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Effects of Temperature on the Acute Toxicity of PCP in the Midge *Chironomus riparius* Meigen

Susan W. Fisher

Department of Entomology, 1735 Neil Ave., Ohio State University, Columbus, OH 43210

The pesticide pentachlorophenol (PCP) acts as an uncoupler of oxidative phosphorylation, a target site common to all aerobic organisms. As such, PCP has a broad spectrum of activity against a large number of pests. For example, PCP is highly phytotoxic and so has found favor as a herbicide (McEwen and Stephenson 1979); it is even more widely used as a wood preservative (Jones 1981). PCP is also highly toxic to mammals.

Because of its widespread use and its resistance to degradation, PCP is routinely detected in fresh water and sediment samples (Konasewich et al. 1978). Moreover, PCP has been shown to bioconcentrate in aquatic biota (Metcalf and Sanborn 1975; Trujillo et al. 1982). The biological availability of PCP coupled with its potent biological activity render it a significant hazard.

The potential hazard associated with aquatic contamination by PCP is a function of various water quality parameters. Hazard is reduced as the pH of the water increases because the hydroxyl proton is dissociated at pHs above 4.8 (Kaiser and Valdanis 1982) and penetration into aquatic organisms is reduced (Fisher 1985a). It is also likely that water temperature will affect the toxicity of PCP by altering absorption or by affecting the rate of oxidative metabolism, both of which are sensitive to changing temperature. The results of studies conducted on the acute toxicity of PCP under different temperature regimes are presented herein. These data have important implications for hazard assessment.

MATERIALS AND METHODS

The aquatic invertebrate, Chironomus riparius, was selected as the experimental organism because of its cosmopolitan distribution and importance in aquatic foodchains. The animal was reared in the laboratory according to the method of Estenik (1978).

Median effective concentrations for PCP to the midge at 15, 25 and 35°C were determined using 4th instar larvae of C. riparius. Groups of 20 midges were held in 1-L beakers containing 500 mL soft standard reference water (EPA 1975) adjusted to pH 7.0. Cold reagent grade PCP (97% pure, Aldrich Chem. Co.) was dissolved in

acetone to give a stock solution of 4 mg/mL; dilutions were made from stock using reagent grade acetone to give a range of 5 doses projected to give 5-95% response. The water in each beaker was treated with 1 mL of a given concentration of PCP; control beakers received 1 mL of acetone. Three replicates of each concentration were performed. Treated animals were held at the appropriate temperature in a Forma Scientific (#37422) environmental chamber on a diel photoperiod of 14 hours. The toxicity of PCP was scored at 24 hours, the criterion for which was failure to execute 3 figure-eight motions when pinched with a pair of forceps. Toxicity data were transformed according to the method of Finney (1971) to obtain EC_{50} values and 95% confidence limits. EC_{50} values were judged to be significantly different when confidence limits did not overlap.

The internal concentrations of PCP in the midge over time at 15, 25 and 35°C were determined by treating 1-L flasks containing 1-L of soft standard reference water with ^{14}C-PCP (98% pure by TLC). The radiocompound was purchased from California Bionuclear Corp. and was determined to have a specific activity of 8 mCi/mMole. Each flask received 25 ug of ^{14}C-PCP in 50 uL of acetone. Following a 1 hour equilibration period, 100 4th instar midge larvae were added to each flask. At intervals of 1,3,5,7,21 and 24 hours, groups of 15 midges were withdrawn, dried, weighed and frozen for later analysis by scintillation counting according to the method of Fisher (1985b). Three replicates of each temperature were performed. The data from uptake studies were analyzed by analysis of variance (SAS 1982) to determine statistical significance; means therefrom were ranked using Duncan's (1951) Multiple Range Test.

RESULTS AND DISCUSSION

The toxicity of PCP varies significantly with temperature (Table 1). The compound is most toxic at 35°C (EC_{50}=631 ppm) and least toxic at 25°C (EC_{50}=1,556 ppm). PCP is of intermediate toxicity at 15°C (EC_{50}= 1,176 ppm). Uptake studies were performed to evaluate the contribution of differential absorption to varying toxicity observed in toxicity tests.

Table 1. Toxicity of PCP to C. riparius as a Function of Temperature.

Temperature (°C)	EC_{50} ppm	95% Confidence Limits
15	1,176	816-1,349
25	1,556	1,500-1,631
35	631	581- 674

Several general patterns in the uptake data are discernable (Fig. 1). The initial rate of uptake of ^{14}C-PCP is rapid for all three temperatures. An apparent maximum is reached at 21 hours following which depuration of the chemical appears to exceed absorption. The 24 hour uptake readings are thus slightly depressed relative to the

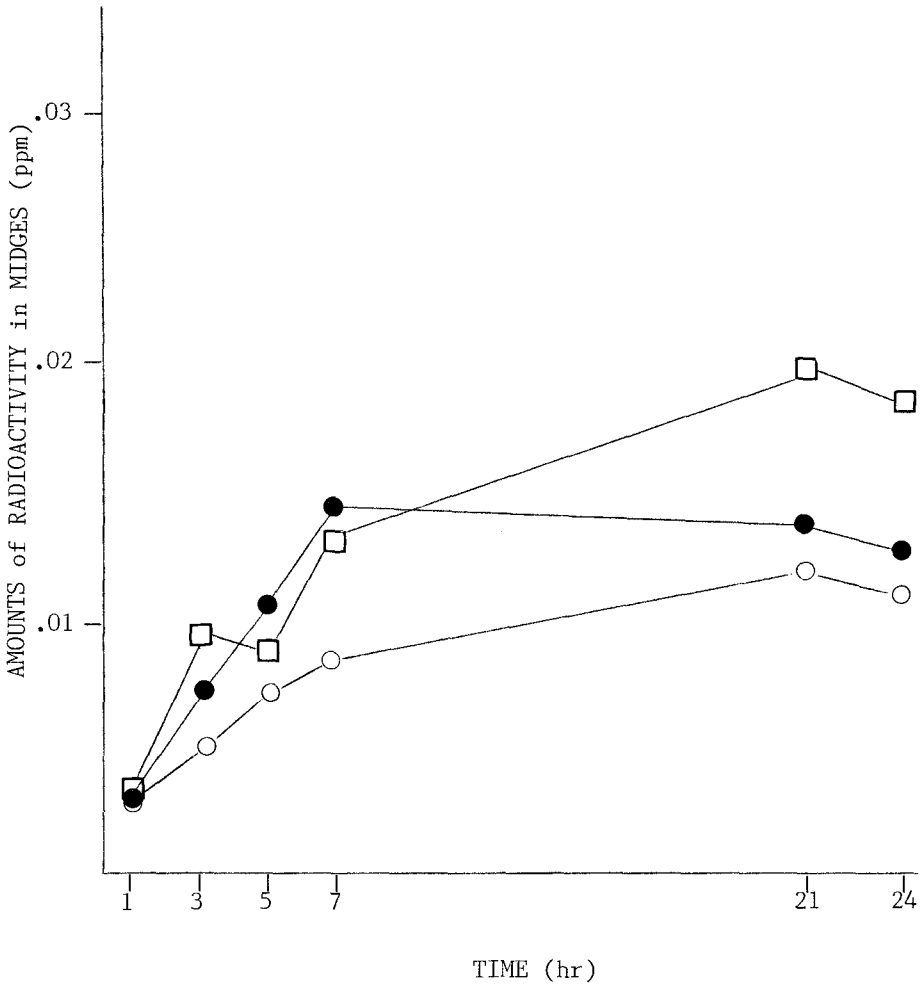

Figure 1. Amounts of Radioactivity (average of three replicates) in Midges held at 15°C, ●–●; 25°C, ○–○; and 35°C, □–□.

respective 21 hour measurements although the latter readings are not significantly different from the former.

Differences seen in toxicity tests are partially explicated by trends arising between treatments in the uptake studies. For instance, the internal concentrations of ^{14}C-PCP at 24 hours decrease in the order 35>15>25 which is completely consistent with the toxicity data. Moreover, significantly higher levels of PCP are present in the midges held at 35°C than those maintained at 25°C ($P<.01$) (Table 2). Thus, the sharp differences observed in the toxicity of PCP at those temperatures may be attributed to differential uptake. While midges absorb more PCP at 35°C than 15°C, the difference is not significant. The statistically different EC_{50}s for the 2 temperatures therefore cannot be due to differential uptake alone.

Table 2. Internal Concentrations of ^{14}C-PCP in Midges at 24 Hours as a Function of Temperature.

Temperature (°C)	Internal Concentration[A] (ppm) ± S.E.
15	$.0126^{ab}$ ± .0020
25	$.0103^{a}$ ± .0015
35	$.0183^{b}$ ± .0020

[A]Numbers followed by the same letter are not significantly different.

Additional causes for observed variation in toxicity at 15 and 35°C may pertain to the sensitivity of the target site to the toxin at the temperatures evaluated in these experiments. Elevated toxicity is common at temperature extremes among nervous system poisons (Norment and Chambers 1970; Reichenbach and Collins 1984) where target site susceptibility as well as absorption respond to variations in ambient temperature. The same may be true for respiratory poisons such as PCP in which case increased uptake coupled with increased respiratory activity at 35°C may act synergistically to produce higher toxicity at this temperature. Thus, statistically indistinguishable doses are absorbed at 15 and 35°C but the effect is quantitatively greater at the latter temperature. A similar situation obtains for the 15 and 25°C comparison. However, in this case, the midge appears to be less sensitive to the toxin at 25°C than it is at 15°C.

These data are of great significance to the development of models for forecasting the hazard associated with aquatic pollutants. In general, hazard is evaluated under a unique set of physical conditions. However, changes in physical parameters can profoundly alter the biological fate of a chemical and its associated hazard. In the case of PCP, acute toxicity is more than twice as high at 35°C than 25°C. Thus, temperature is clearly a factor which must be taken into account when assessing the hazard of aquatic pollutants.

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