Stable Metal Isotopes Reveal Copper Accumulation and Loss Dynamics in the Freshwater Bivalve *Corbicula*

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Characterization of uptake and loss dynamics is critical to understanding risks associated with contaminant exposure in aquatic animals. Dynamics are especially important in addressing questions such as why coexisting species in nature accumulate different levels of a contaminant. Here we manipulated copper (Cu) stable isotopic ratios (as an alternative to radioisotopes) to describe for the first time Cu dynamics in a freshwater invertebrate, the bivalve *Corbicula fluminea*. In the laboratory, *Corbicula* uptake and loss rate constants were determined from an environmentally realistic waterborne exposure to 65Cu (5.7 µg L⁻¹). That is, we spiked deionized water with Cu that was 99.4% 65Cu. Net tracer uptake was detectable after 1 day and strongly evident after 4 days. Thus, short-term exposures necessary to determine uptake dynamics are feasible with stable isotopes of Cu. In *Corbicula*, 65Cu depuration was biphasic. An unusually low rate constant of loss (0.0038 d⁻¹) characterized the slow component of efflux, explaining why *Corbicula* strongly accumulates copper in nature. We incorporated our estimates of rate constants for dissolved 65Cu uptake and physiological efflux into a bioaccumulation model and showed that dietary exposure to Cu is likely an important bioaccumulation pathway for *Corbicula*.

Introduction

Trace element dynamics in aquatic organisms provide insights into species-specific mechanisms of bioaccumulation (1–3) and can explain trophic transfer of metals and metalloids into food webs (3–5). The protocols for determining trace metal dynamics quantify unidirectional uptake rate constants from solution (6), assimilation efficiencies (defined as the fraction of metal incorporated into animal tissue from ingested food (7)), and efflux rate constants (defined as the proportional rate constant for the unidirectional loss of metals from animal soft tissue via physiological elimination (8)). These can be combined into biodynamic models (1,8,9) to determine trace element bioaccumulation from aqueous and dietary sources (6,10). Usually, these metal-specific and species-specific physiological parameters are determined using radioisotopes, because a unique marker is necessary to quantify unidirectional fluxes. However, complicated logistics, handling, and waste issues limit the use of radioisotopes to laboratories that have trained handlers and can maintain permits. In addition to health hazards associated with radioactivity, the lack of a suitable radioisotope can be a problem. For example, the study of copper (Cu) dynamics is impeded by radioisotopes that are either difficult to prepare (e.g., 64Cu) or have relatively short half-lives (e.g., 65Cu). Consequently, despite its prevalence in the environment (11) and its potential toxicity (12–14), only a few studies have specifically quantified Cu accumulation dynamics in marine invertebrates (e.g., refs 15 and 16). None have dealt with freshwater organisms.

Here we present a simple method for determining Cu bioaccumulation dynamics using stable isotopes. Stable isotope manipulations offer many of the advantages of radioisotopes without some of the problems (Table 1). We use this methodology to develop the first estimates, of which we are aware, for Cu biodynamics for a freshwater invertebrate based on unidirectional fluxes. We also present simplified calculations for determining the quantity of spiked 65Cu that was accumulated and justify simplified computations for analyzing multicompartamental loss dynamics. Finally, we incorporated the estimated Cu uptake and efflux parameters into a bioaccumulation model to determine whether diet is an important source of Cu for this animal in nature.

Recent developments in inductively coupled plasma-mass spectrometry (ICP-MS) technologies allow detection of naturally occurring, low abundance stable isotopes. Solutions and standards enriched in individual isotopes are commercially available. Thus, manipulation of stable isotope ratios in exposure media is possible (e.g., ref 18). To provide the equivalent of a radioisotope tracer, it is preferable that an isotope of proportionately low abundance is spiked into the test media. Then it should be possible to follow the spike, independently, by using isotope ratios to account for the “background” concentrations. However, it first must be demonstrated that the challenges of both radioisotope and stable metal methodologies can be overcome. For example, does the organism take up the stable isotope in direct response to a change in the concentration in the media? If so, it should be relatively straightforward to use manipulation of the abundance of one isotope to study uptake. Can changes in isotope ratios in biological tissues be detected after short exposures to the manipulated ratios in water or food? If so, conversions of the data to dynamic rate constants should also be feasible (1). Finally, can interfering fluxes be accounted for by background ratios in media and experimental animals, using easily manageable computations? A major goal of this study is to address these basic questions.

Methods

Experimental Organisms. We collected the freshwater bivalve *Corbicula fluminea* (hereafter referred to as *Corbicula*) from a shallow basin in the Sacramento-San Joaquin River Delta (Franks Tract: 38° 05’N, 121°35’W) using an Ekman grab in June 2003. Clams of 5- to 15-mm shell length were held in acid-washed HDPE containers filled with Franks Tract water and transported to the laboratory in coolers. Clams were acclimatized to deionized water for 2 days at 15 °C in a glass aquarium. Cultures of the freshwater cryptophyte Cryptomonas ozolinii, the chlorophyte Chlorella vulgaris, and the diatom Cyclotella meneghiniana were fed to clams during the acclimation period and during the experiments (see details below). Algal cultures were maintained at 15 °C in a DY–V media that included macronutrients (N, P, and Si), vitamins, trace metals (Fe and Zn), and a chelating agent (EDTA).
**TABLE 1. Some Advantages and Drawbacks Inherent to Using Stable Isotopes and Gamma Emitting Radioisotopes as Tracers in Metal Dynamics Studies**

<table>
<thead>
<tr>
<th><strong>advantages</strong></th>
<th><strong>disadvantages</strong></th>
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</thead>
<tbody>
<tr>
<td>stable isotope</td>
<td>destructive analysis (i.e., spiked tissue needs to be digested prior to be analyzed)</td>
</tr>
<tr>
<td>most elements have 2 or more stable isotopes</td>
<td>suitable radioisotopes not commercially available for some metals (e.g., $^{65}$Cu)</td>
</tr>
<tr>
<td>low detection limits by ICP-MS</td>
<td>difficult to prepare and has short half-lives</td>
</tr>
<tr>
<td>lack of handling/disposal hazards</td>
<td>handling/disposal hazards associated with radioactivity</td>
</tr>
<tr>
<td>can quantify unidirectional efflux by mathematically eliminating biases from background ratios</td>
<td>challenging to obtain and maintain radioisotope handling licenses</td>
</tr>
<tr>
<td>pure stable isotopes are relatively inexpensive (compared to radioisotopes)</td>
<td>experiments restricted to a limited number of elements by overlap in energy peaks or use of high activities (i.e., only a few laboratories have access to germanium–lithium detectors that allow simultaneous measurement of many isotopes).</td>
</tr>
<tr>
<td>allow the simultaneous measurement of uptake and elimination</td>
<td>radioisotopes are relatively expensive (compared to pure stable isotope samples)</td>
</tr>
<tr>
<td>radioisotope$^a$</td>
<td>high specific activity isotopes difficult to obtain (e.g., $^{110}$Ag, $^{203}$Hg)</td>
</tr>
<tr>
<td>very low detection limits (gamma detector highly sensitive)</td>
<td>low specific activity isotopes imply unrealistic exposure concentrations</td>
</tr>
<tr>
<td>allow the assessment of metal dynamics in the same individual over time</td>
<td>uniform labeling will often need to be assumed</td>
</tr>
<tr>
<td>nondestructive analysis</td>
<td>unique tracer eliminates bias from efflux, allowing quantification of unidirectional efflux</td>
</tr>
<tr>
<td>inexpensive, rapid analysis</td>
<td>unique tracer allows experiments and short-term dissolved exposures, that minimize recycling and biases</td>
</tr>
<tr>
<td>easy to discriminate background from added amount of tracer</td>
<td>conduct experiments on individuals that minimize recycling and biases</td>
</tr>
<tr>
<td>stable isotope and radioisotope</td>
<td>allow the labeling of multicompartments (for some elements)</td>
</tr>
<tr>
<td>sensitive detection limits allow pulse-chase experiments and short-term dissolved exposures</td>
<td>sufficient radioisotopes not commercially sensitive</td>
</tr>
</tbody>
</table>

$^a$ See also Cornelis's (17) discussion of these issues.

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**$^{65}$Cu Uptake Experiment.** One hundred five clams were placed individually into 15-mL acid-washed LDPE vials, which had been conditioned (i.e., rinsed with the experimental solution) to reduce loss of $^{65}$Cu onto the walls and filled with deionized water spiked (5.6 μg L$^{-1}$) with a commercially purchased Cu standard isotopically enriched in $^{65}$Cu (99.4%). Clams were fed a 1 h pulse of cultured, unspiked algae every day in order to be more representative of natural conditions; at a biomass (monitored using in vivo fluorescence (a)) representative of Franks Tract (i.e., ~0.4–6 μg L$^{-1}$ chlorophyll-a (19)). One hour of feeding allowed Corbicula to filter the algal solution at least 2 times (assuming a filtration rate of 4 mL mg$^{-1}$ h$^{-1}$ and a mean weight of 8.3 ± 1.0 mg for our experimental Corbicula (20)). Following feeding, each individual was transferred to a new acid-washed vial that had been conditioned and filled with deionized water spiked with $^{65}$Cu. Deionized water was used to maximize exposure to free copper ion, assuming that Corbicula’s $^{65}$Cu uptake would follow the precepts of the free ion activity model (21, 22). Restricting the feeding period to 1 h per day and replacing the $^{65}$Cu-spiked water every day, minimized fecal scavenging and backflux of $^{65}$Cu to the media. After 1, 2, and 4 days of $^{65}$Cu waterborne exposure, 10 to 15 clams were sacrificed, and soft tissues were removed from shells and frozen. We selected these time intervals to determine if detection of uptake was possible from the relatively short-time exposure necessary to ensure that $^{65}$Cu concentrations in Corbicula would be minimally influenced by efflux (1).

**$^{65}$Cu Loss Experiment.** Following the 4-day exposure to $^{65}$Cu, the remaining Corbicula were placed individually into 15-mL acid-washed LDPE vials filled with unspiked deionized water. Clams were once again fed a mixture of algae for 1 h each day, and water was replaced daily after feeding. At 1, 2, 4, 6, 9, and 14 days, 10 clams were sacrificed and frozen.

**Control Organisms.** To provide background concentrations of $^{65}$Cu in clams, 10 clams were sacrificed before the beginning of the experiments. As a control, 20 clams were placed individually into 15-mL acid-washed LDPE vials filled with unspiked deionized water. Clams were fed a mixture of algae for 1 h each day and water was refreshed after feeding as described above. At the end of both the uptake (4 days) and loss experiments (18 days), 10 control clams were sacrificed and frozen.

**Sample Preparation and Analysis.** To minimize inadvertent Cu contamination, labware, vials, and Teflon sheeting were soaked for 24 h in 15% nitric acid, rinsed several times in ultrapure water (Milli-Q system water, > 18 MΩ cm$^{-1}$), and allowed to dry under a laminar-flow hood prior to use. Partially thawed Corbicula were dissected to remove soft tissue, placed individually on a piece of acid-washed Teflon sheeting, and allowed to dry at 40 °C for 3 days. Dried clams were then weighed (Sartorius M 2P electronic microbalance) and digested at room temperature in Teflon vials with concentrated nitric acid (Baker Ultratrace II grade, 100 μL mg dry weight sample$^{-1}$) for 7 days (23). Hydrogen peroxide (Baker Ultratrace II grade, 40 μL mg dry weight sample$^{-1}$) was added prior to final dilution with ultrapure water (760 μL mg dry weight sample$^{-1}$) to a final concentration of 10% H$_2$O$_2$. Samples were digested in a Corning microwave digestion system using an open system, for 30 min at 180 °C. Digests were cooled to room temperature and diluted to 5 mL with 10% H$_2$O$_2$. Samples were analyzed for $^{65}$Cu using ICP-MS. Cu concentrations were calculated using the external standard method. Each experiment was replicated three times.
dry weight sample (-1). Samples of similar weight from the certified reference material TORT-2 (lobster hepatopancreas from National Research Council of Canada, NRCC) were submitted to the same digestion procedures during each analytical run. Copper concentrations measured in TORT-2 were within the certified range.

Water samples and digested clams were analyzed for both naturally occurring stable isotopes of Cu, 63Cu, and 65Cu, by inductively coupled plasma-mass spectrometry (ICP-MS). Specifically, all samples, blanks, and standards were introduced by direct injection (peristaltic pump; spray chamber) into an Elan 6000 ICP-MS (single-detector; quadrupole) by Perkin-Elmer. Two 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 (NRCC; SLRS-4) were analyzed for Cu during each analytical run. Copper concentrations derived from signal intensities for both Cu stable isotopes (i.e., 63Cu and 65Cu) were within the certified range. To check for the instrument drift and change in sensitivity, we reanalyzed one of our standards after every 5 samples. Isotopic composition of Cu in samples was expressed as the ratio of the net signal intensity (ion counts) of 63Cu to 65Cu.

Calculation of Accumulated Tracer Concentrations. We first determined the relative abundance of 65Cu isotope (i.e., p65) using the signal intensities of each isotope in known standards (i.e., those used to calibrate the ICP-MS) (eq 1).

\[ p_{65} = \text{Intensity}_{65Cu} \div \left( \text{Intensity}_{65Cu} + \text{Intensity}_{63Cu} \right) \] (1)

Signal intensity (p65) was calibrated against total copper standards of varying concentration. Concentrations of 65Cu in (the unknown) experimental Corbicula (\([65Cu]_u\)) were calculated as the product of p65 and the total Cu concentration (\([T_{65Cu}]\)), i.e.,

\[ [65Cu]_u = p_{65} \times [T_{65Cu}] \] (2)

Typically, total metal concentrations determined by ICP-MS can be derived from signal intensities for any (or all) metal isotopes set as analytes in the software method, assuming that there is no isotopic fractionation in the sample. For example, Cd and Cu concentrations could be inferred respectively 8 and 2 times, since Cd has 8 stable isotopes and Cu has 2. In practice, concentrations for many multi-isotopic elements are often derived from only one isotope, due to isobaric interference that may render certain isotopes as ineffective analytes. In fact, one original purpose for analyzing multiple isotopes on ICP-MS was to determine if isobaric interferences exist.) For the purposes of this study we determined total Cu from both 65Cu and 63Cu.

To independently follow the spike of 65Cu it is necessary to separate out the concentration of 65Cu that occurred in each sample in the absence of a spike (e.g., the "background" that existed prior to exposure to the spiked solution). For this, we used the total Cu concentrations inferred from the intensity of 65Cu (\([T_{65Cu}]\)) to derive the original load of 65Cu (\([65Cu]_0\); eq 3).

\[ [65Cu]_0 = p_{65} \times [T_{63Cu}] \] (3)

Results

As shown in Figure 1A, Corbicula enrichment in 65Cu was progressive and significantly related to the duration of exposure (\(r^2 = 0.98, p < 0.01\)). After 1 day of exposure, Corbicula’s 65/63Cu ratio was significantly different from those in the unexposed animals (\(p < 0.001\)). Short exposures to environmentally realistic concentrations not only caused a net change in the isotope ratio in the animal but also were detectable within a period reasonable for determining unidirectional uptake dynamics.

Depuration of 65Cu following 4 days of uptake from the dissolved phase occurred exponentially over time (Figure 1B). 65Copper was rapidly lost from Corbicula’s tissue during the first 2 days of depuration (p < 0.05), and then no significant difference was observed in Corbicula’s 65/63Cu ratios from day 2 to 14 (p > 0.1).

Modeling 65Cu Accumulation. To characterize Corbicula’s 65Cu bioaccumulation dynamics, we first used eqs 1–4 to transform Corbicula’s Cu isotope ratios into 65Cu concentrations. We then treated Corbicula as a single compartment.
FIGURE 2. Depuration of $^{65}$Cu in Corbicula following 4 days of exposure to waterborne metal. Values represent percentage of $^{65}$Cu in clams (mean ± 95% CI). Dotted and dashed lines represent the two exponential terms of eq 11, i.e., the dotted line represents the first exponential term with a shorter half-life and a higher rate constant, whereas the dashed line represents the second exponential term with a longer half-life and a lower rate constant. The sum of these two exponential terms gives the model curve (solid line) obtained using eq 11 and the parameters $k_1$ and $k_2$ given in Table 2.

for the accumulation of $^{65}$Cu and assumed that the accumulation of tracer was proportional to the free $^{65}$Cu-ion concentration ([$^{65}$Cu]$^2$) (22) and that overall uptake and loss followed first-order kinetics (e.g., ref 9). The rate of change in Corbicula $^{65}$Cu concentrations was expressed as the difference between $^{65}$Cu entering and leaving the clam

$$\frac{d[^{65}\text{Cu}]_k}{dt} = k_\text{u}[^{65}\text{Cu}^2] - k_\text{e}[^{65}\text{Cu}]_k$$  (5)

where $k_\text{u}$ (L g$^{-1}$ d$^{-1}$) is the uptake rate constant (6) and $k_\text{e}$ is the proportional rate constant of loss (d$^{-1}$). Growth was assumed to be negligible compared to $k_\text{u}$ since $^{65}$Cu uptake and elimination lasted only 18 days and food availability was kept low.

Landrum et al. (9) emphasized that $k_\text{u}$ can only be expressed in units of d$^{-1}$ (allowing generalization to other circumstances) if it is determined from log-transformed, proportional loss ($C_t/C_0$) over time, where $C_t$ is the concentration at a given time and $C_0$ is the concentration when unidirectional loss began. From Figure 2 it is clear that log-transformed, proportional elimination of $^{65}$Cu from Corbicula’s tissues can be reduced to a biphasic exponential trend. Almost half (37%) of the accumulated tracer was lost within the first 4 days of depuration. Then loss was very slow through the following 10 days of depuration (Figure 2).

To simplify treating $^{65}$Cu biodynamics as two compartments, we assumed that all exchanges follow first-order kinetics and that the compartments behaved independently. Uptake and loss for each compartment, separately, then would be given by

$$\frac{d[^{65}\text{Cu}]_k}{dt} = k_\text{u}[^{65}\text{Cu}^2] - k_\text{e}[^{65}\text{Cu}]_k$$  (6)

$$\frac{d[^{65}\text{Cu}]_k}{dt} = k_\text{u}[^{65}\text{Cu}^2] - k_\text{e}[^{65}\text{Cu}]_k$$  (7)

where $[^{65}\text{Cu}]_k$ and $[^{65}\text{Cu}]_e$ (µg g$^{-1}$ d.w.) are the $^{65}$Cu concentrations in compartment 1 ($C_1$) and 2 ($C_2$), respectively, $k_1$ and $k_2$ (per day) are rate constants for $^{65}$Cu efflux for each compartment, and $k_{12}$ (d$^{-1}$) is the rate constant of $^{65}$Cu uptake in $C_2$. To determine $[^{65}\text{Cu}]_k$ at any given time, we summed the integrated form of eqs 6 and 7 (assuming that $[^{65}\text{Cu}]_e$ and $[^{65}\text{Cu}]_k$ are constant over time):

$$[^{65}\text{Cu}]_k = \frac{k_\text{u}[^{65}\text{Cu}^2]}{k_1} (1 - e^{-k_1t}) + \frac{k_{12}[^{65}\text{Cu}]_e}{k_2} (1 - e^{-k_2t})$$  (8)

Because we could follow unidirectional $^{65}$Cu loss (accounting for background $^{65}$Cu influx with the isotope ratios) and assuming no influx of $^{65}$Cu beyond that accounted for by $[^{65}\text{Cu}]_e$ (eq 3), the rate of loss of $^{65}$Cu concentrations within each compartment can be expressed by

$$\frac{d[^{65}\text{Cu}]_k}{dt} = -k_1[^{65}\text{Cu}]_k$$  (9)

$$\frac{d[^{65}\text{Cu}]_e}{dt} = -k_2[^{65}\text{Cu}]_e$$  (10)

Summation of the integrated forms of eqs 9 and 10 gives the overall $^{65}$Cu concentration in Corbicula at a given point in time during the loss experiment, i.e.,

$$[^{65}\text{Cu}]_k = \frac{[^{65}\text{Cu}]_k}{k_1} (1 - e^{-k_1t}) + \frac{[^{65}\text{Cu}]_e}{k_2} (1 - e^{-k_2t})$$  (11)

The rate constants were derived from the “fast” and “slow” phases of $^{65}$Cu elimination as determined by mathematical stripping (Figure 2 (24)). Briefly, the rate constant associated with the slow exchanging pool was first determined from the slope of the straight line drawn through the last few points (days 6–14) of the in-transformed proportional loss data plotted against time (Figure 2, dashed line). Then, the rate constant associated with the fast exchanging pool was determined from the slope of the straight line drawn through the points representing the difference between each earlier measured value and that predicted by the curve for the slow exchanging compartment (Figure 2, dotted line).

The depuration rate constant associate with $C_1$ was $k_1 = 0.319$ d$^{-1}$ compared to $k_2 = 0.004$ d$^{-1}$ (Table 2). The biological half-lives of $^{65}$Cu in $C_1$ and $C_2$ were thus 2.5 and 182 days, respectively. Compartimental analysis of each pool indicates that at the beginning of depuration, 56 and 44% of $^{65}$Cu were in the fast and slow exchanging compartment, respectively. The proportion of total $^{65}$Cu in $C_1$ decreased to 31% after 4 days of depuration and declined to less than 3% after 14 days. After 100 and 360 days of depuration, we estimated that 30 and 11% of the initial load of $^{65}$Cu would remain in Corbicula’s tissues (sequestered in $C_2$), respectively. As shown in Figure 2, the model curve obtained using the above estimated values shows a good fit of Corbicula’s loss of $^{65}$Cu.

The assumption that $^{65}$Cu is compartmentalized into independent pools in Corbicula, upon accumulation, allowed us to simplify the differential equations required to describe bioaccumulation dynamics. Such a treatment is consistent with the traditional approach to this type of transport (e.g., refs 24 and 25). Where others assumed that compartments are not independent (linkages between compartment must be described (26, 27)), resolution of the resulting differential equations requires “integrator software” as well as numerical methods specifically designed to both solve and fit to experimental data (e.g., ref 26).
TABLE 2. Estimated Values (± SE) of Dynamics Parameters Used for or Derived from Modeling 65Cu Accumulation and Elimination in Corbicula*  

<table>
<thead>
<tr>
<th>parameter</th>
<th>symbol</th>
<th>unit</th>
<th>value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rate constant of 65Cu loss</td>
<td>$k_1$</td>
<td>d⁻¹</td>
<td>0.319 ± 0.251</td>
</tr>
<tr>
<td>rate constant of 65Cu uptake from dissolved phase into C₁</td>
<td>$k_2$</td>
<td>d⁻¹</td>
<td>0.224 ± 0.038</td>
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<tr>
<td>free 65Cu ion concentration</td>
<td>$\mu$g</td>
<td>L⁻¹</td>
<td>5.2</td>
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<td>65Cu biological half-life</td>
<td>$\tau_{1/2}$</td>
<td>d</td>
<td>2.5</td>
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<tr>
<td>proportion of 65Cu retained after 4 days of uptake</td>
<td>%$[65Cu]_{te}$</td>
<td></td>
<td>56</td>
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### Fast Exchanging Compartment

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<th>parameter</th>
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<tr>
<td>proportion of 65Cu retained after 4 days of uptake</td>
<td>%$[65Cu]_{te}$</td>
<td></td>
<td>44</td>
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### Slow Exchanging Compartment

<table>
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<tbody>
<tr>
<td>rate constant of 65Cu loss</td>
<td>$k_1$</td>
<td>d⁻¹</td>
<td>0.0038 ± 0.054</td>
</tr>
<tr>
<td>rate constant of 65Cu uptake from C₁ into C₂</td>
<td>$k_{12}$</td>
<td>d⁻¹</td>
<td>0.223 ± 0.038</td>
</tr>
<tr>
<td>65Cu biological half-life</td>
<td>$\tau_{1/2}$</td>
<td>d</td>
<td>182</td>
</tr>
<tr>
<td>proportion of 65Cu retained after 4 days of uptake</td>
<td>%$[65Cu]_{te}$</td>
<td></td>
<td>44</td>
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### 65Cu Exchange Rates

<table>
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<tbody>
<tr>
<td>65Cu–UR</td>
<td>ng 65Cu g⁻¹ d⁻¹</td>
<td>$t_{1/2a} = 38, 79$</td>
</tr>
<tr>
<td>65Cu–LR</td>
<td>ng 65Cu g⁻¹ d⁻¹</td>
<td>$t_{1/2a} = 1432, 772$</td>
</tr>
<tr>
<td>$\Delta$65Cu</td>
<td>ng 65Cu g⁻¹ d⁻¹</td>
<td>$-1394$ to $693$</td>
</tr>
</tbody>
</table>

* Also given are 65Cu exchange rates during our 65Cu loss experiment on the basis of parameter estimates at t = 0 and t = 14.

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FIGURE 3. Temporal changes in accumulated Corbicula's 65Cu concentrations (± 95% C.I.) during short-term waterborne exposure to 65Cu. Experimental data are represented by symbols (mean ± 95% C.I.). Lines represent model curve obtained with eq 12 and the parameters $k_1$ and $k_2$ as well as the value of [65Cu²⁺] given in Table 2.

Using the value of the elimination rate constant for C₁ ($k_1$, Table 2) and the concentration of 65Cu²⁺ estimated by the speciation model WHAM (1.0 (28)), we could determine a rate constant of 65Cu uptake of 0.224 ± 0.038 L g⁻¹ d⁻¹ (± SE) by fitting our experimental data for the first day of measurable uptake (day 1) to the integrated form of eq 5, that is,

$$\Delta [65Cu]_c = \frac{k_1[65Cu^{2+}]}{k_e} (1 - e^{k_1t})$$

(12)

where we assumed that at t = 0, $\Delta [65Cu]_c$ equals zero and that [65Cu²⁺] was constant (i.e., nominal [65Cu²⁺] = 5.2 µg L⁻¹). That is, WHAM estimates that 92% of the 65Cu in the exposure media was present as free ions (assuming a pH 6.5). The model curve obtained using eq 12 and the estimated values of $k_1$ and $k_2$ (Table 2) shows a good fit to Corbicula’s 65Cu uptake data (Figure 3). Then, using the values of $k_1$, $k_2$, [65Cu²⁺], and $\Delta [65Cu]_c$ from Table 2, we estimated the rate constant of 65Cu uptake from C₁ into C₂ (i.e., $k_{12}$) by fitting (using a nonlinear regression model) our data points (Figure 3) to eq 8.

An important assumption for eq 8 is that 65Cu concentration in C₁ is constant over time. This appears to be reasonable for natural conditions, where applications of the model are most important. As shown in Figure 4, 65Cu concentration in C₁ reaches a plateau within 10 days. So it seems reasonable that 65Cu concentration in the fast exchanging pool ($\Delta [65Cu]_e$) would be near steady state, in most exposures under natural conditions (especially since the life span of Corbicula is 4 yrs (29)). Of course, the size of C₁ will vary if $[Cu^{2+}]$ varies, because the rate constants $k_1$ and $k_2$ are both species and metal specific constants (4).

We can also use eq 8 to determine the implications of using short exposures for estimating uptake rates. For this, we inserted in eq 8 the values of our rate constants ($k_1$, $k_2$, $k_{12}$, Table 2) as well as that of [65Cu²⁺] used during our uptake experiment, to determine uptake of 65Cu into C₁ and C₂ for different exposure time. We used a step-by-step procedure to first calculate $\Delta [65Cu]_e^*$, which is needed to determine 65Cu uptake in C₂. The computation made using more than 1500 exposure times (with time steps varying from 6 s to 2 h) showed that C₂ is negligible at the beginning of the 65Cu uptake experiment (Figure 4). The relative proportion of 65Cu accumulated in C₁ was slightly lower than 100% (i.e., 99.1%) after 1 hour of exposure and reached 82% after 1 day. Because the slow exchanging compartment contributed minimally to the overall 65Cu load during the first hours of uptake, a one-compartment model is appropriate to depict Corbicula’s 65Cu uptake (Figures 1A and 3). Similarly, using the first measurable uptake data to estimate $k_1$, (e.g., those for 1 day of exposure) is an appropriate way to estimate the unidirectional flux of metals from solution.

Two compartment loss dynamics complicate analysis of biodynamics, both mathematically and in terms of generalizations (the latter because two compartment analysis carries an implicit assumption about exposure time). However, two compartment analyses may not be necessary to simulate most conditions in nature if, as Cutshall (25) showed C₁ declines in importance as time of exposure increases. Recent biodynamic papers use a single compartment, assuming that exposures in nature are long and that the slow compartment will usually dominate loss (6). We tested the conditions under which biodynamics of Cu in Corbicula are dominated by the physiological turnover of metals in the slowest exchanging pool. As shown in Figure 4, the relative proportion of 65Cu accumulated in C₁ decreased rapidly. At $k_2$ equals 0.004 d⁻¹, the relative proportion of 65Cu accumulated in C₁ is less than 5% after 100 days of exposure; C₁ is proportionately irrelevant after such long exposures. Organisms are chronically exposed to metals in nature, as determined by biological generation times and geochemical factors such as association with sediments that buffer...
fluctuations. On this basis, employing rate constants from the most slowly exchanging compartment is a valid assumption.

In organisms with faster rate constants of loss, the same principle holds, but the time before C₁ becomes of minor importance changes. Increasing the value of the loss rate constant for the slowest exchanging pool increased the proportion of 

\[ ^{65}\text{Cu} \text{ accumulated in the fast exchanging pool.} \]

When \( k_2 = k_1 \), a plateau is reached. This scenario likely represents the case of a unique exchanging pool (see refs 6 and 25) (Figure 4).

We assumed that 

\[ ^{65}\text{Cu} \text{ uptake by Corbicula during our } ^{65}\text{Cu loss experiment was negligible. We tested this assumption by using eq 5 and values of } k_u \text{ and } k_e \text{ to estimate } ^{65}\text{Cu uptake and loss rates during our } ^{65}\text{Cu loss experiment (at both } t = 0 \text{ and } t = 14). \]

Assuming that recycled 

\[ ^{65}\text{Cu} \text{ could increase } [^{65}\text{Cu}^{2+}] \text{ to a maximum concentration of 0.35 } \mu\text{g L}^{-1} \text{ (i.e., concentration calculated from the amount of } ^{65}\text{Cu lost by Corbicula during each day of loss, knowing Corbicula’s weight, considering an exposure volume of 15-mL and assuming a lack of ligands that could complex } ^{65}\text{Cu ions), we calculated that rate constants of loss would have been affected by less than 2-10% of the reported values (Table 2).} \]

**Predicting Cu Accumulation in Corbicula in Nature.** If the only source of uptake were the dissolved metal for clams in a natural system, we can use our model parameters to estimate Cu concentrations that might be expected in Corbicula in Franks Tract (California). Under steady-state conditions, eq 5 becomes

\[ \Delta [^{65}\text{Cu}]_\text{estr} = \frac{k_u}{k_2} [^{65}\text{Cu}^{2+}] \]  

(13)

where \( \Delta [^{65}\text{Cu}]_\text{estr} \) is the 

\[ ^{65}\text{Cu} \text{ concentration in clam soft tissues (} \mu\text{g g}^{-1} \text{) at steady-state. The free } Cu \text{ ion concentration was estimated using the speciation model WHAM (28) assuming that the lake water has a } pH \text{ near 8 (30), a dissolved organic carbon concentration of 2.7 mg L}^{-1} \text{ (31), and a total dissolved } Cu \text{ concentration of } 2 \mu\text{g L}^{-1} \text{ (32). Measured } Cu \text{ concentrations in clams were from 45 to } 155 \mu\text{g g}^{-1} \text{ depending on size (32). Those concentrations were 7 times higher than predicted by eq 13. This suggests that } Cu \text{ accumulation by Corbicula in nature is driven mainly by a dietary-pathway (i.e. cannot be explained by dissolved uptake), which has been demonstrated for other metals and bivalve species (e.g., Se and Cr (III) in Mytilus edulis (6, 33)). Similarly, the importance of food for the uptake of } Cu \text{ by marine copepods has recently been highlighted by Chang and Reinfelder (16).} \]

**Discussion**

In Corbicula, the rate constant \( k_2 \) ultimately acts as a bottleneck for 

\[ ^{65}\text{Cu} \text{ efflux and controls physiological loss (1). If we set } k_2 \text{ equal to } k_u \text{ we can compare Corbicula } ^{65}\text{Cu loss rate constant to those found for other metals in crustaceans and bivalves. We found that the } Cu \text{ efflux rate constant measured for Corbicula was } 15-20 \text{ times lower than that of marine copepods (0.060 } 0.08 \text{ d}^{-1} \text{ (16)). This means that loss of } Cu \text{ is consistent with the body of literature that suggests that metal elimination rates in copepods are, in general, an order of magnitude higher than in marine bivalves (Table 3).} \]

The \( k_b \) for 

\[ Cu \text{ in Corbicula is similar to } k_b \text{'s for } Cd \text{ and } Zn \text{ in the coastal oyster Saccostrea glomerata but somewhat lower to } k_b \text{'s for } Ag, Cd, Co, Se, \text{ and } Zn \text{ found in other bivalves (Table 3), although rate constants of loss show in general no pattern of variation among elements and bivalve species (4).} \]

Extremely low metal efflux rates, like those seen for 

\[ Cu \text{ in Corbicula, typically cause the accumulation of metals to high levels (e.g., } Cd, Se, \text{ and } Zn \text{ in } S. \text{ glomerata (34); } Zn \text{ in barnacles Balanus amphitrite (35, 36)).} \]

Our estimate for \( k_b \) appears to be an order of magnitude lower than the 

\[ Cu \text{ specific uptake rate constant calculated by Chang and Reinfelder (16) for marine copepods (5.1 } \mu\text{g g}^{-1} \text{ d}^{-1}, \text{ Table 3).} \]

The higher surface area ratio of copepods compared to that of bivalves would likely enhance diffusion and transport, which would help to explain in part higher \( k_b \) found by Chang and Reinfelder (16). Ranking the \( k_b \) values reported for marine copepods indicates that uptake rate constants for the dissolved phase increased in the order of 

\[ Se < Co < Cd < Zn < Cu \text{ and } Ag (Table 3). \]

While integrating our \( k_b \) value to those reported for marine bivalves (6, 34), however, we found faster accumulation of dissolved 

\[ Cd \text{ and } Zn \text{ relative to that of } Cu \text{ in bivalves.} \]

The paucity of data on 

\[ Cu \text{ accumulation dynamics in aquatic animals reflects mainly the lack of a suitable radioisotope for this metal. The unidirectional fluxes that drive bioaccumulation are difficult to determine unambiguously without using tracer methodologies (i.e. using stable metals or metalloids) because the background of stable metal in tissues, and the high variability typical of tissue concentrations, make detection of small changes difficult. For} \]
example, rate constants of loss cannot be accurately determined after stable metal spikes, because a background of stable metal occurs within all media and influx from that background cannot be accounted for. Thus rates determined by tissue analysis (or changes in media) will appear slower than unidirectional rates. Landrum et al. (9) called these “elimination rate constants”, representing fractional elimination, while the animal is still exposed to the element. Such “constants” are valid only for the experimental conditions they describe. They represent a combination of mechanisms (influx and efflux) and thus cannot be generalized over a variety of conditions. Unidirectional “deposition” constants (9) or rate constants of loss (the traditional terminology from radioecology (25)) are both generic and mechanistic descriptors, if the loss curves are analyzed correctly.

Some studies have overcome this difficulty using novel methodologies, usually adapted, however, to a narrow range of conditions. For example, Chang and Reinfielder (13) used siliceous tests of diatom cells as an unassimilated tracer (i.e., sample and a spiked sample), which could be problematic while dealing with small samples (e.g., individual invertebrates). The calculations proposed herein (eqs 1–4) directly and concisely convert metal concentrations measured at the ICP-MS into accumulated tracer concentrations. The refinement stems in part from the use of baseline Cu isotopic ratios determined from the ICP-MS into tracer concentrations. Although the details of their conversion are not fully described, isotope dilution typically mandates complicated arithmetic and duplication of the analysis (i.e., sample and a spiked sample), which could be problematic while dealing with small samples (e.g., individual invertebrates). The calculations proposed herein (eqs 1–4) directly and concisely convert metal concentrations measured at the ICP-MS into accumulated tracer concentrations.

Overall, refining an approach that applies metal stable isotopes to trace Cu bioaccumulation dynamics allowed us to determine for the first time unidirectional Cu influx and efflux rate constants in a freshwater organism and to compare these fluxes to other metals. The refinement stems in part from the use of baseline Cu isotopic ratios determined from standards to convert metal concentrations measured at the ICP-MS into tracer concentrations. Our experimental results suggested that a slow rate constant of loss for Cu likely explains the elevated Cu levels found in these molluscs in nature. Metal uptake from diet might act as a major pathway for Cu accumulation in Corbicula in nature. The refined approach described herein expands the access to tracer methodologies and offers potential to help investigating

### TABLE 3. Some Trace Element Uptake Rate Constants from the Dissolved Phase (k_u) as Well as Efflux Rate Constants (k_e) (After Dissolved Uptake)

<table>
<thead>
<tr>
<th>organism</th>
<th>element</th>
<th>k_u (L g⁻¹ d⁻¹)</th>
<th>k_e (d⁻¹)</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>marine copepods (Acarti a sp. and Temora sp.)</td>
<td>Cu</td>
<td>5.1</td>
<td>0.06–0.08</td>
<td>(16)</td>
</tr>
<tr>
<td>marine copepod (Temora longicornis)</td>
<td>Cu</td>
<td>10.4</td>
<td>0.173</td>
<td>(42)</td>
</tr>
<tr>
<td></td>
<td>Cd</td>
<td>0.694</td>
<td>0.108</td>
<td>(42)</td>
</tr>
<tr>
<td></td>
<td>Co</td>
<td>0.606</td>
<td>0.122</td>
<td>(42)</td>
</tr>
<tr>
<td></td>
<td>Se</td>
<td>0.024</td>
<td>0.155</td>
<td>(42)</td>
</tr>
<tr>
<td>estuarine oyster (Crassostrea rivularis)</td>
<td>Cd</td>
<td>0.719</td>
<td>0.014</td>
<td>(34)</td>
</tr>
<tr>
<td></td>
<td>Se</td>
<td>0.060</td>
<td>0.034</td>
<td>(34)</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>2.050</td>
<td>0.014</td>
<td>(34)</td>
</tr>
<tr>
<td>coastal oyster (Saccostrea glomerata)</td>
<td>Cd</td>
<td>0.534</td>
<td>0.004</td>
<td>(34)</td>
</tr>
<tr>
<td></td>
<td>Se</td>
<td>0.064</td>
<td>0.013</td>
<td>(34)</td>
</tr>
<tr>
<td>marine mussel (Mytilus edulis)</td>
<td>Cu</td>
<td>1.79</td>
<td>0.019</td>
<td>(6)</td>
</tr>
<tr>
<td></td>
<td>Cd</td>
<td>0.365</td>
<td>0.011</td>
<td>(6)</td>
</tr>
<tr>
<td></td>
<td>Co</td>
<td>0.124</td>
<td>0.018</td>
<td>(6)</td>
</tr>
<tr>
<td></td>
<td>Se</td>
<td>0.035</td>
<td>0.026</td>
<td>(6)</td>
</tr>
<tr>
<td>marine bivalves (Crassostrea virginica, Macoma balthica, Mercenaria mercenaria, Mytilus edulis)</td>
<td>Cd &amp; Zn</td>
<td>ND*</td>
<td>ND*</td>
<td>(43)</td>
</tr>
<tr>
<td>fresh water mussel (Dreissena polymorpha)</td>
<td>Cu</td>
<td>3.6–7.2</td>
<td>0.084</td>
<td>(44)</td>
</tr>
<tr>
<td>freshwater clam (Corbicula fluminea)</td>
<td>Cu</td>
<td>2.3–3.2</td>
<td>0.011</td>
<td>(44)</td>
</tr>
<tr>
<td></td>
<td>Co</td>
<td>0.05–0.10</td>
<td>0.035</td>
<td>(44)</td>
</tr>
</tbody>
</table>

* ND, not determined.
Literature Cited

(30) Vayssière, M. California Department of Water Resources, personal communication.
(41) Longerich, H. P. At Spectrosc. 1999, 10, 112–115.

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