Bull. Environ. Contam. Toxicol. (1994) 53:426-433

Environmental Contamination and Toxicology

Disposition of Quinoline in the Crayfish *Pacifasticus leniusculus*

D. D. Dauble, R. M. Bean, R. W. Hanf, Jr.[1]

[1]Environmental Sciences Department, Battelle, Pacific Northwest Laboratories, P.O. Box 999, Richland, Washington 99352, USA
[2]Chemical Sciences Department, Battelle, Pacific Northwest Laboratories, P.O. Box 999, Richland, Washington 99352, USA

Received: 31 July 1993/Accepted: 30 January 1994

The fate of nitrogen-heterocyclics in the aquatic environment is of concern because they are found in many industrial products (Weisburger and Williams 1980; Wright and Later 1985) and are bioavailable to fish and aquatic invertebrates (Southworth et al. 1980; Bean et al. 1985). These products may enter aquatic ecosystems from waste effluents or surface water spills, or they could be leached from energy-derived waste materials into the groundwater (Zachara et al. 1986). We previously showed that the nitrogen-heterocycle, quinoline (benzo[b]-pyridine), was readily taken up by fish from water (Bean et al. 1985) and from food (Dauble et al. 1987). Although these studies indicate that rainbow trout metabolized quinoline, biotransformation played only a minor role in storage and excretion of the parent compound (Dauble and Curtis 1989). No known studies have been conducted to determine the toxicity or bioaccumulation potential of quinoline or other nitrogen-heterocycles in decapod crustaceans.

The objective of our study was to estimate relative rates of quinoline uptake and elimination in tissues of the crayfish *Pacifasticus leniusculus*. Additionally, we conducted studies to determine whether tissue concentrations, relative distribution, and metabolism of quinoline differed if crayfish were previously exposed to sublethal concentrations of the parent compound. Studies were part of a larger effort directed at describing the fate of energy-related organic compounds in aquatic biota. Our results showed that highest concentrations of quinoline and metabolites were found in the hepatopancreas and indicated that pre-exposure to sublethal concentrations of the parent compound altered the relative distribution and biotransformation of quinoline.

MATERIALS AND METHODS

Initial tests determined the acute toxicity of quinoline to freshwater crayfish. These tests established sublethal exposure concentrations used

Correspondence to: D. D. Dauble

for subsequent studies determining tissue distribution and metabolism of quinoline.

Adult crayfish (2 to 10 g wet weight) were collected from Bobcat Springs near Richland, Washington, and held in our laboratory in Columbia River water for 2 to 4 wk before exposure. Crayfish were fed fish and chopped spinach during acclimation but not during testing. All tests were conducted at 10±1°C. Crayfish (two replicate aquaria with 5 crayfish each, or $n=10$ each treatment plus control) were held in 20-L aquaria under static conditions for 48 hr. Concentrations of quinoline (ultra-pure grade, Aldrich) were measured at 0, 24, and 48 hr in each test container. Test solutions were solvent-free. Water samples were analyzed for quinoline by liquid chromatography using a Waters Model 510 liquid chromatographic system. Separation of 10 uL samples from test containers was accomplished isocratically with a Waters C-18 Bondapak column, eluting with 50:50 volume percent methanol:water flowing at 1 mL/min. Component detection was by ultraviolet detector (Waters) at 227 nm. Columbia River water was used for all exposures. Water quality variables were determined by standard methods (APHA 1981). Dissolved oxygen concentrations exceeded 8 mg/L in test and control aquaria, pH ranged from 7.8 to 8.0, and hardness averaged 65 mg/L as $CaCo_3$. Average test concentrations were log-transformed, and the lethal concentration of 50% of the population (LC_{50}) was determined by interpolation of relative survival rates among treatments and control animals (APHA 1981).

Pharmacokinetics of quinoline uptake were determined based on 48-hr static exposures to 5 mg/L ^{14}C-quinoline. Crayfish ($n=3$) were dissected and tissues (gills, hepatopancreas, midgut, green gland, tail muscle) sampled after 1, 2, 4, 8, 12, 24, and 48 hr of exposure, and during depuration at 24 and 48 hr post-exposure. The hemolymph was removed by syringe from the pericardial sinus of all specimens at the same intervals. Aqueous concentrations of quinoline averaged 4.9 mg/L based on HPLC measurements at 0, 24, and 48 hr. Concentrations of ^{14}C-quinoline during uptake tests were determined from liquid scintillation counting techniques. Total radioactivity in exposure solutions was 90% and 88% of 0-hr levels at 24 and 48 hr, respectively.

Kinetic model theory (Gibaldi and Perrier 1982) and nonlinear least-squares techniques were used to estimate uptake rates and bioconcentration factors. A two-compartment (fish and water) closed-system model was used to obtain simultaneous estimates of uptake and elimination rates during the uptake phase (Hamelink 1977). Bioaccumulation factors were estimated as the ratio of the uptake to the depuration rate.

For metabolism studies, crayfish were exposed to 5 mg/L quinoline for 0, 1, or 2 wk ($n=6$). Following exposure, three crayfish from each time-series were analyzed for the parent compound in gills, hepatopancreas, gut, and muscle. Remaining crayfish ($n=3$) were transferred to aquaria containing 5 mg/L ^{14}C-quinoline for 24 hr, and their tissues were also analyzed for the parent compound and quinoline plus metabolites. All parent compound measurements were based on HPLC following extraction, while parent compound plus metabolite measurements were based on total radioactivity.

The ^{14}C-quinoline (uniformly labeled in the benzo ring) was synthesized by Pathfinder Labs, Inc., St. Louis, Missouri, and purified (>99%) in our laboratory by liquid chromatography before use. Specific activity was 8.83 mCi/m*M*. Tissue samples were analyzed for quinoline and quinoline metabolite hydrolysis products according to methods described in Bean et al. (1985).

RESULTS AND DISCUSSION

The 48-hr LC_{50} for crayfish was determined to be 102 mg/L quinoline. The EC_{50}, or concentration at which 50% of the test population was immobilized after a 48-hr exposure, was estimated to be 80 mg/L. The acute bioassays indicated that crayfish were about 10 times more tolerant than rainbow trout or daphnids to quinoline during short-term exposures (Bean et al. 1985; Dauble et al. 1985).

Crayfish readily absorbed ^{14}C-quinoline when exposed to sublethal concentrations in water. However, apparent equilibrium concentrations were not observed for most tissue compartments after 48 hr exposure. Tissue concentrations of quinoline plus metabolites (based on total radioactivity) at 48 hr were in the order hepatopancreas>green gland> gills=gut>muscle (Table 1). All tissues, excepting the hepatopancreas, showed steady increases in quinoline plus metabolites over the test interval, with no apparent steady-state after 48 hr exposure. Quinoline elimination rates varied among tissues. For example, concentrations of quinoline plus metabolites in the hepatopancreas, green gland, and muscle after 48 hr depuration were about 50% those measured after 48 hr exposure. Relative elimination rates of quinoline from the gut and gills were slower than for other tissues. Tissue: hemolymph ratios of quinoline plus metabolites were greater than unity (i.e., 1.0) for all tissues sampled after 2 hr of exposure. Crayfish tissue concentration factors (ratio of tissue concentration to aqueous concentration, TCFs) ranged from 2.5 for muscle to 9.5 for the hepatopancreas (Figure 1) and were similar to those reported for rainbow trout by Bean et al. (1985).

Table 1. Relative concentrations of quinoline plus metabolites (total radioactivity) in crayfish tissues during uptake and depuration. All values in $\mu g/g$ (mean ± SE, $n=3$).

	Uptake Interval (hr)						Depuration (hr)		
Tissue	1	2	4	8	12	24	48	72	96
gills	nd[a]	0.3±0.1	3.1±1.4	1.7±1.0	2.3±0.5	6.6±1.1	17.6±8.4	10.3±2.5	21.4±7.4
green gland	nd	0.7±0.4	5.7±3.9	4.3±0.7	8.0±0.2	15.3±0.2	24.7±7.4	16.0±2.4	11.7±2.9
gut	0.3±0.1	0.6±0.1	1.9±1.2	1.1±0.6	8.0±2.0	12.2±1.1	16.7±3.3	16.8±2.2	9.9±1.7
hemolymph	0.7±0.6	nd	0.9±0.4	0.9±0.2	1.5±0.5	4.7±2.3	6.9±2.3	3.6±2.6	4.9±0.3
hepatopancreas	4.2±0.3	5.5±0.3	12.1±3.2	20.9[b]	22.4±1.2	42.8±1.7	41.7±3.0	25.2±1.6	18.6±1.4
muscle	0.4±0.1	0.7±0.3	3.6±1.2	2.1±1.3	3.9±0.7	7.3±0.5	10.9±2.3	6.5±0.4	4.9±0.4

(a) nd = not detectable
(b) single measurement

The crayfish hepatopancreas is functionally similar to the vertebrate liver and is the principal organ concerned with digestion (Barker and Gibson 1977). Thus, it is also likely to be involved in metabolism of xenobiotics and may accumulate contaminants that are taken up by the gills and transported through the general circulatory system via the hemolymph. Berry (1980) reported that highest TCFs of the mono-aromatic ^{14}C-toluene occurred in the hepatopancreas of crayfish following aqueous exposures. TCFs for toluene ranged from about 6 in the muscle to nearly 100 for the hepatopancreas. The green gland is

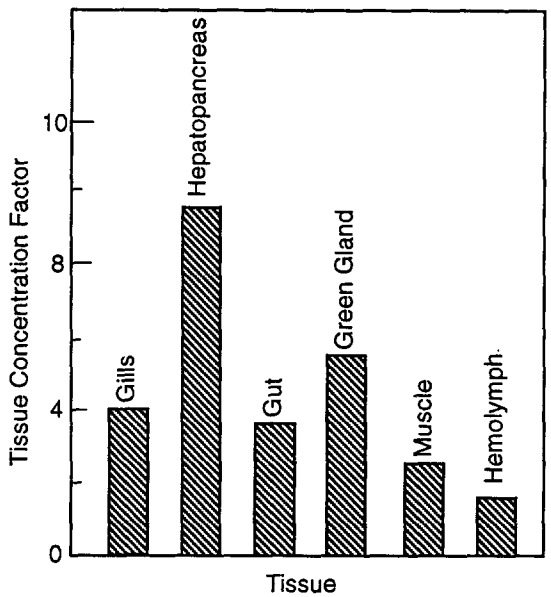

Figure 1. Tissue concentration factors for crayfish exposed to 5 mg/L quinoline for 48 hr.

analogous to the vertebrate kidney and is another major excretory organ in which organic contaminants typically concentrate in decapod crustaceans (James 1982; Little et al. 1985).

Estimated bioconcentration factors (BCFs) of quinoline plus its metabolites (total radioactivity) were determined based on kinetic model theory (Table 2). The highest BCFs were estimated for organs involved with digestion, i.e., green gland, hepatopancreas, and gut. Estimated BCFs for the muscle were about 35% of those found in the green gland and hepatopancreas. Although BCFs for quinoline plus metabolites in crayfish tissue were slightly higher than TCFs (Figure 1), differences among tissues were similar.

Table 2. Estimates of uptake (k_1) and elimination rate (k_2) constants and bio-concentration factors (BCFs) for quinoline plus metabolites in various crayfish tissues

Tissue	Uptake rate constant (k_1)	Elimination rate constant (k_2)	BCF
gill	0.0463	0.0228	no value
green gland	0.1656	0.0156	10.62
gut	0.1315	0.0219	6.00
hemolymph	0.0405	0.0084	4.82
hepatopancreas	0.7108	0.0686	10.36
muscle	0.0854	0.0229	3.73

Elimination rates for quinoline from crayfish tissues appeared slower than those determined for whole-body rates in rainbow trout (Bean et al. 1985). The relatively slow rate of elimination suggests that crayfish do not possess efficient excretory mechanisms for this compound. Gill concentrations after depuration were similar to those measured during exposure, suggesting a potential excretory pathway. Excretion across the gills is the primary elimination pathway for dietary quinoline in rainbow trout (Dauble and Curtis 1989).

Following prior exposure to 5 mg/L quinoline for 1 or 2 wk, crayfish held in 5 mg/L ^{14}C-quinoline had a significantly higher percentage of the parent compound in the hepatopancreas and gut tissue than crayfish with no exposure history (Table 3). With no previous exposure, concentrations of quinoline plus metabolites were significantly lower in the hepatopancreas, but not in the gut or muscle. This suggests that the hepatopancreas may be more effective at eliminating quinoline than are the gut or muscle tissue. This elimination process may be caused by induction of specific enzyme systems that results in increased biotransformation and elimination of the parent compound. There was no apparent pattern of storage for the parent compound and/or metabolites in crayfish muscle. Muscle tissue would be expected to achieve lower equilibrium concentrations than more highly perfused tissues, such as the hepatopancreas and green gland that are actively involved in uptake and elimination of a xenobiotic. While specific metabolites were not identified in our studies, crayfish are known to detoxify some xenobiotics via metabolic transformation (Foster and Crosby 1986).

Our data indicate that crayfish readily take up and store aqueous quinoline, and that highest concentrations are maintained in tissues involved with digestive processes. Additionally, we found that uptake and storage of quinoline and its metabolites are affected if crayfish

Table 3. Concentrations of quinoline (μg/g) and relative amounts of parent compound vs metabolites in crayfish tissues following 0, 1, or 2 wk pre-exposure to 5 mg/L quinoline followed by 24 hr exposure to ^{14}C-quinoline. All values are mean±SE (n=3).

Treatment	Tissue	Quinoline + metabolites	% parent compound
no pre-exposure	hepatopancreas	57.9±17.9	24±9
	gut	15.5±2.6	23±2
	muscle	11.8±0.8	26±2
1 wk pre-exposure	hepatopancreas	24.9±4.2	39±8
	gut	11.3±2.7	34±11
	muscle	8.1±1.4	9±4
2 wk pre-exposure	hepatopancreas	28.7±8.7	60±18
	gut	21.9±16.7	42±2
	muscle	9.8±1.2	26±2

are previously exposed to sublethal concentrations of the parent compound. More detailed studies would be required to determine the relative importance of detoxification mechanisms and to evaluate the role of other processes, including tissue saturation kinetics, in long-term storage of the compound.

Acknowledgments. We thank Deborah Steinkamp for technical assistance with acute toxicity tests, Karyn Kummerfeldt for analysis of tissue samples, and Charles Brandt for analysis of uptake and elimination rates. This work was supported in part by the U.S. Department of Energy, Office of Health and Environmental Research, under Contract DE-AC06-76RLO 1830.

REFERENCES

APHA (American Public Health Association) (1981) Standard methods for the examination of water and waste water, 15th ed. APHA-AWWA-APFC, Washington, DC

Barker PL, Gibson R (1977) Observation on the feeding mechanism, structure of the gut, and digestive physiology of the European lobster Homarus gammarus (L.) (Decapoda: Nephropidae). J Exp Mar Biol Ecol 26:297-324

Bean RM, Dauble DD, Thomas BL, Hanf RW Jr. (1985) Uptake and biotransformation of quinoline by rainbow trout. Aquat Toxicol 7:221-239

Berry WO (1980) A comparative study of the uptake of toluene by bluegill sunfish *Lepomis macrochirus* and crayfish *Orconectes rusticus*. Environ Poll (Ser A) 21:109-119

Dauble DD, Klopfer DC, Hanf RW Jr. (1985) Usefulness of the lipid index for bioaccumulation studies with *Daphnia magna*. In: Bahner RC and Hansen DJ (eds) Aquatic toxicology and hazard assessment. ASTM 891, American Society for Testing and Materials, Philadelphia, pp 350-358

Dauble DD, Bean RM, Carlile DW (1987) Uptake, distribution, and elimination of dietary quinoline by rainbow trout (*Salmo gairdneri*). Comp Biochem Physiol 87C:355-362

Dauble DD, Curtis LR (1989) Rapid branchial excretion of dietary quinoline by rainbow trout (*Salmo gairdneri*). Can J Fish Aquat Sci 46:705-713

Foster GD, Crosby DG (1986) Xenobiotic metabolism of p-nitrophenol derivatives by the rice field crayfish (*Procambarus clarkii*). Environ Toxicol Chem 5:1059-1070

Gibaldi M, Perrier D (1982) Pharmacokinetics. Marcel Dekker, Inc., New York and Basel

Hamelink JL (1977) Current bioconcentration test methods and theory. In: Mayer FL and Hamelink JL (eds) Aquatic toxicology and hazard evaluation, ASTM STP 634. American Society of Testing and Materials, Philadelphia, pp 149-161

James MO (1982) Disposition and taurine conjugation of 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, bis(4-chlorophenyl) acetic acid, and phenylacetic acid in the spiny lobster, *Panulirus argus*. Drug Metab Dispos 10:516-522

Little PJ, James MO, Pritchard JB, Bend JR (1985) Temperature-dependent disposition of [14C] Benzo(a)pyrene in the spiny lobster, *Panulirus argus*. Toxicol Appl Pharmacol 77:325-333

Southworth GR, Keffler CC, Beauchamp JJ (1980) Potential and realized bioconcentration: a comparison of observed and predicted bioconcentration of azzarenes in the fathead minnow (*Pimephales promelas*) Environ Sci Technol 14:1529-1531

Weisburger JH, Williams GM (1980) Chemical carcinogens. In: Caserett and Doull's Toxicology. Macmillan, New York, p 84-138

Wright CW, Later DW (1985) HRI catalytic two-stage liquefaction (CTSL) process materials: chemical analysis and biological testing. PNL-5605, Pacific Northwest Laboratory, Richland, Washington

Zachara JM, Ainsworth CC, Felice LJ, Resch CT (1986) Quinoline sorption to subsurface materials: role of pH and retention of the organic cation. Environ Sci Technol 20:620-627