Toxicity, Accumulation, and Release of Three Polychlorinated Napthalenes (Halowax 1000, 1013, and 1099) in Postlarval and Adult Grass Shrimp, *Palaemonetes pugio*

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INTRODUCTION

Chlorinated hydrocarbons have become ubiquitous in the marine environment, and some of these compounds are readily accumulated to high levels in the tissues of marine organisms (BIDLEMAN and OLNEY, 1974; BAIRD et al., 1975; PEAKALL, 1975). While investigators have directed most attention toward the occurrence and effects of chlorinated pesticides and polychlorinated biphenyls (PCBs) in the environment, polychlorinated naphthalenes (PCNs) have recently been detected in the water and sediments of a South Florida drainage ditch (CRUMP-WEISNER et al., 1974).

The effects of several chlorinated hydrocarbons on marine organisms have been studied. NIMMO et al. (1975) found that the PCB, Aroclor 1254, was toxic to grass shrimp (Palaemonetes pugio), and was accumulated in the tissues to concentrations of 0.2, 1.0, and 10.0 ppm when the shrimp were exposed for 3 to 5 weeks to water concentrations of 0.04, 0.09 and 0.62 ppb, respectively. They also showed that the shrimp lost most of the PCB within 4 weeks after being returned to PCB-free seawater. By comparison, HANSEN et al. (1974) found that P. pugio concentrated Aroclor 1016 to levels of 1.1, 22.0, and 44.0 ppb after a 96 hour exposure to seawater levels of 0.5, 9.4, and 38.0 ppb, respectively.

As chlorinated hydrocarbons are such important environmental pollutants, it is necessary to study the biological effects of compounds of this type which appear to be potential contaminants. With increasing use by industry of PCNs as substitutes for PCBs, their release into the enviornment appears inevitable. For this reason, we report here the toxicity to and accumulation and release of three PCNs by postlarval and adult grass shrimp, Palaemonetes pugio.

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MATERIALS AND METHODS

Bioassays were conducted to determine the acute toxicity of three PCNs to postlarval and adult grass shrimp, Palaemonetes pugio. The shrimp used in these tests were collected from Galveston, Texas, and were held in the laboratory in 20 o/oo salinity artificial sea water (Instant Ocean, Aquarium Systems, Inc.) at room temperature (19-24°C) for at least one week prior to use in experiments. Postlarvae were obtained from gravid females which were held individually in the laboratory. The larvae which hatched were immediately isolated and reared on a diet of brine shrimp nauplii up to the postlarval stage. All postlarvae were used in the bioassays within 4 days after reaching this stage. Their size at this time ranged from 5 to 7 mm.

The PCNs used in these experiments were obtained from Koppers Company, Inc. (Pittsburgh, Pennsylvania), and are registered under the trademark, Halowax. The compounds used were Halowax 1000, 1013, and 1099. These are described in Table 1. In each case, they were administered in solution to the shrimp using reagent grade acetone as the carrier.

TABLE 1

Chemical and physical characters of three PCNs, Halowax 1000, 1013, and 1099. (From, "Halowax® Chlorinated Naphthalene Oils and Waxlike Solids" The Koppers Co., Pittsburgh, Pa.)

Compound	% Chlorination (by wt.)	Number of Chlorine Atoms per molecule	Physical State
Halowax 1000	26	60% monochlorinated 40% dichlorinated	clear liquid
Halowax 1013	56	10% trichlorinated 50% tetrachlorinated 40% pentachlorinated	white solid flake
Halowax 1019	52	40% trichlorinated 60% tetrachlorinated	white solid flake

In the bioassays, the shrimp were exposed to a range of concentrations (10 to 450 ppb) for a duration of 96 hours. Ten shrimp were held at each concentration; postlarvae were held in fingerbowls in a volume

of 500 ml while adults were exposed in small aquaria in a volume of 4 liters. Acetone controls (shrimp exposed to a volume of acetone equal to the maximum volume of carrier solvent which was used) were maintained with each bioassay. All bioassays were conducted at 20 o/oo salinity and room temperature. The water was changed and redosed every 24 hours at which time the survival of shrimp was recorded. Water samples were taken to determine the concentration of Halowax in solution initially and at the end of 24 hours. LC50 values (concentration of the toxicant lethal to 50% of the test shrimp for a given exposure period) were computed using the method of LITCHFIELD and WILCOXON (1949).

Experiments were then conducted to determine the rates of accumulation of the three PCNs by mature shrimp during 15 days of exposure. For each compound, shrimp were exposed at 20 o/oo salinity and room temperature to concentrations of Halowax of about 40 ppb. Three aquaria were used for each exposure, each holding 60 shrimp in a volume of 34 liters of seawater. Each aquarium was redosed daily and the water was completely renewed every third day. Water samples were taken to determine the exact concentration of Halowax in solution over the 3-day cycle. In addition, all shrimp were fed Tetramin commercial fish food each third day immediately prior to changing the water.

Three tissue samples (8 shrimp per sample) were taken after 1 and 3 days of exposure. Thereafter, the shrimp exposed to Halowax 1000 and 1013 were sampled every third day. Due to rising mortalities, shrimp exposed to Halowax 1099 were sampled on the fourth and fifth day of exposure. The samples were quickly frozen for subsequent whole body analysis of Halowax.

A second group of experiments was conducted in order to determine the rate at which the Halowax was released from the tissues. For each Halowax, shrimp were exposed for 3 days to approximately 40 ppb following the same procedure described above. A group of shrimp was then sacrificed for tissue analysis, and the remaining shrimp were transferred to PCN-free seawater. These were sampled daily for three days and again on the fifth day.

The concentrations of Halowax in the tissues were determined according to the procedure of NEFF and ANDERSON (1975). Shrimp were thawed, blotted, and weighed immediately before being homogenized in 10 ml of spectrophotometric grade n-hexane. The hexane

extract was then put over approximately 2 gm of Florisil for at least 48 hours before determining the absorbance maxima using a Pye-Unicam SP-1800 dual beam recording spectrophotometer. The ultraviolet spectrum for each Halowax was scanned between wavelengths of 210 and 250 nm. The absorbance maxima lying at 224, 239 and 234 nm for Halowax 1000, 1013, and 1019, respectively, were used for quantitation.

Concentrations of Halowax in the water samples were also determined by ultraviolet spectrophotometry. The samples were initially acidified by addition of 1 ml concentrated hydrochloric acid for each 100 ml of sample. Each sample was then extracted with 10 ml spectrophotometric grade hexane, and the absorbance spectra of the extracts were measured in the same manner as described for the tissue extracts.

RESULTS

Analysis of the test solutions of each bioassay revealed that the concentrations of Halowax were initially as calculated, but dropped to about one-third of the initial concentration at the end of 24 hours.

The results of the toxicity tests are presented in Table 2. Halowax 1099 (trichloro and tetrachloronaphthalene) was more toxic to grass shrimp than

TABLE 2

Acute toxicity of three PCNs to postlarval and adult grass shrimp, Palaemonetes pugio.

		LC50 (ppb)	
		72 hour	96 hour
Halowax 1000	Postlarvae	-	440
	Adults	370	325
Halowax 1013	Postlarvae	130	74
	Adults	-	-
Halowax 1099	Postlarvae	75	69
	Adults	132	90

Halowax 1013 (primarily tetrachloro and pentachloronaphthalene) and Halowax 1000 (monochloro and dichloronaphthalene). Postlarvae were less tolerant than

adults to Halowax 1099 and 1013, but were more tolerant to Halowax 1000 than the adults. No LC50 values were calculated for adults exposed to Halowax 1013 because mortalities occurred randomly within the range of concentrations tested. It is possible that these shrimp became much more sensitive to this particular Halowax at a certain intermolt stage, and this would result in sporadic deaths at each concentration throughout the exposure period.

The rates of uptake of the three PCNs are shown in Figure 1. Halowax 1099 was accumulated to the highest concentration (12.0 ppm) in whole body tissues while Halowax 1000 was accumulated to the lowest level (2.6 ppm). Halowax 1013 was concentrated to an intermediate level of 8.2 ppm. In each case, accumulation was rapid for the first three days, after which the tissue concentration appeared to plateau at a relatively stable level. Halowax 1099 was concentrated in the tissues

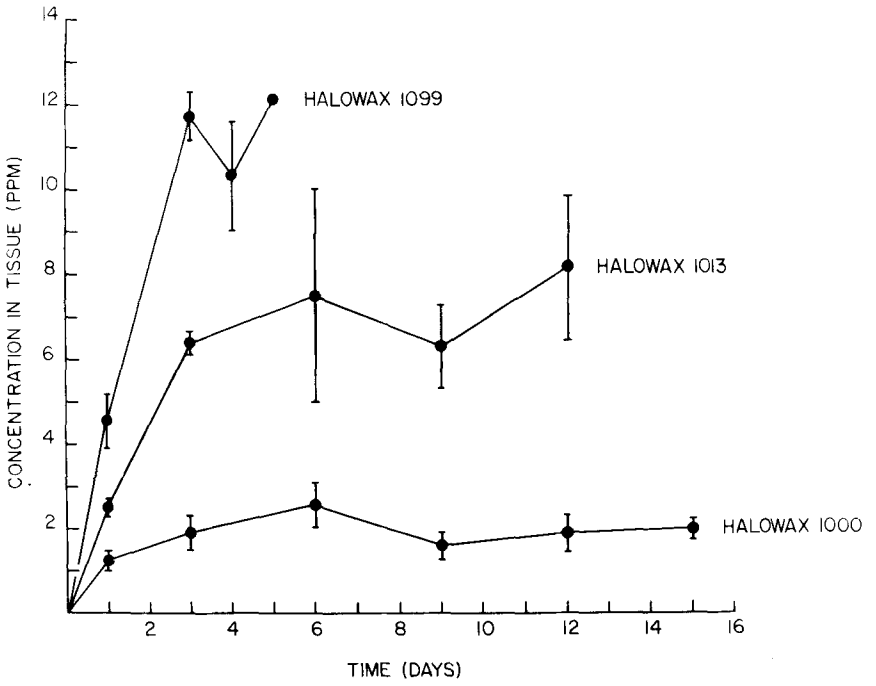

Figure 1. Accumulation of Halowax 1099, 1013, and 1000 in adult Palaemonetes pugio exposed to concentrations of approximately 40 ppb. Vertical lines represent 1 standard deviation.

of adult grass shrimp to a level 257 times greater than the exposure concentration, whereas Halowax 1013 and 1000 reached magnification factors of 187X and 63X the exposure concentration, respectively.

Water analyses were performed to determine the exact concentrations of Halowax in the exposure solutions during the 3-day dosing cycle of these experiments. The results show that the concentrations fell to about one-half of the initial level over each 24 hour period of the cycle (Table 3).

TABLE 3

Analyzed concentrations of Halowax in solution (ppb) during uptake experiments.

		Halowax 1000	Halowax 1013	Halowax 1099
1st day	initial	52.0	49.0	43.5
	final	16.0	17.0	24.0
2nd day	initial	57.5	58.0	59.5
	final	25.0	31.5	37.0
3rd day	initial	64.5	66.5	71.0
	final	31.5	41.0	43.5
Average concentration		41.1	43.8	46.6

Depuration experiments revealed that the accumulated Halowax was greatly reduced after 5 days in Halowax-free seawater (Figure 2). The reates of release of the three PCNs were rapid and appeared to be nearly equal for the first day of depuration. As the concentration of Halowax in the tissues dropped, the rate of depuration decreased, with the release of Halowax 1013 lagging slightly behind that of Halowax 1099.

DISCUSSION

Little information concerning the toxicity and biological effects of PCNs is available in the literature. However, organisms may respond similarly to PCNs and PCBs due to the structural similarities of these compounds. For this reason, biological effects caused by PCBs will be used here for comparison.

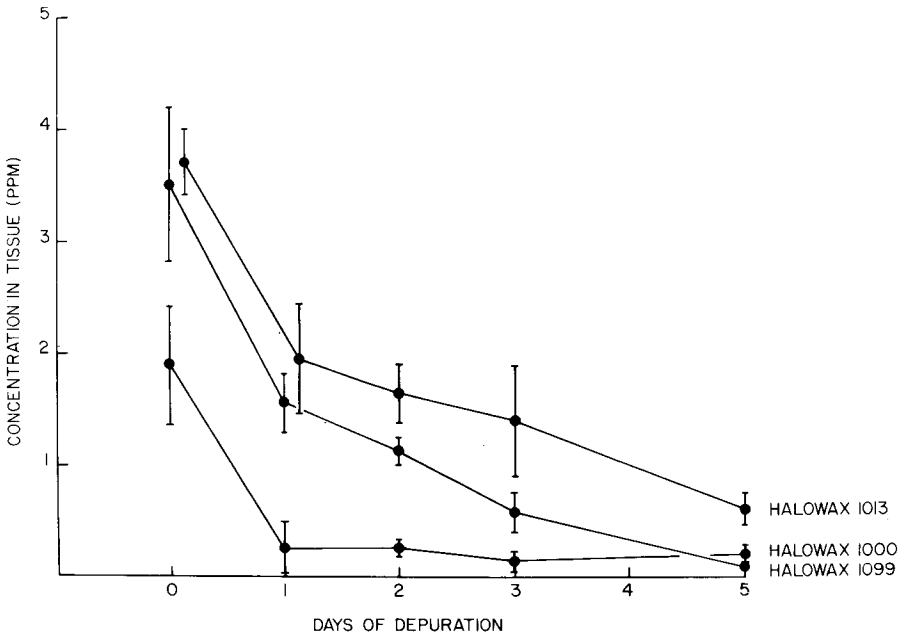

Figure 2. Release of Halowax 1099, 1013, and 1000 from the tissue of adult Palaemonetes pugio were pre-exposed to concentrations of appro approximately 40 ppb for 3 days. Vertical lines represent 1 standard deviation.

Concerning the toxic effects of PCNs, NEFF and GIAM (1976) reported that Halowax 1099 was not acutely toxic to juvenile horsehose carbs (Limulus polyphemus) at concentrations up to 80 ppb. However, they found that exposure to 80 ppb Halowax 1099 produced a 50% mortality to juveniles in the first tailed stage after 27 days of exposure. They were unable, on the other hand, to produce a 50% mortality in the second tailed stage juveniles exposed to this same concentration for 96 days. In addition, they found that the PCB, Aroclor 1016, was slightly more toxic to this organism than Halowax 1099.

Using a continous-flow exposure system, NIMMO et al. (1975) found that a two-week exposure to Aroclor 1254 at a concentration of 4.0 ppb was toxic to P. pugio. They noted that acute tests (48 hour exposure) did not show the true sensitivity of marine species to this PCB and stated that chronic bioassays (one week or more) revealed toxic levels 100 times greater than

acute levels. Also using a continous-flow exposure system, HANSEN et al. (1974) found that grass shrimp (P. pugio) exposed to 0.5, 0.4, and 38.0 ppb Aroclor 1016 for 96 hours exhibited mortality rates of 33, 38 and 93%, respectively. ROESIJADI et al. (1975), on the other hand, conducted static bioassays, exposing P. pugio to Aroclor 1254, and reported 96-hour LC50 values of 64.0 and 7.8 ppb for adults and juveniles, respectively. They also reported a 75% mortality of larval grass shrimp after 6 days' exposure to 15.6 ppb of this toxicant. By comparison, our toxicity values show that the three PCNs studied here are less toxic to grass shrimp than PCBs. Differences in accumulation of the two types of compounds could account for the greater toxicity of PCBs. Whereas grass shrimp accumulated Halowax 1099 in their tissues by a concentration factor of 257X, they were found to concentrate Aroclor 1016 and Aroclor 1254 to levels 1,158 and 16,129 times greater than the exposure concentrations (0.62 and 38.0 ppb, respectively) (HANSEN et al., 1974; NIMMO et al., 1975).

In this study, postlarval grass shrimp were found to be slightly more sensitive than adults to Halowax 1099 and 1013, but were more tolerant to Halowax 1000. In general, it appears that the shrimp are less tolerant to trichloro- and tetrachloronaphthalene (Halowax 1099) than they are to PCNs of lower or higher chlorination (Halowax 1000 and 1013). The results of the accumulation experiments partially account for these differences in toxicity. The degree to which the different compounds were accumulated corresponded to their relative degree of toxicity. This, in turn, could be directly related to the relative ability of the shrimp to metabolize or excrete PCNs of different chlorine content.

CORNISH and BLOCK (1958) found that monochloro- and dichloronaphthalene were more readily metabolized and excreted by rabbits than more highly chlorinated PCNs. GREB et al. (1975) were able to show that monochlorinated, dichlorinated and trichlorinated biphenyls were hydroxylated in vitro by rat liver homogenate. All identified metabolites contained 1 or 2 hydroxyl groups per PCB molecule. However, AKITAKE and KOBAYASHI (1975) showed that pentachlorophenol was accumulated by goldfish (Carassius auratus) and transformed into pentachlorophenylsulfate before being excreted. According to these authors, sulfate conjugation represents a mechanism in fish for the detoxification of chlorophenols.

Increased metabolism and excretion of PCNs with fewer chlorine atoms per molecule could thus account for the low levels of Halowax 1000 in the tissues of the grass shrimp. This greater ability of organisms to detoxify and excrete PCNs with a smaller degree of chlorination would also explain the reduced toxicity of Halowax 1000.

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