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Acute Toxicity of Pentachlorophenol to the Freshwater Snail, *Gillia altilis*

Robert J. Stuart[1] and James B. Robertson[2]*

[1]Science Communication, Boston University, Boston, MA 02215 and
[2]Department of Environmental Health, East Carolina University, Greenville, NC 27834

Pentachlorophenol (PCP) is one of the most versatile pesticides in use today. Over 80 million pounds of the chemical are produced each year, making it one of the most heavily used pesticides in the United States (Cirelli, 1978). PCP is registered by the Environmental Protection Agency for use as a herbicide, fungicide, bactericide, algicide, insecticide, and as a disinfectant. It is used most extensively by the lumber industry as a wood preservative, protecting against wood-rotting and staining microorganisms. The chemical can easily find its way into aquatic ecosystems through wood treatment plant effluents and run-off from agricultural areas.

PCP has been shown to readily absorb light at 320nm and undergo photodegradation into various compounds, including chlorinated phenols, tetrachlordihdroxyl benzenes, and non-aromatic fragments, such as dichloromaleic acid (Wong and Crosby, 1978). Wong and Crosby (1978) found that the total time required for total photolysis of PCP (in aqueous solution) in sunlight is 5-7 days. Since PCP that reaches aquatic environments could be photolysized, the toxicity of the photodegradation products should also be examined.

Technical grade PCP is 86% PCP, with the remaining 14% of the mixture being composed of tetrachlorophenol, tichlorophenol, hydroxychlorodiphenyl ether, and various chlorinated dioxins and dibenzofurans (Williams, 1982). Since the technical formulation is the one used by industry, the toxicity of this mixture should be examined and compared to that of pure PCP to determine whether it is the parent compound (PCP) or the accompanying impurities that are responsible for any observed toxicity.

*To whom correspondence and reprint requests should be sent.

Considerable work has been done determining the effects of PCP on trout (Hattula et al. 1981), fathead minnows (Phipps et al 1981), and goldfish (Kobayashi, 1978). Several studies have been done with aquatic invertebrates. Grass shrimp have been investigated (Rao, 1978), as have other marine invertebrate species (Rao, 1978). The snail was chosen as an experimental organism because of its wide availability, the ease with which the animal can be maintained in a laboratory environment, and the fact that the snail is a common component of the aquatic ecosystem. Gupta and Rao (1982) studied the acute toxicity of PCP to the pulmonate snail, Lymnaea acuminata, and found the 96 hour LC50 to be 0.6uM PCP (0.16mg/l, reported).

MATERIALS AND METHODS

All snails used in these experiments were collected by hand from a local body of water, the Racquette River. The organisms were then transported to the laboratory in five-gallon buckets containing river water. At the laboratory, the snails and river water were allowed to come to room temperature before the snails were transferred to the holding tanks, thereby avoiding any chance of shock to the organisms. One-gallon glass jars containing two liters of dechlorinated tap water were used as holding tanks. The water used in all experiments was dechlorinated with sodium thiosulfate (Hartz Dechlorination Droplets) before being used. The water in all holding tanks was continually aerated and was replaced daily. The test organisms were acclimated to this environment for at least 48 hours prior to use. With the onset of winter, snails were collected in larger numbers and sustained in two laboratory aquariums, each containing 10 gallons of dechlorinated tap water. These large tanks were aerated and the water was replaced bimonthly. The snails' diet included algae from the river, Tetra goldfish food, and lettuce. The snails were not fed during any of the experiments.

All exposures were carried out in rectangular glass containers, each containing one liter of dechlorinated tap water. The water level was about 4cm. Pure PCP (99%) was obtained form the Fluka, AG Chemical Factory, West Germany. One-hundred millimolar stock solutions were prepared as needed in 95% ethanol. These stock solutions were stored in the dark at $0^{o}C$. A stock solution was discarded once its total accumulated exposure to light reached 30 minutes, and then a fresh solution was prepared. The PCP exposure concentrations were all made by serial dilution of the stock solution. The PCP concentrations examined during the 4 hour test ranged from 0 to 200uM, 1uM PCP equals 0.27

mg PCP/l. After adjusting the chemical concentration, 25 snails were added to each tank. Each group of snails was weighed prior to being placed in the exposure tanks to insure that the mean weight per snail was nearly the same in each tank. The mean weight was 3.0 grams with a standard deviation of 0.07. Following the 4 hour exposure, the snails were removed from the tanks, rinsed briefly in tap water to remove any residual PCP, and then were transferred to gallon glass recovery jars, again containing two liters of dechlorinated tap water, where the snails' progress was followed for 7 days. The water level in the recovery jars was 10cm. All recovery jars were aerated and the water was replaced daily. By changing the water daily, the amount of reexposure of the animals to excreted PCP or its metabolites was reduced.

During this seven day period, dead snails were removed and counted at least every 24 hours. Death was determined by the failure of the snail to respond when its foot was prodded with a dissecting probe. The mortality data from 15% to 85% was then plotted and then dose-response curves were constructed and LC50 values calculated from the results of a linear regression analysis of the data (Armitage, 1973). Controls were exposed to both dechlorinated tap water and the highest concentration of ethanol that resulted from solution preparation. The ethanol by itself produced no toxicity. Water temperature and pH were also monitored (Table 1).

Table 1. Mean & Standard Deviation of Water Characteristics

Water Temp(^{o}C)	Mean	Stand. Dev.
River-Spring	19.0	0.8
Fall	21.0	0.9
Laboratory	21.5	0.5
pH		
River-Spring	6.7	0.1
Fall	6.7	0.1
Laboratory*	6.7	0.1

* pH was tested before and after addition of PCP to the exposure tank. No variation in pH was observed.

This procedure was repeated for the 96 hour exposure, however, due to the longer exposure time, the dose range examined was only from 0 to 10uM. A dynamic 96

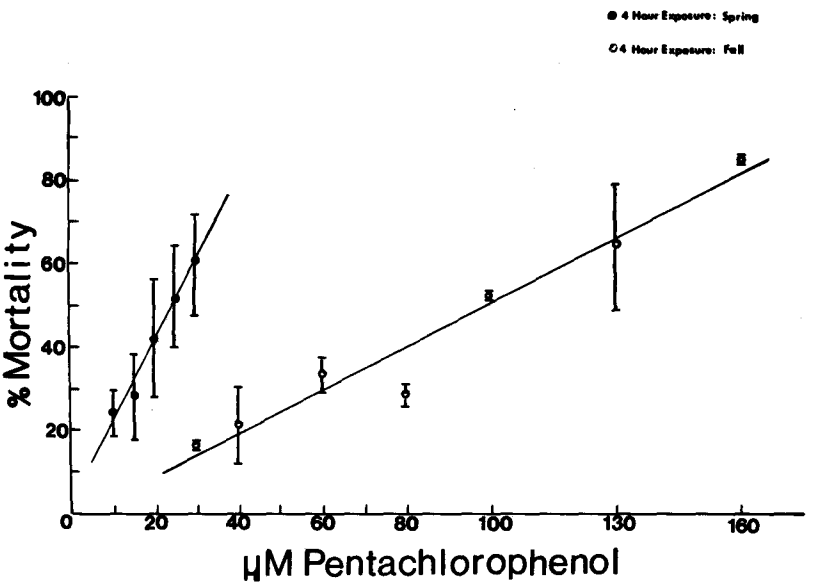

FIGURE 1. DOSE-RESPONSE CURVE AFTER A 4 HOUR EXPOSURE TO PCP. OPEN CIRCLES REPRESENT THE RESPONSE OF SNAILS GATHERED IN THE FALL, DATA POOLED FROM 4 EXPERIMENTS. CLOSED CIRCLES REPRESENT THE RESPONSE OF SNAILS GATHERED IN THE SPRING, DATA POOLED FROM 5 EXPERIMENTS. ERROR BARS REPRESENT ONE STANDARED ERROR. PLOTTED LINE IS THE RESULT OF A LINEAR REGRESSION ANALYSIS AS DESCRIBED IN THE TEXT.

hour test was also performed. In this experiment, the PCP solutions were replaced every 24 hours with a freshly prepared solution. The concentrations used in this dynamic test were 0.8, 1.0, and 1.5uM PCP. Both dose-response curves were plotted and compared as before using simple linear regression analysis (Armitage, 1973).

Since PCP is known to undergo photodegradation (Wong and Crosby, 1978), a 4 hour exposure was performed in the dark. The darkness was achieved by placing an opaque box over the exposure tanks. After the exposure, the snails were allowed to recover in the ambient laboratory light.

Because technical grade PCP is known to contain many impurities, it was compared to pure (99%) PCP using a 4 hour exposure. The 100mM technical grade stock solution was prepared exactly like the 99% stock solution except that the difference in PCP percentage in the technical grade compound was compensated for by adding an extra 16% of technical grade to the stock solution.

RESULTS AND DISCUSSION

It is important to note that we found a large variation in results that seemed to be a function of the time of the year that the snails were collected. This is illustrated in Figure 1. The LC50 value obtained for the

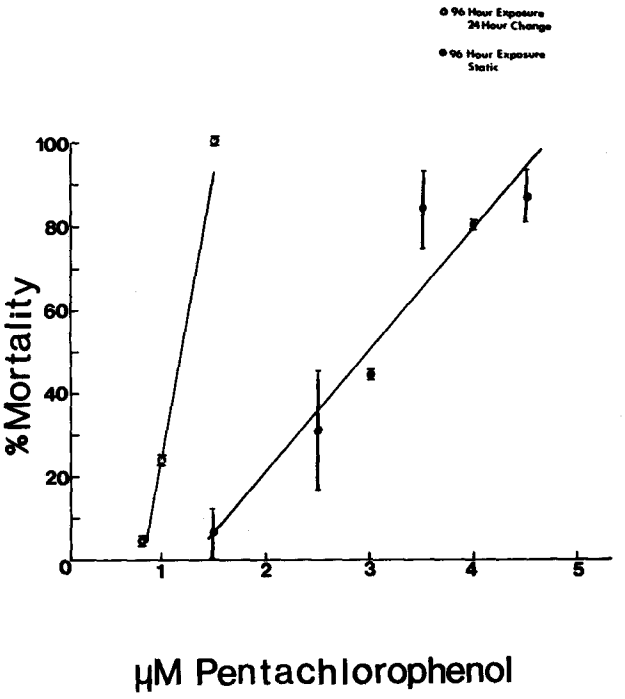

FIGURE 2. DOSE-RESPONSE CURVES AFTER A 96 HOUR EXPOSURE TO PCP. CLOSED CIRCLES REPRESENT THE RESPONSE OF ANIMALS WHOSE EXPOSURE SOLUTION WAS NOT CHANGED. DATA POOLED FROM 5 EXPERIMENTS. OPEN CIRCLES REPRESENT SNAILS WHOSE EXPOSURE SOLUTION WAS CHANGED AT 24 HOUR INTERVALS. DATA POOLED FROM 3 EXPERIMENTS. ERROR BARS REPRESENT ONE STANDARD ERROR. PLOTTED LINE IS THE RESULT OF A LINEAR REGRESSION ANALYSIS AS DESCRIBED IN THE TEXT.

snails tested in the fall (data pooled from 4 replicate experiments) was 100uM PCP (27mg/l) which was four times as great as the spring value (data pooled form 5 replicate experiments) of 24uM PCP (6.5 mg/l). A t-test (Armitage, 1973) of the difference between the slopes of the curves showed them to be significantly different ($P<.01$).

This difference could be attributed to several factors. First, it is possible that the snails, just coming out of dormancy, were less resistant to PCP due to the stress of winter survival when compared to those collected in the fall, which would be expected to be at their peak physical condition. Another possibility could be that differences in the diet and/or lipid content of the animals during the two seasons somehow affected the absorption, distribution or metabolism of PCP, and while we carefully weighed the animals to assure a similar exposure in moles of PCP per gram of snail, no attempt was made to see if wet weight differed from dry weight during the different seasons.

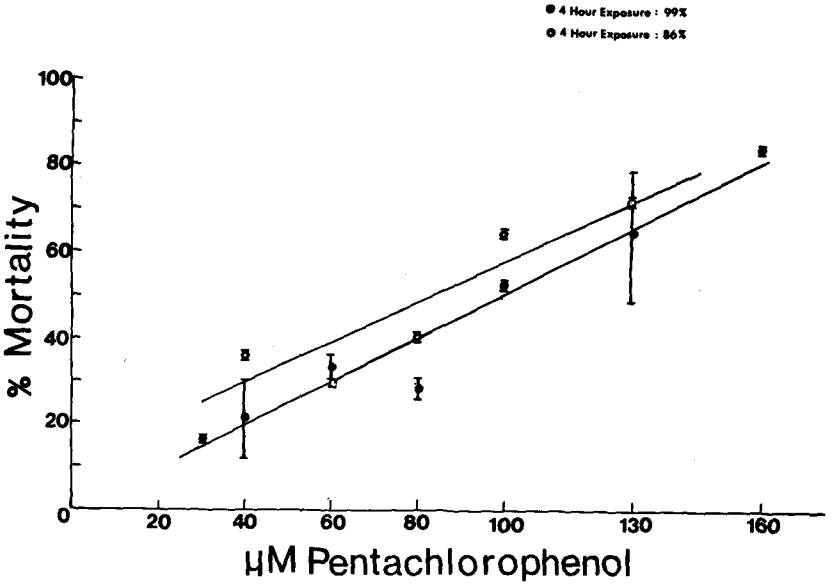

FIGURE 3. EFFECT OF LIGHT ON PCP EXPOSURE. CLOSED CIRCLES REPRESENT THE RESPONSE OF FALL SNAILS EXPOSED UNDER AMBIENT LIGHT CONDITIONS, DATA TAKEN FROM FIGURE 1. OPEN CIRCLES REPRESENT THE RESPONSE OF ANIMALS EXPOSED IN THE DARK AS DESCRIBED IN THE TEXT. ERROR BARS REPRESENT ONE STANDARD ERROR. PLOTTED LINE IS THE RESULT OF A LINEAR REGRESSION ANALYSIS AS DESCRIBED IN TEXT.

It is possible that the actual grams of snail was less in the spring. Finally, one cannot eliminate the possibility of some other environmental parameter that conditioned the early or late season snail.

In the static 96 hour test, the LC50 value obtained was 3uM (0.81mg/1) for the pure PCP. It is important to note that we observed no difference between spring (3 experiments) and fall (2 experiments) animals. This would seem to indicate that the mechanism, target or action of PCP toxicity could be different under these two different, 4 vs. 96 hr., exposure conditions. The data from the 5 experiments was pooled and one graph was constructed, Figure 2. These results were then compared to those obtained in the dynamic test which had an LC50 of 1.1uM PCP (0.3mg/1). A t-test of the difference between the slopes showed them to be significantly different ($P<.01$). The increased toxicity observed when the solutions were changed could have been due to two reasons. One, the changing of the solutions every 24 hours replaced the material lost due to photodegradation. Also, fresh PCP may have saturated the snails' detoxification capabilities. Either could contribute to the increased toxicity observed in the dynamic test. These differences also illustrated the value of flow-through exposure procedures and could explain in part some of the differences

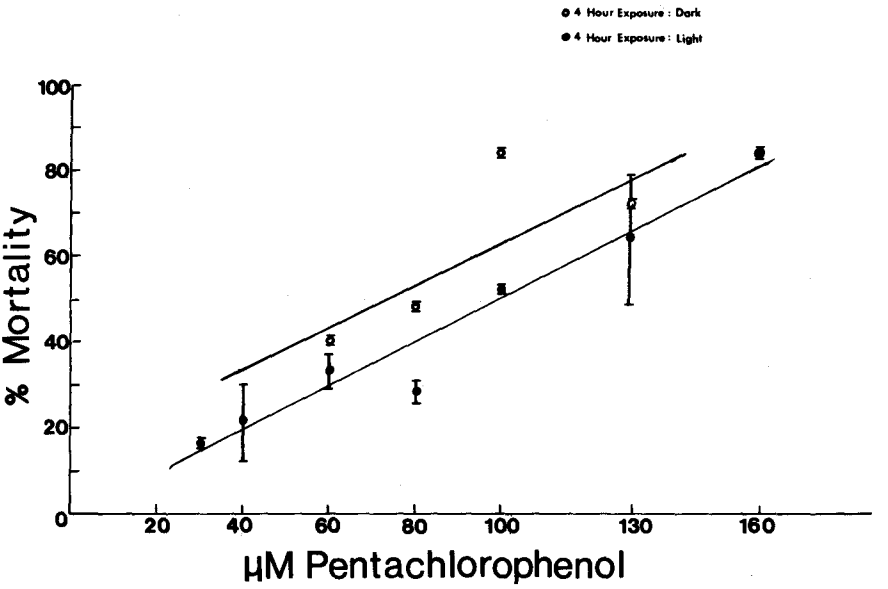

FIGURE 4. COMPARISON OF PURE AND TECHNICAL GRADE PCP. CLOSED CIRCLES REPRESENT THE RESPONSE OF FALL SNAILS EXPOSED TO 99% PURE PCP, TAKEN FROM FIGURE 1. OPEN CIRCLES REPRESENT THE FALL SNAILS EXPOSED TO 86% PCP AS DESCRIBED IN THE TEXT. LINE IS THE RESULT OF LINEAR REGRESSION ANALYSIS AS DESCRIBED IN TEXT. ERROR BARS REPRESENT ONE STANDARD ERROR.

sometimes reported in the aquatic toxicology literature.

The result of our study of photodegradation can be seen in Figure 3. The LC50 for the 4 hour exposure in ambient laboratory light was 100uM, but only 70uM for the exposure carried out in the dark. A t-test of the difference of slopes showed them to not differ significantly from each other ($P>0.2$). The parallelism of the lines indicates that the same mechanism is operating in both experiments and that the concentration of PCP is simply reduced in the light exposed tanks by photodegradation. Also, we can infer that the photodegradation products are less toxic than the parent PCP.

The results obtained in the 4 hour comparative test between pure and technical grade PCP in late season animals is illustrated in Figure 4. The LC50 values observed were 85uM for the 86% PCP and 100uM for 99% PCP. The t-test of the difference between the slopes showed that there was no significant difference between the slopes of the curves ($P>0.2$). This increase in toxicity is presumably due to the impurities in the technical grade PCP. Again, the parallelism of the lines could indicate that the same mechanism was involved in the toxicity of both solutions.

When the LC50 value obtained in the static 96 hour test is compared to values reported for other aquatic organisms, it can be seen that the snail is more sensitive to PCP. Adelman, et al. (1976) reported an LC50 for goldfish of 212uM PCP. The value obtained for fathead minnows by Phipps et al. (1981) was 215uM PCP. The test performed with Lymnaea acuminata, a pulmonate snail, by Gupta and Rao (1982) was a 96 hour dynamic test similar to those performed in this research. The LC50 they obtained, 0.6uM, is certainly in the same range as the 1.1uM reported here for the nonpulmonate, Gillia altilis. In preliminary tests we performed, two types of pulmonate snails were examined, Physa integra and Lymnaea stagnalis. Both of these species of snail were even more sensitive than the two species previously discussed. It is possible that these species could be used as biological monitors of PCP pollution in rivers and streams. Additional research is being conducted along these lines.

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