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Bioaccumulation Kinetics and Organ Distribution of Nickel in the Marine Clam (*Protothaca staminea*)

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Man's activities, primarily fossil fuel combustion, currently introduce about 47 x 10^6 kg of Ni per year into the world's atmosphere (NRIAGU 1979); this rate is expected to increase greatly during the next 20 years (WILSON 1980). Much of this Ni is associated with sub-micron atmospheric particles (NAS 1975). When these particles deposit at the sea surface, about 47% of the associated Ni is released in a soluble form (HODGE et al. 1978) that could enter marine food webs.

Ni is normally present in seawater at 0.1 to 2.4 µg/L (BRULAND 1980; CHESTER & STONER 1974; NAS 1974; DANIELSSON 1980), and Ni concentrations in marine pelycepods range from 0.05 to 3.2 µg/g dry wt., depending upon the location of collection (REISH et al. 1981). Higher concentrations of Ni in seawater may be toxic to marine life. The 48-h LC_{50} for Ni is 1,180 and 310 µg/L for oyster embryos and hard-shell clam embryos, respectively (CALABRESE et al. 1973; CALABRESE & NELSON 1974).

Little information exists regarding the ability of marine shellfish to concentrate Ni from seawater. Our studies were undertaken to determine the degree of bioconcentration, kinetics of accumulation, and tissue distribution of Ni in marine clams exposed to seawater enriched with subtoxic levels of Ni.

METHODS

Littleneck clams (Protothaca staminea) were collected from the intertidal area of Sequim Bay and held in running seawater for 48 h prior to exposure. For baseline Ni concentrations, 10 separate clams or clam gills were depurated in clean flowing seawater for 2 days, dried at 60°C, digested in Ultrex HNO_3 and analyzed on an Instrumentation Laboratories 251 atomic absorption spectrophotometer. For the excised gill exposure, clams were shucked and the gills dissected free. Single gills were placed in polyethylene beakers with 100 or 150 mL of 0.45 µm filtered seawater at 10°C and salinity of 31 °/$_{oo}$. One hundred µL of a $NiNO_3$ solution containing carrier-free ^{63}Ni was added to each beaker to yield total Ni concentrations between 0.5 and 8 µg/L. The gills were incubated at constant temperature with gentle rotary stirring. To measure the rate of release of Ni, gills exposed to 8 µg/L Ni for 48 h were transferred to 130 mL of clean seawater, incubated under the same conditions, and at time intervals of 3 and 24 h were tranferred to new beakers of filtered seawater. During the exposure, 100 µL samples of water were removed at time intervals for counting of radioactivity. All

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radioactivity was corrected for counting efficiency and converted by specific activity to concentrations of Ni. After exposure, gills were rinsed with clean seawater, dried to constant weight at 60°C, and digested in 15 mL of formic acid at 60°C for 48 h. A 0.5 mL subsample of the formic acid digestate was added to 15 mL of Aquasol® and counted in a liquid scintillation counter.

Whole clams were exposed to Ni in individual 1 L polyethylene "dispo" beakers containing 800 mL of 0.45 µm filtered seawater and 8 µg/L Ni plus ^{63}Ni at 10°C with aeration. To avoid depletion of Ni from the water, clams were transferred after 24 h to new media under the same conditions. At the end of the 48-h exposure, clams were removed, rinsed with clean seawater, shucked, dissected into individual organs, and treated as described for gills above. The quantity of Ni eliminated by feces or pseudofeces during the second 24-h period was measured by filtering the total 800 mL through 0.45 µm filters and counting the particulate Ni radioactivity.

RESULTS AND DISCUSSION

Baseline Ni levels in clams collected from Sequim Bay were 1.15 ± 0.09 and 2.38 ± 0.11 (± SEM) µg/g dry wt for whole soft bodies and excised gills, respectively. Both accumulation and release of Ni by excised clam gills exposed to 8 µg Ni/L follow biphasic kinetics with an initial rapid rate followed by a second and slower rate (Fig. 1).

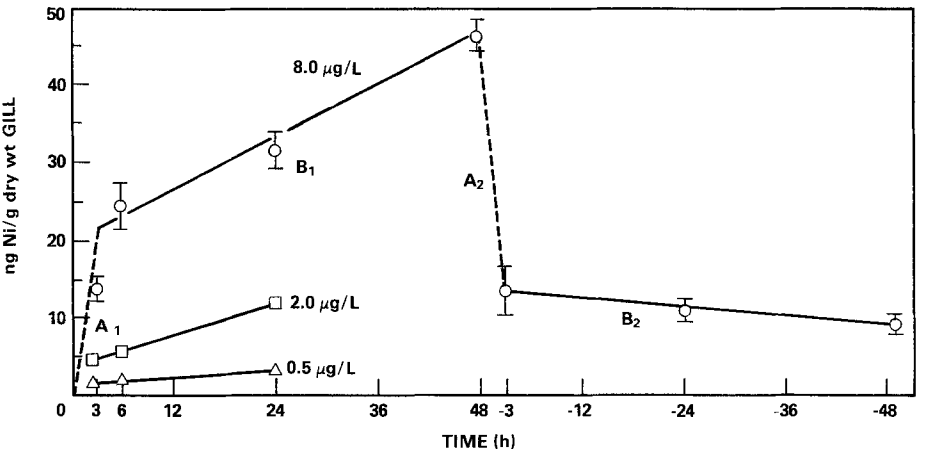

Fig. 1. Accumulation and elimination of Ni by excised clam gills exposed to 8 µg Ni/L seawater. Vertical bars enclose standard errors.

Initial 3 h accumulation (A_1) and release (A_2) occur at similar rapid rates (7 and 10 ng/g/h, respectively). This suggests an easily exchangeable compartment. This could represent either Ni loosely sorbed to the gill surface and/or Ni which is swept into the spaces of the highly convoluted gill epithelium by solvent drag (BERRIDGE & OSCHMAN 1972) and not released by the external

rinsing and blotting of the undepurated gills prior to drying. The slower accumulation (B_1) and release (B_2) rates can be described by the linear relationships:

1) accumulation: $Y_1 = 20.5\ \mu g/g + B_1 t$ $r^2 = 0.99$
2) elimination: $Y_2 = 14.11\ \mu g/g - B_2 t$ $r^2 = 1.00$

where: $B_1 = 0.52\ \mu g/g/h$
$B_2 = 0.12\ \mu g/g/h$
t = time (h)

The half-life of Ni (calculated from B_2) in excised gills is 59 -h. B_1 and B_2 probably represent biological uptake and release of the Ni by less easily exchangeable compartment(s).

Ni accumulation in excised gills (i.e., B_1) increased with Ni levels in seawater. We represent this as a linear function $A = 0.082 + 0.080C$ ($r^2 = 0.95$) where A = gill Ni accumulation during 3 to 24 h in ng Ni/g dry wt/h and C = concentration of Ni in seawater in µg/L (Fig. 2). However, clams were exposed to only three concentrations of Ni in seawater. It is possible that at higher Ni concentrations the uptake rate would become saturated.

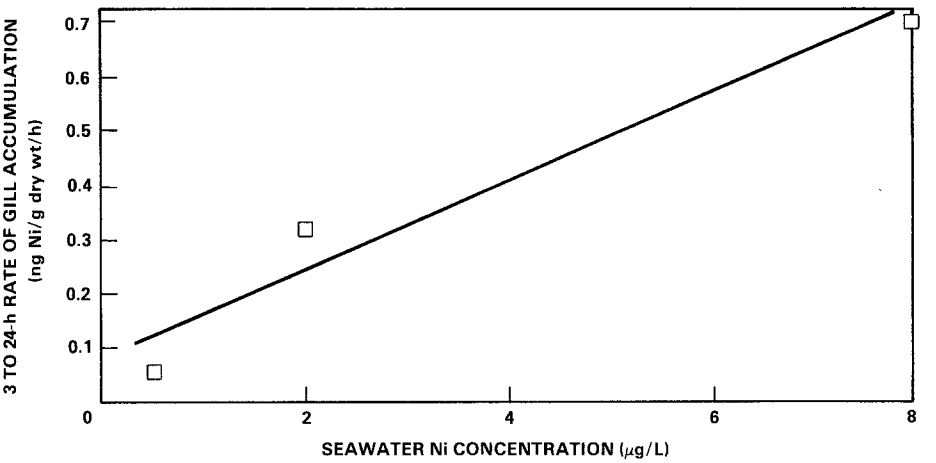

Fig. 2. Three to 24 h accumulation of Ni by excised clam gills increases with Ni seawater concentration. n = 12.

Bioconcentration factors (BCF) for Ni accumulation were determined for gills and whole animals using Ni per gram dry weight of tissue versus Ni per volume of seawater as a basis of comparison (i.e., ng Ni/g dry tissue ÷ ng Ni/mL seawater). Alternative methods for expressing BCF have been discussed by POLIKARPOV (1966). For excised gills, the BCF was estimated using the kinetic approach of HAMELINK (1977) and is calculated by dividing the slope of the Ni accumulation equation (A_1, B_1) by that for release (A_2, B_2). This yielded BCF's of 0.7 and 4.3X for the fast and slow compartments. The value of 0.7 for the fast compartment indicated that the rate for release of Ni by this

compartment was close to or slightly greater than the rate of accumulation and, therefore, no net Ni uptake occurred. These results indicate that the slow compartment with a BCF of 4.3 was responsible for Ni accumulation of excised gills. The BCF of whole animals was determined directly by comparing the Ni concentration of dry tissues to that of seawater. During the exposure of whole clams, the seawater was renewed after 24 h, and the seawater Ni concentration declined from 8.18 to 7.23 µg Ni/L during each of the 24-h intervals of the 48-h exposure (Table 1).

TABLE 1. Distribution of Ni between compartments. Clams exposed 48 h to 8 µg/L Ni, (± standard error).

Compartment (n)	Dry wt (g)	ng Ni/g	Total ng Ni	% of Ni added	% of total soft body
Adductor muscle(6)	0.300 (0.21)	16.5 (1.8)	4.9 (0.6)	0.07	7.4
Neck + mantle(6)	0.675 (0.058)	29.6 (4.2)	19.5 (2.6)	0.30	29.3
Visceral mass(6)	1.054 (0.136)	18.6 (3.6)	18.4 (2.8)	0.28	27.7
Gills(6)	0.181 (0.021)	139.6 (18.4)	23.7 (2.5)	0.36	35.6
a) Total soft body	2.21	30.1	66.5	1.02	
b) Feces(3)	-	-	39.0	0.60	
c) Shell(4)	16.89 (1.74)	30.2 (9.1)	488.8 (148.7)	7.47	735.0
d) Water(3)*	initial	8.18×10^3 (0.08)	6.54×10^3 (0.07)		
	after each 24h	7.23×10^3 (0.15)	5.78×10^3 (0.12)	88.38	
Total(a,b,c,d)				97.47	

* Values for water are ng Ni/mL.

The mean concentration was 7.7 µg/L. The concentration of Ni in the whole clams was 30.1 ng Ni/g dry weight after the entire 48-hour exposure and reflected a BCF of about 4.0. This value showed good agreement with the BCF of 4.3 from the excised gill experiment described above. The BCF for Ni is quite low when compared to other metals. Under similar conditions we have found 24 h BCF's one to two orders of magnitude higher for Cd, Cu and Hg (HARDY 1982).

From the mass balance of Ni added during the exposure, it is evident that, of the total Ni added to the exposure, only about 1% was accumulated by the soft-body tissues of the clam; 0.6% was contained in the feces excreted during a 24 h period, and a large proportion, or 7.5% of the total nickel added, was accumulated by the clam shell. The shell represents a major repository for total Ni with more than 7 times as much Ni as the total soft-body parts (Table 1). In all, 97.5% of the added Ni could be accounted for by the water, shell, feces, and soft-body compartments. The remaining 2.5% may have been adsorbed on the walls of the exposure container, as has been shown for Cd and Zn by HENNIG and GREENWOOD (1981).

Measurements of the Ni contained in individual organs of the clam indicate that concentrations, i.e., ng Ni/g dry wt, increase in the order of adductor muscle < visceral mass < neck + mantle < gills. This order also holds true for the total amount of Ni contained within each organ (Fig. 3). The high concentration of Ni in the gills of the whole clams indicates that gills are a major site for Ni accumulation.

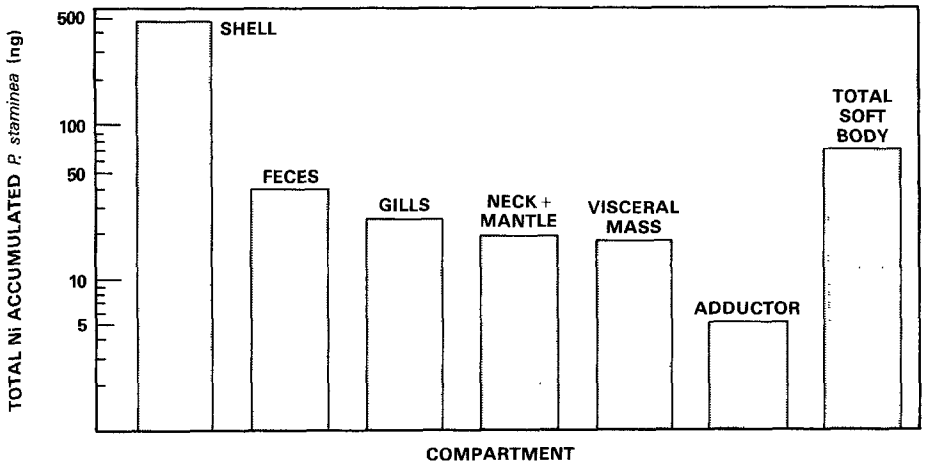

Fig. 3. Compartmental distribution of added Ni.

Ni concentrations in the gills of whole clams exposed to 8 µg/L were 3X greater than those of excised gills exposed to the same Ni concentration for 48 h. Recent studies by WRIGHT et al. (1980) have shown that excised gill preparations exhibit

inhibition of lateral ciliary activity. This would result in decreased movement of seawater over gill membranes, the presence of an unstirred layer, and a reduction in the rate of solute exchange. This may explain the lower Ni accumulation by excised as opposed to intact gills in our study. However, since release and accumulation rates would be affected to a similar degree by the decrease in water circulation, the bioconcentration factor for Ni in excised gills, calculated from kinetic considerations, would not be affected by the reduced exchange rate. The BCF of 4.3X for excised gills was able to accurately predict that for the whole clam.

In summary, these results demonstrated that, in the marine clam P. staminea, both uptake and elimination rates of Ni were biphasic, with an initial rapid phase (probably surface adsorption and desorption) and a second slower phase responsible for net accumulation. Whole clams accumulated substantial quantities of Ni. However, much of this was associated with the shell and bioconcentration of Ni from seawater occured to a much lesser degree than Cd, Cu, or Hg under similar conditions.

ACKNOWLEDGMENTS

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