Environ. Sci. Technol. 1992, 26, 485-491

Determination of Selenium Bioavailability to a Benthic Bivalve from Particulate and Solute Pathways

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■ Particulate organo-Se was assimilated with 86% efficiency by the deposit feeding bivalve *Macoma balthica*, when the clam was fed ^{75}Se-labeled diatoms. Absorption efficiencies of particulate elemental Se were 22%, when the animals were fed ^{75}Se-labeled sediments in which elemental Se was precipitated by microbial dissimilatory reduction. Precipitation of elemental Se did not eliminate biological availability of the element. Selenite was taken up from solution slowly by *M. balthica* (mean concentration factor was 712). Concentrations of selenite high enough to influence Se bioaccumulation by *M. balthica* did not occur in the oxidized water column of San Francisco Bay. However, 98–99% of the Se observed in *M. balthica* could be explained by ingestion of the concentrations of particulate Se found in the bay. The potential for adverse biological effects occurred at much lower concentrations of environmental Se when food web transfer was considered than when predictions of effects were based upon bioassays with solute forms of the element. Selenium clearly requires a protective criterion based upon particulate concentrations or food web transfer.

1. Introduction

Aquatic animals are exposed to trace contaminants in solution and in the material they ingest (*1*, *2*). Separating the relative importance of each pathway of exposure is especially important for those contaminants which are not at thermodynamic equilibrium in nature (*3*, *4*). Where thermodynamic equilibrium prevails, models might be able to predict bioavailability of the contaminant from the activity of a reactive chemical species (*5*, *6*). However, if a contaminant is not at equilibrium, concentrations of the various geochemical forms are independent of one another and bioaccumulation of the various forms is independent and additive (*7*). In the latter case, credible protective criteria would have to be based on contaminant concentrations in the sources that most influence bioavailability.

Equilibrium thermodynamic calculations do not accurately predict the speciation of Se in oxidized natural waters, because of the influences of biological processes (*8*). In such waters, Se exists in a variety of oxidation states. Selenate is the predominant form in solution. However, selenite and organoselenium both exist at concentrations higher than predicted (*9–11*). Biological accumulation by microorganisms (including phytoplankton), reductive bioproduction of organoselenium, and release of the latter contribute to the disequilibrium. Organoselenium is a predominant particulate form of the element, because of its biological production within cells (*10*, *12*, *13*). Selenium also is found in greater concentrations in sediments of natural systems than predicted by the speciation and chemical reactivity of the solute forms (*14*). Recent studies suggest that sedimentary sequestration of selenium occurs principally by microbial dissimilatory reduction of selenate to elemental selenium (*15–17*).

Bioaccumulation of Se is the result of additive uptake from different sources because of the disequilibrium among forms. For that reason we compared the bioavailability of several of the predominant forms of Se that a deposit feeding bivalve (*Macoma balthica*) would be likely to encounter in nature. We assessed bioaccumulation of selenite from solution. Previous studies show that this oxidation state is of much greater bioavailability than selenate (*18*, *19*). We determined uptake of particulate organo-Se by clams from ingestion of diatoms that were exposed to selenite. Diatoms are a principal food of *M. balthica* in nature. We also assessed the bioavailability of the Se converted from selenate to elemental Se by microbial dissimilatory reduction. This could be a predominant form of the element encountered by *M. balthica* (and other organisms) when sediments are ingested. We then employed a simple physiological model to assess the contribution of each of these sources to Se bioaccumulation in nature.

Other studies have considered the bioavailability of particulate selenium (*19–23*). However, no previous studies have compared availability among forms of ingested Se nor has the bioavailability of the potentially large pool of elemental Se in sediments been studied previously. The study also presents an improved approach to predicting the relative importance of exposure pathways. An important part of the methodology is experimental assessment of physiological absorption efficiencies for ingested contaminants. Absorption efficiencies were employed with feeding rates to calculate gross influx rates of Se from food. Gross influx rates of Se from solution were determined directly. By use of known rate constants of loss, bioaccumulation was calculated using fluxes from each source and concentrations of Se in water and particulate material observed in San Francisco Bay. The relative importance of the sources was compared from these calculations. The model predictions were compared to determinations of Se concentrations in *M. balthica* from the bay. The results showed that only bioaccumulation from food could explain the Se levels observed in nature, and that the type of food and the feeding rate had an important influence on bioaccumulation. It was also clear that regulatory criteria that depend solely upon assays of the toxicity of Se in solution would fail to protect an aquatic system like San Francisco Bay.

2. Methods and Materials

A. Choice of Species. *Macoma balthica* is a common inhabitant of temperate estaurine benthic communities (*24–26*). It is a deposit feeding species with suspension feeding capabilities (*27*, *28*). Its principal sources of nutrition are benthic/suspended microalgae (diatoms) and the microbial biomass and nonliving organic materials associated with fine-grained (<100 μm) surficial sediments

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0013-936X/92/0926-0485$03.00/0

(*29, 30*). *M. balthica* is consumed by waterfowl and fish in nature (*31, 32*) and thus is a source of Se to higher trophic levels, where significant toxicological effects have been documented (*33, 34*).

M. balthica were collected from a mudflat in San Francisco Bay where ambient concentrations of Se were low (0.1 μg/g of Se in sediment; station 3 in ref 32). The animals were held for 2–5 days in the laboratory and then their digestive tracts were evacuated prior to the beginning of each experiment. All experiments, acclimations, and evacuations were conducted at 20‰ salinity and 10 °C, conditions the animals typically experience in nature.

B. "Pulse–Chase" Determination of Absorption from Food. "Pulse–chase" procedures were employed to determine Se absorption and gut evacuation time in *M. balthica*. The general protocol involved exposure of animals to ^{75}Se-labeled food for 2.5 h, followed by exposure to unlabeled food (either diatoms or a thin slurry of fine-grained suspended sediment) for sufficient time to allow egestion of the label. To determine mass balances in the experiments, all fecal material was collected and analyzed for ^{75}Se at frequent intervals (3–12 h) during the egestion period and analyses of 0.45-μm filtered water were performed frequently. After each collection of feces the animals were placed in clean seawater with a fresh unlabeled food suspension. Live animals were counted by nondestructive analysis immediately after the exposure and then at subsequent 24-h intervals. Shells were separated from soft tissues at the end of egestion and ^{75}Se was determined in each. After analysis, the soft tissues were dried at 60 °C and weighed. The initial activity of ingested material was measured directly by nondestructive analysis of whole clams at the end of the 2.5-h ingestion period in order to avoid problems such as selective ingestion (*35, 36*) and analytical uncertainties in estimating small losses of label consumed from the experimental media.

C. Determination of Gut Evacuation Time. To determine gut evacuation times, an experiment was conducted with a radiolabel that could not be assimilated. Acrylic beads which encapsulated a ^{51}Cr label (*37*) were fed to 15 animals in a dilute slurry of fine-grained suspended sediment. The beads were the size of small diatoms or large clay particles (15-μm diameter) and were readily ingested by the animals. ^{51}Cr was determined in the 12 individuals that accumulated the largest quantity of label. The egestion period was 168 h.

D. Absorption from Sediment. Microbial dissimilatory reduction of selenate in sediment was employed to generate a slurry that could be used to study the bioavailability of elemental Se in an environmentally realistic sediment matrix. Preparation of ^{75}Se-labeled sediments followed previously established procedures (*17*). A core (sediment volume 3 mL) was obtained from the upper 2.7 cm of sediment from an intertidal mudflat of San Francisco Bay (station M4 in refs 32 and 38) and immediately sealed with a latex rubber serum stopper. $Na_2{}^{75}SeO_4$ (717.8 kBq/100 μL of deaerated aqueous injectate; specific activity 4.06×10^5 MBq/mmol) was injected at several locations along the length of the core, as the needle of the delivering microsyringe was withdrawn. The sediment core was incubated at 25 °C for 48 h to allow the microbial reduction process to proceed and was then extruded and vortexed in 7 mL of isotonic saline solution. After two washes, the sediment pellet containing the elemental ^{75}Se was resuspended in 10 mL (5.8×10^5 Bq; 0.114 μg of Se). That elemental selenium $^{75}Se^0$ was the sole end product of this reduction was previously confirmed by extraction with organic solvents (*15, 17*), by oxidation with sulfite (*16*), and by experiments at elevated pH (*17*).

Table I. ^{75}Se (Bq/Individual) in *M. balthica* after a 2.5-h Exposure to a Radiolabeled Sediment Slurry, after Exposure to the Solution from That Slurry, and after Egestion Periods of 96 and 168 h

	whole animal	soft tissue	shell	foot	mantle	other tissues
2.5-h solute exposure[a]	148 (19)	81 (22)	21 (3)			
2.5-h feeding after exposure[b]	1221 (236)					
after 168-h egestion	314 (69)	233 (84)	26 (4)	12 (5)	77 (48)	238 (20)
C_0	1073 (210)					
C_e[c]	242 (70)					
A	0.225					

[a] Mean and (standard deviation) for five individuals. [b] Mean and (standard deviation) for 12 individuals. [c] Determined after 96 h of egestion.

The labeled sediments were suspended in 1800 mL of seawater and stirred for 2 min. Eighteen animals were placed in the tank for the 2.5-h exposure. Preexposure activity of the seawater suspension was 570 Bq/mL with 38 Bq/mL in solution (0.45-μm filtrate). Postexposure suspension ^{75}Se activity was 475 and 131 Bq/mL in solution. An aliquot of the suspension (250 mL) was filtered (0.45 μm) into a clean beaker and five animals were exposed to this solution for 2.5 h in order to directly assess uptake from solution during the exposure. Whole-body uptake was 12.1% of the activity accumulated during the feeding exposure (Table I). This value was subtracted from total exposure values in all calculations.

The 12 animals that had ingested the most activity (767–1433 Bq per clam) were fed unlabeled food for the 168-h egestion phase of the experiment. Shell, foot, mantle, and other soft tissues were separated for analysis in six individuals at the end of this experiment to assure ^{75}Se incorporation into nondigestive tissues. Activities were below the detection limit for ^{75}Se in water throughout the egestion period. The mass balance suggested ^{75}Se activity in egestion water was <0.25 Bq/mL.

E. Absorption from Diatoms. To determine Se assimilation from ingested phytoplankton, *M. balthica* were fed the small centric diatom *Thalassiosira pseudonana* (clone 3-H) labeled with both ^{75}Se and ^{241}Am. The dual label was employed to assess Se absorption relative to a more inert element as a function of retention time in the digestive tract and to provide an independent check on the absorption results. Details of this method are given in Fisher and Reinfelder (*22*).

To prepare the radiolabeled phytoplankton, an axenic culture of *T. pseudonana* was grown in f/2 medium (*39*) but with f/50 levels of Mo, Co, Mn and Fe; no Cu, Zn, or EDTA was added. The medium was prepared with sterile filtered (0.2 μm) seawater (surface seawater collected 10 km off Southampton, Long Island, NY). ^{75}Se and ^{241}Am were added to the culture, which was incubated for 92 h as in Fisher and Reinfelder (*22*); the ^{75}Se was from a stock solution in 0.5 N Ultrex-HCl, added as selenite to give 148 KBq/L (543 pM Se addition); the ^{241}Am was from a stock solution in 3 N Ultrex-HNO_3, added to give 55.5 KBq/L (1.8 nM Am addition). The addition of the ^{75}Se and ^{241}Am in 7.5 μL of dilute HNO_3 did not affect the pH of the culture, which was 8.0.

The algal cells exposed to radiotracers accumulated 55% of the ^{75}Se and 74% of the ^{241}Am, attaining volume–volume

concentration factors (*40*) of 5.1×10^4 for Se and 1.2×10^5 for Am. Diatom cells, each containing 183 μBq of ^{75}Se and 86.7 μBq of ^{241}Am, were resuspended out of their radioactive water via 1-μm Nuclepore polycarbonate filters into unlabeled sterile-filtered seawater (diluted to 20‰) and added to the chambers containing *M. balthica* at an initial cell density of $(7.7–8.7) \times 10^4$/mL (1.7–2.0 mg dry wt/L or $(47–53) \times 10^8$ μm^3/L). At the end of the exposure period, each cell had 123 μBq of ^{75}Se and the ^{75}Se activity in solution was 7.4 Bq/mL. Cells from an unlabeled culture of *T. pseudonana* in the same physiological state were fed at this same density to *M. balthica* through the egestion phase.

Twenty-one *M. balthica* were exposed in three chambers, each containing seven animals of different size [mean weight ± SD: (A) 184 ± 36 mg dry wt; (B) 44 ± 6 mg; (C) 26 ± 3 mg]. Chamber B contained animals of sizes similar to those employed in gut evacuation and sediment ingestion experiments. Uptake of solute ^{75}Se during exposure was not determined directly, but low concentrations in solution, mass balance calculations, and very low concentrations in shell indicated it was not significant.

In contrast to the previous experiments, whole clams in each chamber were analyzed in aggregate after the exposure and at the end of a 48-h egestion period. Each time fecal material was collected, an aliquot of intact fecal pellets was analyzed to estimate absorption using the dual radiotracer method (eq 3). The remainder of the feces and pseudofeces were then collected on a mesh.

F. Calculation of Absorption Efficiency. In all experiments, absorption efficiency (A) was determined from the ratio

$$A = C_e/C_0 \quad (1)$$

where C_0 is the initial activity determined from whole-body activity after the 2.5-h ingestion period and C_e is the activity in whole clams at the termination of egestion, after >90% of the unassimilated food had been egested. In the experiment where animals were fed ^{75}Se-labeled diatoms, several additional procedures were compared for determining absorption efficiencies. In one, C_0 was determined by loss of activity from the feeding suspension, to compare results with the above approach. For a comparison independent of these approaches, absorption was calculated by comparing C_0 to the total activity egested as fecal material plus the sum of activities in water from the "chase" chambers (E) by

$$A = 1 - (E/C_0) \quad (2)$$

When C_0 was compared to $E + C_e$, the two agreed within 10%. Selenium absorption efficiencies also were estimated by employing the dual-label procedure from Fisher and Reinfelder (*22*):

$$\text{efficiency} = \frac{[^{75}\text{Se}/^{241}\text{Am (food)}] - [^{75}\text{Se}/^{241}\text{Am (feces)}]}{[^{75}\text{Se}/^{241}\text{Am (food)}]} \quad (3)$$

G. Bioconcentration from Solution. ^{75}Selenite exposures were employed to estimate Se bioconcentration from solution. Five individuals were exposed in each of five chambers to [^{75}Se]selenite plus selenite carrier over a range of environmentally reasonable concentrations, from 0.25 to 100 nM. The short exposures were employed to estimate gross influx, which could then be used to model steady-state bioconcentration.

H. Radioactive Counting. The γ emissions of ^{75}Se (264 KeV), ^{51}Cr (320 KeV), and ^{241}Am (60 KeV) in water, feces, algal cells, sedimentary material, live *M. balthica*, or *M. balthica* tissues were determined in γ counters with well-type NaI (Tl) crystals. Activities in live, whole animals were determined while the animals were held in counting chambers with seawater. Counting times ranged from 1 (for the live animals) to 30 min to reach propagated errors of ≤5% (errors were higher for some water samples collected during egestion, where activities were near detection limits). Counts were corrected (with standards for each isotope) for counting efficiency, given different counting geometries, and radioactive decay.

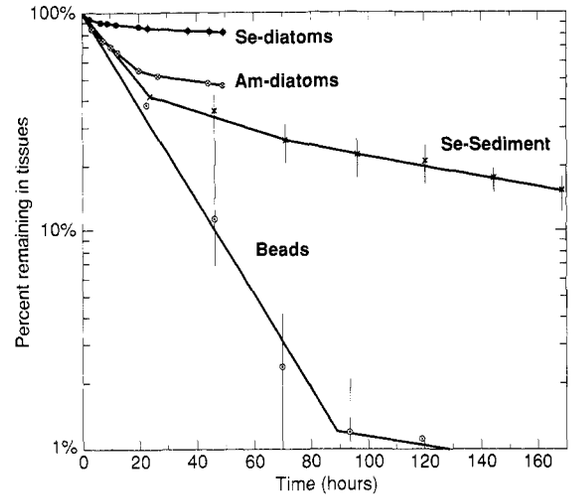

Figure 1. Retention through time of label by *M. balthica* after ingestion of ^{51}Cr-impregnated beads, ^{241}Am- and ^{75}Se-labeled diatoms, or ^{75}Se-labeled sediments.

3. Results

A. Gut Evacuation. Mean egestion occurred as a single-exponential function, after *M. balthica* ingested ^{51}Cr-impregnated beads (Figure 1). Variability among individuals was great in the first 22 h of egestion. Some individuals lost >90% of the label in that time, and others <10%. Eight of 12 individuals had egested >90% of the label within 48 h and all but two individuals had egested >99% of the label within 94 h (those two had lost >95%). These results confirm that *M. balthica* will progressively evacuate 99% of its gut content within 94 h if the food source is inert to digestive processes and suggest that ingested material retained beyond 94 h can be considered absorbed.

B. Bioaccumulation of Particulate Elemental Se. At least some of the elemental ^{75}Se in the sediments was biologically available to *M. balthica*. The average proportion of sedimentary food and inertly labeled beads egested in the first 24 h was similar (Figure 1). However, between 24 and 96 h, a much lower proportion of sedimentary ^{75}Se was egested than of the inertly labeled beads (Figure 1).

Mean absorption efficiency of ^{75}Se from the ingested sediment was 22.5% (determined from whole-animal activities after 96 h of egestion). Fourteen percent of the whole-body ^{75}Se accumulated from solution was associated with the shell (Table I). The activity of ^{75}Se in the shell of animals exposed to the radiolabel in solution was virtually the same as the activity in the shells of feeding animals. Thus feeding had no influence on Se deposition in the shell (see also ref 20). Absorption of the ^{75}Se into tissues as a result of exposure to the bolus of food was verified by the high activities in the soft tissues of feeding animals after gut evacuation, compared to animals from the solute exposure, and by the beginning of redistribution

Table II. Percent Absorption Efficiency of ^{75}Se from Ingested Radiolabeled Diatoms Calculated by Different Methods

chamber	C_e/C_0	C_e/W[a]	$1 - (E/C_0)$	Se/Am	soft tissue/C_0
A	91	83	82	91	91
B	84	77	78	92	83
C	95	70	84	96	94
mean	90 ± 5	77 ± 6	81 ± 3	93 ± 3	89 ± 6

[a] W, quantity of radiolabel lost from the exposure medium during the exposure.

of the label to nondigestive tissues such as the foot and the mantle (Table I).

Recycled ^{75}Se was not detectable (<0.25 Bq/mL) during the egestion phase of this experiment. No detectable ^{75}Se was desorbed from fecal pellets.

C. Bioaccumulation of Se from Diatoms. The organo-Se ingested with the diatoms was retained much more efficiently than elemental Se (Figure 1). Some egestion of unassimilated ^{75}Se occurred early in the experiment. However, the difference between egestion of diatoms and egestion of inert beads indicated Se was absorbed throughout the digestion process. After 48 h, very little ^{75}Se activity was observed in feces, suggesting nearly all the Se was absorbed from food retained for longer than this in the digestive tract. If the rate of egestion between 44 and 49 h was extended to 168 h, only an additional 2% of the ^{75}Se in the clams would have been lost.

Independent procedures gave very similar estimates of absorption efficiency. Absorption efficiency of organo-Se from the diatoms averaged 86% among the different procedures employed for the calculation and varied from 77 to 93% among approaches (Table II). The lowest estimate occurred where the ingested dose was estimated from the loss of activity in the feeding suspension. Uncertainties in determining activities in suspension may have contributed to these differences. The highest values for absorption were from the Se/Am ratio in feces, possibly because this method required relatively large corrections for Se desorption from fecal pellets and Am retention by clams. No significant effect of animal size on Se absorption was evident.

Egestion of ^{241}Am was initially faster than that of ^{75}Se, following a time course similar to the inert beads during the first 12 h of egestion (Figure 1). This suggested little ^{241}Am was absorbed from material with a short gut residence. Egestion slowed after 12 h, and some ^{241}Am was absorbed from material with a longer gut residence time. Overall, absorption efficiency of ^{241}Am was 41%, less than half that of ^{75}Se. The ratio of $^{75}Se/^{241}Am$ through the ingestion period also indicated that Se was assimilated more efficiently later in the digestive process than during the first few hours (Figure 2). Greater than 90% of the Se was absorbed if it was retained in the gut longer than 24 h.

Some recycling of ^{75}Se was possible during egestion. Fecal pellets of *M. balthica* fed diatoms lost 27.7 ± 7% of their ^{75}Se if left for 12 h in unlabeled seawater. Concentrations of ^{75}Se in the egestion chambers were highest during the first 24 h when an average of 0.3 μBq/cell was found associated with phytoplankton and 0.1 Bq/mL in solution; ^{75}Se was virtually undetectable thereafter. Because concentrations in the media were very low relative to the exposure, it is unlikely that recycling affected the interpretations.

D. Bioconcentration of Selenite from Solution. The uptake rate of [^{75}Se]selenite from solution varied

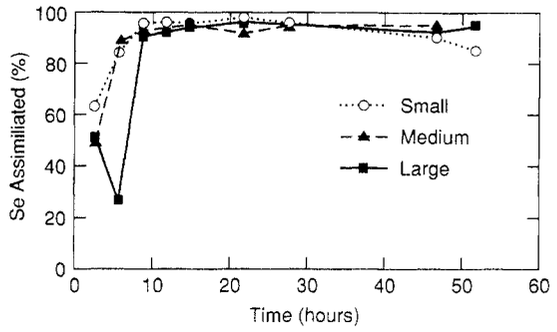

Figure 2. Absorption efficiency of Se in three size classes of *M. balthica* at different time intervals after ingestion of labeled diatoms, as determined by the ratio $^{75}Se/^{241}Am$ in fecal pellets.

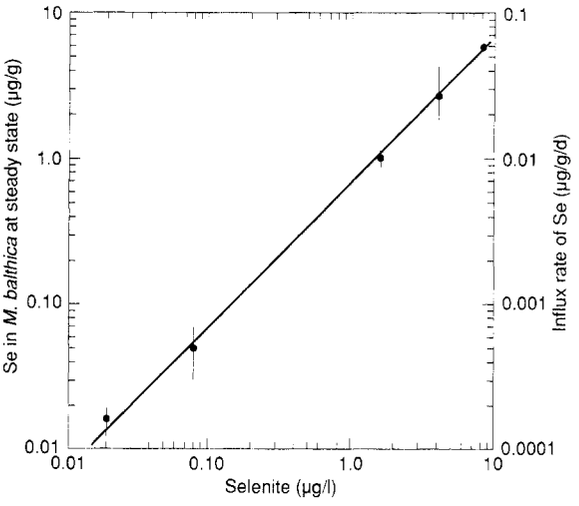

Figure 3. Influx rates and concentrations at steady state (from eq 7) in *M. balthica* exposed to five concentrations of selenite in solution.

linearly with concentration between 0.25 and 100 nM (Figure 3).

4. *Model Determinations*

A "kinetic" bioaccumulation model (*7*) was employed to calculate steady-state uptake from food and water using the rates determined experimentally and concentrations of Se observed in San Francisco Bay. The bioaccumulation estimates from the model were validated by comparison to tissue concentrations of Se observed in *M. balthica* from the bay.

A kinetic model assumes that the concentration of contaminant achieved by an organism is a balance between influx and efflux, and that influx from food and water is independent and additive. For our study, bioaccumulation was expressed as

$$dC_M/dt = (I_w + I_f) - C_M k_e \quad (4)$$

where dC_M/dt was the concentration of Se at time t in *M. balthica*, I_w was the influx from water [μg of Se (g of tissue)$^{-1}$ day^{-1}], I_f was the influx from food, and k_e was the rate constant of loss. Efflux was described by the rate constant of first-order, single-compartment isotope-substitution kinetics as employed in other studies of whole-organism trace-element efflux in bivalves (*41–45*). More than one exponential component has been employed in studies where uptake times were short (*23, 41, 44*), but we assumed that chronic Se exposures would prevail in nature, and that single-component loss was appropriate. Whole-organism rate constants of loss for Se are similar in clams, mussels, shrimp, mosquitofish, eel, leeches, and oysters, varying between 0.007 and 0.012 day^{-1} (*20, 23, 46*). The

Table III. Comparison between Model-Projected Se Bioaccumulation in *M. balthica* ($C_{Mf,ss}$) and Se Concentrations Observed in Nature at Three Levels of Contamination, Four Different Feeding Rates, and Two Assimilation Efficiencies (A)

station	[Se] sediment[a]	feed rate[b]	A	$C_{Mf,ss}$[a]	Nature[a]
M5					
uncontam	0.1	1.0	0.86	8.6	
	0.1	0.5	0.86	4.3	2.95
	0.1	0.25	0.86	2.2	±0.5
	0.1	1.0	0.23	2.3	
	0.1	0.5	0.23	1.1	
	0.1	0.25	0.23	0.6	
M4					
9/85	0.2	0.25	0.86	4.3	4.98 ±0.7
2/86	0.4	0.25	0.86	8.6	6.7 ±0.8

[a] Units: μg/g dry wt. [b] Units: g of sediment (g of tissue)$^{-1}$ day^{-1}.

value we chose for k_e in our evaluation was 0.01 day^{-1}.

The Se concentration in *M. balthica* obtained from food alone ($C_{M,f}$ in μg/g dry wt) or water alone ($C_{M,w}$) can be expressed as

$$dC_{M,f}/dt = I_f - Ck_e \quad (5)$$

Equation 5 solves to

$$C_{M,t} = I_f/k_e + (1 - e^{-k_e t}) \quad (6)$$

where $C_{M,t}$ is the concentration of Se at any time (t) in *M. balthica* and I_f/k_e is the concentration of Se at steady state in *M. balthica* or

$$C_{Mf,ss} = I_f/k_e \quad (7)$$

Thus, the steady-state concentration obtained from food or water, $C_{Mw,ss}$, may be determined if influx rates can be determined.

Influx rates from water were determined directly, thus steady-state concentrations could be calculated from eq 7 (Figure 3). They varied from 0.02 μg/g at 0.25 nM to 6 μg/g at 100 nM. The mean bioconcentration factor was 712 ± 69 for selenite uptake.

In a deposit feeding animal, the influx from ingestion (I_f) is dependent upon the concentration of Se in sediment (E in μg of Se/g of food), the rate of ingestion [R in g of food (g of tissue)$^{-1}$ day^{-1}], and the absorption efficiency of Se (A) by

$$I_f = FRA \quad (8)$$

Data from Hummel (*28*) suggest a deposit feeding rate of 0.5 g of sediment (g of tissue)$^{-1}$ day^{-1} for *M. balthica* (12–22-mm shell length) under a variety of food concentrations. Other studies have shown that feeding rates in this species vary from 0.25 to 1.0 g of sediment (g of tissue)$^{-1}$ day^{-1} with sediment characteristics, for animals of the sizes employed in this study (*36*, *47*, *48*). For suspension feeding, eq 8 was modified such that

$$R = (\mathrm{SPM})V \quad (9)$$

where SPM is the concentration of suspended particulate material solids (in g/L) and V is the ventilation rate [in L (g of tissue)$^{-1}$ day^{-1}]. Estimates of time-integrated ventilation rates for *M. balthica* vary from 1.0 (*28*) to 2.5 L (g of tissue)$^{-1}$ day (ref 49; our filtering rates plus activity estimates of 50). In the model we use 2.0 L (g of tissue)$^{-1}$ day. It was assumed that the suspended Se was principally associated with organic material (absorption efficiency of 0.86), a reasonable assumption for oxidized waters (*10*).

The model calculations predicted that bioaccumulation of Se via deposit feeding would vary by 10-fold across the range of feeding rates reported for *M. balthica* and the absorption efficiencies reported above (Table III). Such variability can occur independent of Se concentrations in sediments, illustrating the potential importance of environmental conditions that affect feeding rate or the food type.

Steady-state concentrations predicted for uptake via deposit feeding were within the range of Se concentrations reported for *M. balthica* in nature (Table III; ref 32). Table III shows that the concentration of Se predicted in *M. balthica* in uncontaminated conditions (0.1 μg/g in sediment) was similar to that observed at such station in San Francisco Bay. Earlier field studies reported Se concentrations in individual clam samples ranged from 1 to 3.5 μg/g among several uncontaminated locations (*32*). Such variability is consistent with influences of feeding rate and food type predicted by the model. The model also predicted increases in bioaccumulation with small increases in Se concentrations in sediments. The predicted bioaccumulation response was of a magnitude similar to that observed in nature (Table III). Most important, bioaccumulation via ingestion alone was sufficient to explain the concentrations of Se observed in *M. balthica* from San Francisco Bay. Results similar to Table III could also be achieved by assuming intermediate feeding rates and that the animals feed on an even mix of sediments and benthic diatoms.

Comparisons of bioaccumulation from food and solution rarely consider the concentrations in the two sources that occur in nature (*2*). Food was a much more important source than solute selenite when bioaccumulation was compared between solution and suspended material using concentrations typical of contaminated and uncontaminated localities in San Francisco Bay (Table IV). Very little Se was accumulated from solution by *M. balthica* at selenite concentrations typical of San Franciso Bay (Table IV). Selenite concentrations more than 10 times higher than observed anywhere in the bay would be necessary to reach tissue concentrations of even 1 μg/g of Se (Figure 3). On the other hand, the model showed that *M. balthica* could reach the tissue concentrations observed in the bay

Table IV. Predicted Bioaccumulation of Se via Food ($C_{Mf,ss}$) and Water ($C_{Mw,ss}$) at the Concentrations of Se in Suspended Particulate Material (SPM) and Selenite in Solution at Three Stations in San Francisco Bay[a]

	conditions			bioaccumulation			
location	Se in SPM, μg/L	Se in water, μg/L	A	$C_{Mf,ss}$, μg/g	$C_{MW,ss}$, μg/g	$C_{M,ss}$, μg/g	% from food
Sacramento	0.011	0.024	0.86	1.90	0.02	1.92	99
Suisun[b]	0.021	0.073	0.86	3.67	0.06	3.73[c]	98
So. Bay	0.026	0.069	0.86	4.47	0.05	4.52	99

[a] Water column data from Cutter (*11*). Units converted to micrograms per liter to facilitate model calculation. [b] This is the highest value observed by Cutter (*11*) in April 1986, near Carquinez Straits. [c] *M. balthica* collected from near this station in 9/85 and 5/87 had a mean Se concentration of 3.8 ± 0.3 μg/g (*32*).

(see footnote in Table IV) via suspension feeding, as well as via deposit feeding. This occurred despite the fact that suspended concentrations of Se were often lower than concentrations of selenite. The low suspended concentrations of Se reflected low densities of suspended particulate material (10–50 mg/L) (*11*). Estimates of Se concentrations on particulates at the stations in Table IV ranged from 0.5 to 0.9 μg/g [calculated from Cutter (*11*)].

5. Discussion

This study illustrates the importance of understanding the pathways that determine Se bioavailability in nature. Most assessments of Se toxicity have employed selenite, the most bioreactive form of the element in solution (*19, 51–54*). However, selenite contributed little to Se bioaccumulation by *M. balthica* in San Francisco Bay because of slow uptake and low concentrations. The selenite concentration factor of 712 predicted for *M. balthica* was within the same magnitude as the values of 100–200 observed in 120-day studies of selenite uptake with mussels and shrimp (*18, 20*), so the low efficiency of selenite bioaccumulation is not unique to *M. balthica*. The toxicity of selenite begins at concentrations of >100 nM in most studies. Concentrations of selenite in oxidized estuarine waters are typically much lower than the level that causes toxicity in bioassays (*9–11*) and are unlikely to reach such levels under conceivable environmental circumstances. However, adverse biological effects are conceivable in natural waters via food web transfer.

Selenium enrichment has been observed in the tissues of diving ducks (scaup and scoters) in San Francisco Bay. The low concentrations of Se in solution [0.1–1.0 nM of selenite (*11*)] coincide with concentrations up to 1.0 μg/g of Se in particulate materials (*11*). Our results demonstrate that this is enough particulate Se to contaminate particulate-feeding consumer organisms such as *M. balthica*. Previous studies show that the principal pathway of Se transfer to fish and waterfowl is via Se-contaminated food (*33, 34, 59*). Thus transfer of Se from clams to waterfowl is the most reasonable explanation of the observed contamination in the upper trophic levels. The most severe Se contamination occurs in Suisun Bay and has been documented in birds, in mussels, and in clams in addition to *M. balthica* (*32*). Concentrations of 9–10 μg/g (dry wt) occur in the most contaminated individuals of the clam *Corbicula fluminea* in Suisun Bay. This is the concentration at which dietary toxicity is observed in fish and at which Se-treated food is avoided by mallard ducks (*33, 55–58*). Most notably, recent studies have shown reduced growth and survival in fall run Chinook salmon (a threatened species in the bay) fed a diet with this level of Se for 90 days (*57*). Thus Se threatens upper trophic level organisms in San Francisco Bay at concentrations in solution far lower than protective criteria levels proposed from solute bioassays. In order for regulatory criteria to protect food webs from the adverse effects of Se, particulate concentrations and food web transfer of the element must be considered. The potential for adverse effects via food web transfer occurs at much lower concentrations in solution than indicated by direct solute toxicity tests.

Previous studies demonstrated bioaccumulation of Se via ingestion in zooplankton (*21, 22, 60*), clams (*Puditapes philippnarum*) (*23*), shrimp (*Lysmata seticaudata*), and mussels (*Mytilus galloprovincialis*) (*20*). In contrast, daphnia (*Daphnia magna*) did not accumulate appreciable concentrations of Se from ingestion of the green alga *Selenastrum capricornutum* (*19*). None of these studies quantitatively compared solute and ingested sources of the element, although Fowler and Benayoun (*20*) suggested that uptake of Se from solution was too slow to explain tissue concentrations observed in *M. galloprovincialis*. These studies considered only food sources where organo-Se was the predominant form of the element. Detrital material and sediments are also ingested by many benthos; therefore consideration of the bioavailability of the forms of Se in sediment is also important. One of the most important of those forms is elemental Se precipitated by microbial dissimilatory reduction of selenate (*15–17*). In *M. balthica* the absorption efficiency of biologically produced elemental Se was approximately 4-fold lower than absorption from ingested diatoms. The highly efficient absorption of Se from ingested microalgae observed in *M. balthica*, zooplankton (*Arcartia tonsa*) (*22*), and clams (*23*) is not applicable to all food types. The nature of the particulate Se must be considered when bioaccumulation via particle ingestion is assessed.

The efficient food web transfer that apparently occurs in Suisun Bay may be facilitated by anthropogenic discharges of selenite into that system (*11*). Selenite discharge would facilitate efficient bioaccumulation by phytoplankton and enhance efficient transfer of Se to *M. balthica* and other consumers (*22, 23*). If Se were discharged as selenate, it not only would be less available from solution but, more importantly, the biogeochemically generated particulate forms would be of lower bioavailability. Oxidation of selenite to selenate might be studied as an initial, minimum step in remediation of selenite-rich wastestreams, because of the accentuated adverse effects of even small anthropogenic selenite discharges into nature.

Although Se may be immobilized in contaminated environments when selenate is precipitated in sediments as elemental Se (*15–17*), and transferred to animals less efficiently in this form than as organo-Se in food, this process does not eliminate food web exposures or the potential for toxic effects. Some of the elemental Se in sediments is assimilated by *M. balthica*. According to our model, *M. balthica* ingesting sediments with >1.5 μg/g of Se in this form could achieve steady-state tissue burdens approaching the level toxic to fish. Such results must be considered in remediation efforts that attempt to reduce hazardous exposures to Se at highly contaminated sites by stimulation of microbial dissimilatory reduction of selenate from water [i.e., "wet flux" methods (*61*)]. Treatment systems of this type must be isolated from the ecosystem to ensure that Se is not bioaccumulated in food webs (*16*).

Absorption efficiencies are a critical piece of missing information in realistically evaluating the bioavailability of particulate contaminants. Our experiments point to the usefulness of pulse–chase in determining absorption efficiencies. Such procedures (1) minimize recycling within the experiment (*62*) and uptake of desorbed solute label (*63, 64*), (2) eliminate changes in specific activity that cause absorption efficiency determinations to decrease with time (*65*), (3) eliminate the necessity of considering efflux of previously assimilated contaminant when absorption efficiency is determined (*35, 66*), and (4) reduce the possibility of behavioral anomalies that can occur in long-term handling of bivalves (*67*). The values generated by the pulse–chase experiments are appropriate for relatively simple physiological models. These models can employ concentrations of contaminants from natural systems to assess bioavailability and to assess the validity of the model calculations. This combined field/laboratory approach offers an opportunity to reduce sources of uncertainty that have long plagued assessments of trace contaminant bioaccumulation from multiple pathways.

Registry No. Se, 7782-49-2.

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Received for review April 8, 1991. Revised manuscript received October 15, 1991. Accepted October 16, 1991. The MSRC work was supported in part by NSF Grant OCE8810657 to N.S.F. This is MSRC Contribution No. 824.