Acute Toxicity Modeling of Rainbow Trout and Silver Sea Bream Exposed to Waterborne Metals

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ABSTRACT: Of three proposed acute toxicity models, the uptake–depuration (UD) model, the time-integrated concentration (TIC) model, and the concentration–time (CT) model are derived and verified with acute toxicity data to estimate the internal residues of waterborne metals in fish as a function of a few constants and variables. The main factors are the exposure time, the external exposure concentration, the bioconcentration factor (BCF), and the depuration rate constant (k_2). The UD model is based on the concept of residue levels at the cell membrane well correlating with the whole-body concentrations, whereas the TIC and the CT models are based on the idea of irreversible inhibition of the enzyme acetylcholinesterase (AChE) governing the metal acute toxicity in that metals in the entire fish or in the aqueous phase can be described by the critical area under the time–concentration curve that is associated with a critical TIC of toxicant in the target tissue. A highly significant correlation ($r^2 > 0.9$) was found between predictions and $LC_{50}(t)$ data for both the TIC and the CT models, indicating successfully describe 4- to 18-d $LC_{50}(t)$ data of arsenic (As), cobalt (Co), copper (Cu), and Co/Cu mixture in rainbow trout (*Oncorhyuchus mykiss*) and of Cu in fingerlings and subadults of silver sea bream (*Sparus sarba*). The time-dependent lethal internal concentration at the site of action that causes 50% mortality is also predicted for a given compound and species. It concludes that the TIC and the CT models can be applied to regulate the acute toxicity and to estimate incipient LC_{50} values and internal residues of waterborne metals in fish. Environ Toxicol 16: 349–360, 2001

Keywords: acute toxicity; fish; modeling; waterborne metals

INTRODUCTION

Many heavy metals such as arsenic, cobalt, copper, lead, and mercury can be found in the environment in organic forms originating from either natural or anthropogenic sources. These compounds exhibit many of the characteristics of organic chemicals. There is organism- and species-specific variability in accumulation as well as in response. Connolly (1985), Peterson et al. (1989), McGeachy and Dixon (1990, 1992), Enserink et al. (1991), Borgmann et al. (1991), and Liao et al. (1999) found that waterborne metals such as zinc, aluminum, arsenic, and cadmium all appear to exhibit residue-effect relationship in fish/shellfish. If residue-effect relationships can be better defined and modeled, the ability to interpret existing laboratory and field data, as well as predict situations of impact in advance, will be substantially improved.

Based on the toxicological principles, toxic chemicals elicit their toxicity by a specific/nonspecific reversible/irreversible disturbance of the cell membrane caused by their accumulation within the aquatic organisms. McCarty and Mackay (1993) suggested that the whole-body concentration of chemicals at the time of death, referred to as the lethal body burden, critical body residue, or internal response concentration, is constant. The expressions "internal concentration,"

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"body burden," and "residue" are used synonymously (Hendriks, 1995) in that the concept is based on the idea that residue levels at the cell membrane are well correlated with the whole-body concentrations.

McCarty and Mackay (1993) and Hendriks (1995) have reviewed the literature and showed that internal response concentrations of toxic chemicals are indeed fairly constant. Moreover, it has been shown that toxic chemicals exhibiting the same mode of action are associated with a specific range of internal concentrations. These findings lead to the application of the internal concentration as a relevant parameter for the risk assessment of chemicals among mode of actions. Mancini (1983), Connolly (1985), Menzel (1987), Bartell et al. (1988), and others modeling the environmental fate, the bioaccumulation, and toxicity have recognized the need for residue-effect relationships and advocated a body-residue-based approach in environmental toxicity and risk assessment.

The adverse effects of metals depend on the site of exposure. Generally, the toxicity of heavy metals is largely due to their reactions with sulfhydryl groups (Bast, 1996). Lipophilic organometallic compounds (such as methylmercury and triethyltin) easily cross the blood-brain barrier, whereas inorganic metallic compound also reach the brain tissue (Bast, 1996). Bast (1996) also indicated that for many axonal toxins, deregulation of intracellular energy production is the main cause of toxicity. Arsenic is a good example. In experimental animals, arsenic has been shown to affect hepatic mitochondria enzymes (Fowler and Woods, 1979). It can also pass the blood-brain barrier, be accumulated in the brain, and can exert neurochemical effects (Nagaraja and Desiraju, 1993; Valkonen et al., 1983). Tas et al. (1991) noted that the residue level and mode of action (neurotoxicity) of tributyl and triphenyltins in fish were similar to those of the pyrethroid insecticides.

The inhibition of acetylcholinesterase (AChE) in the nervous tissue and other target organs is generally considered to be the critical effect leading to the acute toxicity of many toxic chemicals (Maxwell et al., 1988; Gearhart et al., 1990; McCarty and Mackay, 1993; Abbas and Hayton, 1997). Nagaraja and Desiraju (1994) further indicated that AChE activity in rats was inhibited in some regions of the brain following inorganic arsenic intake. Gearhart et al. (1990) and Abbas and Hayton (1997) indicated that AChE inhibition and mortality due to toxic chemical exposure have been shown to be dependent on both exposure concentration and duration for a variety of aquatic organisms.

In this study, we derive three models that predict acute toxicity of metals in fish. All the proposed models are developed based on a well-established one-compartment bioaccumulation model. The applicability of these proposed models is compared with each other to predict toxicity to aquatic organisms during continuous exposures to waterborne metals based on acute toxicity data obtained from published studies by McGeachy and Dixon (1992), Marr et al. (1998), and Wong et al. (1999).

We test the proposed models using published acute toxicity data for two species of fish, rainbow trout (*Oncorhynchus mykiss*) and silver sea bream (*Sparus sarba*), exposed continuously to waterborne metals, arsenic (As), cobalt (Co), copper (Cu), and Co/Cu mixture, to compare observed and predicted median lethal concentrations (LC_{50}). As a result, the proposed models could be tested extensively for continuous exposure regimes, and the accuracy of its predictions could also be verified through the chemical being tested, the test species, and the exposure scenario being tested.

MATHEMATICAL MODELS

Uptake-Depuration Toxicity Model

Mancini (1983) has been proposed a mechanistic model for predicting toxicity of time-varying exposure of a chemical to an aquatic organism based on simple first-order kinetics of the uptake–depuration model. Testing the mathematical model with experimental data, Mancini (1983) found a good agreement between predicted and observed mortality of rainbow trout and golden shiners exposed to time-varying concentration of zinc. Based on the data for small fish as well as the internal concentration estimates for large fish by McKim and Schmieder (1991), who indicated that whole-body residues are reasonable first approximations of the amount of chemical present at the toxic action sites. For acutely toxic exposures, data obtained from Hickie (1990), Meyer et al. (1995), and Marr et al. (1998) also confirmed this conclusion for both continuous and intermittent exposure regimes.

Thus based on the whole-body residue concept, an aquatic organism dies at a constant internal threshold concentration of a toxicant. McCarty and Mackay (1993) indicated that using whole-body residues as surrogates for target tissue residues in the organism has shortcomings in dealing with the external exposure approach such as metabolic breakdown or activation, internal distribution, lipid types and content, temperature, and general biological factors such as species, sex, life stage, and season; however, a class of chemicals for which the concept of the whole-body residue model has been originally derived and successfully applied.

A well-established first-order one-compartment bioaccumulation model generally can be used to estimate the internal whole-body concentration of chemi-

cal in aquatic organism and has the form (Lin and Liao, 1999)

$$\frac{dC_{\mathrm{wb}}(t)}{dt} = k_1 C_w - k_2 C_{\mathrm{wb}}(t), \tag{1}$$

where C_{wb} is the internal whole-body concentration of chemical in aquatic organism (mg kg^{-1}), C_w is the external aqueous concentration of the chemical ($\mu\mathrm{g\ L}^{-1}$), k_1 (L kg^{-1} d^{-1}) and k_2 (d^{-1}) are the uptake and depuration rate constants, respectively, and t is the exposure time (h). The solution of Eq. (1) subjects to constant C_w and zero initial concentration of toxicant at the site of action is

$$C_{\mathrm{wb}}(t) = \mathrm{BCF} C_w (1 - e^{-k_2 t}), \tag{2}$$

where BCF (L kg^{-1}) is the steady-state bioconcentration factor that is defined as the ratio between the uptake and depuration constants or between the internal whole-body chemical concentration and water chemical concentration as $\mathrm{BCF} = C_{\mathrm{wb}}/C_w = k_1/k_2$.

Two model assumptions are inherent in our analysis: (1) An organism dies when it accumulates an internal lethal concentration (C_L) at the site of action, and (2) $m\%$ of the organisms have died when $C_{\mathrm{wb}} = C_{L,m}$, i.e., the toxicant that the concentration at the site of action that causes $m\%$ mortality, is a constant. Based on these assumptions, LC_{50} can then be predicted from knowledge of the exposure time, depuration rate constant, BCF, and $C_{L,50}$ followed by Eq. (2) as:

$$\mathrm{LC}_{50}(t) = \frac{C_{L,50}}{\mathrm{BCF}(1 - e^{-k_2 t})}. \tag{3}$$

Equation (3) is referred to as the uptake–depuration (UD) toxicity model. If the UD model is appropriate, the data will lie along a convex curve that approaches a horizontal asymptote in a plot of $\ln(\mathrm{LC}_{50})$ vs. $\ln(t)$. The curvature is determined by the depuration rate (k_2), and the horizontal asymptote is the incipient lethal level of LC_{50} that is denoted as $\mathrm{LC}_{50}(\infty)$.

When the exposure time approaches infinity, Eq. (3) gives a relation among $\mathrm{LC}_{50}(\infty)$, $C_{L,50}$, and BCF as:

$$C_{L,50} = \mathrm{LC}_{50}(\infty)\mathrm{BCF}. \tag{4}$$

According to the UD model, the $C_{L,50}$ will be constant and thus independent of exposure concentration and time of death. The LC_{50} will reach its incipient value when the internal body concentration has reached an equilibrium with the external constant aqueous concentration.

According to the receptor theory (Musch, 1996), the intensity of a toxic effect exhibited by a toxicant depends on the degree of receptor occupation. Musch (1996) indicated that receptor interactions could be divided into reversible and irreversible receptor interactions. According to Hermens (1989), the application of the internal whole-body concentration regarded as a surrogate target concentration, i.e., the concept of the UD model, only in the case for reversibly acting compounds that have their target located in the lipid phase.

Time-Integrated Concentration Toxicity Model

de Vries (1996) suggested that in pharmacokinetic modeling, the area under the plasma concentration–time curve is commonly applied to estimate the total amount of substance eliminated from the body over a certain time period. Analogously, over a certain time period, the total amount of inhibited AChE molecules in the target tissue that can be seen equals the amount of toxic compound that has been removed from the target tissue. This amount is dependent on both the inhibition rate constant (k_i) and the time course of the concentration or time-integrated concentration (TIC) of the toxic compound in the target tissue. Therefore, this TIC can be estimated from the area under the curve, which describes the concentration of the toxic compound in the target tissue as a function of time.

The concept of the TIC model thus could be stated as follows: The amount of inhibited molecules per mass unit of target tissue at the time of death, $C_{I,b}$ (mg kg^{-1}), would be determined by an inhibition rate constant, k_i (d^{-1}), and the critical area under the time–concentration curve, which describes the toxicant concentration at the target tissue of action [$C_b(t)$] until the time of death t (de Vries, 1996):

$$C_{I,b}(t) = k_i \int_0^t C_b(t)\, dt = k_i A_{C,b}, \tag{5}$$

where $A_{C,b} \equiv \int_0^t C_b(t)\, dt$ is expressed as mg d kg^{-1}.

Although the brain and the skeletal muscle are known to be the main target tissues for heavy-metals poisoning, it is impossible to assign in precise location where AChE inhibition is critical for mortality. Furthermore, it is not exactly known which organ is responsible for the enzymatic formation of the toxic compound that will eventually reach the critical target.

Therefore, we simplify the behavior of chemical in the organism. We assume the aquatic organism as a single compartment and regard the entire aquatic organism as a reference compartment for the target tissue. If we approximate the aquatic organism as a single compartment, $A_{C,b}$ could be directly related to a

critical area under time–concentration curve in the entire organism. The $C_{I,b}$ in this case is defined as the amount of inhibited molecules per unit mass of organism and described as follows:

$$C_{I,\mathrm{wb}}(t) = k_i \int_0^t C_{\mathrm{wb}}(t)\, dt = k_i A_{C,\mathrm{wb}}, \qquad (6)$$

where $A_{C,\mathrm{wb}}$ can be derived from the first-order one-compartment bioaccumulation model in Eq. (2) as follows:

$$A_{C,\mathrm{wb}} \equiv \int_0^t C_{\mathrm{wb}}(t)\, dt = \int_0^t \mathrm{BCF} C_w (1 - e^{-k_2 t})\, dt$$

$$= \frac{\mathrm{BCF}}{k_2} C_w (k_2 t + e^{-k_2 t} - 1). \qquad (7)$$

The $\mathrm{LC}_{50}(t)$ can then be calculated from Eq. (7) by regarding C_w as $\mathrm{LC}_{50}(t)$:

$$\mathrm{LC}_{50}(t) = \frac{A_{C,\mathrm{wb}}}{\mathrm{BCF}} \left(\frac{k_2}{k_2 t + e^{-k_2 t} - 1} \right). \qquad (8)$$

Equation (8) implies that $\mathrm{LC}_{50}(t)$ will reach zero at infinite exposure duration. Because organisms can actively regulate some metals as essential micronutrients (McCarty and Mackay, 1993), therefore, the organisms will put compensating mechanisms into action. Hence, the $\mathrm{LC}_{50}(t)$ is eventually expected to reach an incipient value, $\mathrm{LC}_{50}(\infty)$, as:

$$\mathrm{LC}_{50}(t) = \frac{A_{C,\mathrm{wb}}}{\mathrm{BCF}} \left(\frac{k_2}{k_2 t + e^{-k_2 t} - 1} \right) + \mathrm{LC}_{50}(\infty). \quad (9)$$

Equation (9) is then referred to as the TIC toxicity model.

Therefore, the TIC toxicity model is based on a direct relationship between adverse effects and extent of AChE inhibition in the target tissue, i.e., mortality is assumed to occur at a fixed AChE inhibition percentage. The TIC model assumes that the AChE concentration in the target tissue is constant, i.e., the lethal AChE inhibition percentage is related to a critical amount of occupied target sites. In contrast to reversible toxic effect in the UD model, the inhibition of AChE due to the waterborne metals is considered to be an irreversible interaction (Bast, 1996).

Substitution of C_w in Eq. (2) by $\mathrm{LC}_{50}(t)$ in Eq. (9) leads to the following description of the internal lethal concentration as a function of time $[C_{\mathrm{wb}}(t) = C_{L,50}(t)]$:

$$C_{L,50}(t) = A_{C,\mathrm{wb}} \left[\frac{k_2 (1 - e^{-k_2 t})}{k_2 t + e^{-k_2 t} - 1} \right]$$

$$+ \mathrm{BCF}(1 - e^{-k_2 t}) \mathrm{LC}_{50}(\infty). \qquad (10)$$

Concentration–Time Toxicity Model

Because it is difficult to measure the amount of toxicant at target sites within the organisms, a surrogate measurement, such as concentration in the exposure medium, is normally used. Barron (1990), McCarty and Mackay (1993), Meyer et al. (1995), and Musch (1996) suggested that the external exposure water as a reference compartment for the internal aqueous phase could be applied in modeling the concentration kinetics of chemicals in aqueous phases of aquatic organisms. Their suggestion, based on the idea that for a specified percentage mortality, for some aquatic organisms exposed continuously to constant concentrations of a toxicant, the arithmetic product of external exposure concentration (C_w) and time to death (t_d) approximately reaches a constant value as:

$$C_w t_d = \text{constant}, \qquad (11)$$

where $C_w t_d$ denotes the product of the toxicant concentration in exposure water and duration at death (t_d, h). We designate $A_{\mathrm{CT}} \equiv C_w t_d$, which has the unit as mg d kg^{-1}.

In comparing Eqs. (7) and (11), the values of k_2 and BCF with respect to Eq. (11) become $k_2 \to \infty$ and BCF = 1. That is to say, the instantaneous equilibrium between the external and internal compartments is kinetically represented by an infinite k_2 value, where k_2 represents the total depuration rate constant for the aqueous compartment of the organism. The BCF, defined as C_{wb}/C_w, thus is assumed to have a value of unity, indicating that the concentrations in the external and internal aqueous phases are not expected to deviate significantly.

Therefore, incorporating with the concept was shown in Eq. (11), the $\mathrm{LC}_{50}(t)$ could be calculated from Eq. (9) with $k_2 \to \infty$ and BCF = 1 as:

$$\mathrm{LC}_{50}(t) = \frac{A_{\mathrm{CT}}}{t} + \mathrm{LC}_{50}(\infty). \qquad (12)$$

Equation (12) is referred to as the concentration–time (CT) toxicity model. The CT toxicity model gives a very simple model describing time-dependent LC_{50} values of waterborne metals in fish. In comparing the UD and the TIC models, the CT model does not require kinetic input parameters.

The CT model assumes that the concentration of the chemical in the aqueous compartment that is represented by blood plasma of fish or by the hemolymph of molluscs is now considered as a reference tissue for the target tissue and for the tissue where chemicals are biotransformed. The chemical concentration in the aqueous phase of the organism instantaneously reaches equilibrium with its concentration in the external aqueous exposure phase.

The internal lethal concentration in the entire organism at time of death ($C_{L,50}$) can subsequently be described as follows by substituting C_w in Eq. (2) by $LC_{50}(t)$ in Eq. (12) and acknowledging that $C_{wb}(t) = C_{L,50}(t)$ as:

$$C_{L,50}(t) = \mathrm{BCF}(1 - e^{-k_2 t})\left(\frac{A_{CT}}{t} + \mathrm{LC}_{50}(\infty)\right). \quad (13)$$

Comparing the UD model in Eq. (4), Eqs. (10) and (13) reflect a time-dependent toxicity with which we may give a reason to explain the fact that time dependency was also observed after relatively long-term exposure regarding the time required to reach a steady-state concentration in the organisms.

The integrated scheme of principal algorithms for the UD model, the TIC model, and the CT model are schematically illustrated in Figure 1.

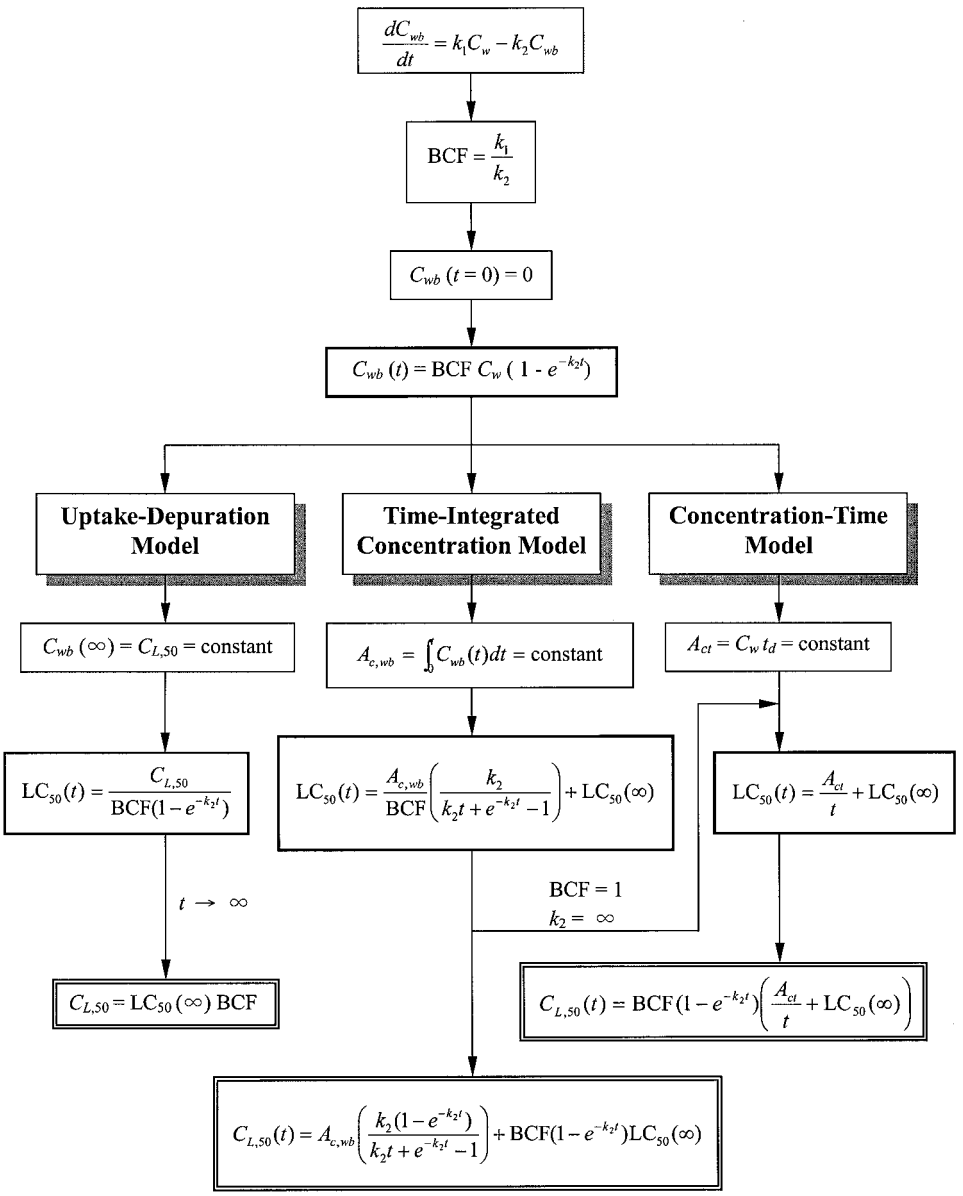

Fig. 1. Integrated scheme of principal algorithms for the uptake–depuration (UD) toxicity model, the time-integrated concentration (TIC) toxicity model, and the concentration–time (CT) toxicity model.

MODEL IMPLEMENTATION

Input Parameters

The majority of the data used in this investigation were taken from published works on the acute toxicity of waterborne arsenate to rainbow trout (*Oncorhyuchus mykiss*) (McGeachy and Dixon, 1992), of Cu, Co, and Co/Cu mixture to rainbow trout (*Oncorhyuchus mykiss*) fry (Marr et al., 1998), and of Cu to fingerlings and subadults of sliver sea bream (*Sparus sarba*) (Wong et al., 1999). Table I lists the main experimental conditions used in rainbow trout and silver sea bream acute toxicity tests.

To evaluate the UD model, $LC_{50}(t)$ values were fitted according to Eq. (3), whereas the $LC_{50}(t)$ data were fitted based on Eq. (9) for the TIC model and Eq. (12) for the CT model, respectively. The input parameters of the depuration rate constant (k_2) for the Eqs. (3), (9), and (12) are given in Table II. The k_2 value for the whole-body arsenic concentrations in the rainbow trout was estimated using a nonlinear regression fitting according to Eq. (2) as: $C_{wb}(t) = \mathrm{BCF}C_w[1 - \exp(-k_2 t)]$, based on a 77-d uptake–depuration exposure experiment by McGeachy and Dixon (1990). Depuration rate constant for Cu, Co, and Co/Cu mixture in the rainbow trout were obtained from the given values by Marr et al. (1998) based on an acute model nonlinear fitting to the $LC_{50}(t)$ data. The k_2 values for Cu in the fingerlings and subadults of the silver sea bream were also estimated by a nonlinear fitting to Eq. (2) based on the experimental results of the measured Cu concentrations in tissues and in seawater at the end of a 30-d exposure period by Wong et al. (1999).

TABLE I. Experimental conditions used in rainbow trout and silver sea bream acute toxicity tests

Species	Compound	Duration (d)	Temperature (°C)	pH	Fish Size		Reference
					Weight (g)	Length (mm)	
Rainbow trout	As	17.5	5, 15	7.98 ± 0.03[a]	1.5[b]	ND[c]	McGeachy and Dixon (1992)
	Co	6	9.8 ± 0.3	7.50 ± 0.11	ND	29–33	Marr et al. (1998)
	Cu	6	9.8 ± 0.3	7.50 ± 0.10	ND	29–33	
	50 μg L^{-1} Co + Cu	6	10.0 ± 0.3	7.47 ± 0.09	ND	29–33	
	250 μg L^{-1} Co + Cu	6	10.0 ± 0.3	7.49 ± 0.90	ND	29–33	
Silver sea bream							
Fingerlings	Cu	4	28 ± 2	ND	9.4 ± 2.1	7.2 ± 0.5	Wong et al. (1999)
Subadults	Cu	4	28 ± 2	ND	85.5 ± 27.1	14.3 ± 1.1	

[a] Mean value ± standard deviation.
[b] Mean value.
[c] Not determined.

TABLE II. Input parameters and parameter estimates for the UD, TIC, and CT models applied to the $LC_{50}(t)$ data of rainbow trout and silver sea bream

Species	Compound	Input Parameter	Parameter Estimates				
			UD Model	TIC Model		CT Model	
		k_2 (d^{-1})	$LC_{50}(\infty)$ (mg L^{-1})	$A_{c,wb}$ BCF (mg d L^{-1}/L kg^{-1})	$LC_{50}(\infty)$ (mg L^{-1})	A_{CT} (mg d L^{-1})	$LC_{50}(\infty)$ (mg L^{-1})
Rainbow trout	As	0.018	29.239	14.722	57.308	226.754	34.163
	Co	0.583	0.679	2.405	0.267	3.815	0.236
	Cu	0.259	0.010	0.005	0.013	0.026	0.011
	50 μg L^{-1} Co + Cu	0.520	0.017	0.019	0.009	0.053	0.007
	250 μg L^{-1} Co + Cu	0.898	0.017	0.035	0.004	0.065	0.001
Silver sea bream							
Fingerlings	Cu	0.367	0.663	0.167	0.986	1.294	0.698
Subadults	Cu	1.134	1.418	0.511	1.091	1.513	0.827

The values of lethal body concentration ($C_{L,50}$) of As, Cu, Co, and Cu/Co mixture in rainbow trout and of Cu in silver sea bream were predicted for different exposure times according to Eq. (4) (the UD model), Eq. (10) (the TIC model), and Eq. (13) (the CT model). The input parameters used in Eqs. (4), (10), and (13) are presented in Table III. The BCFs of arsenic in the rainbow trout and of Cu in the silver sea bream were estimated by a nonlinear regression fitting to the Eq. (2), using measured exposure concentrations (C_w's) and whole-body concentrations [$C_{wb}(t)$'s] based on the exposure experiments by McGeachy and Dixon (1990) and Wong et al. (1999), respectively.

All curve fittings were performed using the nonlinear regression option of the Statistica software package (StatSoft, Tulsa, OK, USA). The coefficient of determination (r^2) of the optimal fits of the $LC_{50}(t)$ data were also calculated by the Statistica.

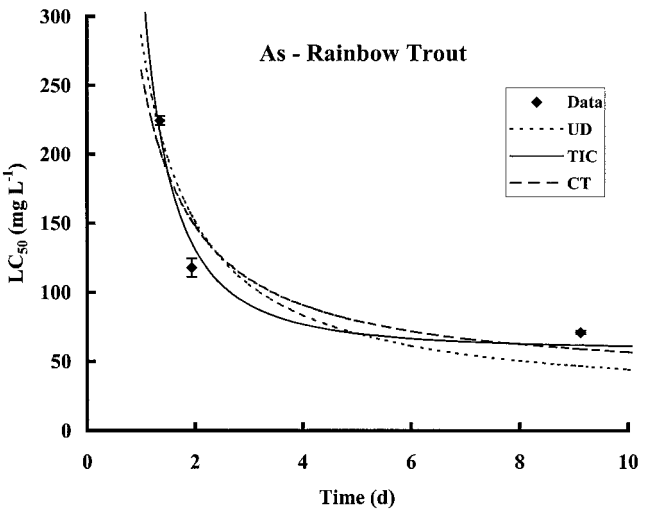

Fig. 2. Optimal fits of the UD model, the TIC model, and CT model to the $LC_{50}(t)$ data of As in rainbow trout (*Oncorhyuchus mykiss*).

Fitting Proposed Models to $LC_{50}(t)$ Data

The optimal fits of the UD model, the TIC model, and the CT model to the $LC_{50}(t)$ data of As in rainbow trout, of Cu in fingerlings and subadults of silver sea bream, and of Cu, Co, and Co/Cu mixture in rainbow trout are presented in Figures 2, 3, and 4. Estimated values for $LC_{50}(\infty)$, $A_{c,wb}/BCF$, and A_{CT} are presented in Table II for the different metal–species combinations. Statistical data associated with the optimal fits of the various mode ls is given in Table IV.

As can be seen from Figures 2–4, neither the $LC_{50}(t)$ data for As, Co, Cu, and Co/Cu mixture in rainbow trout nor the data for the Cu in fingerlings and subadults of silver sea bream are fitted accurately by the UD model. Generally, the UD model consistently overestimates $LC_{50}(t)$ values at exposure times and substantially underestimates $LC_{50}(t)$ values at the beginning of exposure times. The low quality of the $LC_{50}(t)$ fits of the UD model are further expressed by the low coefficient of determination (average $r^2 = 0.615 \pm 0.32$) and by the model estimates of $LC_{50}(\infty)$, which are inaccurate since they are higher than the LC_{50} values at $t = 10$ d with respect to Co and Co/Cu mixture in rainbow trout [Figs. 4(A), (C), and (D)] and Cu in subadults of silver sea bream at $t = 2$ d [Fig. 3(B)].

Table IV also shows that the discrepancy between the UD model and the TIC and CT models is most

TABLE III. Input parameters for $C_{L,50}$ (mg kg^{-1}) predictions of As in rainbow trout and of Cu in silver sea bream

Species	Compound	Input Parameter	UD Model	TIC Model	CT Model
Rainbow trout	As	BCF (L kg^{-1})	0.083	0.083	0.083
		k_2 (d^{-1})		0.108	0.108
		$LC_{50}(\infty)$ (mg L^{-1})	29.239	57.308	34.163
		$A_{C,wb}$ (mg d L^{-1})		1.217	
		A_{CT} (mg d L^{-1})			226.754
Silver sea bream					
Fingerlings	Cu	BCF (L kg^{-1})	2.143	2.143	2.143
		k_2 (d^{-1})		0.367	0.367
		$LC_{50}(\infty)$ (mg L^{-1})	0.663	0.986	0.698
		$A_{C,wb}$ (mg d L^{-1})		0.359	
		A_{CT} (mg d L^{-1})			1.294
Subadults	Cu	BCF (L kg^{-1})	8.552	8.552	8.552
		k_2 (d^{-1})		1.134	1.134
		$LC_{50}(\infty)$ (mg L^{-1})	1.418	1.091	0.827
		$A_{C,wb}$ (mg d L^{-1})		4.367	
		A_{CT} (mg d L^{-1})			1.513

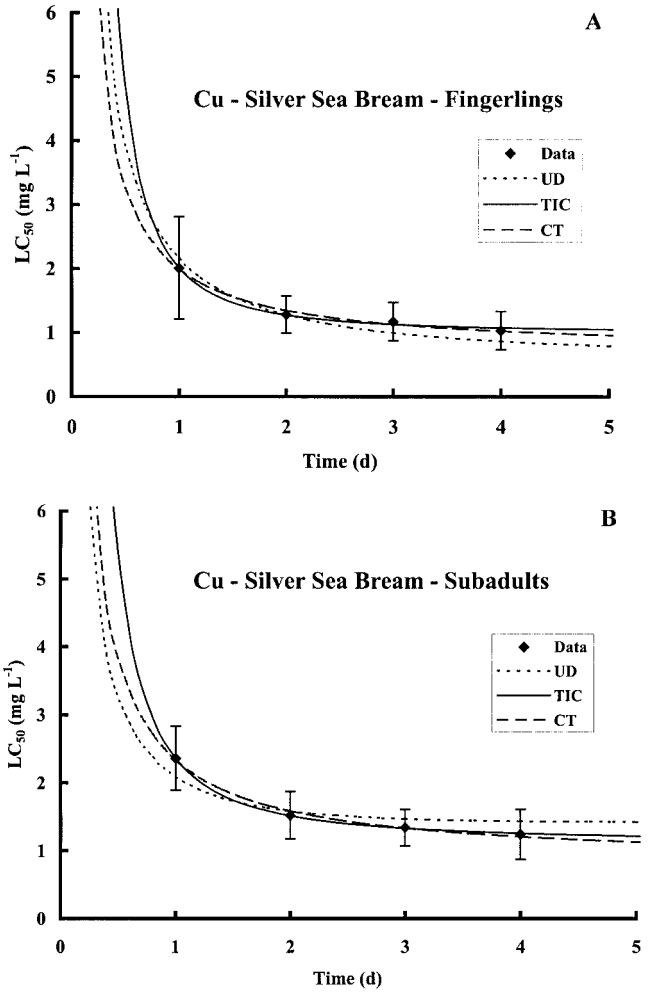

Fig. 3. Optimal fits of the UD model, the TIC model, and CT model to the $LC_{50}(t)$ data of Cu in (A) fingerlings and in (B) subadults of sliver sea bream (*Sparus sarba*).

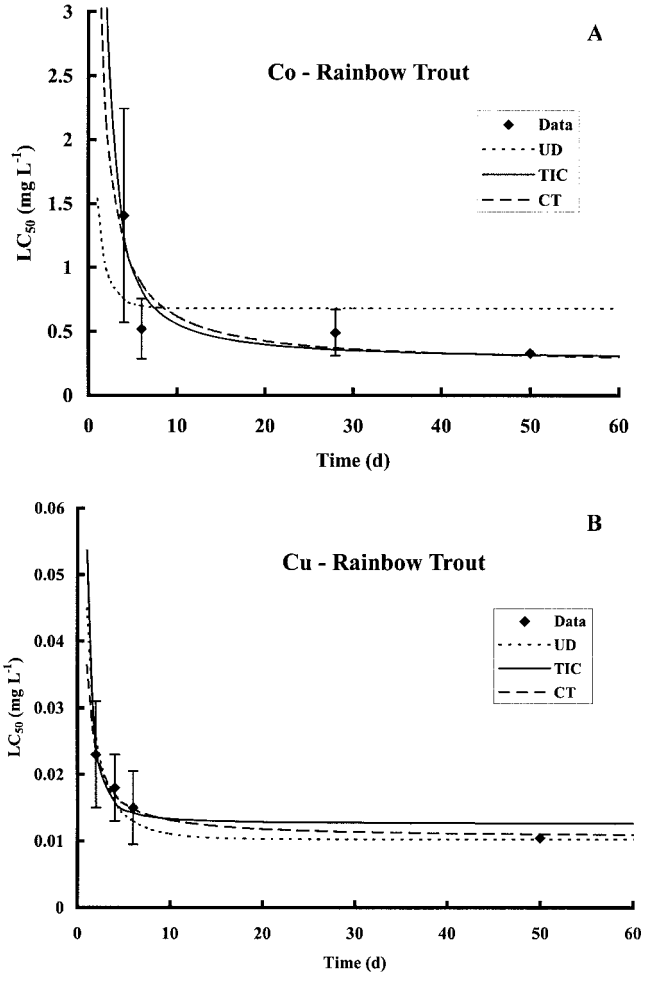

Fig. 4. Optimal fits of the UD model, the TIC model, and CT model to the $LC_{50}(t)$ data of (A) Co, of (B) Cu, of (C) 50 μg L^{-1} Co + Cu, and of (D) 250 μg L^{-1} Co + Cu in rainbow trout (*Oncorhyuchus mykiss*).

significant in the data from the experiments using Co or Co/Cu mixtures, indicating the mechanisms of Co toxicity will affect the predictive ability of the UD model that is based on the reversible receptor interaction. Marr et al. (1998) further pointed out that in Co/Cu mixtures Co acted as an antagonist during the first 48–96 h (i.e., Co^{2+} acted as a competing ion for binding of Cu^{2+} on the fish gills, thus allowing less Cu to accumulate), but later acted as an additive or slightly synergistic toxicant, making it difficult to predict short-term mortality of fish in Co/Cu mixtures.

Both the TIC and the CT models describe the data in a much more accurate way and are in correspondence with the observed toxicity. Both r^2 values indicate that the quality differences between the fits of the TIC (average $r^2 = 0.939 \pm 0.07$) and the CT (average $r^2 = 0.905 \pm 0.09$) models are small (Table IV and Figs. 3 and 4).

The estimated incipient LC_{50} values by the TIC and the CT models seem accurate since they are reasonably in agreement with the observed LC_{50} values at $t = 6$ d for Co, Cu, and Co/Cu mixture in rainbow trout [Figs. 4(A)–(D)]. The differences in incipient LC_{50} estimates between the TIC and the CT models are also small (Table II).

The fit of a model might be strongly determined by the input parameters. Therefore, uncertainties in the k_2 value, which is an input parameter in both the UD and TIC model (Table II), affect the validation of the models. The experimental LC_{50} data for the rainbow trout and silver sea bream exposure to waterborne metals support the validity of the TIC and the CT models, despite the uncertainties in the input parameter k_2.

Due to the low-quality fits of the UD model to acute toxicity data, the present study suggests that the use of constant internal body residues for each individual

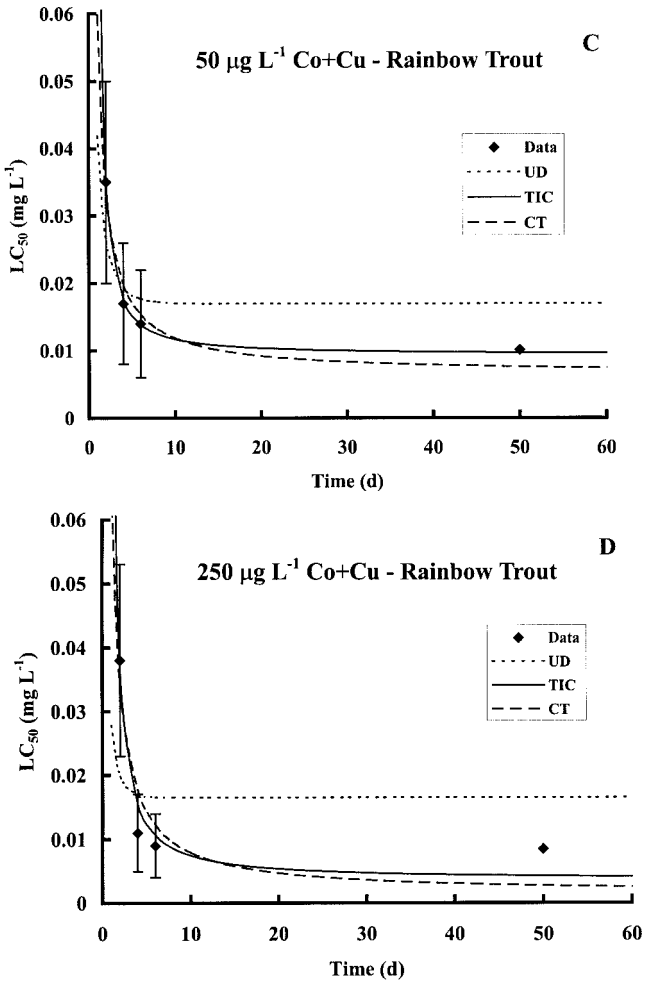

Fig. 4. (*Continued*)

mode of action as an interpretive and regulatory tool in the environmental risk assessment of chemicals is limited to mode of actions that have a reversible receptor interaction. Hermens (1989) and McCarty and Mackay (1993) also indicated that the concept employed in the UD model may not hold for chemicals exhibiting an irreversible adverse effect or a specific model of action could also be complicated and misleading in estimating ecosystem concentrations and comparing these concentrations with LC_{50} data.

As described by Meyer et al. (1995) and Musch (1996), the concept of the CT model in their expression has the form as $C_w^n t_d = \text{constant}$, where the exponent n is usually greater than 1. The conclusion drawn by Meyer et al. (1995) indicated that if $n \neq 1$ and error of $\pm 50\%$ is acceptable, the $C_w^n t_d$ type model appears to adequately predict pulse–exposure LC_{50} data for common shiners (*Notropis cornutus*) and rainbow trout (*Oncorhyuchus mykiss*) to monochloramine. In the present CT model $n = 1$ appears to predict the 4–18-d $LC_{50}(t)$ data successfully for waterborne metals in rainbow trout and silver sea bream. However, whether the acceptable accuracy of the CT model for other specific compound–species combinations and exposure regimes are confined to such exponent ranges remains to be seen.

The CT toxicity model [Eq. (12)] that does not require kinetic input parameters offers a simple model to estimate incipient LC_{50} values. The CT model, however, might be restrictively applicable to situations where an internal steady-state concentration of the chemical has been approximately reached. If the CT model does not succeed in the prediction of $LC_{50}(t)$ data, the more complex TIC toxicity model [Eq. (9)] is likely to be a candidate to employ. The results thus suggest that constant $A_{C,\text{wb}}$'s or A_{CT}'s for chemicals that act irreversibly with a specific receptor may have future potential as a tool in environmental risk assessment.

Prediction of Lethal Internal Concentration $C_{L,50}$

The predicted lethal internal concentrations at the site of action that causes 50% mortality (i.e., the $C_{L,50}$ values) by the UD, the TIC, and the CT models for As

TABLE IV. Coefficient of determination (r^2) of the optimal fits of UD, TIC, and CT models to LC_{50} (t) data for rainbow trout and silver sea bream

Species	Compound	Coefficient of Determination (r^2)		
		UD Model	TIC Model	CT Model
Rainbow trout	As	0.843	0.974	0.859
	Co	0.131	0.812	0.740
	Cu	0.830	0.871	0.981
	50 μg L^{-1} Co + Cu	0.605	0.998	0.946
	250 μg L^{-1} Co + Cu	0.202	0.927	0.828
Silver sea bream				
Fingerlings	Cu	0.857	0.993	0.989
Subadults	Cu	0.834	0.999	0.993

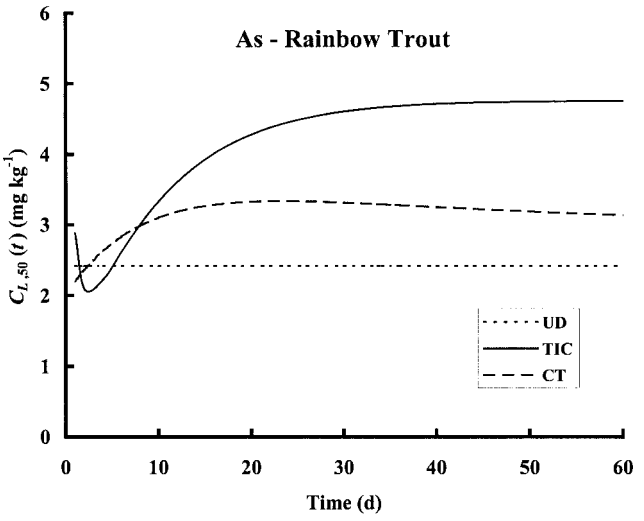

Fig. 5. Prediction of lethal internal residues at the site of action that causes 50% mortality ($C_{L,50}$ values) by the UD model, the TIC model, and the CT model for As in rainbow trout (*Oncorhyuchus mykiss*).

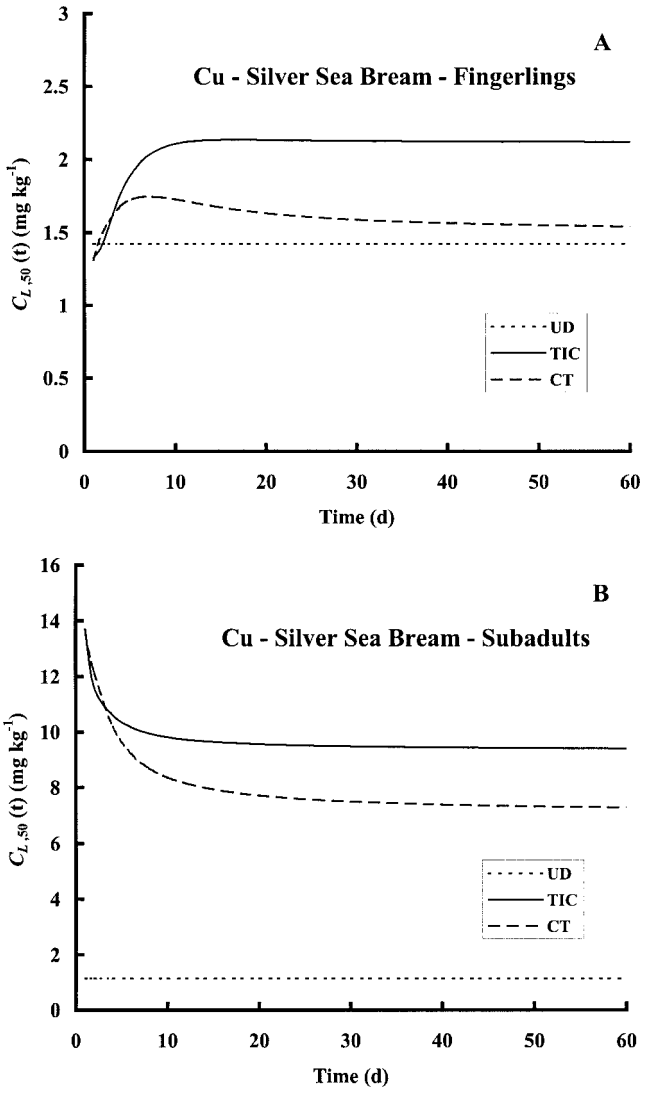

Fig. 6. Prediction of lethal internal residues at the site of action that causes 50% mortality ($C_{L,50}$ values) by the UD model, the TIC model, and the CT model for Cu in (A) fingerlings and in (B) subadults of sliver sea bream (*Sparus sarba*).

in rainbow trout and for Cu in the fingerlings and subadults of silver sea bream are presented in Figures 5 and 6, respectively, based on the input parameters given in Table III. Table V lists the estimated LC_{50} values with 95% confidence limits (CL) and predicted $C_{L,50}$ values ($\pm$SE) of As in rainbow trout and of Cu in fingerlings and subadults of silver sea bream at different exposure times.

As can be seen from Figures 5 and 6, the UD model fails to describe the apparent time-dependent concentration of the $C_{L,50}$ values. Figures 5 and 6(A) indicate that both the TIC and the CT models predict the $C_{L,50}$ values to increase slightly in time, then reach a steady-state concentration in As–rainbow trout and Cu–silver sea bream–fingerlings combinations, whereas in Figure 6(B) the predicted $C_{L,50}$ values decrease in time during the time scope of dead fish in Cu–silver sea bream–subadults combination.

Figure 5 shows that $C_{L,50}$ values of As in rainbow trout are calculated to reach an equilibrium at about 40 and 30 d for the TIC and the CT models, respectively. For $C_{L,50}$ values of Cu in fingerlings of silver sea bream, the times to reach an equilibrium are about 20 and 45 d [Fig. 6(A)], whereas in subadults are about 15 and 20 d for the TIC and the CT models, respectively [Fig. 6(B)]. Therefore, the most accurate prediction of the $C_{L,50}$ values in rainbow trout and silver sea bream seems to be given by the TIC and the CT models. Although the predictions of the UD model may be in reasonable agreement with the $C_{L,50}$ values at the low exposure regimes, however, they do not explain the time-dependent $C_{L,50}$ values.

Figures 5 and 6 also show that different chemical–species–exposure scenario combinations result in different shapes of time–course profiles. The possible reasons for the shape differences in relation to the postulated relevant biological mechanisms could be explained as follows: (1) According to the TIC toxicity model, mortality occurs at a critical TIC of the toxic agent in the target tissue, whereas in the CT model, the time to death of fish can be determined by the aqueous exposure concentration of the chemicals; indicating that for a given $A_{C,\mathrm{wb}}$ or A_{CT}, different exposure concentrations followed by different times to deaths are associated with different internal lethal concentrations. Thus, the TIC and the CT models reflect the

TABLE V. Estimated LC_{50} value with 95% confidence limits and average $C_{L,50}$ values ($\pm$SE) of As in rainbow trout (*Oncorhyuchus mykiss*) and Cu in sliver sea bream (*Sparus sarba*) at different exposure times

Species	Compound	t (d)	$LC_{50}(t)$ (mg L^{-1})	$C_{L,50}(t)$ (mg kg^{-1})
Rainbow Trout	As	1.34	224.50 $\pm$ 3.32[a]	2.07 $\pm$ 0.63
		1.93	117.88 $\pm$ 6.60	2.29 $\pm$ 0.16
		9.13	71.00 $\pm$ 1.15	2.89 $\pm$ 0.42
Silver sea bream				
Fingerlings	Cu	1	2.01 (1.69 − 3.29)[b]	1.35 $\pm$ 0.06
		2	1.28 (0.98 − 1.56)	1.45 $\pm$ 0.05
		3	1.17 (0.86 − 1.47)	1.88 $\pm$ 0.47
		4	1.03 (0.71 − 1.31)	1.63 $\pm$ 0.18
Subadults	Cu	1	2.36 (2.14 − 3.07)	9.47 $\pm$ 7.22
		2	1.52 (1.18 − 1.88)	8.30 $\pm$ 6.21
		3	1.34 (1.00 − 1.53)	8.04 $\pm$ 5.12
		4	1.24 (0.84 − 1.57)	7.32 $\pm$ 5.36

[a] Mean value $\pm$ standard deviation.
[b] Value of 95% confidence limits.

dependency of $C_{L,50}$ values on both exposure concentration and time. (2) The $C_{L,50}$ values depend on the chemical characteristics of the waterborne metals, on species, and on exposure concentration and duration [Eqs. (10) and (13)]. McCarty and Mackay (1993) also pointed out the constant internal lethal concentrations with respect to time and species for chemicals indicating the same mode of action; thus the UD model differs from the concept of the TIC or the CD models employed.

In summary, assessment of aquatic toxicity issues regarding waterborne metals toxicity in aquatic organisms requires the consideration of different control strategies and pollution control options. The decision-making process leading to the optimal control strategy now heavily relies on quantitative computer models of acute toxicity. These approaches in turn call for model development of the pertinent physiological and biological processes. Thus we need a detailed understanding of the phenomena controlling waterborne metals toxicity, and we must be able to rely on quantitative models, which are able to capture the main features of these processes.

Additionally, because of the large number of chemicals, high cost, and long duration of toxicity tests, resources are insufficient to obtain experimental information about long-term environmental impacts for all these chemicals. Thus it is of great interest to develop a general mathematical model that can predict toxicity based on data arising from acute toxicity experiments. Predictive models for the acute toxicity of waterborne metals in aquatic organisms are important tools for testing our understanding of ecotoxicological phenomena and for designing management strategies to provide a healthy aquatic ecosystem.

We believe that this work could contribute to integrated models of bioaccumulation and toxicity and give some insight into whether the concept of internal concentration or whole-body residues can be applied as a surrogate parameter for residue levels to explain time-dependent toxicity. Time dependency is also observed after relatively long exposure times with respect to the time required to reach a steady-state concentration in the organism.

CONCLUSIONS

This work uses acute toxicity models in predicting internal metal residues in fish as a function of the concentration in water, bioconcentration factor, depuration rate constant, exposure duration, and incipient LC_{50} value. Although these factors are likely to govern the internal concentration level, correlated chemical (e.g., molecular size, persistence) and biological (e.g., physiology, trophic level) factors may contribute to the simulated patterns.

A highly significant correlation (average coefficient of determination > 0.9) was found between predictions and $LC_{50}(t)$ data for both the TIC and the CT models. Thus, the study highly suggests the applicability of the TIC or the CT models since they demonstrate that the toxicity is indeed dependent on the time-integrated concentration $[A_{C,\mathrm{wb}} \equiv \int_0^t C_{\mathrm{wb}}(t)\,dt]$ of waterborne metals in the entire fish or on the product of the metal concentration in exposure water and duration at death ($A_{\mathrm{CT}} \equiv C_w t_d$) in the fish aqueous phase.

Therefore, the estimated $A_{C,\mathrm{wb}}$ or A_{CT} values for waterborne metals in fish may be coupled with the whole-body AChE inhibition percentage in enhancing

the ecotoxicological modeling in toxicokinetics, the time course of accumulation of the chemical, and in toxicodynamics, the time course of the adverse biological response by fish to the accumulated chemical.

The proposed technique is highly useful in the preliminary assessment of acute toxicity of chemicals. It is especially useful in predicting internal residues for survival with fish species that are difficult to culture under toxicity testing conditions. One of the applications of this work is that using acute toxicity data and the proposed models, one can obtained information quickly about long-term exposure and use the results to assign priorities to chronic studies. Further work is needed to use the concept and models developed for predicting chronic lethality and to provide a framework that enables transfer of this knowledge to the other aspect of acute/chronic toxicity.

REFERENCES

Abbas, R.; Hayton, W. L. Toxicol Appl Pharm 1997, 145, 192–201.

Barron, M. G. Aquat Toxicol 1990, 18, 61–86.

Bartell, S. M.; Gardner, R. H.; O'Neill, R. V. In Aquatic Toxicity and Hazard Assessment; Adams, W. J.; Chapman, G. A.; Landis, W. G., Eds.; ASTM STP 971: Philadelphia, 1998; Vol. 10, pp 261–274.

Bast, A. Anatomy and toxicological pathology of the nervous system. In Toxicology: Principles and Applications; Niesink, J. M., de Vries, J., Hollinger, M. A., Eds.; CRC Press: New York, 1996; pp 974–1001.

Borgmann, U.; Norwood, W. P.; Babirad, I. M. Can J Fish Aquat Sci 1991, 48, 1055–1060.

Connolly, J. P. Environ Toxicol Chem 1985, 4, 573–582.

de Vries, J. Toxicokinetics: Quantitative aspects. In Toxicology: Principles and Applications; Niesink, J. M., de Vries, J., Hollinger, M. A., Eds.; CRC Press: New York, 1996; pp 136–183.

Enserink, E. L.; Maas-Diepeveen, J. L.; van Leeuwen, C. J. Wat Res 1991, 25, 679–687.

Fowler, B. A.; Woods, J. S. Toxicol Appl Pharm 1979, 50, 177–187.

Gearhart, J. M.; Jepson, G. W.; Clewell III, H. J.; Anderson, M. E.; Conolly, R. B. Toxicol Appl Pharma 1990, 106, 295–310.

Hendriks, A. J. Ecotoxicol Environ Safety 1995, 32, 103–130.

Hermens, J. L. M. Quantitative structure-activity relationships of environmental pollutants. In Handbook of Environmental Chemistry, Vol. 2E; Hutzinger, O., Ed., Springer-Verlag: Berlin, 1989; pp 111–162.

Hickie, B. E. Ph.D. Thesis, University of Waterloo, Waterloo, ON, Canada, 1990.

Liao, C. M.; Lin, M. C.; Chang, C. H.; Chen, B. C.; Chiang, H. C. J Environ Sci Health A 1999, 34, 1945–1966.

Lin, M. C.; Liao, C. M. Aquaculture 1999, 178, 89–101.

Mancini, J. L. Wat Res 1983, 17, 1335–1362.

Marr, J. C. A.; Hansen, J. A.; Meyer, J. S.; Cacela, D.; Podrabsky, T.; Lipton, J.; Bergman, H. L. Aquat Toxicol 1998, 43, 225–238.

Maxwell, D. M.; Vlahacos, C. P.; Lenz, D. E. Toxicol Lett 1988, 43, 175–188.

McCarty, L. S.; Mackay, D. Environ Sci Tech 1993, 27, 1719–1728.

McGeachy, S. M.; Dixon, D. G. Can J Fish Aquat Sci 1990, 47, 2228–2234.

McGeachy, S. M.; Dixon, D. G. Ecotoxicol Environ Safety 1992, 24, 300–308.

McKim, J. M.; Schmieder, P. K. In Bioaccumulations in aquatic systems: Contributions to assessment. Proceedings of an International Workshop, Berlin 1990; Nagel, R.; Loskill, R., Eds.; VCH Verlagsgesellschaft, Weinheim: New York, 1991; pp 161–188.

Menzel, D. B. Environ Sci Tech 1987, 21, 944–950.

Meyer, J. S.; Gulley, D. D.; Goodrich, M. S. Environ Toxicol Chem 1995, 14, 165–175.

Musch, A. Dose-time-effect relationships. In Toxicology: Principles and Applications; Niesink, J. M., de Vries, J., Hollinger, M. A., Eds.; CRC Press: New York, 1996; pp 184–237.

Nagaraja, T. N.; Desiraju, T. Bull Environ Contam Toxicol 1993, 50, 100–107.

Nagaraja, T. N.; Desiraju, T. Human Exper Toxicol 1994, 13, 353–356.

Peterson, R. H.; Bourbonniere, R. A.; Lacroix, G. L.; Martin-Robichaud, D. J.; Takats, P.; Brun, G. Wat Air Soil Poll 1989, 46, 399–413.

Tas, J. W.; Seinen, W.; Opperhuizen, A. Comp Biochem Physiol 1991, 100C, 59–60.

Valkonen, S.; Savolainen, H.; Jarvisalo, J. Environ Contam Toxicol 1983, 30, 303–308.

Wong, P. P. K.; Chu, L. M.; Wong, C. K. Environ Int 1999, 25, 417–422.