Subcellular Partitioning Links BLM-Based Toxicokinetics for Assessing Cadmium Toxicity to Rainbow Trout

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ABSTRACT: The purpose of this article was to develop an integrated-scale toxicological model to investigate the impact of cadmium (Cd) toxicity on rainbow trout (*Oncorhynchus mykiss*) based on recent published experimental data. This model was generated from three different types of functional relationship: biotic ligand model (BLM), damage assessment model (DAM), and subcellular partitioning model (SPM), both of key toxicological determinants involved and of functional connections between them. Toxicokinetic parameters of uptake rate constant (k_1) and elimination rate constant (k_2) in gill, liver, and subcellular fractions were derived. A negative correlation between gill binding fraction of Cd and bioaccumulation factor was found. Detoxifying ability (% detoxified in liver metabolically detoxified pool (MDP)) and k_2 were negatively correlated, indicating that increasing % detoxified in MDP can compensate for lower k_2. This finding suggests a potential tradeoff between the abilities of elimination and detoxification for Cd. Yet, compensation between the ability to eliminate Cd and the ability to recover Cd-induced damage was not found. However, changes in k_2 and recovery rate constant (k_r) can shift the dynamics of Cd susceptibility probability. This analysis implicates that once k_2 is determined experimentally, the values of k_r and % detoxified in MDP can be predicted by the proposed k_2-k_r and k_2-% detoxified relationships. This study suggests that the mechanistic linking of BLM-based DAM and SPM can incorporate the organ- and cell-scale exposure experimental data to investigate the mechanisms of ecophysiological response for aquatic organisms exposed to metal stressors. © 2010 Wiley Periodicals, Inc. Environ Toxicol 00: 000–000, 2010.

Keywords: cadmium; rainbow trout; subcellular partitioning; detoxification; toxicokinetics; biotic ligand

INTRODUCTION

Cadmium (Cd) is a nonessential trace metal that has a tendency to accumulate in aquatic organisms (Wang and Rainbow, 2006; Wang and Wang, 2008). In the potentially metal-sensitive sites of cell, Cd can compete effectively with Zn and displace this essential cellular metal from sulfhydryl groups of enzymes, altering their functions and inducing toxic effects (Wang and Rainbow, 2006; Wang and Wang, 2008). The recommended water quality criterion for Cd is 2.2 μg L^{-1} (0.020 μM) (USEPA, 1999). USEPA (2001) reported that Cd exposure concentrations for acute effects in freshwater organisms were estimated to be $\leq$0.34 μg L^{-1} (0.003 μM). Therefore, bioaccumulation and toxicity of Cd in aquatic organisms are worth studying.

In the last decade, the biotic ligand model (BLM) has been widely used to predict the toxicological effects of metals on aquatic organisms (Niyogi and Wood, 2004; Bielmyer et al., 2007). The BLM is derived from the gill surface interaction model and the free ion activity model. The BLM proposes that the free metal ions reacting with the binding sites at the biotic ligand result in metal toxicity for aquatic organisms. The surface membrane of the gill, a negatively charged ligand, is widely recognized as the biotic ligand of fish (De Schamphelaere and Janssen, 2002; Paquin et al., 2002; Morgan and Wood, 2004). Practically,

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the BLM has been successfully applied to predict both acute and chronic toxicities of metals and their bioavailability in aquatic organisms (De Schamphelaere and Janssen, 2002; Schwartz and Vigneault, 2007).

Recently, a biologically based damage assessment model (DAM) has been used to describe the mode of toxic action of contaminants with rapid reversible binding to target sites and to those that act with irreversible binding (Lee et al., 2002; Ashauer et al., 2007). The DAM assumes that death occurs when cumulative damage reaches a critical level. Damage is assumed to accumulate in proportional to accumulated residue, whereas damage recovery is in proportional to cumulative damage while damage is reversible. When initial damage overwhelms threshold damage, damage is irreversible. Therefore, recovery rate in the DAM is species and metal specific, ranging from 0 to infinity. The DAM thus provides a more comprehensive framework to investigate time-dependent toxicity of chemicals incorporating both chemical and damage accumulations. This is particularly true for real field exposures.

Recent studies of subcellular Cd compartmentalization in aquatic organisms have led to conclusions regarding the significance of subcellular fates of metals to potential biological consequences of accumulated metals (Wallace et al., 2003; Wang and Rainbow, 2006; Steen Redeker et al., 2007; Buchwalter et al., 2008; Gimbert et al., 2008; Wang and Wang, 2008; Dubois and Hare, 2009; Kamunde, 2009). They suggested that subcellular partitioning of metals in the organisms may serve as another suitable toxicity predictor compared with accumulation-based determinants. Subcellular Cd compartmentalization can also be referred to as the subcellular partitioning model (SPM) (Wang and Rainbow, 2006). The SPM considers the complex binding of metals in different subcellular pools with different metal-binding ligands.

Generally, accumulated metals can approximately separate into five subcellular fractions: (i) cellular debris and membrane, (ii) heat-labile (sensitive) proteins (HLP), (iii) organelle including nucleus, mitochondria, and microsomes–lysosomes, (iv) metallothionein-like proteins (e.g., heat-stable proteins, HSP), and (v) metal-rich granules (Vijver et al., 2004; Wang and Rainbow, 2006; Kamunde, 2009). Cd associated with HLP and organelle reflects greater potential for Cd to interfere with vital physiological processes, whereas Cd associated with HSP and metal-rich granules can be accounted for a detoxifying ability (Wang and Rainbow, 2006; Buchwalter et al., 2008; Kamunde, 2009). It has been noted that toxicological significance was not found in Cd associated with cellular debris (Buchwalter et al., 2008). The ability to protect the cells is species specific by using metal-binding proteins such as metallothionein-like (heat stable) proteins and metal-rich granules that combined serves as the metabolically detoxified fractions (Wang and Rainbow, 2006; Kamunde, 2009).

Understanding the processes of exposure on aquatic organisms response will require viewing it on several levels, including bioavailability, bioaccumulation, internal damage, and detoxification in individual species (Rainbow, 2002; Vijver et al., 2004). To better understand the processes driving metal toxicity and assess their potential impact on ecophysiological response after Cd exposures, a linkage of the DAM in the BLM scheme with internal metal partitioning is capable of examining the bioaccumulation and coping mechanisms in species. The present approach is a step in this direction and can be enhanced by existing ecotoxicological modeling methods and relevant experimental data. Thus, the proposed integrated-scale toxicological model, formulated by understanding of inherent interactions among metal stressors, receptors, and metal regulations of organisms, can be used to quantify metal bioavailability, damage accumulation, and subcellular metal distribution under a broad range of metal stressor-driven environment.

The purpose of this article was to examine mechanistically the impact of Cd toxicity on rainbow trout (*Oncorhynchus mykiss*) based on recent published experimental data by linking the BLM-based DAM with the SPM. The biological model chosen, rainbow trout, is a commonly used model fish in ecotoxicological studies (Kamunde and MacPhail, 2008; Kamunde, 2009). The present approach can provide a window for the ecotoxicological scheme and opens the way for understanding how rainbow trout show ecophysiological responses to Cd exposure.

MATERIALS AND METHODS

Study Data

A valuable dataset provided by Kamunde (2009) gave us the unique opportunity to examine the linkages and correlations between toxicokinetics and subcellular partitioning. Kamunde (2009) conducted organ-level accumulation and subcellular fractionation experiments to better understand the importance of intracellular Cd partitioning for accurate tissue burden-based toxicity assessment. Thus, the published data adopted from Kamunde (2009) were reanalyzed. The data covered a wide range of physiological characterization such as uptake, elimination, and detoxification, which allowed the present analysis.

Briefly, Kamunde (2009) carried out an exposure experiment to determine Cd accumulation by using juvenile rainbow trout (10–15 g wet wt) exposed to low-to-near-lethal concentrations of 5, 25, and 50 μg L^{-1} Cd (as $Cd(NO_3)_2{\cdot}4H_2O$) for 96 h without feeding at temperature 12.5°C ± 0.5°C, pH 7.5, and dissolved oxygen 9.22 ± 0.04 mg L^{-1}. The characteristics of water chemistry were measured to be: Na 47.1, Ca 58.8, Mg 27.6, K 2.26, Cl 137.3, and sulfate 17.2 mg L^{-1}. The gill and liver were chosen as the target organs with measurements of 0.48 ± 0.07 and 0.17 ± 0.05 g wet wt, respectively.

Kamunde (2009) separated the homogenates of livers and gills from three rainbow trout to determine subcellular Cd fractionation by differential centrifugation. The chemical and heat treatments were separated into five operationally defined subcellular fractions. These fractions include cell debris, cellular organelles (including nuclei, mitochondria, and microsomes–lysosomes), cytosolic proteins denatured by heat treatment (heat-labile proteins), heat-stable proteins, and metal-rich granules (expressed as NaOH resistant). Data from subcellular Cd partitioning were summed into metal-sensitive and detoxified metal compartments or referred to as metabolically active pool (MAP) and metabolically detoxified pool (MDP). Thus, two summed pools have recently been proposed to represent bioactive and detoxified fractions in subcellular Cd partitioning. MDP comprises HSP- and NaOH-resistant fractions. On the other hand, MAP comprises HLP, nuclei, mitochondria, and microsomes–lysosomes, each containing sites potentially vulnerable to Cd binding.

Proportions of accumulated Cd in the potentially sensitive subcellular compartments (i.e., MAP) were adopted to represent fish physiological response exposed to waterborne Cd. Thus, an exposure time-specific concentration-response profile can be constructed. Based on the constructed concentration-response curves, the exposure time-specific 50% effect concentration (EC50(t)) can then be estimated.

Mechanistic Models

Here, the BLM was used to predict the degree of Cd binding at the site of action causing toxicity in gill of rainbow trout. In the BLM scheme, the effect concentration for 50% response over time ($EC50_{BLM}(t)$) can be expressed in terms of time course fraction of total number of Cd-binding sites occupied by Cd at 50% effect (i.e., $f_{CdBL}^{50\%}(t)$) (De Schamphelaere and Janssen, 2002),

$$EC50_{BLM}(t) = \frac{f_{CdBL}^{50\%}(t)(1+[a])}{(1-f_{CdBL}^{50\%}(t))K_{CdBL}}. \quad (1)$$

where $[a] = K_{CaBL}\{Ca^{2+}\} + K_{MgBL}\{Mg^{2+}\} + K_{NaBL}\{Na^{+}\} + K_{HBL}\{H^{+}\}$ in that K_{CaBL}, K_{MgBL}, K_{NaBL}, and K_{HBL} represent the affinity constants for binding of these cations to biotic ligand (M^{-1}), {ions} denotes the activity of each ion of water chemistry characteristics (M), and K_{CdBL} represents the Cd binding to biotic ligand (M^{-1}). This analysis focused on pH, Na^{+}, Ca^{2+}, and Mg^{2+} because they have significant effects on Cd toxicity (Niyogi and Wood, 2004).

In this study, the DAM can be described by three dynamic variables: the internal cumulative damage ($D(t)$), the cumulative hazard ($H(t)$), and the susceptibility probability ($S(t)$). First, $D(t)$ can be solved by incorporating the one-compartment toxicokinetic model $dC_b(t)/dt = k_1C_w - k_2C_b(t)$ into the damage accumulation model $dD(t)/dt = k_aC_b(t) - k_rD(t)$ as (Lee et al., 2002)

$$D(t) = k_a\frac{k_1}{k_2}C_w\left(\frac{e^{-k_rt}-e^{-k_2t}}{k_r-k_2}+\frac{1-e^{-k_rt}}{k_r}\right), \quad (2)$$

where $C_b(t)$ is the Cd burden in tissue in time t ($\mu g\ g^{-1}$), k_a is the damage accumulation rate ($g\ \mu g^{-1}\ day^{-1}$), k_r is the damage recovery rate constant (day^{-1}), k_1 is the rainbow trout uptake rate constant ($mL\ g^{-1}\ day^{-1}$), k_2 is the elimination rate of Cd (day^{-1}), and C_w is the dissolved Cd concentration in water ($mg\ L^{-1}$). It suggested that the recovery rate constant characterizes all processes leading to recovery such as repair mechanisms on a cellular scale or adaptation of the physiology and other compensating processes (Ashauer et al., 2007).

Second, an important DAM parameter referred to as the killing rate constant k_k ($g\ \mu g^{-1}\ day^{-1}$) is introduced. The killing rate constant is the proportionality factor describing the relations between cumulative damage and cumulative hazard as

$$H(t) = (k_k/k_a)D(t). \quad (3)$$

Lastly, the susceptibility probability $S(t)$ can be derived directly from an exponential relationship of cumulative hazard as (Lee et al., 2002; Ashauer et al., 2007)

$$S(t) = 1 - \exp(-H(t)) = 1 - \exp\left[-k_k\frac{k_1}{k_2}C_w\left(\frac{e^{-k_rt}-e^{-k_2t}}{k_r-k_2}+\frac{1-e^{-k_rt}}{k_r}\right)\right]. \quad (4)$$

A relation for estimating time-dependent $f_{CdBL}^{50\%}(t)$ and bioconcentration factor (BCF(t)) can be constructed by linking the BLM and the DAM (Tsai et al., 2009). This assumed that the free ion activity concentration resulting in 50% effect calculated by the DAM ($EC50_{DAM}(t)$) equals to that predicted by the BLM ($EC50_{BLM}(t)$)

$$\frac{D_{E,50}/k_a}{\left(\frac{e^{-k_rt}-e^{-k_2t}}{k_r-k_2}+\frac{1-e^{-k_rt}}{k_r}\right)}BCF^{-1}(\{ions\},t) = \frac{f_{CdBL}^{50\%}(t)(1+[a])}{(1-f_{CdBL}^{50\%}(t))K_{CdBL}}, \quad (5)$$

where $D_{E,50}$ is the cumulative damage for 50% effect (−), $D_{E,50}/k_a$ is a coefficient reflecting the compound equivalent toxic damage level required for 50% effect ($\mu g\ d\ g^{-1}$), BCF({ions},t) is the BCF of Cd to organism considering the competition of cations {ions} at time t. The killing rate constant can be calculated as $k_k = \ln 2/(D_{E,50}/k_a)$ (Lee et al.,

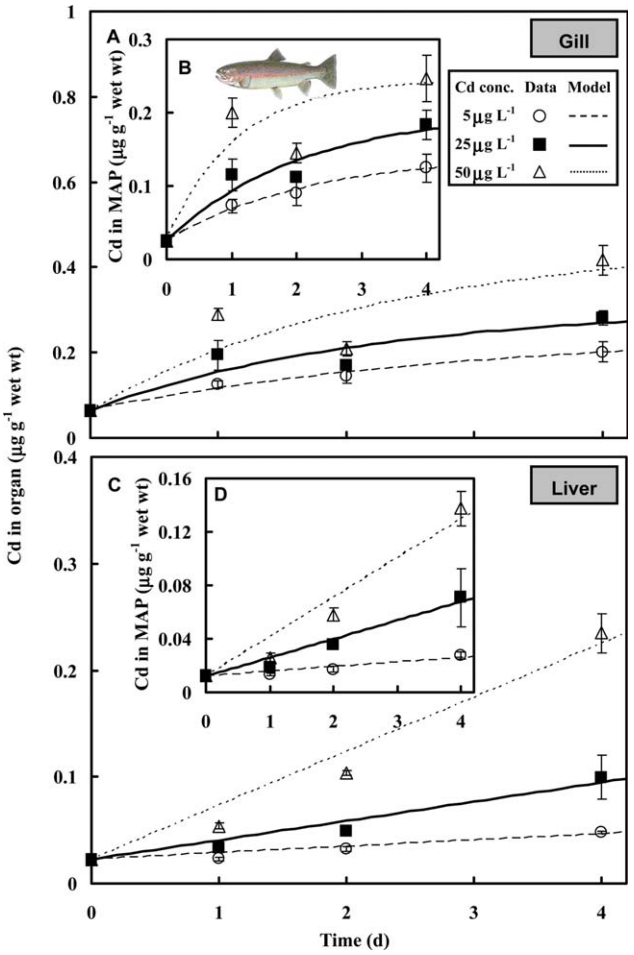

Fig. 1. Four-day bioaccumulation experiment and model fitting of Cd in (A) gill and (C) liver and in metabolically active pool (MAP) of (B) gill and (D) liver of rainbow trout exposed to 5, 25, and 50 μg L^{-1} waterborne Cd. Error bars represent standard deviation from mean. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

2002). BCF is defined as the ratio of metal concentration in organism or tissue to the concentration of metal in the media at steady state. Furthermore, BCF can also be calculated from the ratio of uptake rate constant to elimination rate constant.

Equation 5 is based on the BLM assumption of water chemistry-independent $f^{50\%}_{\mathrm{CdBL}}(t)$. Equation 5 is rearranged to solve BCF({ions},t) for obtaining a mechanistic model that can be used to predict the temporal change of BCF

$$\mathrm{BCF}(\{\mathrm{ions}\},t)=\frac{(1-f^{50\%}_{\mathrm{CdBL}}(t))K_{\mathrm{CdBL}}(D_{\mathrm{E},50}/k_{\mathrm{a}})}{f^{50\%}_{\mathrm{CdBL}}(t)(1+[a])\left(\frac{e^{-k_{\mathrm{r}}t}-e^{-k_2t}}{k_{\mathrm{r}}-k_2}+\frac{1-e^{-k_{\mathrm{r}}t}}{k_{\mathrm{r}}}\right)}. \quad (6)$$

Therefore, the BLM was mechanistically linked with the DAM to refine the traditional one-compartment toxicokinetic model.

Data Analysis

A Hill model was used to fit the constructed concentration-physiological response curve to estimate EC50 value as

$$R(C_{\mathrm{w}})=\frac{R_{\max}}{1+\left(\frac{\mathrm{EC50}}{C_{\mathrm{w}}}\right)^n}, \quad (7)$$

where R is the physiological response (%), $R_{\max}$ is the maximum response (%), and n is the Hill coefficient. Toxicokinetic parameters of uptake and elimination rate constants (k_1 and k_2) can be determined by fitting the integrated form of the toxicokinetic rate equation to exposure data under constant waterborne Cd

$$C_{\mathrm{b}}(t)=C_{\mathrm{b}0}e^{-k_2t}+\frac{k_1}{k_2}C_{\mathrm{w}}(1-e^{-k_2t}), \quad (8)$$

where $C_{\mathrm{b}0}$ is the initial concentration of Cd in target tissue of rainbow trout (μg g^{-1} wet wt).

TableCurve 2D (Version 5.0) (AISN Software, Mapleton, OR) was used to perform the model fittings. A value of $P < 0.05$ judged significant. The WHAM (Windermere humic aqueous model) Version 6 (WHAM VI, Center for Ecology and Hydrology, Lancaster, UK) was performed to calculate the activities of competing and complex ions considered in the BLM scheme. A Monte Carlo technique was performed to generate 2.5 and 97.5 percentiles as the 95% confidence interval (CI) for all fitted models. Largely because of limitations in the data used to derive model parameters, inputs were assumed to be independent. The result showed that 5000 iterations were sufficient to ensure the stability of results. The Crystal Ball® software (Version 2000.2, Decisionerring, Denver, CO) was used to implement the Monte Carlo simulation.

TABLE I. Fitted toxicokinetic parameter values (mean ± SE) for gill, liver, and metabolically active pool (MAP)

	Cd in Water (μg L^{-1})	k_1 (mL g^{-1} day^{-1})	k_2 (day^{-1})	r^2
Organ				
Gill	5	11.85 ± 2.26	0.32 ± 0.14	0.98
	25	4.47 ± 2.31	0.46 ± 0.44	0.86
	50	3.46 ± 2.41	0.44 ± 0.57	0.77
Liver	5	1.18 ± 0.69	1.01×10^{-5} ± 0.32	0.92
	25	0.72 ± 0.30	6.08×10^{-6} ± 0.23	0.95
	50	1.01 ± 0.34	1.19×10^{-6} ± 0.18	0.97
MAP				
Gill	5	12.97 ± 1.85	0.46 ± 0.10	0.99
	25	3.90 ± 1.67	0.48 ± 0.34	0.92
	50	4.56 ± 0.58	0.93 ± 0.17	0.79
Liver	5	0.69 ± 1.1	4.52×10^{-6} ± 0.30	0.93
	25	0.56 ± 0.17	1.26×10^{-7} ± 0.10	0.96
	50	0.59 ± 0.30	2.92×10^{-6} ± 0.23	0.95

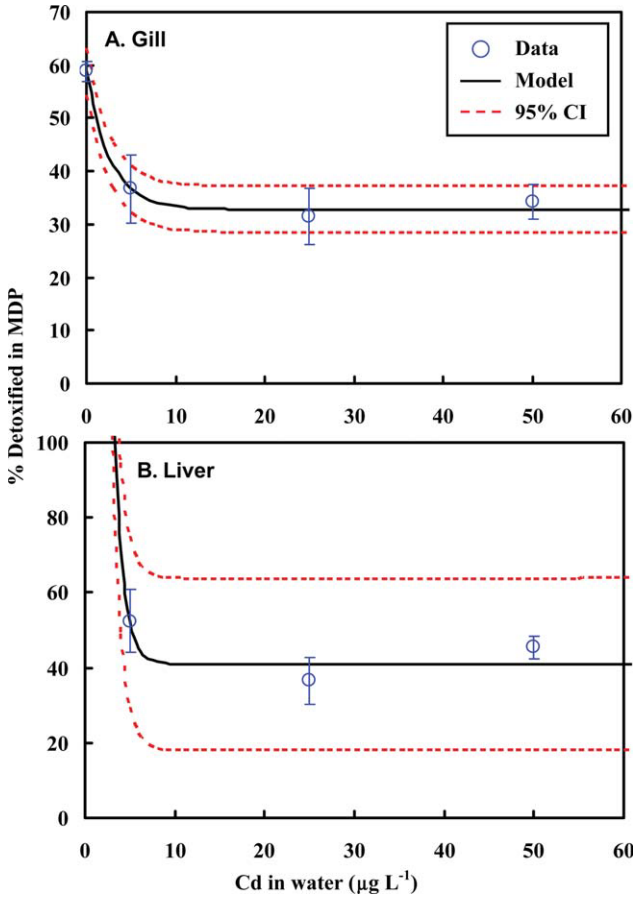

Fig. 2. Best-fitting exponential models describing the relationships between % detoxified in (A) gill and (B) liver and waterborne Cd. Error bars represent standard deviation from mean. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

RESULTS

Toxicokinetics and Subcellular Partitioning

The biphasic uptake fashions were found in gill and gill-MAP when rainbow trout were exposed to waterborne Cd ranging from 5 to 50 μg L^{-1} over the course of exposure experiment [Fig. 1(A,B)]. On the other hand, the linear uptake patterns were found in liver and liver-MAP [Fig. 1(C,D)]. The toxicokinetic equation in Eq. 8 was fitted to exposure data to obtain the concentration-specific estimates of uptake rate and elimination rate constants k_1 and k_2 in gill, liver, and MAP, respectively (Table I). Overall, k_1 estimates of gill and gill-MAP increased with decreasing Cd concentrations, ranging from 3.46 to 11.85 and 4.56 to 12.97 mL g^{-1} day^{-1}, respectively. However, k_2 of gill and gill-MAP increased with increasing of Cd concentrations, ranging from 0.32 to 0.46 and 0.46 to 0.93 day^{-1}, respectively (Table I).

For liver and liver-MAP, k_1 estimates did not change significantly with Cd concentrations (nearly 1 mL g^{-1} day^{-1} for liver and 0.6 mL g^{-1} day^{-1} for liver-MAP). Very low elimination rates with order of magnitudes 10^{-6}–10^{-5} were found, indicating that Cd in liver and liver-MAP can only be eliminated very slowly (Table I). The relationships between % detoxified in gill-MDP and liver-MDP and waterborne Cd were analyzed, showing that the parsimonious exponential models of $y = 32.80 + 26.02\exp(-x/2.62)$ ($r^2 = 0.99$) for gill and $y = 40.98 + 1690.52\exp(-x)$ ($r^2 = 0.69$) for liver-MDP best described the relations (Fig. 2).

Correlations Between Bioavailability and Bioaccumulation

A Hill model was used to fit reconstructed exposure time-specific concentration-physiological response relationships

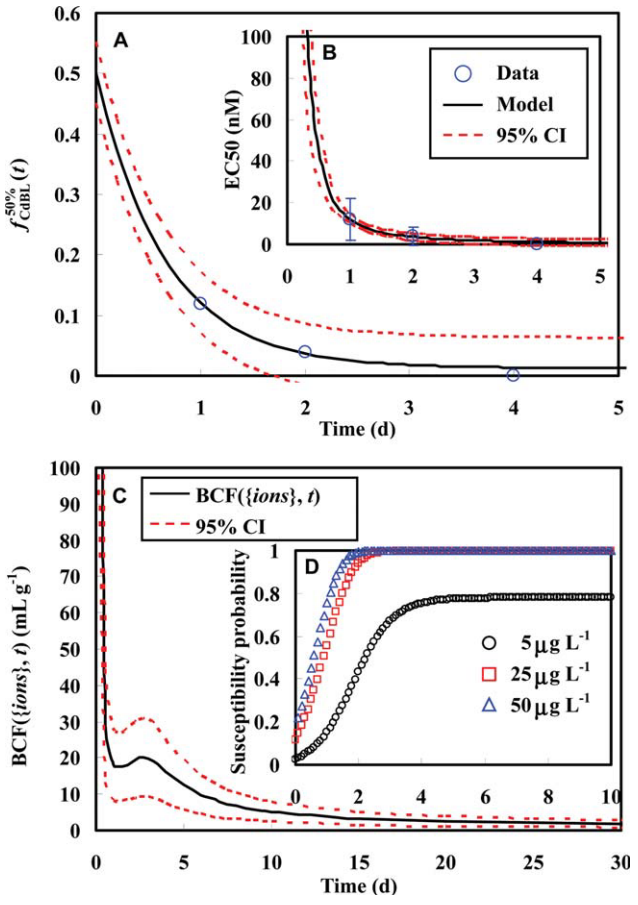

Fig. 3. (A) Relationship between predicted $f_{CdBL}^{50\%}(t)$ and exposure time. (B) Using EC50(t) model in the left-hand side of Eq. 5 to fit $EC50_{BLM}(t)$ estimates for exposure times of 1, 2, and 4 days. Error bars represent standard deviation from mean. (C) Time course of BLM-based bioaccumulation factor followed by Eq. 6. (D) BLM-based DAM-derived susceptibility probability over time in different waterborne Cd concentrations. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE II. Affinity constants, BLM parameters, and BLM-based DAM parameter values used in this study

Affinity Constants (M^{-1})	
log K_{CdBL}	8.6[a]
log K_{CaBL}	4.5[b]
log K_{HBL}	6.7[a]
log K_{NaBL}	3[b]
log K_{MgBL}	3.2[b]
BLM parameters	
[a]	34.65
BLM-based DAM parameters	
Input parameter[c]	
k_1(mL g^{-1} day^{-1})	6.59 ± 2.64
k_2 (day^{-1})	0.41 ± 0.04
BCF (mL g^{-1})	19.20 ± 9.43
Fitted parameter[d]	
$D_{E,50}/k_a$ (pmol day g^{-1})	39.14 ± 1.65
k_r (day^{-1})	0.0314 ± 0.105
k_k (g $pmol^{-1}$ day^{-1})[e]	0.0177
$f^{50\%}_{CdBL}(\infty)$	0.0128 (0–0.0634)[f]
BCF({ions},∞) (mL g^{-1})	1.88 (0.88–2.88)[f]

[a] Adopted from Playle et al. (1993).
[b] Adopted from Santore et al. (2002).
[c] Mean ± SD.
[d] Mean ± SE.
[e] $k_k = \ln 2/(D_{E,50}/k_a)$.
[f] Mean (95% CI).

in MAP. This resulted in EC50(t) estimates of 12.18 ± 10.14 (mean ± SE), 3.55 ± 4.33, and 1.10×10^{-5} ± 2.46 $\times 10^{-4}$ nM, R_{max} of 27.34, 29.32, and 36.73%, and n of 0.61, 0.53, and 0.09 for exposure time 1, 2, and 4 days, respectively. Equation 1 together with EC50(t) estimates can be used to calculate $f^{50\%}_{CdBL}$ (t) with known values of affinity constants, BLM parameters [a], and K_{CdBL} (Table II). The $f^{50\%}_{CdBL}$ (t) values were calculated to be 0.12, 0.038, and 1.23×10^{-7}, respectively, for exposure time 1, 2, and 4 days. Figure 3(A) shows $f^{50\%}_{CdBL}$ (t) over time with a best-fitting model of $f^{50\%}_{CdBL}$ (t) = 0.0125 + 0.49exp(−t/0.66) (r^2 = 0.97, $P < 0.05$).

The right-hand side of Eq. 5 can be used to estimate $EC50_{BLM}(t)$ values, resulting in 12.21, 3.36, and 1.24 nM for exposure time 1, 2, and 4 days, respectively. The function of $EC50_{DAM}(t)$ in the left-hand side of Eq. 5 was used to fit $EC50_{BLM}(t)$ estimates to obtain the BLM-based DAM key parameters of k_r and k_k [Fig. 3(B)]. This resulted in k_r and k_k estimates were 0.0314 ± 0.105 day^{-1} and 0.0177 g $pmol^{-1}$ day^{-1}, respectively, based on concentration-averaged toxicokinetic parameters (Table II).

The equilibrium BCF({ions},∞) was estimated to be 1.88 (95% CI, 0.88 – 2.88) mL g^{-1} followed by Eq. 6 [Fig. 3(C)]. The 50% tolerable time was calculated to be 2.33, 1, and 0.57 days for rainbow trout exposed to waterborne Cd of 5, 25, and 50 μg L^{-1}, respectively, based on the derived susceptibility probability ($S(t)$) [Fig. 3(D)]. Table II summarizes the estimates of the BLM and the BLM-based DAM parameters.

Linkages Between Bioaccumulation and Detoxification

A relationship between % detoxified (the proportion of Cd bound to MDP) of gill and liver and waterborne Cd is depicted in Figure 4(A). Figure 4(A) demonstrates the changes of average proportion of Cd in subcellular fractions of gill and liver comprising nuclei, mitochondria, microsomes–lysosomes, HLP, HSP, and metal-rich granules exposed to 5, 25, and 50 μg L^{-1} waterborne Cd. The % detoxified in gill-MDP decreased with increasing of Cd concentrations, whereas a biphasic fashion was found in liver-MDP [Fig. 4(A)]. It is noted, however, that % detoxified in liver-MDP increased at the relative higher waterborne Cd of >25 μg L^{-1}.

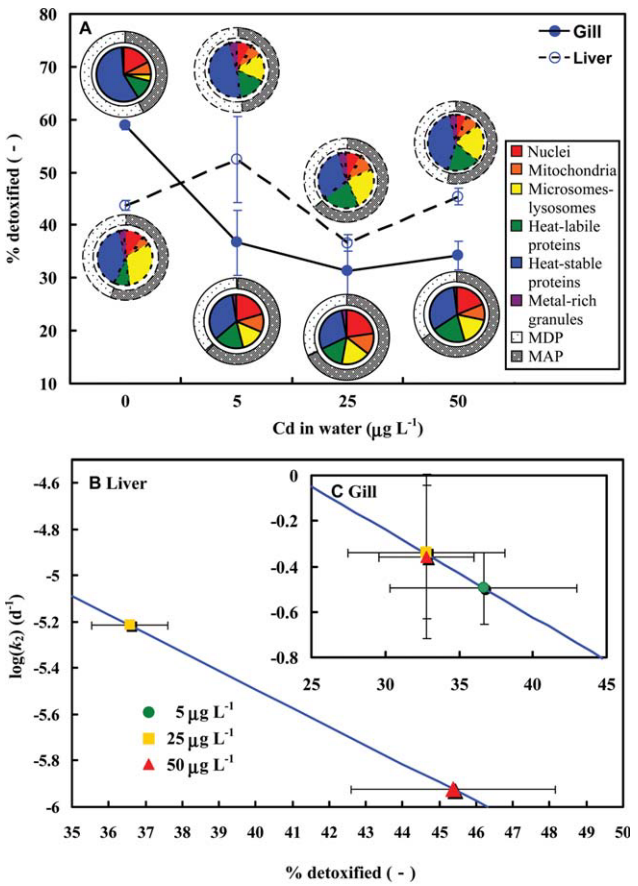

Fig. 4. (A) Relationships between % detoxified of gill and liver and waterborne Cd. The pie charts represent the average proportion of accumulated Cd in subcellular fractions of gill and liver. (B) The relationships between k_2 and % detoxified in (B) liver and (C) gill under varied Cd concentrations. Error bars represent standard deviation from mean. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

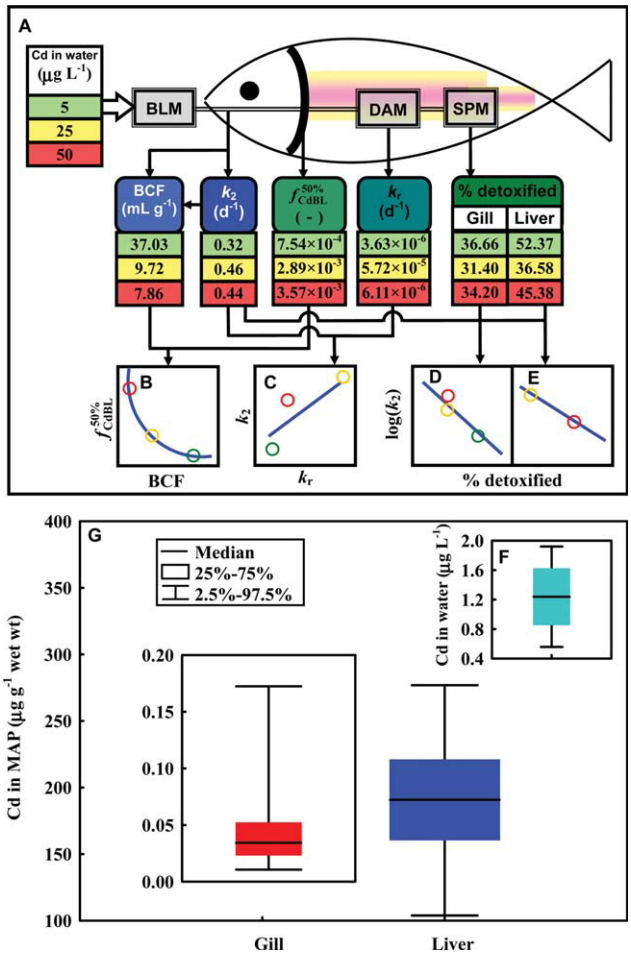

Fig. 5. (A) Schematic of linking subcellular partitioning with BLM-based DAM to estimate the concentration-specific key parameters and the associations between key parameters (B, C, D, and E). (F) Environmentally relevant Cd concentration distribution in Taiwan showing a normal distribution with mean of 1.25 μg L^{-1} and standard deviation of 0.29 μg L^{-1}. (G) Cd distributions in MAP of gill and liver showing a lognormal distribution with a geometric mean (GM) of 0.042 μg g^{-1} and geometric standard deviation (GSD) of 0.025 μg g^{-1} for gill-MAP and GM of 190.86 μg g^{-1} and GSD of 44.47 μg g^{-1} for liver-MAP. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

A negative relationship between toxicokinetic parameter k_2 and proportional intracellular distribution of Cd bound to MDP in liver and gill was found [Fig. 4(B,C)]. Figure 4(B) indicates that liver k_2 decreased and MDP % detoxified increased, with increasing of Cd concentrations higher than 25 μg L^{-1}. In contrast to liver, gill k_2 increased and MDP % detoxified decreased, with increasing of waterborne Cd [Fig. 4(C)]. These results suggested that liver did not have an effective ability to eliminate Cd at the relative higher waterborne Cd; however, they performed a good ability to detoxify Cd. The results also revealed that subcellular Cd partitioning not only varied in gill and liver but also varied in their ability to protect vulnerable cellular sites.

Linking Subcellular Partitioning with BLM-Based DAM

To explore the substantial interactions among the BLM, the DAM, and the SPM in enhancing Cd toxicity assessment, calculation was performed to estimate the concentration-specific key parameters [Fig. 5(A)]. The results revealed (i) a negative association between BCF({ions},∞) and $f^{50\%}_{CdBL}(\infty)$ and (ii) a positive linear relationships between k_2 and k_r [Fig. 5(B,C)]. The finding indicates that rainbow trout with a lower $f^{50\%}_{CdBL}(\infty)$ value in gill can give a higher ability to accumulate bioavailable Cd. Moreover, once k_2 is determined experimentally, the values of k_r and % detoxified in MDP can be calculated by the k_2-k_r and k_2-% detoxified relationships as depicted in Figure 5(C–E).

To understand the sensitivity differences between gill and liver, Cd accumulated in potentially sensitive subcellular compartments (i.e., MAP) was predicted subjected to environmentally relevant Cd concentration in Taiwan region (1.24 μg L^{-1}, 95% CI 0.56–1.92) [Fig. 5(F)]. Here, a parsimonious relation was used to predict Cd sensitivity or susceptibility in gill and liver as follows: Cd in MAP is equal to $k_{1,MAP}/k_{2,MAP}$ times, environmentally relevant Cd distribution in that toxicokinetic parameter values of $k_{1,MAP}$ and $k_{2,MAP}$ are listed in Table I. The results indicated that liver significantly accumulated a larger amount of Cd in liver-MAP (190.8 μg g^{-1}, 95% CI 103.88–276.85) than that in gill-MAP (0.034 μg g^{-1}, 95% CI 0.011–0.173) at a long-term low-dose exposure [Fig. 5(G)]. This is due in part to liver having very slow elimination ability in MAP compared with gills having relatively fast uptake and elimination abilities (Table I). Moreover, according to uptake and elimination rate constants that were determined by fitting the toxicokinetic equation to exposure data, showing that very low elimination rates were found in liver-MAP. Therefore, liver-MAP significantly accumulated Cd. On the contrary, gill elimination rate was relatively higher and resulted in $k_{1,MAP}/k_{2,MAP}$ value was smaller than that of liver.

DISCUSSION

Gill Cd Binding and Bioaccumulation

A negative correlation between the BLM parameter $f^{50\%}_{CdBL}(\infty)$ and the BCF was found. The potential explanation can be given as follows. The BLM suggests that fraction of the biotic ligand sites occupied by Cd ions, f_{CdBL}, can provide an approximation of Cd activity at target sites and toxicity. The increasing fraction of the biotic ligand sites occupied by Cd indicates the organism accumulating more Cd toxicities. This study quantifies the relationships

between water chemistry-specific BCF({ions}) and $f_{CdBL}^{50\%}$. Here, $f_{CdBL}^{50\%}$ is related to Cd-binding proportion and represented the ability that induced a 50% effect when organisms are exposed to a toxicant. Therefore, low value of Cd-binding proportion in the biotic ligands at 50% effect represents a relatively higher toxicity. Moreover, toxicity can increase with increasing BCF({ions}) and decreasing $f_{CdBL}^{50\%}$ values. Therefore, a negative association was revealed in the relationships between BCF({ions}) and $f_{CdBL}^{50\%}$.

Gill is the first interaction site with the trace metal, which has a finite interaction capacity. Given the direct contact with ambient water, gills are proposed to be the first and most important targets of fish exposed to waterborne metals (Tao et al., 2000). Several studies also indicated that the major route of uptake for the chemicals that concentrate in fish was across the gill epithelium (Pelgrom et al., 1997; Bury et al., 1999; Szebedinszky et al., 2001). Hence, responses of fish to metals are mediated by physiological regulation mechanisms at gill. Moreover, gill regulation is a time-dependent acclimation.

Damage Assessment and Intracellular Cd Partitioning

A positive correlation between the toxicokinetic parameter k_2 and the DAM parameter k_r was found. Notably, a tradeoff between the ability to eliminate Cd and the ability to recover the Cd-induced damage was not found. The k_2 can be estimated experimentally. However, direct measurement of k_r via the DAM is difficult: not much is known about the central mechanisms underlying damage recovery. Once k_2 is estimated, however, the value of k_r can be predicted by the proposed BLM-based DAM algorithm. Here, the BLM-based DAM can be applied to analyze how the physiological response characterizing by the concentration-specific susceptibility probability. This analysis indicates that changes in elimination and recovery rate constants may shift the dynamics of susceptibility probability.

In this analysis, two key parameters are determined to link subcellular Cd distribution and bioaccumulation: the ability to eliminate Cd (k_2) and the ability to detoxify Cd (% detoxified). A negative correlation between the toxicokinetic parameter k_2 and SPM parameter % detoxified was found, indicating that increasing % detoxified in MDP can compensate for lower k_2. This finding suggests a potential tradeoff between the abilities of elimination and detoxification for Cd. This study also reveals that k_r and % detoxified in MDP can be predicted by the proposed k_2-k_r and $k_2-\%$ detoxified relationships in terms of the known k_2.

Kamunde (2009) reported that the rank of Cd accumulation in subcellular fractions in gill of rainbow trout was as follows: HSP > HLP > nuclei > microsomes–lysosomes ≥ mitochondria > resistant fraction. For liver, it was HSP > HLP > microsomes–lysosomes > mitochondria > nuclei > resistant fraction. Generally, Cd-induced toxicity could be linked to the increase of Cd in structures with essential metabolic roles in MAP such as mitochondria, nuclei, microsomes–lysosomes, and HLPs. Detoxification may not bring into play when relative contribution of a metal-sensitive fraction to total cellular Cd burden remains constant or increasing. On the other hand, if the relative proportion decreases, some protection of this fraction may be evident. In this analysis, the relative contribution of liver-MDP to total Cd content increased, at least for lower waterborne Cd, and remained unchanged for higher Cd concentrations. These results suggest that a partial protection of some metal-sensitive sites is achieved by initiation of cellular detoxifying mechanisms in liver. In contrast, Cd binding in gill-MAP increased as Cd in exposure medium increased, suggesting that gill tissue may be more susceptible to waterborne Cd toxicity relative to liver due to high amount of bioavailable Cd (Kamunde, 2009).

In the recent series of studies, Cd bound to metabolically detoxified pool in freshwater fish liver ranged between 30 and 76.5%, whereas Cd associated with metabolically active pool and cellular debris fractions ranged from 21.52 to 59.85% and 10.21 to 33.68%, respectively (Olsson and Hogstrand, 1987; Kraemer et al., 2006a,b). The percent cadmium distribution differed among subcellular partitioning in the following order: metabolically detoxified pool > metabolically active pool > cellular debris. Thus, the metabolically detoxified pool may play an important role for aquatic fish in response to Cd toxicity.

Liver is the main detoxifying organ in all the animals and is the dominant accumulating organ for metal in fish. The metal concentration in fish hepatic tissue seems to be highly dependent on metal concentration in medium and exposure time. This analysis, however, indicates a biphasic fashion in the relationships between % Cd in MDP and Cd in water. The distinct phenomenon may be caused by spillover of MDP or exposure period too short to synthesize detoxified protein. The mechanism of Cd distribution in subcellular partitioning is still not fully understood based on the limited published data. However, Cd concentrations in HSP fraction increased with Cd, reaching high proportions of intracellular Cd (nearly 40–50%) when rainbow trout were exposed to high Cd (50 $\mu g\ L^{-1}$). Therefore, the important sequestration of Cd in liver-MDP fraction of rainbow trout strongly suggests that metal-binding HSP plays an important role in metal detoxification and can account for the partial protection of metal-sensitive sites discussed above.

Implications

This analysis suggests that recent developed toxicological modeling approaches for predicting metal toxicity to aquatic organisms are readily amenable to an integrated-

scale analysis. This study shows that such models can be derived from an integrated-scale analysis generated from three different types of functional relationship: the BLM, the DAM, and the SPM, both of key toxicological determinants involved and of functional connections between them.

We anticipate that the model predictions can be coupled quantitatively with the essential experiments where direct determinations of toxicokinetic rate constants and manipulation of environmental conditions would be achieved. Well-performed metal exposure experiments associated with integration of toxicokinetic model and subcellular partitioning in investigating physiological response after metal exposures in particular are capturing the essence of dynamics of several model systems. The model systems included freshwater bivalves (Wallace et al., 2003; Adam-Guillermin et al., 2009), yellow perch (*Perca flavescens*) (Campbell et al., 2008), aquatic insect (Buchwalter et al., 2007, 2008; Dubois and Hare, 2009), marine diatom (*Thalassisra nordenskioeldii*) (Wang and Wang, 2008), freshwater snails (*Lymnaea stagnalis*) (Croteau and Luoma, 2009), terrestrial snail (*Helix aspersa*) (Gimbert et al., 2008), and earthworm (*Eisenia fetida*) (Huang et al., 2009; Li et al., 2009).

Looking forward, given that physiological parameters of aquatic organisms and geochemistry parameters of ambient water, this analysis is potentially useful to develop and refine the ambient water quality criteria (Buchwlater et al., 2007; Campbell et al., 2008). Hence, the integration between the BLM-based DAM and the SPM can be used to describe metal–gill binding interactions and metal detoxification strategies and further to predict metal toxicities to aquatic organisms in the field situations (Campbell et al., 2008; Lavoie et al., 2009). Although the current model is used for fish, the underlying principle of linking metal bioavailability and internal damage accumulation to environmental sensitivity caused by metal exposures is broadly applicable across aquatic species.

In conclusion, the mechanistic linking of the BLM-based DAM and the SPM can incorporate the organ- and cell-scale exposure experimental data to investigate the mechanisms of ecophysiological response for aquatic organisms exposed to metal stressors (Cain et al., 2004; Vijver et al., 2006; Gimbert et al., 2008). It would be of interest to investigate similar parameters related to detoxification rate constants via dietary exposure routes (Croetau and Luoma, 2009), which may open up additional perspectives on these sophistication of coping mechanisms in response to environmental metal stressors.

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