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Changes in the Toxicity of Three Pesticides as a Function of Environmental pH and Temperature

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Accurate predictions of pesticide fate and toxicity in aqueous environments are hindered by lack of information on how site-specific water quality parameters affect the biological activity of these compounds. At present, there is a small data base which describes the effects of individual water quality parameters such as pH (Jones 1984; Watson and Maly 1987), temperature (Sparks et al. 1982; Reichenbach and Collins 1984) and water hardness (Clements et al. 1989; Persoone et al. 1989) on pesticide fate. Indeed, where possible, the USEPA water quality criteria are based on pH or temperature adjusted measurements. However, there is little information on what influence simultaneous changes in two or more parameters could have on pesticide toxicity. Since these factors vary simultaneously in nature, it is important to have some estimate of what influence these parameters will exert when varied in concert. In the present study, the influence of simultaneous variation in pH and temperature on the toxicity of three pesticides, pentachlorophenol (PCP), aldicarb and benzene hexachloride (BHC), is considered.

MATERIALS AND METHODS

Fourth instar midge larvae of the benthic invertebrate, *Chironomus riparius,* were obtained from laboratory cultures maintained according to the methods of Estenik and Collins (1979). The pesticides were purchased from Chem. Services (West Chester, Pennsylvania) and were of the following purities: PCP (99%); the gamma isomer of BHC (97%); aldicarb (95%). The toxicity of each compound was measured at pHs 4, 6 and 8. In addition, each pH level was held at one of three different temperatures: 15, 25 or 35°C. For all tests, groups of 20 midges were treated in 1-L beakers containing 500 mL of soft (40-48 mg/L) standard reference water (USEPA 1975) adjusted to pH 4, 6 or 8 with 1 M NaOH, 1 M HCl or 1 M KH_2PO_4 as appropriate. The pH levels in each beaker were checked at 8-hr intervals and, in each case, fell within 0.2 pH units of the original value. Thereafter, midges were added to the water and placed in a Forma Scientific environmental chamber (Marietta, Ohio) and allowed to acclimate to the proper test temperature (15, 25 or 35°C) over a 4-hr period. Following acclimation, the beakers were treated with a pesticide dissolved in 1 mL reagent grade acetone. For each

compound and set of conditions, a range of concentrations, determined to yield 5-95% toxicity in range finding tests, was used. Three replicates of each concentration were made. Toxicity was scored at 24 hr, the criterion for which was failure to execute three figure-eight motions when pinched with a pair of forceps. Since this was a behavioral assay, the data are reported as EC_{50} values.

Toxicity data were subjected to probit analysis (Finney 1971) to determine EC_{50} values and 95% confidence limits. EC_{50} values were considered significantly different when the 95% confidence limits did not overlap.

RESULTS AND DISCUSSION

Variations in pH and temperature significantly affected the toxicity of the three pesticides (Tables 1-3). When the two parameters were varied simultaneously, the results were frequently different than when a single factor was varied.

Table 1. The toxicity of PCP as a function of pH and temperature.

pH	T°C	EC_{50}* (ug/L)	95% confidence limits
4	15	526[a]	535 - 588
	25	384[d]	295 - 435
	35	253[f]	242 - 265
6	15	782[b]	730 - 828
	25	465[e]	446 - 496
	35	415[d]	394 - 441
8	15	2,046[c]	1,976 - 2,098
	25	2,052[c]	1,915 - 2,335
	35	1,263[g]	1,207 - 1,316

*EC_{50} values followed by the same letter are not significantly different

The most striking example of how water quality parameters can affect the toxicity of pesticides was illustrated with PCP (Table 1). An increase of pH from 4 to 8 was associated with a dramatic decrease in toxicity. This change in toxicity is attributable to ionization of PCP as pH goes up. That is, PCP is a weak acid; the pKa of the alcoholic proton is 4.8 (Kaiser and Valdanis 1982). Thus, as pH changed from 4 to 8, increased ionization to the dissociated form occurred. Because the latter is charged, the dissociated species is less lipophilic than the unionized form and it is less likely to bioaccumulate (Fisher and Wadleigh 1986). As a result, toxicity was reduced as pH went up.

Increases in temperature also affected the toxicity of PCP (Table 1). As temperature rose from 15°C to 35°C, toxicity increased significantly at all 3 pH levels. The reasons for this increase in toxicity are not clear. Increased uptake due to increased respiratory activity at higher temperatures may have been responsible. In addition, the target site i.e., electron transport may be more sensitive to the toxicity of PCP at the relatively extreme temperature of 35°C (Norment and Chambers 1970).

Changes in toxicity as a function of environmental parameters for aldicarb and BHC were less clearcut than for PCP. For both of these chemicals, interactions between pH and temperature were evident (Tables 2 and 3).

Table 2. The toxicity of BHC as a function of pH and temperature.

pH	T°C	EC_{50}* (ug/L)	95% confidence limits
4	15	7.3[a]	6.1 - 8.2
	25	29.0[c]	28.0 - 30.0
	35	7.6[a]	6.8 - 8.2
6	15	9.1[b]	9.1 - 9.9
	25	11.2[d]	10.3 - 12.2
	35	9.3[b]	8.7 - 9.9
8	15	6.7[b]	5.1 - 7.8
	25	28.7[c]	17.8 - 63.8
	35	9.6[b]	9.2 - 10.2

*EC_{50} values followed by the same letter are not significantly different.

In the case of BHC, the influence of temperature was fairly straightforward; BHC was most toxic at the temperature extremes (15°C and 35°C) and least toxic at 25°C. This was true for all three pH levels (Table 2). In single factor studies of BHC toxicity, absorption of BHC by midges from water was highest at 25°C, significantly lower at 35°C and lowest at 15°C (Fisher and Wadleigh 1985). Accentuated toxicity at 25°C could thus be due to increased accumulation. Although the accumulation tests were conducted only at pH 6, the present data suggest that the outcome would be the same at pHs 4 and 8 (Table 2). When pH and temperature were changed simultaneously, the results were highly variable. At 25°C, BHC was most toxic at pH 6. However, when the systems were held at 15°C or 35°C, there was very little change in toxicity when pH changed and what little alteration was observed was probably not of any biological consequence. Although an increase in degradative dehydrochlorination of BHC has been observed at alkaline pHs (Metcalf and Sanborn 1975), such dehydrochlorination should be faster at higher temperatures and this was not reflected in the toxicity data.

Aldicarb is an ester of carbamic acid which undergoes pH-mediated hydrolysis to innocuous products (Kuhr and Dorough 1976) and pH-mediated sulfoxidation to the equitoxic sulfoxide and sulfone forms (Hansen and Spiegel 1983). In both cases, the rates of reaction have been shown to increase as pH goes up. Since these reactions are biologically mediated, they should also be responsive to changes in temperature.

Table 3. The toxicity of aldicarb as a function of pH and temperature.

pH	T°C	EC_{50}* (ug/L)	95% confidence limits
4	15	9.9[a]	9.1 - 10.5
	25	8.4[b]	7.8 - 8.9
	35	13.9[d]	13.3 - 15.0
6	15	9.9[a]	9.4 - 10.4
	25	8.0[b]	7.4 - 8.5
	35	11.4[c]	10.9 - 11.9
8	15	9.7[a]	9.3 - 10.1
	25	8.5[b]	7.9 - 9.0
	35	11.4[c]	10.7 - 12.0

*EC_{50} values followed by the same letter are not significantly different.

As can be seen from Table 3, the effect of changing temperature on aldicarb intoxication contrasted with the results obtained for temperature and BHC (Table 2). That is, aldicarb was most toxic at 25°C and least toxic at 35°C. In addition, no pH effect was visible at any specific temperature. The general reduction in toxicity at 35°C across the pH range may reflect increased hydrolysis to nontoxic products although increased sulfoxidation and accumulation at 35°C would be expected to offset the reduction in levels of biologically active compounds. The failure of changing pH to exert an effect on toxicity is difficult to explain. However, it is clear that no combination of temperature and pH dramatically altered toxicity of aldicarb. Indeed, toxicity values ranged from a low of 8.0 ug/L (pH 6, 25°C) to a high of 13.9 ug/L (pH4, 35°C). While narrow confidence limits rendered these values significantly different, it is doubtful that the biological consequences are significant.

In evaluating the mechanisms by which toxicity is altered under changing environmental conditions, it is difficult to separate effects attributable to altered uptake and depuration, increased stability and availability of pesticide and target site sensitivity of the animal. Midges were used in these studies because they survive readily in a variety of settings, many of which are highly polluted, and across a range of environmental variables (Davies and Hawkes

1981). Thus, midges have a natural tolerance for environmental extremes and this should have minimized effects attributable to target site sensitivity. The fact that no mortality occurred in controls held under any of the experimental conditions underscores the midges' tolerance for surviving in a range of environmental conditions. However, it is apparent from the lack of clear-cut trends in toxicity for BHC and aldicarb that interactions between biological and physical variables ultimately determined toxicity of the pesticides.

Changes in temperature and pH both affected the toxicity of the pesticides used in this study. When the two variables were considered individually, temperature emerged as the primary determinant of toxicity for BHC and aldicarb while temperature and pH were both important in dictating the toxicity of PCP. Interactions between temperature and pH altered the patterns of toxicity established by changing single variables. These data emphasize the importance of environmental variables in determining the toxicity of pesticides and suggest that for some chemicals, a hazard assessment will be incomplete unless key site-specific variables are analyzed for their influence on toxicity both individually and in concert with other water quality parameters.

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