Bull. Environm. Contam. Toxicol. 20, 275-279 (1978)

Toxicity of Sodium Pentachlorophenate (Na-PCP) to the Grass Shrimp, *Palaemonetes pugio*, at Different Stages of the Molt Cycle

Philip J. Conklin, and K. Rango Rao
Faculty of Biology, University of West Florida, Pensacola, Fla. 32504

Pentachlorophenol (PCP) and its salt, sodium pentachlorophenate (Na-PCP) are widely used as biocides (BEVENUE AND BECKMAN 1967). Previous investigations indicate that adult crustaceans are more tolerant than fish to PCP and Na-PCP (GOODNIGHT 1942; KAILA AND SAARIKOSKI 1977). The toxicity data for crustaceans are based on short term (usually 96 hours or less) bioassays on individuals whose physiological status in relation to the molt cycle was unknown.

Since changes in the permeability of cuticles are expected to occur in relation to the cyclic shedding, secretion and hardening of the exoskeleton in crustaceans (PASSANO 1960) it is important to evaluate the toxicity of pesticides at known stages of the molt cycle. Although there are reports of an apparent increase in the sensitivity of adult crustaceans during or soon after molting (ecdysis) to toxicants such as copper (HUBSCHMAN 1967), Aroclor^{R} 1254 (DUKE *et al.*1970; NIMMO *et al.* 1971) and Methoxychlor (ARMSTRONG *et al.* 1976) there have been no toxicological evaluations in relation to the different stages of the molt cycle. The crustacean molt cycle is divided into five major stages (A through E) and several substages (DRACH 1939) which can be identified by distinct morphological criteria. The aim of this investigation was to evaluate the toxicity of Na-PCP to the grass shrimp, *Palaemonetes pugio*, at specific stages of the molt cycle using standard 96 hour bioassays.

MATERIALS AND METHODS

Grass shrimp (*Palaemonetes pugio* Holthuis) were collected from grass beds in Santa Rosa Sound, Gulf Breeze, Florida. Shrimp were used within two weeks of collection as it has been indicated that holding the animals in the laboratory for longer periods of time before use may have adverse effects on their response (TATEM *et al.* 1976).

0007-4861/78/0020-0275 $01.00

Test conditions were the same for all experiments. Salinity was 10 ± 1 ‰ while temperature was maintained at 20 ± 1°C with a controlled temperature chamber. These values are well within optimal ranges for *Palaemonetes pugio* (WOOD 1967). The pH of the sea water (natural sea water diluted with distilled water) varied between 7.7 and 8.0. Animals were kept on a 12 hour light-12 hour dark photoperiod with fluorescent illumination.

Animals for the experiments were selected for uniformity of size. Rostrum-telson length averaged 25 mm with a range of about 22-28 mm. Only non-gravid shrimp were used. Selected shrimp were randomly distributed to the test groups and feeding was discontinued during the 96 hour test period.

In order to determine how close the shrimp were to ecdysis, they were individually staged. The staging technique used was similar to one used for lobster larvae (RAO *et al.* 1973). Animals were placed on a standard glass microscope slide, with a drop of seawater under the telson. The telson was gently spread apart with a teasing needle. Generally, water tension was sufficient to prevent the animal from moving or jumping off the slide. The outer edges of the uropods were then examined under 100X magnification. With practice, staging could be done swiftly and without adverse effects on the shrimp.

Using this method, the outer edges of uropods were examined for any sign of epidermal retraction or formation of new setae. Identification of molt stages was based largely on the classification scheme of DRACH and TCHERNIGOVTZEFF (1967). For this study, animals were selected from three general molt stages. Group one, intermolt animals (stage C), showed no evidence of epidermal retraction or new setae formation. Group two, premolt animals (stage D_0), showed obvious signs of epidermal retraction, but had not initiated the formation of setae. The third group consisted of very late premolt animals (stage D_3 - D_4) which completed the formation of new setae beneath the old setae. This last group of animals generally were very close (48 hours or less) to molting.

Na-PCP (supplied by Gulf Breeze Environmental Research Laboratory, Florida) was dissolved in filtered sea water. Solutions of Na-PCP were prepared daily, immediately prior to use, and diluted to the desired concentrations. Standard 96 hour toxicity tests (APHA 1975) were used to determine the acute toxicity of Na-PCP to grass shrimp at known stages of the molt

cycle. Fresh solutions of Na-PCP were used daily and the medium for controls (sea water) was also changed concurrently. Mortality was recorded at 24, 48, 72 and 96 hours after the initiation of the test. A minimum of 20 shrimp in each molt stage were exposed to each concentration of Na-PCP tested. Median lethal concentrations (LC-50) were computed using probit analysis (FINNEY 1971). Confidence intervals (95% level) were computed for the different LC-50 values.

RESULTS

The LC-50 values obtained for the intermolt (stage C) and early premolt (stage D_0) shrimp were similar (Table 1). Substantially lower LC-50 values were noted for late premolt (stage D_3 - D_4) animals at all exposure times. Visual observations indicated that for late premolt animals mortalities occurred shortly after they molted (stage A of the molt cycle). If the animals were able to survive about the first six hours following molt, then they generally survived the test. No deaths were recorded for control animals during the 96 hour tests.

TABLE 1

Toxicity of Na-PCP to *Palaemonetes pugio* at different molt stages

Toxicity of Na-PCP		Molt stage at beginning of exposure C	D_0	D_3-D_4
Exposure				
24 hr	LC-50 (ppm)	4.226	5.886	0.499
	95% C.I.	2.770-7.668	3.623-20.988	0.415-0.583
	slope	2.72	2.19	9.82
48 hr	LC-50 (ppm)	3.536	3.627	0.444
	95% C.I.	2.185-6.261	2.067-7.097	0.371-0.507
	slope	2,52	2.18	15,05
72 hr	LC-50 (ppm)	3.319	3.064	0.436
	95% C.I.	1.958-5.975	1.781-5.086	0.361-0.498
	slope	2.36	2.48	16.53
96 hr	LC-50 (ppm)	2.632	2.743	0.436
	95% C.I.	1.407-4.396	1.378-4.764	0.361-0.498
	slope	2.35	2.18	16.53

DISCUSSION

The toxicity of Na-PCP to *Palaemonetes varians*, *Palaemon elegans* and *Crangon crangon* was reported recently (VAN DIJK *et al.* 1977). Tests on adults revealed that *Crangon crangon* was the most sensitive species (96 hour LC-50: 1.79 ppm) while *Palaemon elegans* was the least sensitive (LC-50: 10.39 ppm). VAN DIJK *et al.* (1977) reported an LC-50 value of 5.09 ppm for adult *Palaemonetes varians*. Our studies show that the sensitivity of adult *Palaemonetes pugio* varied with the stage in the molt cycle. At the most sensitive stage of the molt cycle the 96 hour LC-50 value is 0.436 ppm (95% confidence interval: 0.361-0.498 ppm). This LC-50 value for adult *Palaemonetes pugio* is comparable to that reported by VAN DIJK *et al.* (1977) for the larvae of *Palaemonetes varians* (LC-50: 0.363 ppm; 95% confidence interval: 0.200-0.680) and is less than one-tenth of the value reported for adults of this species.

The relative changes in permeability of the cuticle during the molt cycle may be responsible for the observed increases in the sensitivity of adult grass shrimp to Na-PCP soon after molting. When the old exoskeleton is cast off, the new thin, uncalcified cuticle is exposed. At this time the overall permeability is greater than during the hard shell (intermolt) stage (PASSANO 1960). Preliminary results of the experiments being carried out in our laboratory indicate that the uptake of ^{14}C-PCP is substantially increased immediately after molting.

In contrast to our observations on adult *Palaemonetes pugio*, the larvae of *Palaemonetes varians* did not appear to show increased sensitivity in relation to molting (VAN DIJK *et al.* 1977). The latter may be related to the fact that larvel crustaceans molt frequently and possess much thinner cuticles compared to adults. Therefore, the magnitude of changes in the permeability of cuticle of larval forms in relation to molt cycle may not be as great as that in adults.

The generalization that crustaceans are less sensitive than fish to Na-PCP may not be applicable to all species. The 96 hour LC-50 value obtained in this study with grass shrimp (0.435 ppm) and those obtained by VAN DIJK *et al.* (1977) for decapod crustacean larvae (0.084 to 0.363 ppm) are comparable to the range of LC-50 values reported for fish (0.037 to 0.247 ppm: DAVIS and HOOS 1975; ADELMAN *et al.* 1976).

ACKNOWLEDGMENTS

This investigation was supported by Grant R-804541 -01 from the U. S. Environmental Protection Agency.

REFERENCES

ADELMAN, I.R., L. L. SMITH, JR., and G. D. SIESENNOP: Water Res. 10, 685 (1976).
A.P.H.A.: Standard Methods for the Examination of Water and Wastewater Including Bottom Sediments and Sludges, 14th ed. Am. Publ. Health Assn. Washington, D.C., 1193 pp. (1975).
ARMSTRONG, D. A., D. V. BUCHANAN, M. H. MALLON, R. S. CALDWELL, and R. E. MILLEMANN: Marine Biol. 38, 239 (1976).
BEVENUE, A., and H. BECKMAN: Residue Rev. 19, 83 (1967).
DAVIS, J.C. and R. A. W. HOOS: J. Fish. Res. Bd. Canada 32, 411 (1975).
DRACH, P.: Ann. Inst. Oceanogr. (Paris)(N.S.) 19, 103 (1939).
DRACH, P., and C. TCHERNIGOVTZEFF: Vie Millieu A18, 595 (1967).
DUKE, T. W., J. I. LOWE, and A. J. WILSON, JR.: Bull. Environ. Contam. Toxicol. 5, 171 (1970).
FINNEY, D.J.: Probit Analysis, 3rd ed. Cambridge Univ. Press. London. 333 pp. (1971).
GOODNIGHT, C. J.: Industr. Eng. Chem. 34, 868 (1942).
HUBSCHMAN, J. H.: Crustaceana 12, 33 (1967).
KAILA, K., and J. SAARIKOSKI: Environ. Pollut. 12, 119 (1977).
NIMMO, D. R., R. R. BLACKMAN, A. J. WILSON, JR., and J. FORESTER: Marine Biol. 11, 191 (1971).
PASSANO, L. M.: In: The Physiology of Crustacea, Vol. 1, T. H. Waterman (ed.), p. 473. Academic Press, New York (1960).
RAO, K. R., S. W. FINGERMAN and M. FINGERMAN: Comp. Biochem. Physiol. 44A, 1105 (1973).
TATEM, H. E., J. W. ANDERSON, and J. M. NEFF: Bull. Environ. Contamin. Toxicol. 16, 368 (1976).
VAN DIJK, J. J., C. VAN DER MEER, and M. WIJNANS: Bull. Environ. Contam. Toxicol. 17, 622 (1977).
WOOD, C. E.: Contrib. Mar. Sci., Univ. Texas 12, 54 (1967).