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A Biodynamic Understanding of Dietborne Metal Uptake by a Freshwater Invertebrate

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Aquatic organisms accumulate metals from dissolved and particulate phases. Dietborne metal uptake likely prevails in nature, but the physiological processes governing metal bioaccumulation from diet are not fully understood. We characterize dietborne copper, cadmium, and nickel uptake by a freshwater gastropod (*Lymnaea stagnalis*) both in terms of biodynamics and membrane transport characteristics. We use enriched stable isotopes to trace newly accumulated metals from diet, determine food ingestion rate (IR) and estimate metal assimilation efficiency (AE). Upon 18-h exposure, dietborne metal influx was linear over a range encompassing most environmental concentrations. Dietary metal uptake rate constants (k_{uf}) ranged from 0.104 to 0.162 g g^{-1} day^{-1}, and appeared to be an expression of transmembrane transport characteristics. Although k_{uf} values were 1000-times lower than uptake rate constants from solution, biodynamic modeling showed that diet is the major Cd, Cu, and Ni source in nature. AE varied slightly among metals and exposure concentrations (84–95%). Suppression of Cd and Cu influxes upon exposure to extreme concentrations coincided with a 10-fold decrease in food IR, suggesting that feeding inhibition could act as an end point for dietary metal toxicity in *L. stagnalis*.

Introduction

Aquatic organisms are exposed to both dissolved and particulate metals. Bioaccumulation occurs from both phases, although the dietary uptake pathway appears more important under most circumstances (*1*). Metal uptake corresponds to the outcome of metal binding and subsequent metal transfer across the biological membrane. Conceptually, metal must first bind to receptor sites on the biological membrane and then be internalized to initiate a biological effect. Metal internalization fluxes are directly related to the bioavailable metal species outside the membrane, including those bound to "sensitive" uptake sites (as assumed by the Biotic Ligand Model (*2*)) and those that are free (as assumed by the Free Ion Activity Model (*3*)). Mechanistically, transport site affinity and binding site capacity pose a limit to the extent of metal accumulation, as shown in several waterborne exposures studies (*4–8*). But the role of site affinity and site capacity on dietary metal influxes is not fully understood.

Like waterborne uptake, dietborne metal uptake is species- and metal-specific (*9*). Species with fast uptake rates from diet are expected to efficiently accumulate metals highly partitioned to solid phases (*10*). Metal uptake from food is directly proportional to metal assimilation efficiency (AE) (*11*), which is a measure of metal bioavailability from diet (*12*). AE is greatly impacted by the digestive processes occurring in an animal's gut. For example, prolonged retention of food in the digestive tract increases the uptake of metal due to more assimilation (*13, 14*).

The biodynamic model uses empirically determined species-specific and metal-specific physiological parameters to describe bioaccumulation in terms of fluxes (*9, 15*). Specifically, it predicts that metal body burden increases as long as influxes from all exposure routes (e.g., food and water) exceed loss rates. Metal influx from solution is expressed as a function of k_{uw}, a unidirectional metal uptake rate constant from solution, and the dissolved metal concentration. Conversely, metal influx from food varies as a function of k_{uf}, a unidirectional metal uptake rate constant from food, and the dietborne metal concentration. Both k_{uw} and k_{uf} represent first-order accumulation processes. They also both reflect the affinity of each transport site on the membrane for a metal, the number of transport sites, and the competitive effects on ligand binding. For instance, Croteau and Luoma (*16*) showed that k_{uw} values for Cd in a freshwater snail correlate with changes in site affinity, whereas k_{uw} values for Cu correlate with changes in both site affinity and capacity. Inference of membrane characteristics from dietary metal influxes is, however, technically and biologically challenging. In addition to the difficulties inherent in preparing metal-contaminated diets, unpalatability and impaired feeding affect metal influxes from diet. As a result, very few studies have determined conditional binding constants for dietborne metals.

Here, we use a novel approach that combines pulse-chase feeding and a multilabeled stable isotope technique (*17*) to characterize unidirectional Cu, Cd and Ni uptake rates from diet, both in terms of membrane transport characteristics and biodynamics. Specifically, we expose freshwater snails to a wide range of enriched ^{65}Cu, ^{106}Cd, and ^{62}Ni concentrations through diet for a time period shorter than food gut residence time (GRT (*17*)). We also amend diet with an inert tracer (i.e., enriched ^{53}Cr) to allow determination of metal AEs and food ingestion rates (IR). Specifically, we ask: can dietborne metal influxes be quantified using enriched stable isotope tracers? If so, how do they vary with exposure? Can transmembrane transport characteristics, or biodynamic parameters, or both, explain differences in influxes among metals and between exposure routes? Finally, we evaluate the usefulness of transmembrane transport characteristics and biodynamic parameters as insights into toxic stress responses to dietborne metal exposures.

Materials and Methods

Experimental Organisms. Freshwater snails (*Lymnaea stagnalis*) were reared in laboratory in moderately hard water (MOD, hardness of 80–100 mg of $CaCO_3$ L^{-1}; pH 7.8 (*18*)) at 15 °C. Three days prior to each experiment, snails of a restricted size range (mean shell size of 16.6 ± 0.3 mm 95% CI, $n = 136$) were transferred to a 10-L glass aquarium (~40 snails per aquarium) filled with MOD water. Food was withheld during this period.

Labeling of Food. Lettuce was exposed to ^{65}Cu, ^{106}Cd and ^{62}Ni, either simultaneously (to simulate natural conditions) or to each tracer individually (to determine whether influxes of these elements are independent of one another), for 3 days at 15 °C (Table S1, Supporting Information). Lettuce was also amended with an inert tracer (*17*). Specifically, pieces

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of lettuce (~1 cm^2) were placed into a 148-mL acid-washed polypropylene vial filled with 100 mL of deionized water spiked with different concentrations of commercially purchased standards isotopically enriched in ^{53}Cr (92.8%), ^{65}Cu (99.4%), ^{106}Cd (96.5%) and ^{62}Ni (96.1%). Because the tracer stock solutions were prepared using HNO_3, or HCl, or both, the pH of the exposure media was increased to reach neutrality with NaOH soon after adding the lettuce. Coincidently, the pH rise enhanced the tracers' adsorption onto lettuce surfaces (*19*). We did not distinguish between absorbed or adsorbed metal. After exposure, lettuce pieces were thoroughly washed with ultrapure water (Milli-Q system, > 18 Mohm cm^{-1}) for 1 h (to remove weakly adsorbed metals) and offered as food to *L. stagnalis*. Samples of labeled and unlabeled lettuce ($n = 5$ per treatment) were also individually placed on a piece of acid-washed Teflon sheeting and dried for 24 h at 50 °C.

Dietborne Tracer Uptake and Depuration Experiments. For each treatment, 8 acclimated snails were transferred to an acid-washed 2-L HDPE container filled with MOD water. Snails were allowed to feed on the labeled lettuce for 18 h at 15 °C. Exposure was shorter than food GRT (~22.5 h (*17*)), which minimizes the confounding influences of efflux and isotope recycling (*15*). Short exposures are also required to determine gross uptake rates (*15*) and to infer transmembrane transport processes (*16*). Dietborne metal concentrations ranged from 30 to 41 400 nmol g^{-1} for ^{65}Cu, from <0.1 to 1400 nmol g^{-1} for ^{106}Cd, and from 0.8 to 1744 nmol g^{-1} for ^{62}Ni. The lowest dietborne tracer concentrations were at least an order of magnitude lower than those found in epiphytes collected in a freshwater lake of the San Francisco Bay (SFB) delta (*20*). Low exposure concentrations are required to derive metal uptake rates that follow first-order kinetics (i.e., linear function of concentrations).

After feeding on the labeled food, snails were removed, rinsed with ultrapure water, and placed individually in an acid-washed enclosure for a 24-h depuration period. Each enclosure was made of a 150 mL polypropylene vial, for which the bottom had been removed and covered with 100 μm acid-washed nylon net (to allow exchanges with the surrounding media). Enclosures for each treatment were partially submerged in an acid-washed polyethylene tray (32 × 45.7 × 5.7 cm) filled with 5-L of MOD water. Air was bubbled in each tray. Unlabeled lettuce was provided ad libidum during depuration. All feces were collected periodically throughout depuration (i.e., after 2, 4, 6, 9, 12, and 24 h). Feces for each snail were cumulatively placed on a piece of acid-washed Teflon sheeting, thereby allowing mass-balance calculation for each experimental organism. Feces were dried for 24 h at 50 °C. Snails were frozen at the end of depuration.

Aliquots of water ($n = 3$ per treatment) were taken immediately after labeled feeding and throughout depuration (e.g., after 2, 6, 12, and 24 h). Water samples were filtered through a 0.45 μM Millex-HV filter (Millipore) and acidified with concentrated nitric acid (Baker Ultrex II grade, 2% final concentration). Because loss of tracers in the media during depuration was expected to be negligible (*17*), media was changed once during depuration (i.e., after 10 or 12 h), except for one experiment (no renewal).

Sample Preparation and Analysis. To minimize inadvertent metal contamination, we soaked labware, vials, and Teflon sheeting for 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 snails 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 lettuce samples were weighed and digested at room temperature in Teflon vials with concentrated nitric acid (100 μL per mg of dry wt sample) for 7 days. Hydrogen peroxide (Baker Ultrex II grade, 40 μL per mg of dry wt) was added prior to heating samples to dryness the following day (*17*). Dried samples were reconstituted with HNO_3 (5%). 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, Cd, Ni, and Cr (Table S2, Supporting Information) by inductively coupled plasma-mass spectrometry (ICP-MS). Specifically, all samples, blanks and standards were introduced by direct injection into the ICP-MS (single-detector; quadrapole). Two replicates were measured for each sample. A replicate consisted of 32 individual measurements that were averaged. Single-element, high-purity standards (Charleston, SC), serially diluted, 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, Ni, and Cr 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, except the calibration blanks. We also reanalyzed one of our standards after every 10 samples. Deviations from standard value were in general less than 5% for all metals. Background Cr concentrations in snail and lettuce samples were adjusted using the correction factors determined by Croteau et al. (*17*).

Calculation of Accumulated Tracer Concentrations. We used an isotope tracing technique that allows tracking newly accumulated tracers independently from background levels (*21*). Briefly, the relative abundance of each tracer (e.g., ^{65}Cu, ^{106}Cd, ^{62}Ni, and ^{53}Cr) is determined using the signal intensities of each isotope in the calibration standards, i.e.

$$p^i = \left(\frac{\text{intensity } {}^iE}{\sum_{j}^{jj} \text{intensity } {}^jE} \right)_{\text{Standard}} \quad (1)$$

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 (see Croteau and Luoma (*16*) for an example of calculation with ^{106}Cd). Equation 1 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 ($[{}^iE]_{\hat{e}}$) are calculated as the product of p^i and the total metal concentrations inferred by the ICP-MS software from tracer intensity ($[T^iE]$), i.e.,

$$[{}^iE]_{\hat{e}} = p^i[T\,{}^iE] \quad (2)$$

The original load of tracer ($[{}^iE]_{\hat{e}}{}^0$) 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 ($[T^kE]$), i.e.,

$$[{}^iE]_{\hat{e}}^0 = p^i[T\,{}^kE] \quad (3)$$

The net tracer uptake ($\Delta[{}^iE]_{\hat{e}}$) is derived from the total experimental metal concentration ($[{}^iE]_{\hat{e}}$, eq 2) minus the pre-existing concentration of tracer ($[{}^iE]_{\hat{e}}{}^0$, eq 3), which gives after algebraic simplifications

$$\Delta[{}^iE]_{\hat{e}} = p^i([T\,{}^iE] - [T\,{}^kE]) \quad (4)$$

Ranking Metal Exposures. To facilitate discriminating the effects of exposure on the measured parameters (see

below), cluster analysis (K-means) was used to partition the amount of label accumulated by the lettuce (eq 2) into 4 "exposure" groups, i.e., low, moderate, high and extreme (Figure S1, Supporting Information).

Biodynamic Calculations and Membrane Transport Characterization. Rate constants for dietary metal uptake (k_{uf}) were determined from the slope of the regression between the metal uptake rate into *L. stagnalis* soft tissue (data from the linear portion of the curve) and the dietborne exposure concentrations. 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}.

Metal AE (%) was calculated by the ratio method (*22*), i.e.

$$\text{AE}\ (\%) = \left[1 - \frac{({}^{i}E/{}^{53}\text{Cr})_{\text{feces}}}{({}^{i}E/{}^{53}\text{Cr})_{\text{food}}}\right] \times 100 \qquad (5)$$

where $({}^{i}E/{}^{53}\text{Cr})_{\text{feces}}$ is the ratio of the tracer ${}^{i}E$ and ^{53}Cr net signal intensities in the feces (after depuration), and $({}^{i}E/{}^{53}\text{Cr})_{\text{food}}$ is the ratio of the tracer ${}^{i}E$ and ^{53}Cr net signal intensities in the enriched lettuce. The ratio method assumes that ^{53}Cr is not bioavailable for snails, which has been verified by Croteau et al. (*17*).

Food IR (g of ingested food per g of body tissue per day) was derived from uptake rates for each tracer using the tracer concentrations in the labeled food and the tracer AE. We also determined IR by mass-balance calculations using ^{53}Cr as an inert tracer (*17*). Specifically, food IR during the labeled feeding phase was calculated for each experimental snails using the total amount of ^{53}Cr egested in the feces during depuration ($\Sigma^{53}\text{Cr}_{\text{feces}}$), the enriched lettuce ^{53}Cr concentration ($[{}^{53}\text{Cr}]_{\text{LL}}$), the snail's dry weight (wt_{snail}), and the exposure duration (T in day), i.e.

$$\text{IR} = \frac{\sum {}^{53}\text{Cr}_{\text{feces}}}{[{}^{53}\text{Cr}]_{\text{LL}} \times \text{wt}_{\text{snail}} \times T} \qquad (6)$$

The conditional membrane transport characteristics were characterized by fitting a Michaelis–Menten equation to plots of accumulated metal concentrations in the snail (eq 4, in nmol g^{-1}) upon 18-h exposure versus total metal concentrations in the labeled food (eq 2, in nmol g^{-1}). For this, we assume that food breakdown does not limit the transport of metal toward the gut membrane receptors, which appear reasonable given the 18-h period of ingestion. The nonlinear regression fit was used to determine the saturation point of the curve, which is reflective of binding site capacity in the gut (B_{max} in nmol g^{-1}). If we assume that metal internalization rate is negligible compare to the rate of metal dissociation from the receptor (back into the gut lumen), then the reciprocal value of the metal concentration at half-saturation (K_{metal} in nmol g^{-1}) can be indicative of gut site affinity (log K) (*23*). To make log K comparable with waterborne exposure studies, units of nmol kg^{-1} were used for K_{metal}. It is important to note that site affinity and capacity are traditionally inferred from rates, which should be expressed as a function of time (*5*). If expressed as concentration accumulated, then different conditional constant will result from different exposure times (*6, 24*).

Results

Dietborne Tracer Uptake. Snails exposed for 18 h to dietborne metal concentrations ranging from 16 to 1397 nmol g^{-1} for ^{106}Cd, from 0.34 to 41.4 μmol g^{-1} for ^{65}Cu, and from 55 to 1744 nmol g^{-1} for ^{62}Ni accumulated significant amounts tracers compared to controls ($p < 0.001$, Figure 1). Dietborne metal influx was linear over a range of concentration that would encompass most environmental exposures (*20, 25*), i.e., < 793 nmol g^{-1} for Cd, < 5.45 μmol g^{-1} for Cu, and <1.74

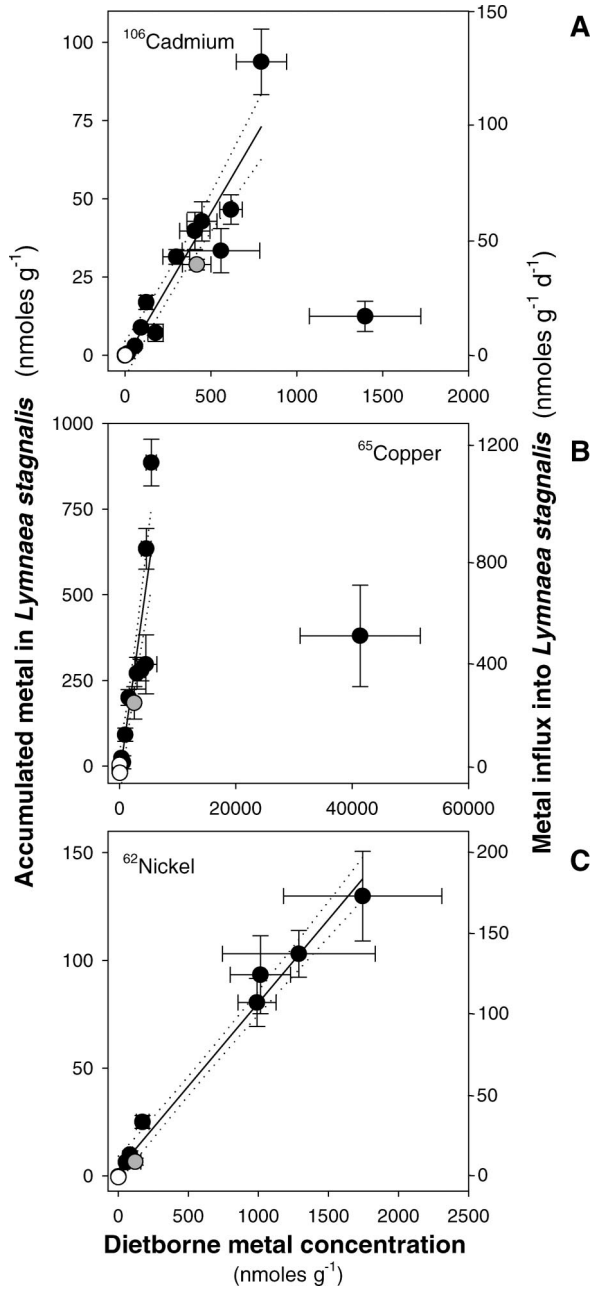

FIGURE 1. Metal uptake rates (in nmol g^{-1} day^{-1} ± 95% C.I.) in *L. stagnalis* (soft tissue) exposed for 18 h to a range of dietborne metal concentrations (in nmol g^{-1} ± 95% C.I., eq 2). Each symbol represents metal concentrations of 8 individuals and 5 labelled lettuce samples (±95% C.I.). Open circles are for controls; solid circles are for the exposed snails; gray circles are for snails exposed to a single tracer (in addition to ^{53}Cr). Solid lines represent linear regression relationships ($p < 0.001$, $r^2 = 0.90$ for ^{106}Cd, 0.84 for ^{65}Cu, and 0.98 for ^{62}Ni); dotted lines are for the 95% C.I. surrounding each slope.

μmol g^{-1} for Ni (Figure 1). The metal uptake rate constant from food (k_{uf} ± 95% C.I. in g g^{-1} d^{-1}) was significantly higher for ^{65}Cu (0.162 ± 0.039) than for ^{62}Ni (0.104 ± 0.012). k_{uf} for ^{106}Cd was intermediate between that for ^{65}Cu and that for ^{62}Ni (0.125 ± 0.023, Table 1). The k_{uf} for ^{53}Cr averaged 0.014 ± 0.008 g g^{-1} d^{-1}. Thus Cr uptake was essentially negligible especially compared to uptake of other metals (as observed earlier (*17*)).

Single-tracer exposure elicited uptake rates comparable to those found upon multitracer exposure, suggesting that influxes of these elements were independent of one another.

TABLE 1. Metal Binding Characteristics (±S.E.) and Rate Constants (±95% C.I.) for Dietary Metal Uptake by *L. stagnalis*[a]

metal	B_{max} (nmol g^{-1})	K_{metal} (nmol g^{-1})	log K	k_{uf} (g g^{-1} day^{-1})
nickel	639 ± 309	4651 ± 2888	2.3 ± 0.18	0.104 ± 0.012
copper	1181[b] ([124– 196][d])	3914[b]	2.4[c] ([7.2–8.6][d])	0.162 ± 0.039
cadmium	125[b] ([211–289][d])	514[b]	3.3[c] ([6.0–7.0][d])	0.125 ± 0.023

[a] Metal binding characteristics for dissolved Cd and Cu uptake (from ref (*16*)) are also given in parenthesis. [b] Lower boundary. [c] Upper boundary. [d] Data range.

TABLE 2. Metal AE (±95% CI) Determined by the Ratio Method for Each Exposure Category[a]

	AE Cu	AE Cd	AE Ni
low	82 ± 4 (46)	88 ± 2 (38)	96 ± 1 (24)
moderate	87 ± 2 (15)	82 ± 4 (22)	93 ± 2 (23)
high	90 ± 2 (30)	78 ± 4 (23)	93 ± 2 (8)
extreme	98 ± 1 (8)	95 ± 2 (8)	n.d.[b]
mean	86 ± 2 (99)	84 ± 2 (83)	95 ± 1 (55)

[a] *n* value is in parenthesis. [b] n.d. = Not determined.

As shown in Figure 1, influxes resulting from each single metal exposure (gray circle) fall within the confidence interval of the corresponding linear regression, which was determined upon multimetal exposure (i.e., data for the single-exposure treatment were excluded when the regression fit was calculated).

Membrane Characteristics. Tracer concentrations in *L. stagnalis* increased linearly over exposure concentrations that varied 100 to 1900-times above that for controls (Figure 1). At extreme exposure concentrations, Cd and Cu influxes were substantially suppressed, i.e., Cu and Cd influxes were respectively 2.3 and 7.5-times lower than those for the high exposures (panels A and B in Figure 1 and Figure 1S in the Supporting Information). Because the plunge in influxes likely represents a behavioral response (i.e., feeding inhibition, see "Biodynamic parameter section" below) rather than a toxic response that would have impaired the functionality of the gut, we can reasonably assume that B_{max} would exceed the highest measured uptake rate. The measured metal concentrations preceding the plunge could thus provide lower boundaries for B_{max} (i.e., 886 nmol g^{-1} for Cu and 94 nmol g^{-1} for Cd), from which could be derived the corresponding lower boundaries for K_{metal} (i.e., 3904 nmol g^{-1} for Cu and 514 nmol g^{-1} for Cd). In contrast, Ni influx into *L. stagnalis* appeared to demonstrate saturation uptake kinetics. However, because B_{max} (374 nmol g^{-1}) and K_{metal} (3317 nmol g^{-1}) exceed the experimental domain, the conditional binding characteristics for Ni remain to be validated.

The differences in B_{max} among metals suggest that, even at the lower boundary, Cu uptake sites are at least 2−9 times more abundant than those for Ni and Cd in the gut of *L. stagnalis*, respectively. Although Ni binding is similar to the lower boundary for Cu, Cd binds to receptors with 10 times more intensity than Cu or Ni (Table 1).

Biodynamics Parameters. Metal AE (% ± 95% C.I.) was 85 ± 2 for Cd, 89 ± 1 for Cu, and 95 ± 1 for Ni (Table 2). These AE values are comparable to those found earlier in *L. stagnalis* using a shorter exposure time (2 h) (*17*). Correspondingly high Cd AEs have also been reported for predaceous marine gastropods (*26, 27*). The high AEs for Cd, Cu, and Ni in *L. stagnalis* are not just a function of metal absorption on the lettuce because Cr AE was essentially 0.

Food IRs inferred from uptake rates were similar among exposure concentrations ($p > 0.5$), except for the extreme exposure ($p < 0.05$, Table 3). Specifically, IR (±95% C.I.) averaged 0.145 ± 0.018 g g^{-1} d^{-1} for the low, moderate and high exposures. Shorter exposure to dietborne Cd and Cu yielded a similar IR, i.e., 0.16 ± 0.07 g g^{-1} d^{-1} (*17*). At the extreme exposure concentrations, food IR decreased by 10-fold (i.e., 0.013 g g^{-1} d^{-1}). Inference of IR from ^{65}Cu, ^{106}Cd and ^{62}Ni using mass-balance calculations show generally good agreement (Table 3).

Discussion

Enriched stable metal isotopes can trace newly accumulated metals, independently from background levels. The technique involves manipulating stable metal isotope ratios in exposure media with standards enriched in individual isotopes. Among the greatest advantages of the technique is the possibility to study metals that lack suitable radiotracers. The enriched isotope methodology has been used to describe Cu biodynamics (*21*), to delineate Cu uptake pathways (*28*), to characterize unidirectional dissolved Cd and Cu influxes and to infer transmembrane transport characteristics (*16*). Prior to extending the approach to the study of dietborne metal uptake, we have first to demonstrate that dietborne tracers can be detected upon short exposure to low dietborne tracer concentrations (*15*). Figure 1 shows that tracer uptake is detectable when *L. stagnalis* are fed for 18 h on lettuce labeled with increasing amounts of enriched ^{65}Cu, ^{106}Cd and ^{62}Ni.

Metal Uptake Rate Constants. Dietborne metal influx rate constants increase in the order of Ni ⩽ Cd ⩽ Cu from this food source. But the differences among metals are small and not statistically significant between Cd and Ni, as well as between Cd and Cu. Uptake rate constants from solution (k_{uw}) also are not different between Cd and Cu for *L. stagnalis* when water hardness varies from 40 to 100 mg $CaCO_3$ L^{-1} (*16*).

We tested the validity of our biodynamic parameters by comparing bioaccumulation observed in nature to that predicted from laboratory-derived constants and field-collected variables (*9*). Under steady-state conditions, Cu and Cd concentrations in *Physa gyrina* ($[M]_{ss}$), a pulmonate snail inhabiting the SFB delta, can be described as

$$[M]_{ss} = \frac{k_{uf}[M]_{food}}{k_e} \quad (7)$$

where $[M]_{food}$ (in nmol g^{-1}) is the metal concentration in epiphytic algae found in the SFB delta and k_e (in d^{-1}) is the rate constant of metal loss (Table S3, Supporting Information). Bioaccumulation of dissolved Cu and Cd is likely negligible in this system in which dissolved organic matter is abundant (*28*). We predicted steady state concentrations of ∼1090 nmol g^{-1} for Cu and 3.7 nmol g^{-1} for Cd. These predictions fall within the range of concentrations measured in *P. gyrina* collected from the SFB delta, i.e., from 677 to 5665 nmol g^{-1} for Cu, and from 1.2 to 13 nmol g^{-1} for Cd depending on size (*20*). Dietary uptake alone, therefore, fully explains metal bioaccumulation by the snail. Dietborne metal uptake is increasingly recognized as an important pathway for metal accumulation, as shown for Cu bioaccumulation in marine copepods (*29*), in the bivalve *Corbicula fluminea* (*28*), and now in the snail *Physa sp.* Bioaccumulation of Cd is often split between dissolved and dietary uptake in freshwaters, but dominance of dietary uptake for Cd bioaccumulation is

TABLE 3. Food IR (g g^{-1} day^{-1} ± 95%CI) Inferred from Metal Uptake Rates and Estimated by Mass Balance Calculations Using ^{53}Cr, ^{65}Cu, ^{106}Cd, and ^{62}Ni as tracers[a]

	^{53}Cr[b]	^{65}Cu	^{106}Cd	^{62}Ni	influx rates
low	0.163 ± 0.031 *(48)*	0.104 ± 0.018 *(23)*	0.143 ± 0.024 *(24)*	0.182 ± 0.017 *(16)*	0.132 ± 0.033 *(10)*
moderate	0.185 ± 0.055 *(24)*	0.192 ± 0.013 *(16)*	0.149 ± 0.011 *(24)*	0.131 ± 0.012 *(32)*	0.147 ± 0.021 *(8)*
high	0.121 ± 0.040 *(23)*	0.140 ± 0.019 *(31)*	0.144 ± 0.018 *(23)*	0.105 ± 0.016 *(8)*	0.151 ± 0.040 *(8)*
extreme	0.056 ± 0.018 *(8)*	0.014 ± 0.005 *(8)*	0.015 ± 0.005 *(8)*	n.d.[c]	0.013 ± 0.001 *(2)*

[a] *n* value is in parenthesis. [b] Categories for ^{53}Cr are based on clusters for Cu. [c] n.d. = Not determined.

known in the freshwater insect *Chaoborus* (*30*) and now in *Physa sp*.

Exposure Routes. Metal uptake rate constants in *L. stagnalis* vary also between exposure routes. When compared in similar units (i.e., in nmol g^{-1} day^{-1} per nmol kg^{-1} for k_{uf} and nmol g^{-1} day^{-1} per nmol L^{-1} for k_{uw}), the k_{uf} value for Cd and Cu are 3-orders of magnitude lower than the k_{uw} values. For instance, the rate constants for Cu are 0.74 L g^{-1} day^{-1} and 0.00016 kg g^{-1} day^{-1} for water and food, respectively (Table S3, Supporting Information). However, differences in concentrations of these elements in the dissolved and particulate phases counterbalance the divergence in uptake rates. Dissolved Cd and Cu concentrations in freshwaters range between <0.09 – 9 nmol L^{-1} and <16–100 nmol L^{-1} for Cd and Cu respectively, when pollution is not extreme (*31*). In contrast, Cd and Cu concentrations in sediments, periphyton and organisms in the same water bodies are more than 1000-times higher than in water. For instance, Cd concentrations in water, in the lake-dwelling insect *Chaoborus*, and in sediment of a lake located far from mining activities are, respectively, 5.8 nmol L^{-1}, 5800 nmol kg^{-1}, and 47 000 nmol kg^{-1} (i.e., Lake St-Joseph (*25*)). Both particles and water are thus potentially important uptake vectors depending on environmental conditions and species traits. Dietary metal influxes can be expected to dominate where speciation reduces the bioavailability of dissolved metals as in the complexation of Cu by organic ligands, major cations and protons competition for uptake sites, or chloro-complexation of Cd in marine waters (*4, 32, 33*).

Relating Uptake Rate Constants to Membrane Transport Characteristics. Given that dietborne Ni uptake in *L. stagnalis* might indicate saturation, inference of membrane characteristics was thus possible using a ligand-binding equation. We also assumed that the measured metal influxes preceding the plunge could provide lower boundaries for B_{max}, and K_{metal}.

Under these assumed conditions, transporter characteristics in the gut of *L. stagnalis* vary among metals. For instance, site affinity for Cu and Ni would be weaker than for Cd, whereas density of Cu transporters would be an order of magnitude higher than for Cd. Thus, the relatively high k_{uf} for Cu likely reflects the high abundance of Cu binding sites. Conversely, the high affinity of Cd transporters seems to be the dominant influence on k_{uf}. The relative influence of site capacity and affinity on k_{uf} thus appears to be metal-specific, as was also shown for k_{uw} (*16*).

Transporter characteristics in *L. stagnalis* also vary widely between exposure routes. For instance, site affinity for dietborne Cu is 6-orders of magnitude lower than that found for dissolved Cu (Table 1), but binding site density appears 6–10 times higher for dietborne Cu than for dissolved Cu. Similar differences were found for Zn in rainbow trout. Affinity for branchial Zn uptake varies between 3.6 and 7.9 μmol l^{-1} (*34, 35*) whereas in the gut K_m is 309 μmol l^{-1} (*36*). Capacity for Zn uptake is also greater in the gut with a maximal rate of 933 nmol kg^{-1} h^{-1} (*36*) compare to 240 – 410 nmol kg^{-1} h^{-1} for the gill (*34, 35*). Glover and Hogstrand (*36*) concluded that the gut acted as the main pathway for Zn uptake in rainbow trout. If so, the same seems true for Cu in *L. stagnalis*. However, dissolved metal uptake might supplement absorption of essential elements when food availability is low (*37*).

The animal gut plays a central role in absorption and assimilation of nutrients, such as amino acids, as well as metals (*38*). Ions and organic solutes are concentrated by processes occurring in the gut lumen. Such conditions favor the occurrence of low-affinity and high-capacity transport processes. In contrast, higher affinity and lower capacity sites are expected on membranes exposed to waterborne concentrations, consistent with lower environmental concentrations. Furthermore, metals such as Cu, Fe, and Zn may be cotransported across the gut epithelium on transport proteins for organic nutrients like amino acids. For instance, histidine can enhance intestinal uptake of Zn in rainbow trout (*36*). Thus, the high site density observed for dietborne Cu in *L. stagnalis* might also reflect the abundance of amino acid-Cu chelates that cotransport Cu across the epithelial surface (*39*).

Biological Toxicity End Points. Mass-balance calculations indicate that food IR decreased by 1 order of magnitude at the extreme metal exposure concentrations. As a result of slow feeding, Cd and Cu influxes into *L. stagnalis* were greatly reduced. Feeding inhibition is a typical response reflecting dietary metal stress, as reported for several crustacean and mollusk species (*40*). Impaired feeding has serious implications that can propagate from species to populations and ecosystems (e.g., reduced growth, fecundity and species diversity (*41, 42*)). Our results suggest that food IR could act as a biologically relevant end point for dietary metal toxicity in *L. stagnalis*. Enriched stable isotope tracers provide a way to quantify food ingestion and thereby quantify this effect. However, identifying the thresholds at which significant adverse effects begin is challenging. Enriched Cr isotopes offer potential to help characterize toxic responses. For instance, the small amounts of ^{53}Cr retained by snails in some treatments could be indicative of changes in gut processes at concentrations below those that caused the most extreme response. However, it is unclear whether a very small amount of ^{53}Cr was assimilated or whether egestion was slightly impeded by the high dietborne metal concentrations. Longer term studies are probably necessary to further understand those responses.

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Note Added after ASAP Publication

The assignment of copyright for the version published ASAP February 1, 2008 was in error; the corrected version was published ASAP February 28, 2008.

Supporting Information Available

Nominal dissolved tracer concentrations used to label the food offered to *L. stagnalis*, relative abundance of metal isotopes, biodynamic parameters used to predict bioaccu-

mulation, and tracer concentrations in the labeled food as a function exposure categories (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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