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Predicting Dietborne Metal Toxicity from Metal Influxes

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Dietborne metal uptake prevails for many species in nature. However, the links between dietary metal exposure and toxicity are not well understood. Sources of uncertainty include the lack of suitable tracers to quantify exposure for metals such as copper, the difficulty to assess dietary processes such as food ingestion rate, and the complexity to link metal bioaccumulation and effects. We characterized dietborne copper, nickel, and cadmium influxes in a freshwater gastropod exposed to diatoms labeled with enriched stable metal isotopes. Metal influxes in *Lymnaea stagnalis* correlated linearly with dietborne metal concentrations over a range encompassing most environmental exposures. Dietary Cd and Ni uptake rate constants (k_{uf}) were, respectively, 3.3 and 2.3 times higher than that for Cu. Detoxification rate constants (k_{detox}) were similar among metals and appeared 100 times higher than efflux rate constants (k_e). Extremely high Cu concentrations reduced feeding rates, causing the relationship between exposure and influx to deviate from linearity; i.e., Cu uptake rates leveled off between 1500 and 1800 nmol g^{-1} day^{-1}. *L. stagnalis* rapidly takes up Cu, Cd, and Ni from food but detoxifies the accumulated metals, instead of reducing uptake or intensifying excretion. Above a threshold uptake rate, however, the detoxification capabilities of *L. stagnalis* are overwhelmed.

Introduction

The accumulation of a metal by an organism involves several steps, among which are the metal association to a binding site on the plasma membrane and its internalization (*1*). Bioaccumulation is a biological end point of that interaction and is, thus, a prerequisite for toxicity. Upon entering the cell, metals can be used for metabolic purposes, excreted, or stored (*2*). However, some accumulated metals can exert toxic effects by binding to inappropriate biologically sensitive molecules or by forming dangerous free radicals (*3*). The potential toxicity of an accumulated metal, thus, depends on its fate upon entering the cell.

Conceptually, toxicity manifests when the rate of metal uptake exceeds the combined rates of loss and detoxification (*4*). Specifically, metal toxicity is related to a threshold concentration of "metabolically available metal". Metals stored in detoxification pools represent no threat to metabolism. For instance, barnacles accumulate very high concentrations of Zn but store most of it in a very slow exchanging form, i.e., detoxified pyrophosphate granules (*5*). The link between dietborne metal toxicity and dietborne exposure concentration varies among species and metals because uptake, loss, and detoxification rates are species and metal specific (*6*). Understanding the underlying mechanisms controlling the inward and outward metal fluxes, as well as those governing metal movements within an organism, appears crucial for predicting the risks and effects of metals.

The biodynamic model uses empirically determined species-specific and metal-specific parameters to describe bioaccumulation in terms of fluxes (*6*). It predicts that metal body burden increases when metal influxes from all exposure routes exceed loss rates. The model takes into account metal influx from water, metal influx from food, metal efflux, and body growth dilution. Simplifying the model is possible if, for example, one uptake pathway prevails (e.g., diet for many aquatic species (*7*)) or when the influence of growth is negligible compared to that of loss (e.g., during short-term exposure experiments (*8*)). While the biodynamic model successfully forecasts metal bioaccumulation in several species and environments (*6*), it does not necessarily provide insights into metal movements within an organism. For instance, the rate at which an organism stores its accumulated metal in a detoxification pool remains undetermined in most studies. Protocols are available for determining operationally defined detoxified metals from subcellular partitioning within organisms (*9*), but determining rates from those approaches is complex (*10*).

We employ biodynamic principles and enriched stable metal isotope tracers to investigate the relationships between metal uptake rates from food and toxicity. Specifically, we expose freshwater snails to a wide range of concentrations of enriched ^{106}Cd, ^{65}Cu, and ^{62}Ni through diet for a time period shorter than food gut residence time (*11*). We ask if metal-specific influxes vary between single-metal and multimetal exposures. We also test whether a metal-specific "influx threshold" can be reached upon short-term exposures to a wide range of dietborne metal concentrations. We show that dietborne Cu toxicity occurs in the form of reduced ingestion rates at high Cu influx rates. Dietborne metal toxicity is better predicted from metal influx than from either tissue concentration or concentration in the food. By quantifying rate constants of metal detoxification (k_{detox}), we show that under acute exposures the excess of uptake rate over detoxification rate results in toxicity at a body concentration lower than that at which toxicity occurs during chronic exposures.

Methods

Biodynamic Model. The biodynamic model predicts the change in metal concentration in an organism ($[M]_{org}$) over time as a function of metal influx from water (I_w), metal influx from food (I_f), metal efflux, and body growth dilution, i.e.,

$$\frac{d[M]_{org}}{dt} = (I_w + I_f) - (k_e + k_g)[M]_{org} \quad (1)$$

I_w is expressed as a function of k_{uw}, a unidirectional metal uptake rate constant from solution, and the dissolved metal concentration. I_f is governed by the dietborne metal concentration and k_{uf}, a unidirectional metal uptake rate constant from food. k_{uf} integrates the influences of food ingestion rate (IR) and metal assimilation efficiency (AE) (*8*). By capturing potential interactions if more than one metal is present (e.g., competitive inhibition), k_{uf} also reflects metal bioavailability. Conversely, metal effluxes vary as a function of the rate constant for physiological loss (k_e) and the accumulated metal concentration. When important,

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growth dilution can be expressed as a function of the rate constant of growth (k_g) and $[M]_{org}$. When metal efflux is dominated by loss from the metabolically available pool, the detoxification rate is controlled by a rate constant of metal detoxification (k_{detox}) and the metabolically available metal concentration. This assumption seems reasonable, for example, for very slow metal efflux (e.g., Cu in the clam *Corbicula fluminea* (*12*)) and for excretion that occurs via metal-rich concretions (Cu in the amphipod *Orchestia gammarellus* (*13*)). Numerous studies properly parametrized k_e (see summary tables in refs *6* and *12*), but there is no published report of k_{detox}.

Experimental Organisms. Freshwater snails (*Lymnaea stagnalis*) were reared in the laboratory in moderately hard water (MOD, hardness of 80−100 mg of $CaCO_3$ L^{-1}; pH 7.8 (*14*)). Three days prior to each experiment, snails of a restricted size range (mean soft tissue dry weight of 5.34 ± 0.53 mg 95% CI, $n = 158$) were transferred to a 10 L glass aquarium filled with MOD water. Food was withheld during this period.

Labeling of Food. The benthic diatom *Nitzschia palea* was exposed for 24 h to ^{65}Cu, ^{106}Cd, and ^{62}Ni, simultaneously, or to ^{106}Cd and ^{62}Ni, or to ^{65}Cu alone. Specifically, diatoms were grown axenically for several generations in an S-diatom medium (*15*). Diatoms were harvested onto a 1.2 μm Isopore membrane filter (Millipore) and rinsed with soft water (SO, hardness of 40−48 mg of $CaCO_3$ L^{-1}; pH 7.6 (*14*)). Algae were resuspended into a 20 mL acid-washed glass scintillation vial filled with SO water spiked with different concentrations of commercially purchased standards, isotopically enriched with ^{65}Cu (99.4%), ^{106}Cd (96.5%), and ^{62}Ni (96.1%) (Table S1, in the Supporting Information). The pH of the exposure media varied between 6.4 and 6.7. We did not distinguish between absorbed and adsorbed metal. After exposure, labeled diatoms were harvested onto a 1.2 μm Isopore membrane filter (Millipore) and rinsed with SO water. Five small sections of the filters holding the labeled diatoms were sampled and dried for 24 h at 50 °C. The remaining filters (onto which were collected the labeled algae) were offered as food to *L. stagnalis*. Typically, *L. stagnalis* (a generalist herbivore (*16*)) avidly graze films of deposited diatoms.

Dietborne Tracer Uptake and Depuration Experiments. For each treatment, 8−10 acclimated snails were transferred to a 150 mL acid-washed polypropylene vial partially submerged in a 20 L glass tank filled with MOD water. Each exposure vial had two 4 cm diameter holes (on opposite edges), covered with 100 μm acid-washed nylon mesh to allow exchanges with the surrounding media. Exposure was shorter than food gut residence time (*11*), which minimizes the confounding influences of efflux and isotopes recycling (*17*). Short exposure times are also required to determine unidirectional metal influxes (*8, 17*).

Snails were exposed to labeled-diatoms for either 6 h (experiments 1 and 2) or 5 h (experiment 3). Dietborne metal concentrations ranged from 1150 to 117 000 nmol g^{-1} for ^{65}Cu, from 13 to 4130 nmol g^{-1} for ^{106}Cd, and from 32 to 7200 nmol g^{-1} for ^{62}Ni. The lowest dietborne tracer concentrations for each metal were similar to those found in epiphytes collected in a freshwater lake of the San Francisco Bay delta (*18*). Low exposure concentrations are required to derive metal uptake rates that follow first-order kinetics (*8*). After feeding on the labeled food, snails were removed, rinsed with ultrapure water (Milli-Q system, >18 MΩ·cm), and placed individually in an acid-rinsed enclosure for a 24 h depuration period. Each enclosure was made of a 15 mL polypropylene vial that had two 1 cm^2 circular holes punched on opposite sides and were covered with 100 μm mesh net (to allow exchanges with the surrounding media). Enclosures were partially submerged in a 20 L glass tank filled with MOD water. Unlabeled lettuce was provided ad libitum during depuration. After depuration, snails were removed from the enclosures and frozen. The feces produced by each snail were also collected either manually or harvested by filtration onto a 1.2 μm Isopore membrane filter (Millipore). Feces were cumulatively placed on a piece of acid-washed Teflon sheeting in the former case. Feces were dried for 24 h at 50 °C. Aliquots of water ($n = 3-5$ per treatment) were taken immediately after labeled feeding, as well as at the beginning and the end of depuration. Water samples were acidified with concentrated nitric acid.

Sample Preparation and Analysis. To minimize inadvertent metal contamination, labware, vials, and Teflon sheeting were soaked for at least 24 h in acid (15% nitric and 5% hydrochloric), rinsed several times in ultrapure water, and dried under a laminar-flow hood prior to use.

Partially thawed *L. stagnalis* were dissected to remove soft tissue, placed individually on a piece of acid-washed Teflon sheeting, and allowed to dry at 50 °C for 3 days. Dried snails, feces, and diatoms were weighed and digested at room temperature in Teflon vials with concentrated nitric acid (100 μL mg of dry wt $sample^{-1}$) for 5−7 days (*19*). Hydrogen peroxide (Baker Ultrex II grade, 40 μL mg of dry wt $sample^{-1}$) was added prior to final dilution with ultrapure water (5−10% HNO_3). Similar weight samples of the certified reference material NIST-2976 (mussel tissue from National Institute of Standards and Technology) were submitted to the same digestion procedure during each analytical run.

Water and digested samples were analyzed for the naturally occurring stable isotopes of Cu (^{63}Cu and ^{65}Cu), Cd (^{106}Cd, ^{108}Cd, ^{110}Cd, ^{111}Cd, ^{112}Cd, ^{113}Cd, and ^{114}Cd), and Ni (^{58}Ni, ^{60}Ni, ^{61}Ni, ^{62}Ni, and ^{64}Ni) by inductively coupled plasma-mass spectrometry (ICP-MS). Specifically, all samples, blanks, and standards were introduced by direct injection (peristaltic pump; spray chamber) into the ICP-MS (single detector; quadrupole). Two analytical replicates were measured for each sample. A replicate consisted of 32 individual measurements that were averaged. External standards, serially diluted from ultrapure, single-element stock, were used to create calibration curves for each isotope. Certified reference riverine water samples (National Research Council Canada; SLRS-4) were analyzed for Cu, Cd, and Ni during each analytical run. To account for instrument drift and change in sensitivity, internal standardization was performed by addition of germanium (^{74}Ge) to all samples and standards but the calibration blanks. We also reanalyzed one of our standards after every 10 samples. Deviations from the standard value were in general less than 10% for all metals 95% of the time.

Calculation of Accumulated Tracer Concentrations. We used an isotope tracing technique that allows tracking of newly accumulated tracers, independently from background levels ((*12*), see also example for ^{106}Cd in the Supporting Information). Briefly, the relative abundance of each tracer (e.g., ^{65}Cu, ^{106}Cd, and ^{62}Ni) is determined using the signal intensities of each isotope in the calibration standards, i.e.,

$$p^i = \left(\frac{\text{Intensity } {}^iE}{\sum_j^{jj} \text{Intensity } {}^jE} \right)_{\text{Standard}} \quad (2)$$

where p^i is the relative abundance of the natural isotope ^iE (an element E of atomic weight i), j and jj are the lightest and heaviest isotopes of the metal E, respectively. Equation 2 works because p^i is constant across the range of standard concentrations; i.e., the intensities measured for the various isotopes do not increase disproportionately as the total concentration of the element increases.

Concentrations of tracer in the experimental organisms ($[^iE]_e$) are calculated as the product of p^i and the total metal

concentrations inferred by the ICP-MS software from tracer intensity ([T^{i}E]), i.e.,

$$[^i\mathrm{E}]_{\hat{e}} = p^i \times [\mathrm{T}^i\mathrm{E}] \quad (3)$$

The original load of tracer ($[^i\mathrm{E}]^0_{\hat{e}}$) that occurred in each sample in the absence of a spike is calculated as the product of p^i and the total metal concentrations inferred from the intensity of the most abundant isotope ([T^{k}E]), i.e.,

$$[^i\mathrm{E}]^0_{\hat{e}} = p^i \times [\mathrm{T}^k\mathrm{E}] \quad (4)$$

The net tracer uptake ($\Delta[^i\mathrm{E}]_{\hat{e}}$) is derived from the total experimental metal concentration ($[^i\mathrm{E}]_{\hat{e}}$, eq 3) minus the pre-existing concentration of tracer ($[^i\mathrm{E}]^0_{\hat{e}}$, eq 4), which gives

$$\Delta[^i\mathrm{E}]_{\hat{e}} = p^i([\mathrm{T}^i\mathrm{E}] - [\mathrm{T}^k\mathrm{E}]) \quad (5)$$

Biodynamic Calculations. Rate constants for dietary metal uptake (k_{uf}) were determined from the slope of the regression between metal uptake rate into *L. stagnalis* soft tissue and the dietborne exposure concentrations (data from the linear portion of the curve). The rate constant of metal uptake represents first-order accumulation processes (i.e., metal influx solely depends on the exposure concentration). k_{uf} is expressed as nmol of metal (g of tissue)$^{-1}$ day^{-1} per nmol of metal (g of food)$^{-1}$, or g of food (g of tissue)$^{-1}$ day^{-1}. Short exposure time is required to minimize the influence of efflux (*17*).

Food IR during the labeled feeding phase (g of ingested food per g of body tissue per day) was determined by mass-balance calculations using the total amount of tracer (i.e, ^{65}Cu, ^{106}Cd, or ^{62}Ni) retained in the snails after depuration ($\mathrm{Tracer}_{\mathrm{snail}}$ in ng), the amount of tracer egested in the feces during depuration ($\mathrm{Tracer}_{\mathrm{feces}}$ in ng), the tracer concentration in the enriched diatoms ($[\mathrm{Tracer}]_{\mathrm{diatoms}}$ in ng g^{-1}), the snail's dry weight ($\mathrm{wt}_{\mathrm{snail}}$ in g), and the exposure duration (T in day), i.e.,

$$\mathrm{IR} = \frac{(\mathrm{Tracer}_{\mathrm{snail}} + \mathrm{Tracer}_{\mathrm{feces}})}{[\mathrm{Tracer}]_{\mathrm{diatoms}} \times \mathrm{wt}_{\mathrm{snail}} \times T} \quad (6)$$

Metal AE (%) for each experimental organism was calculated using mass-balance calculations (eq 7) and then averaged by treatment ($n = 8-10$).

$$\mathrm{Tracer\ AE}(\%) = \frac{\mathrm{Tracer}_{\mathrm{snail}}}{\mathrm{Tracer}_{\mathrm{snail}} + \mathrm{Tracer}_{\mathrm{feces}}} \times 100 \quad (7)$$

Results

Dietborne Tracer Influxes. Metal uptake rates into *L. stagnalis* increased linearly with exposure concentrations ranging from 1150 to 23 000 nmol g^{-1} for ^{65}Cu, from 10 to 4100 nmol g^{-1} for ^{106}Cd, and from 30 to 7200 nmol g^{-1} for ^{62}Ni (Figure 1). The metal uptake rate constant from food (k_{uf}) increased in the order Cu < Ni < Cd (Table 1). Specifically, k_{uf} (±SE) averaged, among all experiments, 0.075 ± 0.008 g g^{-1} day^{-1} for Cu, 0.173 ± 0.010 g g^{-1} day^{-1} for Ni, and 0.247 ± 0.021 g g^{-1} day^{-1} for Cd. As a result, dietborne Cd and Ni influxes in *L. stagnalis* are, respectively, 3.3 and 2.3 times faster than that for Cu when diatoms are offered as food. Snails fed on labeled lettuce had a higher k_{uf} for Cu (0.162 ± 0.039 g g^{-1} day^{-1}) and lower k_{uf} for Cd (0.125 ± 0.023 g g^{-1} day^{-1}) and Ni (0.104 ± 0.012 g g^{-1} day^{-1}) (*8*). These metal- and food-specific differences in k_{uf} suggest that metal bioavailability is higher in diatoms than in lettuce for Cd and Ni but lower in diatoms than in lettuce for Cu. For instance, Cu influx in snails feeding on Cu-labeled lettuce is expected to be twice (*8*) that of snails feeding on Cu-labeled diatoms at a similar exposure concentration (Figure 1).

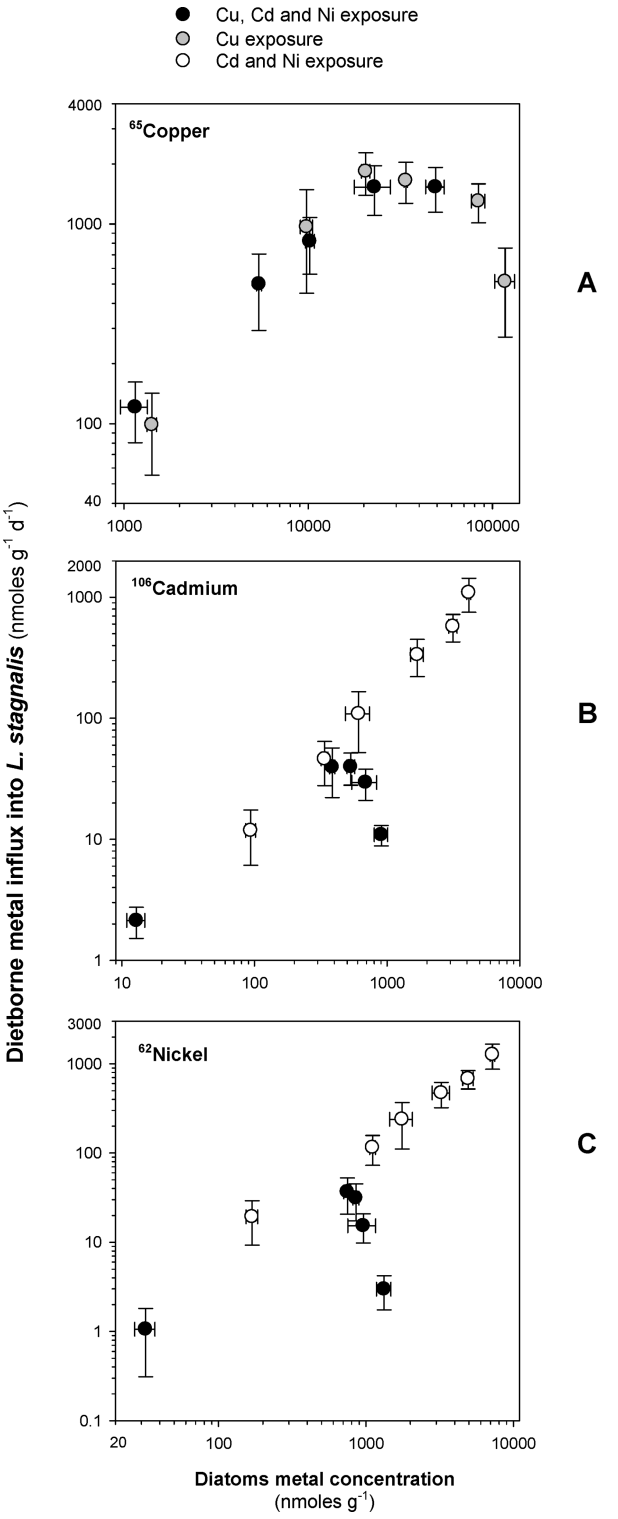

FIGURE 1. (A) Copper, (B) cadmium, and (C) nickel uptake rates (nmol g^{-1} day^{-1} ± 95% CI) in *L. stagnalis* (soft tissue) exposed for 5 or 6 h to a range of dietborne metal concentrations (nmol g^{-1} ± 95% CI). Each symbol represents metal concentrations of 8−10 individuals and 5 labeled lettuce samples (±95% CI). Open circles are for Cd + Ni exposure, solid circles are for Cu + Cd + Ni exposure, and gray circles are for snails exposed to Cu only.

Single tracer exposure elicited Cu influxes (gray symbols, Figure 1A) comparable to those found upon multitracer exposure (solid symbols, Figure 1A). Regardless of the presence of other metals in the exposure, Cu influxes increased linearly with exposure concentrations up to 20 000

TABLE 1. Estimated Parameter (±SE) and Measured Variable (±95% CI) Values for the Biodynamic Modeling of Cu, Cd, and Ni Accumulation, Loss, and Detoxification for *L. stagnalis*

parameter/variable	symbol	unit	Cu[a]		Cd	Ni
metal influx threshold	MIT	nmol g^{-1} day^{-1}	1530 ± 426	1833 ± 438	1093[c] ± 340	1266[c] ± 395
metal concentration at the influx threshold	$[M]_{IT}$	nmol g^{-1}	382 ± 107	382 ± 91	273[c] ± 85	317[c] ± 99
exposure concentration at the influx threshold	$[M]_{food}$	nmol g^{-1}	22864 ± 5084	20523 ± 1052	4130[c] ± 141	7209[c] ± 258
rate constant of dietborne metal uptake	k_{uf}	g g^{-1} day^{-1}	0.063 ± 0.005	0.090 ± 0.006	0.247 ± 0.027	0.173 ± 0.013
rate constant of metal loss	k_e	day^{-1}	0.026[b] ± 0.014		0.021[b] ± 0.006	0.173[e] ± 0.017
rate constant of metal detoxification	k_{detox}	day^{-1}	3.9 ± 0.04	4.8 ± 0.002	3.7[d] ± 0.002	3.8[d] ± 0.001

[a] Values are for the multimetal exposure and for the Cu only exposure, respectively (unless indicated). [b] Croteau and Luoma (*8*). [c] Maximal observed value; no threshold reached. [d] Minimal observed value; no threshold reached. [e] Unpublished results.

nmol g^{-1} in the two experiments. Thereafter, fluxes deviated from linearity to level off between 1500 and 1800 nmol g^{-1} day^{-1}. In contrast, Cd and Ni influxes increased linearly across all concentrations in the absence of Cu (open symbols, Figure 1B,C). Influxes as high as 1100 and 1300 nmol g^{-1} day^{-1} were observed for Cd and Ni, respectively. Influxes for Cd and Ni were significantly correlated (Pearson $r = 0.99$). In the presence of Cu, however, both Cd and Ni influxes were suppressed at high exposure concentrations (solid symbols, Figure 1B,C), although uptake rates were the same at lower concentrations. Suppression of Cd and Ni influxes coincided with the leveling off of Cu uptake rates.

Biodynamic Parameters. Food ingestion rates (IR) for snails exposed to dietborne Cd and Ni were similar among exposure concentrations (Figure 2B). Specifically, IR (±95% CI) averaged 0.19 ± 0.02 g g^{-1} day^{-1} when the total metal concentrations in spiked diatoms ($[^{106}Cd]_{diatoms} + [^{62}Ni]_{diatoms}$) ranged from 260 to 11300 nmol g^{-1}. Shorter and longer exposures to metal-labeled lettuce yield similar food IR for *L. stagnalis*, i.e., 0.16 ± 0.07 g g^{-1} day^{-1} upon a 2 h exposure to dietborne Cd, Cu, and Cr (*10*) and 0.15 ± 0.02 g g^{-1} day^{-1} upon an 18 h exposure to dietborne Cd, Cu, and Ni (*8*). Food IR was, however, significantly suppressed when snails were exposed to diatoms labeled with high levels of Cu (Figure 2A,C). For example, food IR decreased from 0.13 to 0.02 g g^{-1} day^{-1} when the total metal exposure concentrations varied from 1400 to 60 000 nmol g^{-1} (Figure 2A).

The IR inferred from ^{65}Cu and ^{106}Cd showed, in general, a good agreement (Table S2 in the Supporting Information), which is consistent with previous studies (*8, 11*). Inference of IR from ^{62}Ni yielded, however, consistently lower estimations. Preliminary experiments showed that quantification of ^{62}Ni can be complicated by analytical biases from the cone of the ICP-MS at low ^{62}Ni concentrations.

Metal AE (% ± 95% CI), averaged among all treatments, was 76 ± 3 for Cu, 78 ± 3 for Ni, and 81 ± 3 for Cd (Table 2). Exposure concentrations did not influence metal AE ($p > 0.1$), except for Cd and Ni in the multitracer exposure experiments ($p = 0.02$). On a physiological basis, however, variation in metal AE among exposure concentrations within an experiment was considered too small to be important.

Discussion

Cadmium, copper, and nickel are all highly bioavailable to *L. stagnalis* from food (high metal influx), as shown here and in earlier studies (*8, 11*). Bioavailability differed between the two food types we have studied. In contrast to Cd and Ni, Cu seems more bioavailable from lettuce than from a natural food source (diatoms). Differences in metal assimilation efficiencies between food types might explain, in part, why Cu bioavailability is lower from diatoms.

Whatever the food source, it appears that Cu can reduce feeding when organisms are exposed to high concentrations in the diet, but Ni or Cd does not cause such disruptions at

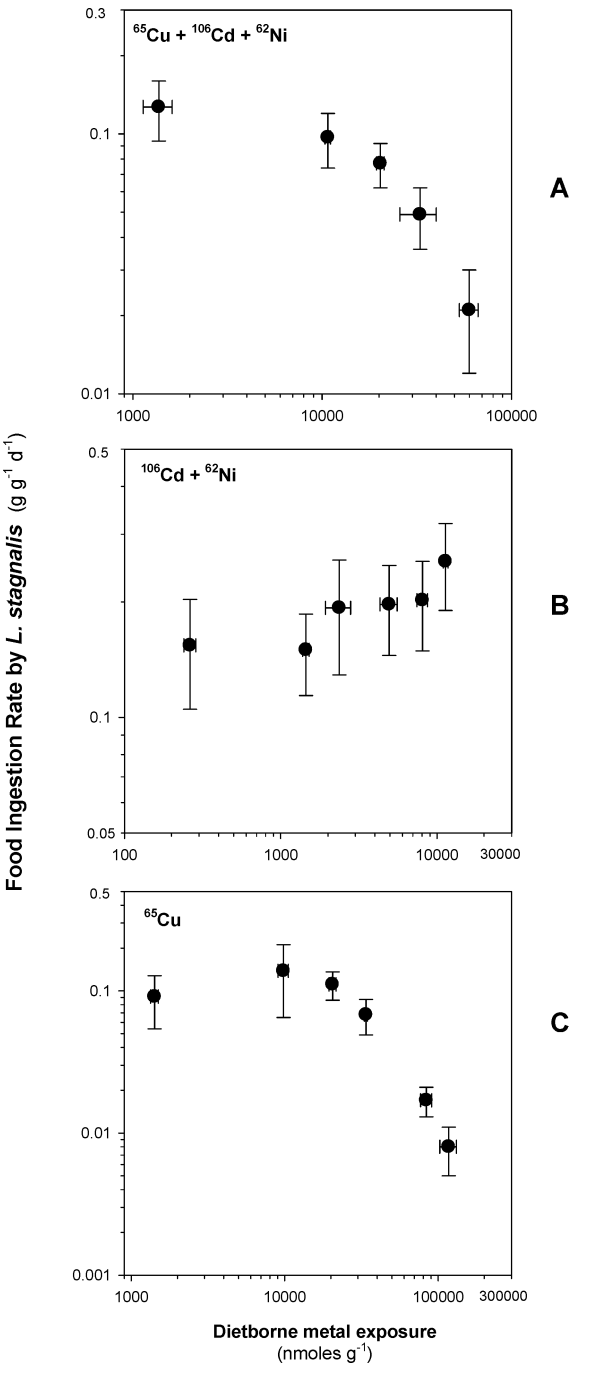

FIGURE 2. Food ingestion rates (g g^{-1} day^{-1}) as a function of exposure concentrations (Σ metal concentrations): (A) Cu + Cd + Ni exposure; (B) Cd + Ni exposure; (C) Cu only exposure.

TABLE 2. Metal AE (% ±95% CI) Determined by Mass-Balance Calculations for Each Exposure Concentration (±95% CI)[a]

metal exposure (nmol g^{-1})	AE Cu	AE Cd	AE Ni
	Cu + Cd + Ni		
1373 ± 240	78 ± 15 (6)	92 ± 3 (8)	79 ± 10 (7)
10718 ± 354	88 ± 8 (8)	93 ± 5 (8)	88 ± 10 (8)
20324 ± 922	83 ± 11 (8)	84 ± 8 (8)	79 ± 17 (7)
32946 ± 7154	88 ± 15 (7)	95 ± 6 (6)	91 ± 9 (6)
59923 ± 6813	72 ± 14 (7)	83 ± 9 (7)	63 ± 25 (6)
mean	**82 ± 5 (36)**	**89 ± 3 (37)**	**80 ± 6 (34)**
	Cd + Ni		
263 ± 23		80 ± 7 (10)	66 ± 14 (9)
1449 ± 69		79 ± 8 (10)	77 ± 8 (10)
2369 ± 434		76 ± 5 (10)	79 ± 4 (10)
4940 ± 622		74 ± 5 (10)	82 ± 3 (10)
8073 ± 632		67 ± 12 (9)	79 ± 9 (10)
11339 ± 399		75 ± 9 (10)	74 ± 9 (10)
mean		**75 ± 3 (59)**	**76 ± 3 (59)**
	Cu only		
1420 ± 83	73 ± 13 (8)		
9808 ± 744	69 ± 8 (8)		
20523 ± 1052	79 ± 7 (10)		
33847 ± 757	73 ± 13 (9)		
83848 ± 6977	69 ± 8 (10)		
117057 ± 14377	65 ± 9 (8)		
mean	**72 ± 3 (53)**		

[a] *n* value is in parentheses.

the concentrations tested here. Taylor et al. (*20*) demonstrated that feeding inhibition in *Daphnia magna* was due to Cd adsorbed to algal cell surfaces, but they could not determine if the cause was disruption of gut function or an effect on behavior. Copper similarly affects food processing in *L. stagnalis*, but snail metal assimilation efficiencies remain comparable among exposure concentrations. Therefore, disruption of the gut at very high Cu concentrations, as evidenced by reduced food ingestion rates and metal uptake rates from food, represent a behavioral response rather than impaired functionality of the gut. Despite dietborne Cd and Ni concentrations 3 orders of magnitude higher than those found in epiphytes collected in a relatively pristine freshwater lake of the San Francisco Bay delta (*18*), snails did not show signs of dietary metal stress in acute exposures to these metals. Specifically, food IR remained comparable among exposures, as did the metal AEs. Both disruption of gut function and reduced feeding activity were observed in trout (*21, 22*) and were found to be of significance for higher levels of biological organizations (e.g., populations (*23*) and ecosystems (*24*)).

Detoxification Rates. The relationship between Cu influx and dietborne Cu concentrations ultimately deviated from linearity. As a result of reduced feeding, Cu uptake rates plateaued between 1500 and 1800 nmol g^{-1} day^{-1} when exposure exceeded 20 000 nmol g^{-1} (Figure 1A). Conceptually, a metal influx threshold (MIT in nmol g^{-1} day^{-1}) occurs when Cu influx equals or begins to exceed the combined rates of Cu loss and detoxification (Figure 3). This translates in terms of biodynamics into

$$\mathrm{MIT} = \underbrace{k_{uf}\,[\mathrm{Cu}]_{\mathrm{food}}}_{\text{Influx}} = \underbrace{[\mathrm{Cu}]_{\mathrm{IT}}\,(k_e + k_{detox})}_{\text{Loss + Detoxification}} \qquad (8)$$

where $[Cu]_{food}$ (in nmol g^{-1}) is the Cu concentration in food, $[Cu]_{IT}$ (in nmol g^{-1}) is the Cu concentration in the organism at the influx threshold, k_e (day^{-1}) is the rate constant for the physiological loss of Cu, and k_{detox} (day^{-1}) is the rate constant for Cu detoxification (Table 1). Solving eq 8 for k_{detox} (by nonlinear regression) yields,

$$k_{detox} = \left(\frac{k_{uf}[\mathrm{Cu}]_{\mathrm{food}}}{[\mathrm{Cu}]_{\mathrm{IT}}}\right) - k_e \qquad (9)$$

The rate of loss ($k_e \times [Cu]_{IT}$) at the Cu influx threshold is only 0.026 × 382 or 6 nmol g^{-1} day^{-1}, compared to a rate of uptake of 1530 nmol g^{-1} day^{-1} (Figure 3). Up to the MIT, we can assume that the net metal accumulating in the organism (the difference between the two rates above) must have gone to the metabolically available pool (MAP) to satisfy metabolic requirements that can be as high as 400 nmol g^{-1} for Cu (*25*). All the excess accumulated metal must have been transferred to the detoxification pool (DP) to avoid causing toxicity. If we assume that all metal lost comes from the metabolically active pool, then k_{detox} can be estimated by fitting our data points (Figures 1A,C) to the eq 9, using the values of k_{uf} and k_e, the accumulated Cu concentration in snails, and the exposure concentrations (Table 1). Under these assumed conditions, k_{detox} for Cu in *L. stagnalis* varies from 3.8 to 4.8 day^{-1}, which is 2 orders of magnitude higher than the rate

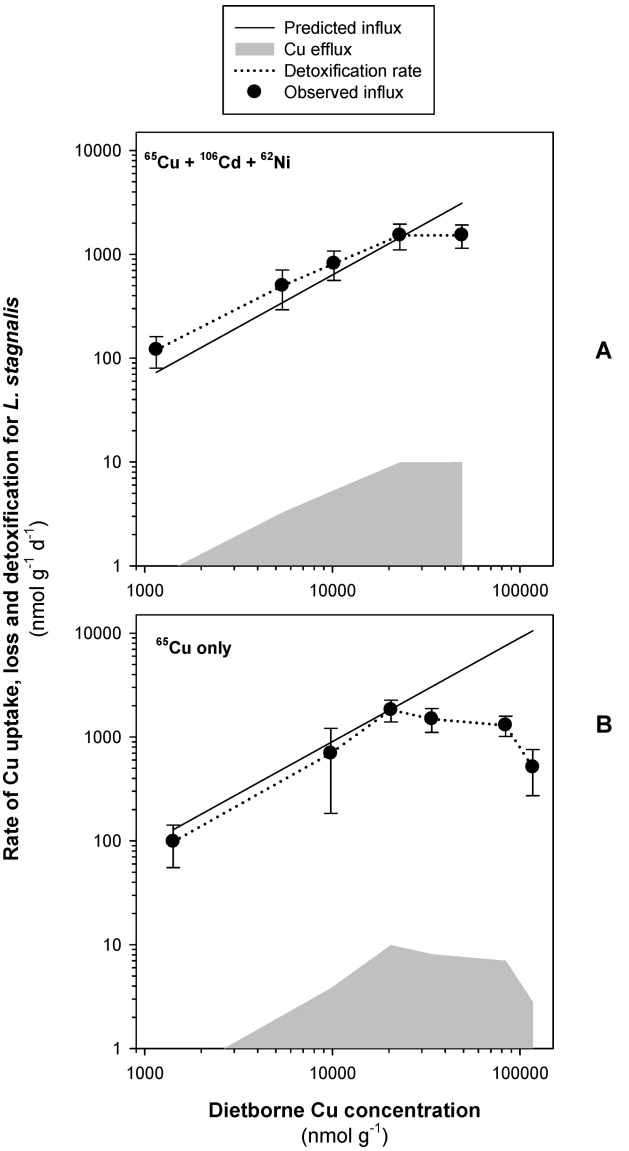

FIGURE 3. Rates of Cu uptake, loss, and detoxification (nmol g^{-1} day^{-1}) for *L. stagnalis* exposed to a range of dietborne Cu concentration (nmol g^{-1}): (A) Cu + Cd + Ni exposure; (B) Cu only exposure. Solid circles are for the measured Cu influx (±95% CI), solid lines represent predicted Cu influxes (eq 8), dotted lines represent Cu detoxification rates (eq 8), and the shaded areas illustrate the elimination rates.

constant of physiological loss (k_e for Cu is 0.026 day^{-1}, (*7*)). If it is assumed that metal lost comes from both the MAP and DP (*25*), the result is similar. In addition to the influence of k_{uf}, the proportion of metal accumulated in the MAP relative to DP depends on whether the metal plays an essential metabolic role (*25*), which should be captured in k_{detox} and k_e. Although the proportion of metal bound to ligands associated with nondetoxified pools can be quite high (even higher than in DP), decreasing the proportion of Cu accumulated in MAP relative to DP would increase k_{detox}. For instance, assuming that 50% of the accumulated Cu resides in MAP would increase k_{detox} by a factor 2 (i.e., 7.9 day^{-1}). Under either set of assumptions (i.e., 100% and 50% of the accumulated metal in MAP), loss of Cu appears insignificant relative to detoxification.

The sequestration of a large proportion of the Cu in *L. stagnalis* in the slowly exchanging detoxified form explains why the organism is a "net accumulator" of Cu. This trace metal accumulation strategy has been reported for many organisms, although the way the organism survives that strategy is rarely quantified (*4*). In particular, rates of Cu sequestration are rarely assessed nor have rates of compartmentalization of Cu into "metabolically available metal" and "detoxified metal" been previously quantified. Subcellular fractionations (*9*) provide, for example, only crude indicators of metal−ligand binding and toxicological risk. Our approach that combines enriched stable isotope tracers and biodynamic modeling provides the first quantification of metal detoxification rate constants.

Additional experimental work is required to determine if Cd and Ni influx thresholds occur in *L. stagnalis*; in any case, they would exceed the highest uptake rates measured for each metal in this study. That means the lower boundaries for k_{detox} for Cd and Ni are 3.7 and 3.8 day^{-1}, respectively (Table 1). As for Cu, these results suggest that basically all the Cd and Ni accumulated in *L. stagnalis* is held in a detoxification "pool". Further work is, however, warranted to evaluate these rate constant estimates and to test the model assumptions by running, for example, biodynamic and subcellular partitioning concurrently.

Predicting Toxicity. Equation 8 can be used to determine the dietborne metal concentration at which toxicity is likely to occur. For instance, we predict that toxicity (i.e., feeding inhibition) would manifest if *L. stagnalis* feed on lettuce labeled with Cu at concentrations equal to or higher than 9200 nmol g^{-1}, i.e., MIT divided by the k_{uf} for Cu in lettuce (*8*). This prediction matches the 10-fold decrease in food IR observed in snails exposed to lettuce labeled with Cu concentrations greater than 9200 nmol g^{-1} (*8*). These dietborne Cu concentrations are half those found for diatoms in this study (i.e., 20 500−22 800 nmol g^{-1}, Table 1). The difference in the concentration of Cu between lettuce and diatoms suggests that the bioavailable fraction of metal in the food better determines dietborne metal toxicity, rather than the total metal concentration. However, toxicity from both foods is first manifested at a similar influx of Cu. Geochemically, determining dietborne metal bioavailability is complex (*9, 10*); uptake rates might provide a simpler indicator of toxicity thresholds.

We can also use eq 8 to determine the highest Cu body concentration to be expected in *L. stagnalis* before toxicity ensues. For this, we used the Cu uptake rate at the point where food ingestion is first inhibited and the corresponding k_{detox} and k_e (Table 1). Upon acute exposure to dietborne Cu concentrations up to 23 000 nmol g^{-1}, we predict that snail Cu concentration would not exceed 400 nmol g^{-1}. This predicted concentration is almost 1 order of magnitude lower than the Cu concentration measured in the snail *Physa gyrina* collected in the San Francisco Bay delta (*18*) where Cu concentrations in epiphytic algae (snail purported food) averaged 200 nmol g^{-1}. Thus, under acute metal exposure, the excess of metal influx over detoxification rates results in toxicity at a lower body concentration than that observed in nature where chronic exposures mostly prevail. Organisms in the field can only attain high body concentrations if exposed to sufficiently low metal concentrations and if metal influxes do not exceed metal detoxification rates, allowing the excess metal to be accumulated in detoxified form. This explains why "total" bioaccumulated metal concentration will be a poor predictor of toxicity, and influxes are a crucial consideration.

Our results corroborate the concept that toxicity manifests when metal influx exceeds the combined rates of loss and detoxification. Organisms like the freshwater snail *L. stagnalis*, which are strong bioaccumulators of toxic metals, can cope with the extremely high levels of metals they accumulate to the extent that rapid influx is accompanied by rapid detoxification.

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Supporting Information Available

Nominal dissolved tracer concentrations used to label *N. palea* offered as food to *L. stagnalis*, equations to calculate the accumulated amount of ^{106}Cd, and food IR determined by mass-balance calculations for each tracer. This material is available free of charge via the Internet at http://pubs.acs.org.

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