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Bioaccumulation and Tissue Distribution of a Quarternary Ammonium Surfactant in Three Aquatic Species

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Quaternary ammonium compounds (QACs) are commonly used as surfactants in drilling muds and fabric softeners and as biocides in antiseptics and disinfectants (Boethling 1984; Lewis and Wee 1983). QACs and cationic polyelectrolytes elicit acute toxic effects in aquatic organisms by disrupting the structure and function of gill tissues, which may result in the suffocation of the organism (Biesinger and Stokes 1986). Little information is available, however, on the relative availability and distribution of QACs in the tissues of aquatic organisms. Information of this nature is required to understand the potential consequences of releases of sublethal concentrations of QACs into the aquatic environment. In this study, hexadecylpyridinium bromide (HPB; CAS #140-72-7) was selected as a compound for initial study because it belongs to a chemical class (alkylpyridinium QACs) that includes the most toxic and environmentally persistent QACs (Baleux and Caumette, 1977; Ruiz Cruz and Dobarganes Garcia 1979). Clams, minnows, and tadpoles were chosen as test organisms to define the relative availability of HPB to organisms that occupy distinctly different ecological niches.

MATERIALS AND METHODS

HPB was obtained from the Eastman Kodak Co. (Rochester, New York) and was reported by the manufacturer to be > 95% pure. Tritiated HPB was prepared from this standard source by catalytic exchange labeling (Du Pont NEN Research Products, Boston, Massachusetts). Radiolabeled HPB (specific activity 576 mCi/mM) was purified immediately before use in experiments according to the high performance liquid chromatography method of Wee and Kennedy (1982).

Tadpoles (Rana catesbeiana) were obtained from Nasco Co. (Fort Atkinson, Wisconsin) and had an average wet weight of 4.9 ± 2.6 g. These animals had the morphological characteristics of premetamorphic stages VI through IX as described by Taylor and Kollros (1946). These stages are characterized by the emergence

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of hind paddles and respiration by gills. Tadpoles were maintained on a diet of dry food that was supplied by Nasco and were fed every other day. Fathead minnows (Pimephales promelas), obtained from the Thomas Fish Co. (Novato, California), had an average wet weight of 0.57 ± 0.17 g and were fed TetraMin (TetraWerke, Melle, West Germany) daily. Clams (Corbicula fluminea), obtained from a local bait shop, had an average soft-body wet weight of 4.3 ± 0.3 g and were held unfed. All animals were held in aquaria that received a constant flow of dechlorinated tapwater (18 ± 1°C, pH = 8.7).

The uptake and depuration of HPB in tissues of organisms exposed to the chemical via the water column were determined in static experiments. Reconstituted water (0.272 mM $CaCl_2 \cdot 2 H_2O$, 0.166 mM $MgSO_4 \cdot 7H_2O$, and 0.476 mM Na_2CO_3 in distilled water, pH = 8.8) was used in all experiments as a standard water source. Because preliminary experiments indicated that HPB at concentrations > 200 µg/L was acutely toxic to each of the test organisms over a 96-h period, a concentration of 10 µg/L was selected for use in all studies. Tadpoles were exposed to HPB three at a time, and minnows and clams were exposed five at a time. Each species was exposed separate from the others in 2-L glass beakers that contained 1-L solutions of radiolabeled HPB (10 µg/L, 2 µCi/L). The water was aerated and the animals were held, unfed, at room temperature (18 ± 1°C) for 24 h. Water samples (1 mL) were taken at the end of the 24-h exposure period and pipetted directly into scintillation vials that contained 15 mL of scintillation cocktail (Instagel, Packard Instrument Co., Downers Grove, Illinois). All radioactive samples were counted in a Packard Model 4530 liquid scintillation counter, and the counts were corrected for background radioactivity and quenching by an external standard. Whole-animal tissue concentrations of HPB were calculated from the amount of chemical that was removed from the water. Bioconcentration factors (BCFs) were calculated by dividing the estimated wet-weight concentration of HPB in the whole animals by the concentration of chemical in the water at 24 h. Water samples taken from aquaria that did not contain animals were used to check for losses of HPB due to volatilization or sorption to beaker walls or clam shells over a 24-h period. Losses of HPB due to these processes were very low (< 5%) and did not affect experimental results significantly.

All organisms were removed from the beakers at the end of the 24-h exposure period and rinsed with distilled water. Clams were dissected immediately, while tadpoles and minnows were first anesthetized in a 0.1% solution of tricaine methanesulfonate (Crescent Research Chemicals, Inc., Paradise Valley, Arizona). Gill tissues were removed from both minnows and clams and were analyzed apart from the remainder of the bodies. Tadpoles were dissected into seven tissue categories: gills, stomach, intestine, liver, skin, kidneys, and fat bodies. Food that remained in the gastrointestinal (GI) tract of tadpoles was also removed and analyzed. All samples were weighed, oven dried overnight at 80°C, and analyzed for residual

[^{3}H]-HPB activity. Dried samples were combusted in a Packard Tri-Carb Sample Oxidizer (Model 306) and analyzed for radioactivity by liquid scintillation. For recovery studies, minnow and clam tissues were spiked with a known amount of [^{3}H]-HPB before analysis and experimental tissue residues were corrected for percent recovery, which was always > 88%. Three replicate experiments were conducted for each test species and results were converted to picograms HPB per milligram wet tissue.

Two replicate experiments were conducted to determine the active transport of water into the GI tract of tadpoles. This investigation was undertaken because preliminary results indicated that water-borne HPB was available to these tissues. Tadpoles were exposed, three at a time, to a 1-L solution of tritiated water (Amersham Corp., Arlington Heights, Illinois) at a specific activity of 4 μCi/L. Following a 24-h exposure, intact GI tracts were removed from each animal, rinsed with distilled water, and solubilized in 2 mL of Soluene (Packard Instrument Co., Downers Grove, Illinois). Fifteen mL of scintillation cocktail (Dimilume, Packard Inst. Co.) was then added to each sample and the amount of tritiated water was determined by liquid scintillation. The extent of water infiltration was subsequently determined by dividing the concentration of radiolabeled water present in the GI tract by the average amount of water that was present in the GI tract of control animals (determined from the weight of wet and dry tissues).

Two replicate experiments were conducted to examine the redistribution of HPB in the tissues of the test organisms during depuration. Organisms were exposed to [^{3}H]-HPB for 24 h as in the uptake experiment described previously except that 20 clams, 15 minnows, and 12 tadpoles were used and each group was placed in separate 2-L solutions of 10 μg HPB/L. Following the 24-h exposure period, the animals were allowed to depurate the HPB. Minnows and clams were separated each into groups of five animals, and tadpoles into groups of three; each group was placed into 2 L of clean, aerated water. The water in each beaker was exchanged daily, and the organisms were not fed during the course of the experiment. For each species, one group was sacrificed and tissues were analyzed at 1, 2, and 3 days. Additionally, clams were sacrificed at 4 days and tadpoles at 7 days. Tissue residues of [^{3}H]-HPB were analyzed as before, and the results of replicate experiments were averaged.

RESULTS AND DISCUSSION

The bioaccumulation of HPB by clams, minnows, and tadpoles following a 24-h exposure is presented in Table 1. Whole-body BCFs were 21 $\pm$ 7, 22 $\pm$ 8, and 13 $\pm$ 4 for clams, minnows, and tadpoles, respectively. These BCFs are very low in comparison to many neutral organic compounds (e.g., PCBs and PAHs) of environmental concern. The relative distributions of the

Table 1. The bioaccumulation of hexadecylpyridinium bromide in clam, minnow, and tadpole tissues following a 24-h exposure to 10 µg HPB/L.

	HPB (pg/mg wet wt)		
Tissue	Clams	Minnows	Tadpoles
Gills	1653(578)[a]	1298(195)	1726(305)
Body	111(54)	266(138)	-[b]
Liver	-	-	17(12)
Kidneys	-	-	49(42)
Fat bodies	-	-	56(69)
Stomach	-	-	31(18)
Intestine	-	-	236(337)
Skin	-	-	312(81)

[a] (Standard deviation)
[b] Not determined

residues within the organisms are important, however, because they indicate the tissues that are at the greatest risk from an acute exposure of a water-borne QAC. In each organism studied, gill tissues accumulated the highest concentrations of HPB. These results are consistent with the observed effects of acute toxicity (e.g., gill pathology and suffocation) that result from acute exposures of fish to cationic polyelectrolytes (Biesinger and Stokes, 1986). Tadpoles also accumulated relatively high concentrations of HPB in intestinal tissues and skin and on food particles (141 $\pm$ 182 pg/mg) that were present in the stomach and intestine. The high variability in the concentration of HPB that was sorbed to the intestinal tissue and food particles was most likely due to the wide range in the amount of food that was present in the tadpoles' GI tract (46-648 mg/tadpole). Results from experiments to determine the extent of water infiltration through the GI tract confirmed that water was rapidly transported through this system as > 80% of the water present in these tissues was replaced by [^{3}H]-H_2O within 24 h. Because the tadpoles were not fed during the exposure period, it is apparent that water-borne HPB entered the GI tract where it became sorbed to food particles. HPB was subsequently available for sorption to the mucoid cells of the intestinal epithelia following its desorption from food particles. This pathway is in addition to the direct absorption of HPB from water in the intestine.

The relative distributions of HPB in the tissues of the test organisms during depuration (Table 2) closely parallel those obtained following the 24-h uptake period (Table 1). Gill tissue retained the highest concentrations of HPB in each species at every depuration period but these tissue burdens declined significantly with time only in minnows and tadpoles ($p < 0.05$). The body burdens of clams and minnows, while remaining relatively low, did not decrease significantly with time ($p > 0.05$). A similar trend was observed in tadpole intestine that retained essentially all of its HPB burden until day three, after which time it lost 42% of the burden that was present following uptake. The amount of food present in the GI tracts of tadpoles at day seven (207-572 mg/tadpole) was only slightly less than on day one (309-827 mg/tadpole) and retained concentrations of HPB that did not differ significantly ($p > 0.05$) from the level accumulated during exposure (day seven = 391 $\pm$ 79 pg/mg; day one = 337 $\pm$ 58 pg/mg). These results indicate that intestinal exposure to HPB can be prolonged by the retention of contaminated food in the intestine, even during periods of prolonged fasting. This same process may be responsible for the retention of HPB in clam and minnow bodies. Lewis and Wee (1983) determined the accumulation of a QAC (ditallow dimethyl ammonium chloride, DTDMAC) in bluegill (Lepomis macrochirus) viscera and muscle tissues. They found that only the visceral tissues accumulated DTDMAC (BCF = 256), but they did not differentiate the accumulation between the associated tissues and organs. The results of our study imply that in a natural environment, where the sorption of cationic surfactants to suspended solids and dissolved organic matter can reduce their overall bioavailability (Cary et al. 1987; Lewis and Wee 1983), they may continue to be available to intestinal tissues.

The distribution of HPB within tadpoles to tissues other than gills and intestines was limited. Tissues of toxicological interest (e.g., liver and kidneys) and large lipid reserves (fat bodies) accumulated only trace amounts of HPB, indicating that the transport of HPB across membranes was limited. These results are consistent with the chemical properties of organic cations that are not sufficiently lipophilic to partition across membranes. Organic cations may, however, bind preferentially to negatively charged tissues that are exposed directly to contaminated water. In this study, gill and intestinal tissues were two principle sites for the accumulation of HPB because they contain epithelial mucoid cells that excrete negatively charged mucopolysaccharides. In addition, QACs that enter the GI tract and sorb to food particles may ultimately be available for accumulation in intestinal tissues. The impacts of such accumulations are not clear, but acute oral doses of QACs to mammals have been shown to result in delayed death and tissue pathology (Cutler and Drobeck 1970). Similar impacts on aquatic organisms should therefore be considered in the assessment of chronic QAC toxicity.

Table 2. The distribution of hexadecylpyridinium bromide (pg/mg wet wt) in the tissues of clams, minnows, and tadpoles during depuration.[a]

Tissue	Depuration period (days)				
	1	2	3	4	7
Clams					
Gills	1030(630)[b]	840(510)	720(170)	570(310)	-[c]
Body	70(35)	53(22)	57(13)	47(22)	-
Minnows					
Gills	984(186)	884(114)	460(62)[d]	-	-
Body	98(36)	91(23)	73(43)	-	-
Tadpoles					
Gills	1405(441)	1312(401)	852(187)[d]	-	508(171)[d]
Liver	23(13)	20(5)	38(32)	-	13(7)
Kidneys	55(25)	35(9)	80(40)	-	45(6)
Fat bodies	25(23)	16(8)	7(4)	-	4[e]
Stomach	35(14)	54(8)	63(16)	-	54(11)
Intestine	242(135)	270(62)	258(6)	-	138(28)
Skin	133(35)	94(33)	82(16)	-	-

[a] Following a 24-h exposure to 10 µg HPB/L
[b] (Standard deviation)
[c] Not determined
[d] *Significantly different from 24-h uptake concentrations* ($p < 0.05$)
[e] Average of two pooled samples

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