol $kg^{-1}$), $R_0$ is a toxicological threshold called as internal No-Effect-Concentration (NEC) based on body residue, and $(R-R_0)_+$ is $R-R_0$ if $R>R_0$ and zero if $R<R_0$. Finally, the survival probability is given by

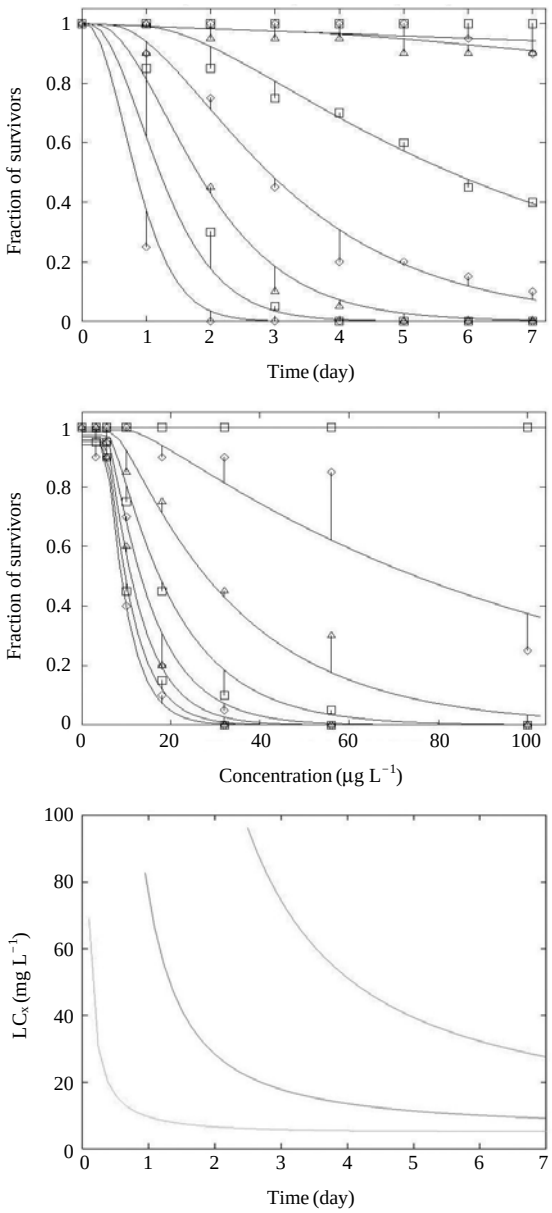


**Fig. 4.** The time and concentration profiles of the DEB-tox model (Source: Kooijman *et al.*, 2003). The resulting parameter estimates are: control hazard rate=0.0083 1/d, NEC=5.2 μg/L, killing rate 0.037 (μg · d)$^{-1}$, elimination rate=0.79 d$^{-1}$. From the last three parameters, LCx-time curves were calculated with DEBtox and DEBtool (http://www.bio.vu.nl/thb/deb/deblab/debtool/index_tox.html), curves for the $LC_0$, $LC_{50}$ and $LC_{99}$ are shown. For long exposure times, the $LC_x$ curves will tend towards the NEC, for all x, in absence of blank mortality.

$$S(t)=\begin{cases}\exp\left(\frac{k_\dagger}{k_e}C_w(e^{-k_e t_0}-e^{-k_e t})-k_\dagger(C_w-C_0)(t-t_0)-h_0 t\right) & \text{if } C_w>C_0 \text{ and } t>t_0\\ \exp(-h_0 t) & \text{otherwise}\end{cases} \quad (29)$$

where $k_\dagger$ is killing rate coefficient based on the external concentration (mol$^{-1}$ L h$^{-1}$), $C_0$ is external No-Effect-Concentration (NEC) based on water concentration, and $t_0=-\{\ln(1-C_0/C_w)\}/k_e$, $h_0$ is a hazard rate in control (h$^{-1}$) (Fig. 4). Unfortunately, $LC_{50}(t)$ and $CBR_{50}(t)$ are not given by an explicit function. In contrast to the constant CBR model, CAUC model, and DAM, the DEB-tox model can estimate the toxicodynamic parameter such as killing rate and NEC directly from the time-dependent survival data (Fig. 4).

Constant CBR model and CAUC model are derived for non-polar narcotic compounds and irreversibly reactive compound to target sites, respectively. Until now, the DEB-tox model was not tested against measured critical body residue data. Especially, according to the DEB-tox model, the elimination rate constant can be estimated from time-dependent toxicity data. However, the estimated value using the DEB-tox model is not similar to the measured value (Gerritsen *et al.*, 1998; Lee *et al.*, 2002a). Recently, DEB-tox model was extended with a simple mechanistic model to deal with receptor interactions (Jager and Kooijman, 2005). In the case of DAM, $LC_x(t)$ and $CBR_x(t)$ have not been described, and the dose-response relationship at a fixed exposure time is over-simplified, because the slope in the dose-response curve is always unity (Lee *et al.*, 2002a).

### 3) Derivation of the Quantitative Structure-Activity Relationship (QSAR) models from toxicokinetic-toxicodynamic models

In the field of aquatic toxicology, quantitative structure-activity relationships (QSARs) were developed as scientifically credible tools for predicting the acute, and in some instances sub-chronic toxicity of chemicals when little or no empirical data are available. When toxicity data are related to physico-chemical

properties for chemicals with as similar mechanism of toxic action, often relationships can be found: inert organic chemicals or narcotic chemicals, polar narcotic chemicals and reactive organic chemicals (Herman, 1989).

In the case of non-polar narcotic compounds, QSAR model is generally given as a function of $K_{OW}$ as follows:

$$\log LC_{50} = -m \log K_{OW} + a \quad (30)$$

where $LC_{50}$ is the median lethal concentration, $K_{OW}$ is the *n*-octanol and water partition coefficient, m and a are constants. Since this relationship is based on the relationship between bioconcentration factor (BCF) and $K_{OW}$, this type of QSAR models can be considered as a body residue-based approach. Here, different types of QSAR models can be derived from different TK-TD models; species-specific, time-independent, and time-dependent QSAR models.

(1) QSAR model derived from the Constant CBR model

According to McCarty (1992), the relationship between the $LC_{50}$ (mmol $L^{-1}$) and $K_{OW}$ for fish is approximately

$$\log LC_{50} = -\log K_{OW} + 1.7 \quad (31)$$

The bioconcentration factor (BCF) also varies with $K_{OW}$

$$\log BCF = \log K_{OW} - 1.3 \quad (32)$$

Since, at steady state of body residue,

$$CBR = LC_{50} \times BCF, \quad (33)$$

CBR is given by

$$\begin{aligned} \log CBR &= \log BCF + \log LC_{50} \\ &= \log K_{OW} - 1.3 - \log K_{OW} + 1.7 \\ &= 0.4, \end{aligned} \quad (34)$$

therefore

$$CBR = 2.5 \text{ mmol kg}^{-1} \text{ wet weight.}$$

The CBR values experimentally determined indicate that the predicted constant CBR values are proper for the first approximation for the threshold concentration for each species (Van Hoogen and Opperhuizen, 1988; Sijm *et al.*, 1993).

(2) Target Lipid Model (TLM)

Recently, Di Toro *et al.* (2000) suggested a new type of QSAR model called as 'Target Lipid Model' for narcotic compounds for the estimation of species-specific CBR value. The TLM was based on several assumptions as follow

① A target lipid (a lipid fraction in test animals) is the site of action in the organism

② Target lipid concentration ($C_L$) can be predicted by water concentration ($C_W$) and partition coefficient ($K_{LW}$) between water phase and lipid phase in membranes assuming steady state conditions

$$K_{LW} = C_L / C_W \quad (35)$$

③ Target lipid has the same lipid-octanol linear free energy relationship for all species.

$$\log K_{LW,k,j} = m_L \log K_{OW,j} + a_{L,k} \quad (36)$$

where k is species and j is individual chemicals.

④ CBR is constant for each chemical group

$$CBR_{k,j} = CBR_k$$

Finally, it was assumed that

$$LC_{50k,j} = CBR_k / K_{LW,j} \quad (37)$$

From $LC_{50}$ data base (156 chemicals and 33 species) and measured CBR values (Di Toro *et al.*, 2000), it was deduced as follows:

① The slope ($m_L$) in $\log(LC_{50}) - \log(K_{OW})$ is the same for all species ($-0.945 \pm 0.014$), but individual species may have varying target lipid body burdens that cause mortality

$$\begin{aligned} \log LC_{50k,j} = &-0.945(\pm 0.014) \log K_{OW,j} \\ &- a_L + \log CBR_k \end{aligned} \quad (38)$$

In contrast to the body burden model (McCarty *et al.*, 1992), the target lipid model reflects the

species sensitivity and chemical differences in the model equation as well as the common narcotic mode of action.

② The body burdens deduced from the target lipid model ($10^{(-a_L+\log CBR_k)}$) are not significantly different from the measured concentrations in extract lipid for five species. Thus $a_L$ is negligible.

Finally, the TLM is summarized as follows

$$\log LC_{50,k,j} = -0.945\ (\pm 0.014) \log K_{OW,j} + \log CBR_k \quad (39)$$

The TLM can estimate species-specific CBR using only the chemical's octanol-water partition coefficient $K_{OW}$ and $LC_{50}$ data, assumed CBR values are constant within similar chemical groups. The species-specific CBR values were used as water or tissue quality criteria for non-polar narcotic chemicals (Di Toro *et al.*, 2000).

(3) Time-dependent QSAR model derived from DAM

From the DAM (Lee *et al.*, 2002b), $LC_{50}(t)$ for chemical j is given by

$$LC_{50}(t)_j = CBR(t)_j / \{K(t)_j BCF_j\} \quad (40)$$

with $CBR(t)_j$ is a chemical-specific time-dependent critical body residue and $K(t)_j$ is a toxicokinetic time-scale function as a function of j and equals $(1-e^{-k_{e,j}t})$ for the one-compartment first-order kinetics.

If

$$\log BCF_j = m_B \log K_{OW,j} + a_B \quad (41)$$

then, the $LC_{50}(t)_j$ is given by

$$\log LC_{50}(t)_j = -m_B \log K_{OW,j} - a_B + \log CBR(t)_j - \log K(t)_j \quad (42)$$

If the body residue is at steady state, the $LC_{50}(t)_j$ is given by a function of time and j as follows:

$$\log LC_{50}(t)_j = -m_B \log K_{OW,j} - a_B + \log CBR(t)_j. \quad (43)$$

It is noticeable that the intercept of the above equation $(-a_B + \log CBR(t)_j)$ is given as a function of time and j. Only after the toxicodynamic steady state, the $LC_{50}(t)_j$ is given by a function of $K_{OW,j}$ as follows:

$$\log LC_{50\infty,j} = -m_B \log K_{OW,j} - a_B + \log CBR_\infty \quad (44)$$

where $CBR_\infty$ is time-independent critical body residue. In the case of non-polar narcotic compounds, $CBR_\infty$ for individual chemicals are similar. It is, therefore, apparent that the time-independent QSAR model for acute toxicity data can relatively underestimate the toxicity for chemicals with high $K_{OW}$ compared with those with low $K_{OW}$.

## 2. Experimental design to estimate toxicokinetic and toxicodynamic parameters

The purpose of TK-TD model is to describe and predict the toxicity time course with estimated toxicokinetic and toxicodynamic parameters. Therefore, TK-TD model must be supported by experimental designs to determine the TK-TD parameters. Generally, TK and TD parameters can be estimated from time-dependent body residue and toxicity data. It should be noted that the time-dependent toxicities based on the external concentration and body residue should be, at the same time, explained by toxicokinetic parameters such as bioconcentration factor (BCF) and the elimination rate constant ($k_e$) estimated in another independent toxicokinetic experiment. Therefore, experimental design and data analysis methods to determine the time-dependent toxicity based on external concentration and body residue should be defined before the experiment starts.

What should be reported for the analysis of time dependent toxicity is not acute and chronic toxicity parameter such as 96-h $LC_{50}$ or 21-d NOEC, but time-to-death curves at different exposure concentrations. From the time-to-death curves, time-dependent toxicity can be described by $LC_{50}(t)$ or $LR_{50}(t)$, which the median lethal concentration or the median body residue for a given exposure time t. The relationship between $LC_{50}(t)$ and $LR_{50}(t)$ is given by

$$LR_{50}(t) = BCF\ K(t)\ LC_{50}(t) \quad (45)$$

where K(t) is dimensionless time-scaled toxicokinetic function and equals $(1-e^{-k_{e,j}t})$ for the one-compartment first-order kinetics. BCF and $k_e$ should be estimated from time-dependent body residue at a given exposure time measured in toxicokinetic experiment or bioconcentration bioassay. Here, three different types of experimental designs are suggested, which includes toxicity bioassay to determine $LC_{50}(t)$, bioconcentration bioassay to determine BCF and $k_e$, and toxicity bioassay to determine $LR_{50}(t)$.

1) Toxicity bioassay: mortality monitoring

At the 'threshold' concentration causing 'incipient' toxicity, when the system is most likely to be in equilibrium, the use of the external surrogate to compare the potency of chemicals is assumed to be valid, since all modifying factors other than potency are equal (Sprague, 1969; Filov *et al.*, 1979; Cox, 1987). It is assumed that there is a normal distribution of the logarithm of tolerance (log-normal) in the exposed population, is commonly made to facilitate statistical analysis of toxicity bioassay data (Finney, 1978). Several types of distributions, including probit, logistic, and Weibull distribution are virtually indistinguishable over most (5% to 95%) of the range of toxicity (Finney, 1978; Christensen, 1984; Hanes and Wedel, 1985).

Time-dependent toxicity can be measured by daily monitoring of mortality. Two different methods such as the median lethal concentration $LC_{50}(t)$ and the median lethal time $LT_{50}(C_w)$ can be used to estimate the toxicodynamic parameters (Lee *et al.*, 2002b). The $LC_{50}(t)$ is determined using concentration-response relationship at a fixed exposure time, whereas the $LT_{50}(C_w)$ is determined using a time-to-death curve in each treatment concentration. Theoretically, $LC_{50}(t)$ and $LT_{50}(C_w)$ are the same. Data analysis method using $LC_{50}(t)$ has a statistical problem called as autocorrelation problem (van den Heuvel *et al.*, 1991). Mortality data in each exposure time are not independent for each other, whereas time-to-death curve in different treatments are independent for each other. $LC_{50}(t)$ and $LT_{50}(C_w)$ can be extended into the lethal concentration or lethal time corresponding to the mortality level x different from 50% such as $LC_x(t)$ and $LT_x(C_w)$ (Kooijman *et al.*, 2003).

2) Bioconcentration bioassays and toxicokinetic modeling

Bioconcentration bioassays focus explicitly on the kinetic aspect of the toxicological process: toxicant uptake, distribution, biotransformation, and elimination. The bioavailability aspect of toxicological process can be examined with bioconcentration bioassays. Most of bioconcentration bioassays are designed to be interpreted in terms of compartmentalized, kinetic-based models (Spacie and Hamelink, 1982) such as one-compartment, first-order kinetic (1CFOK) models (Spacie and Hamelink, 1982; Mancini, 1983; Hawker and Connell, 1985). Although uptake and elimination are often used in a non-specific whole-organism manner (the classic 'black box' approach), rate constants for several pathways, as well as for the impacts of growth dilution, dietary uptake pathway, and metabolism, can be included.

Although the simplification of a complex multi-compartment system to a 1CFOK model has many advantages, there are also several disadvantages. Approximating multi-compartment system with a single-compartment model is reasonable when the rate of distribution is so much faster than the elimination rate, and when the character of the compartment is similar. Another problem is that the single uptake and elimination rate constants of the 1CFOK model are in fact composites of several factors, which act simultaneously. For example, elimination may occur through both the gill and the gut, with contribution of each to the total rate varying as a function of temperature, body size, diet, and ration level. Similarly, growth and metabolic detoxification may appear to be elimination processes in 1CFOK terms. Finally, it is possible to apply 1CFOK model under situations where growth is minimal, where toxicant uptake from food is minimized, and where the test chemical is poorly metabolized.

Usually, two different types of experimental options

can be used to estimate the uptake and elimination rate constant; a long-term uptake phase experiment, where the body residue reaches the steady state, and a short-term uptake and depuration phase experiment. In the case of rapid eliminated compounds, the short-term experiment actually includes the long-term uptake phase experiment. Since, in the case of slowly eliminated compounds, during the test period test animals can significantly grow, a growth dilution term should be included in toxicokinetic model as follows:

$$\frac{dR}{dt}=\frac{k_u}{l}C_w-R\left(\frac{k_e}{l}+\frac{d}{dt}\ln l^3\right) \quad (46)$$

where l is the scaled body length defined by $L/L_m$ where L is body length and $L_m$ is the maximum body length, and defined by $dl/dt=(f-1)\dot{r}_B$, f is the scaled food level and equal 1 if food is enough for growth, and $\dot{r}_B$ isvon Bertalanffy growth rate (Kooijman, 2000). The above toxicokinetic model is one compartment toxicokinetic model with time-varying parameters, because l is given by a function of time. Therefore, the body length should be measured during the experiment and the growth rate should be estimated.

### 3) Determination of critical body residue in toxicity bioassay

In a typical toxicity bioassay, the body residue is essentially being measured indirectly by the mortality of the exposed population. This indirect method of estimating body residue will introduce errors into the estimated value since the variability associated with the tolerance distribution of the exposed population will be included. For many commonly studied organic chemicals the ratio of exposure concentration to body residue is relatively constant over a range of exposure concentrations. The ratio follows the well-established positive relationship between body residue at steady state and the lipophilicity of the bioassay chemical (Mackay, 1982). From the above discussion it appears clear that bioconcentration and toxicity bioassays look alike (McCarty *et al.*, 1989). Both provide information on the kinetic phase of the toxicological process, bioconcentration bioassays by directly estimating rate constants and body residues, and toxicity bioassays by using mortality-time information to indirectly estimate rate constants.

Time-dependent toxicity based on body residue can be measured by two different methods such as the median lethal residue $LR_{50}(t)$ (Landrum *et al.*, 1994) and the mean lethal residue $MLR_{50}(LT_{50,i})$, which can be used to estimate the toxicodynamic parameters (Lee *et al.*, 2002a). The $LR_{50}(t)$ is determined using body residue and response relationship at a fixed exposure time, whereas the $MLR_{50}(LT_{50,i})$ is determined by a mean value of body residue in dead animals from each treatment concentration i. Theoretically, $LR_{50}(t)$ and $MLR_{50}$ $(LT_{50,i})$ are the same if the body residue in dead and live animals are similar. Similar to the $LC_{50}(t)$, data analysis method for $LR_{50}(t)$ has the same problem of $LC_{50}(t)$ ('the autocorrelation'). In contrary to $LT_x$, $MLR_{50}(LT_{50,i})$ cannot be applied to different effect size such 20% or 80% mortality, because $MLR_{50}(LT_{50,i})$ is given by a mean value of body residue from each treatment concentration. Recently, determination methods for the lethal body residue for x% of mortality $LBR_x(t)$ are suggested (Lee and Landrum, 2006b) as follows:

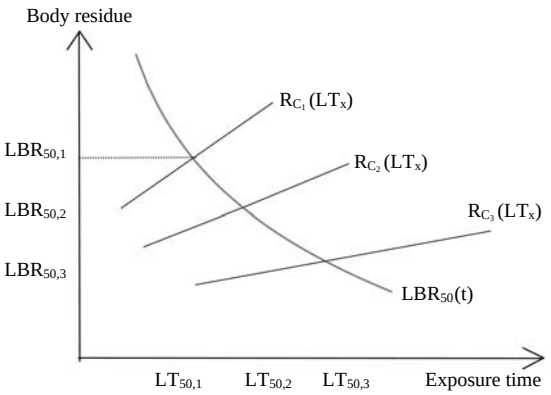


**Fig. 5.** Hypothetical graph showing the relationship between lethal residue and time-to-death. $Rc_i(LT_x)$ is the lethal residue of dead animal with x percent mortality level in each treatment i (i=1, 2, 3), $LBR_{50,i}$ (●) is the mean value of lethal residues of all dead animals within each treatment, $LT_{50,i}$ is the median lethal time in each treatment.

$$LBR_x (t=LT_{x,i}) = R_i (t=LT_x(C_{w,i})) \quad (47)$$

where i is a treatment group with exposure concentration $C_w$, $LT_x(C_w)$ is the lethal time for x% mortality in treatment group with $C_w$, $R_i(t)$ is body residue at exposure time t in each treatment level i. The $LBR_x$ $(t=LT_{x,i})$ can give a schematic picture for the concentration-time-response relationship based on body residue approach (Fig. 5). This new definition reflects variation of lethal body residue in individual test animals within and between treatments.

## FURTHER STUDY NEEDS

For application of toxicokinetic and toxicodynamic models to the effect assessment in field conditions, some issues need to be further investigated. First of all, one-compartment first-order toxicokinetic model needs to be modified to include biotransformation process of organic compounds (Lee and Landrum, 2006a) and two compartments such as target lipid and storage lipid (Escher and Hermens, 2002) as well as growth dilution term (Kooijman, 2000). In addition, for site-specific exposure assessment multiple uptake pathways including sediment particle ingestion and food uptake as well as water uptake should be included (Luoma and rainbow, 2005).

Toxicodynamic model needs to be extended to predict the time-dependent toxicity of a mixture. Recently, Multi-component Damage Assessment Model (MDAM) with toxicokinetic interactions for metabolized organic compound was developed and applied to PAHs (Lee and Landrum, 2006a, b). Further, a new type of toxicodynamic model for mixture toxicity with chemical interaction and toxicodynamic interaction needs to be developed. This type of mixture toxicity model should be based on dynamic interaction between different modes of toxic action.

For more site-specific effect assessment, toxicodynamic model needs to be applied to time-varying exposure condition. Delayed effect, adaptation, and tolerance induction resulting from the time-varying exposures are required to be analyzed quantitatively (Newman and McCloskey, 2000; Reinert, 2002). The long-term ecological consequences of the time-varying exposures depend on the intensity and frequency of the exposures relative to the rates of recovery of the exposed populations (Barnthouse, 2003).

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