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Studies on the Environmental Fate of Carbaryl as a Function of pH

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Abstract. The effects of pH upon the environmental fate of carbaryl were determined in acute toxicity tests, microcosm analyses, and abiotic water stability studies. The toxicity of carbaryl varied significantly with pH in 24-hr toxicity tests with *Chironomus riparius*. Toxicity was greatest at pH 4; the chemical was equitoxic at pH's 6 and 8. Greater amounts of carbaryl were detected in water at pH 4, both in the microcosm and abiotic studies, than at pH's 6 and 8. In spite of the marked persistence of carbaryl in water at pH 4, only minor differences were seen in the amount of parent compound in the microcosm organisms as a function of pH because of the facility with which carbaryl was degraded. The hazard associated with aquatic contamination by carbaryl is affected by pH, but is most significant when contamination of the water exceeds the capacity of aquatic biota to metabolize the chemical. These data underscore the need to consider physical factors which affect environmental fate, particularly in environments in which biotic degradation is minimal.

The insecticide carbaryl (1-naphthyl-*N*-methyl carbamate) is widely used as a replacement for its more persistent organochlorine counterparts. The chemical is highly valued for its rapid insecticidal action and its relatively short environmental persistence (Kuhr and Dorough 1976). Because of its widespread and intensive use (45 million lbs produced in 1971 alone) (NAS 1975), residues of carbaryl have the potential to contaminate surface waters via run-off, movement of contaminated soil, direct dumping and spills. Certain physical conditions, *e.g.*, pH, can affect the rate at which carbaryl is transformed by hydrolysis. Thus, the half-life and, consequently, the hazard associated with aquatic contamination by carbaryl, is dependent upon pH.

The consensus of the literature is that carbaryl is substantially more stable at acidic pH levels than it is under alkaline conditions where hydrolysis occurs rapidly (Wolfe *et al.* 1978; Aly and El-Dib 1971; Sharom *et al.* 1980; Chapman and Cole 1982). Hydrolysis at any pH, furthermore, is primarily a physical process rather than a biological one (Wolfe *et al.* 1978; Liu *et al.* 1981). Thus, changes in pH directly affect the rate of hydrolysis and such influences are operative in any aquatic environment regardless of biotic composition. Changes in aqueous pH do alter the toxicity of carbaryl to various aquatic species including cutthroat trout, amphipods and stoneflies (Woodward and Mauck 1980). Other nontarget organisms are directly affected by carbaryl, although the influences of pH have not been investigated (Murray and Guthrie 1980). It is possible that a change in carbaryl stability or availability at a particular trophic level, as a function of pH, will affect fate throughout an aquatic foodchain. Thus, the significance of pH upon carbaryl fate should be examined, using several trophic levels.

Traditionally, microcosms have been used to examine foodchain effects, transformation and processing of pesticides in multi-species studies. However, Rodgers (1983) recommends the use of fate models in conjunction with microcosms when trying to extrapolate from microcosm data, determined under a unique set of biological and physical conditions, to the various conditions which prevail in the field. An equally valid approach is to test empirically the effects of altered physical conditions on pesticide fate in the microcosms. Fisher (1985a) has shown that the effects of pH on pesticide fate can be examined directly in mirocosms adjusted to

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different pH levels. The fate of carbaryl, its stability, bioavailability, and toxicity are reported herein.

Materials and Methods

Toxicity Tests

The toxicity of carbaryl (99.7% pure, Chem Services, West Chester, PA) to 4th instar larvae of the aquatic midge, *Chironomus riparius* (Diptera: Chironomidae) was determined at pH's 4, 6, and 8. Groups of 20 midges were held in 1-L beakers containing 500 ml of soft standard reference water (US EPA 1975) adjusted to the appropriate pH with 1M HCl or NaOH. The pH of the water was checked at 24 hr and, in every case, fell within 0.1 pH unit of the original value. Carbaryl was dissolved in acetone to give a stock solution of 10 mg/mL; serial dilutions were made from stock to give a range of five test concentrations. The water in each beaker received 1 mL of a given concentration of carbaryl; control beakers were treated with 1 mL of reagent-grade acetone. Three replicates were made of each concentration. Experimental animals were held at 20°C on a photoperiod of 14 hr. Mortality was scored at 24 hr, the criterion for which was failure to execute three figure-eight motions when pinched with a pair of forceps (Detra 1982). Mortality data were subjected to probit analysis (Finney 1971) to determine LC_{50} values and 95% confidence limits. LC_{50} values were significantly different when confidence limits did not overlap.

Microcosm Studies

Microcosm organisms, selected for their compatibility with the pH levels used, were the alga, *Oedogonium cardiacum;* the midge, *Chironomus riparius;* the snail, *Helisoma* spp; the fish, *Gambusia affinis;* the mosquito, *Aedes aegypti*. The following foodchain links were established: excreta → algae → snail; excreta → algae → midge; plankton (introduced with microcosm organisms) → mosquito larvae → fish. All organisms were reared in the laboratory according to the method of Metcalf (1977).

The abiotic component of the microcosm consisted of 3-L of soft standard reference water adjusted to pH 4, 6, or 8 contained in 9.4-L aquaria. Three replicates of each pH level were conducted.

The mirocosms were assembled by adding 300 2nd instar mosquito larvae, 0.5–1.0 g algae, 3 snails, and 50 3–4th instar midge larvae to the water on day 1. The microcosms were treated with 120 μg of ring-labeled ^{14}C-carbaryl in 0.5 mL acetone. The radiochemical, purchased from California Bionuclear Corp (San Diego, CA), was determined by thin layer chromatography (TLC) to be >98% pure and had a specific activity of 20 mCi/mMole. Treated systems were covered and maintained at 20°C on a photoperiod of 14 hr, 5000 foot candles in a Forma Scientific (Marietta, OH) (#37422) environmental chamber. Duplicate 1-mL water samples were removed daily from each microcosm for scintillation counting in 5 mL ^{14}C-cocktail (dioxane/naphthalene/PPO 1000:100:5). Two fish were added on day 5; the experiment was terminated on day 7.

Upon termination of the experiment, fish, snails, and midges were withdrawn, dried by blotting on filter paper, weighed and frozen. Algae was separated from the water with a 6 cm × 6 cm wire mesh screen, washed to remove visible debris, dried by blotting on filter paper, weighed and frozen. The microcosm water was filtered and held at 10°C.

Organisms were extracted by grinding each species with a mortar and pestle in 3 mL acetonitrile. Whole body homogenates were centrifuged in 15 mL conical centrifuge tubes at high speed in a Fisher clinical centrifuge for 5 minutes. The supernatant was decanted and collected; the pellet was resuspended in 3 mL acetonitrile, centrifuged and collected two more times. Combined supernatants for each organism were dried over Na_2SO_4 and filtered. The Na_2SO_4 was washed three times with 3 mL-aliquots of acetonitrile; washes and filtered extracts were combined and the final volume recorded. Duplicate 1.0 mL samples of each extract were removed for scintillation counting in 5 mL ^{14}C-cocktail. The remaining extract was evaporated to a small volume and reconstituted to 3 mL with acetone.

Pellets from each organism were dried and weighed. Weighed samples of each pellet were combined in scintillation vials with distilled water and 0.5 mL NCS tissue solubilizer to dissolve each pellet, following which each sample was neutralized with acetic acid and combined with 5 mL ^{14}C-cocktail for scintillation counting.

In a 1-L separatory funnel, 500 mL of microcosm water were extracted three times with 250 mL aliquots of diethyl ether. Extracts were combined, dried over Na_2SO_4 and filtered. The Na_2SO_4 was washed with 30 mL aliquots of ether; washes and filtrates were combined and the volume recorded. The extract was evaporated to a small volume and reconstituted to 5 mL with acetone, and saved for thin layer chromatography.

A 50 μL volume of each extract and standard (analytical grade standards of carbaryl and 1-naphthol were obtained from USEPA) was applied to a silica gel GF-254 TLC plate. The plates were developed in a moving phase of chloroform/methanol (49:1). Spots were visualized with iodine vapor.

Sections of silica plates containing the visible spots were scraped from the plate into scintillation vials containing 5 mL ^{14}C-cocktail. In addition, all sections corresponding to spots which comigrated with standards were scraped for counting. The remaining silica was divided into 1 cm sections, scraped and collected for counting in a Beckman LS 6800 scintillation counter for 5 min per vial. Compounds which ascended the TLC plate with solvent were considered to be nonpolar; compounds which remained at the origin were considered to be polar.

Data from the microcosm studies were subjected to analysis of variance (SAS 1982) to determine statistical differences; means therefrom were separated by Duncan's (1951) Multiple Range Test. The ecological parameters, ecological magnification (EM) and biotransformation index (BTI) were calculated by the following formulae: EM = amount of parent compound in organism divided by the amount of parent compound in water; BTI = amount of total metabolites (nonpolar and polar) divided by amount of parent compound.

Water Stability Study

The relative persistence of carbaryl was studied in water adjusted to pH 4, 6, or 8 by applying 7.0 μg of ^{14}C-carbaryl in 1 mL acetone to brown glass bottles containing 100 mL of standard reference water. Bottles were capped and held at 20°C in a Forma Scientific environmental chamber. At intervals of 1 day,

Table 1. Toxicity of carbaryl to *C. riparius* as a function of pH

pH	ppb[a] (μg/L)	LC_{50} 95% Confidence limits	Slope (SE)
4	106[a]	79–115	2.63 (0.120)
6	133[b]	120–170	2.48 (0.320)
8	127[b]	120–134	2.57 (0.220)

[a] LC_{50} values with the same letter are not significantly different

14 days, and 28 days, 3 bottles maintained at each of the three pH levels were extracted and subjected to TLC and scintillation analyses in the manner previously described. Water samples (1 mL) were also taken from each bottle before extraction to determine total radioactivity present. The residual water which remained after extraction was analyzed for the presence of radioactivity; the bottles which contained the water were also rinsed with acetone. The latter was subjected to scintillation counting to detect any compound which adhered to the glassware. Data from the water stability studies were analyzed by ANOVA as previously described.

Results

Toxicity Tests

The toxicity of carbaryl to *C. riparius* varies with pH. Within 24 hr, significant differences in LC_{50} values between pH levels were discernable (Table 1). At pH 4, the compound was most toxic; carbaryl was equitoxic at pH levels 6 and 8. No differences were noted in the slopes of the log-concentration probit lines (Table 1).

Microcosm Analyses

Daily water samples from each microcosm (Figure 1) indicated that the levels of radioactivity varied significantly over time ($P < 0.001$) but not because of pH. The levels of radioactivity were highest, immediately after treatment on day 1, and declined thereafter as partitioning into organisms, volatilization, and adhesion to glassware occurred. While the amount of radioactivity present varied significantly over time, the levels of radioactivity did not vary significantly between pH levels on any day. Thus, the microcosm organisms were exposed to equivalent level of radioactivity at all three pH levels. The form of the radioactivity present did, however, vary with pH. Thin layer chromatographic analysis of the microcosm water on day 7 revealed that significantly ($P < 0.02$) more carbaryl was present at pH 4 (Table 2) than at pH's 6 or 8. The results of the abiotic water stability study (Figure 2) confirm that the stability of carbaryl decreases in the order pH 4 < pH 6 < pH 8. Half-lives of 104, 71.6, and 1.4 days for pH's 4, 6, and 8 respectively, were calculated. These values are in accord with previously published results (Aly and El-Dib 1971; Chapman and Cole 1982).

The compound 1-naphthol, a product of carbaryl hydrolysis, was detected in the Day 7 microcosm water at pH's 4 and 6. No 1-naphthol was detected at pH 8.

Two generalizations arise from analysis of microcosm organisms (Table 3). First, in every case except for the fish at pH 8, greater than 50% of the radioactivity measured was unextractable and remained in the pellet. Since unextractable radioactivity is completely metabolized and incorporated into tissues (Metcalf and Sanborn 1975), carbaryl was extensively degraded by all organisms at all pH levels. Secondly, carbaryl represents only a minor fraction of extractable radioactivity, present in any organism. Statistically greater amounts of carbaryl ($P < .05$) were detected in the algae and snails held at pH 4 when an analysis of variance was conducted on carbaryl alone. However, if all metabolites were included in the analysis, the levels of carbaryl present in the organisms did not vary significantly as a function of pH.

Among the individual organisms, the following observations were noted: 1) algae and midges had significantly more total radioactivity ($P < .05$) than did fish and snails. In both the cases of the algae and midges, the very high total radioactivity was contributed by inflation of unextractable radioactivity. That is, the amounts of carbaryl, polar and nonpolar metabolites in algae and midges were not significantly different from fish and snails; 2) the proportion of extractable radioactivity was highest in the fish. However, the amount of carbaryl in fish was not significantly different than that found in other microcosm organisms; 3) the amount of total radioactivity present in the snails and midges decreased significantly ($P < .05$) with pH.

The ecological parameters ecological magnification (EM) and biotransformation index (BTI) reflect the ease of carbaryl degradation. Because of the extensive metabolism of carbaryl at all three pH levels, no statistical differences were seen in EM values between organisms or pH levels (Table 4). The BTI values were greater than 1.0 with the exception of snails held at pH 4.0. No statistical differences in BTI between pH levels were measured but BTI values for snails were significantly lower ($P < .05$) than for the other organisms. Since the parent compound must be present in order to cal-

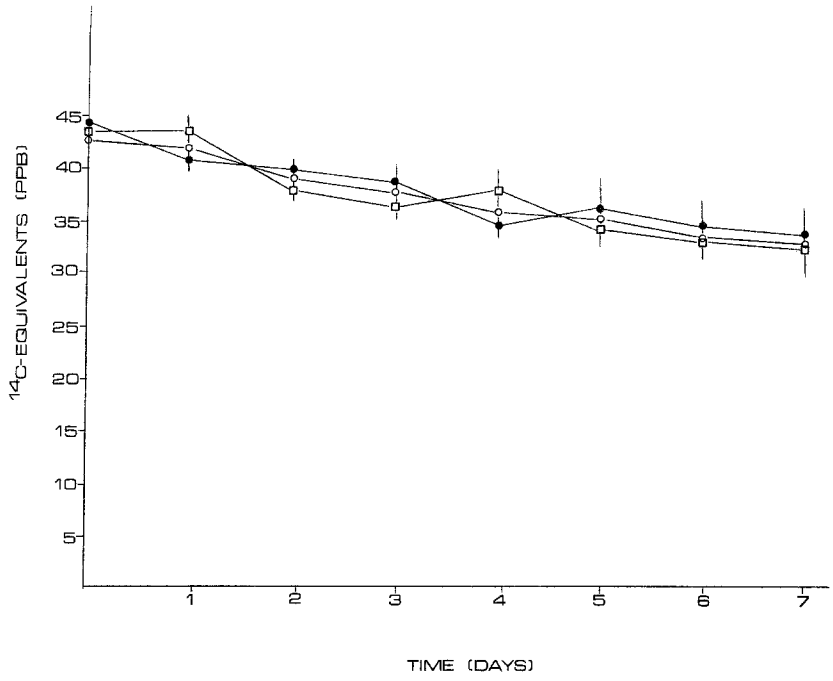

Fig. 1. Amounts of total radioactivity (average of three replicates) in the daily water samples of aquatic microcosms held at pH 4 (●), pH 6 (□), and pH 8 (○). Standard errors are given by vertical lines

Table 2. Distribution of extractable radioactivity in day-7 water of aquatic microcosms treated with ^{14}C-carbaryl

Compound	pH	Average ppm (mg/L)[a]	% Total
Carbaryl	4	0.023[a]	69.2
1-naphthol	4	0.002	6.9
Polar metabolites	4	0.002	7.1
Nonpolar metabolites	4	0.006	17.0
Carbaryl	6	0.015[a]	47.3
1-naphthol	6	0.001	1.7
Polar metabolites	6	0.014	44.9
Nonpolar metabolites	6	0.002	6.1
Carbaryl	8	0.009[a]	26.9
1-naphthol	8	0.000	0.0
Polar metabolites	8	0.022	67.5
Nonpolar metabolites	8	0.002	5.5

[a] $P < .02$

culate EM or BTI, it was not possible to determine these values for snails at pH 8 and fish at pH 6.

Discussion

Acute Toxicity and pH

The mechanism by which pH affected the toxicity of carbaryl to *C. riparius* remains obscure. It is logical to posit that the more rapid breakdown of carbaryl at pH 6 and 8 accounted for the lower toxicity of the chemical, even in 24 hr toxicity tests. Analysis of the microcosm organisms following 7 days of exposure to carbaryl (Table 3) showed that levels of carbaryl in midges were highest at pH 4.0 and nearly equal at pH's 6 and 8. Moreover, Fisher (1985b) and Fisher and Wadleigh (1986) have shown that the pH-specific toxicities of lindane and PCP were directly attributable to pH-specific changes in absorption. However, it is noted that changing pH may have had some deleterious effect upon the physiology of the organism which rendered it more susceptible to the toxicant. Woodward and Mauck (1980), for instance, reported that the toxicity of carbaryl to stoneflies increased 2.6 times when the pH of the water was lowered from 8.5 to 6.5 but amphipods were six times more susceptible at pH 8.5 than at 6.5. Thus, both biological and physical factors may determine the toxicity of carbaryl.

Ecological Effects

The finding that the toxicity of carbaryl to *C. riparius* varied significantly with pH suggests that pH may dictate the ecological fate of carbaryl in aquatic systems generally. Indeed, the increased persistence of carbaryl in water, both in the microcosm study (Table 2) and the water stability study (Figure 2) at pH 4 supports the concern that increased persistence at pH 4 may lead to a general increase in the body burden of aquatic biota either through direct absorption or food chain transfer. Evidence for food chain magnification was not

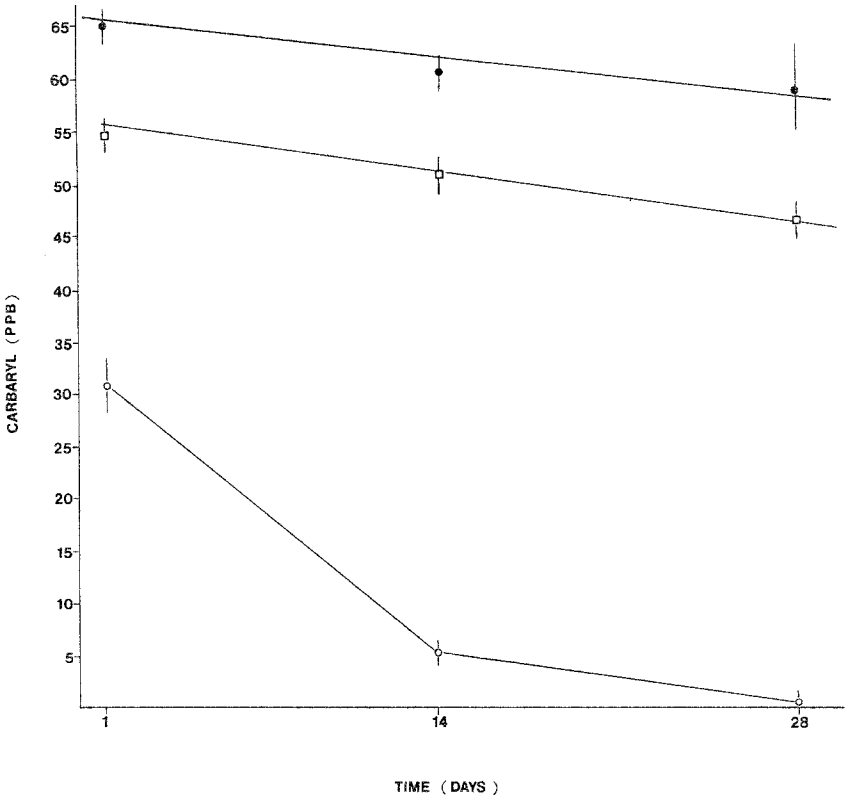

Fig. 2. Amounts of carbaryl in water of abiotic water stability study held at pH 4 (●), pH 6 (□), and pH 8 (○). Standard errors are given by vertical lines

Table 3. Distribution of ^{14}C-equivalents in organisms of aquatic microcosms

	Average ppm (mg/kg)				
Source	pH	Algae	Fish	Snail	Midge
Extractable ^{14}C	4	0.891	0.375	0.254	1.099
Carbaryl	4	0.067	0.013	0.128	0.076
Polar metabolites	4	0.297	0.106	0.008	0.440
Nonpolar metabolites	4	0.526	0.257	0.119	0.583
Unextractable ^{14}C	4	5.365	0.592	0.524	2.549
Extractable ^{14}C	6	0.701	0.457	0.296	0.791
Carbaryl	6	0.058	0.000	0.080	0.036
Polar metabolites	6	0.241	0.227	0.084	0.369
Nonpolar metabolites	6	0.402	0.229	0.132	0.385
Unextractable ^{14}C	6	2.420	0.532	0.433	1.312
Extractable ^{14}C	8	0.625	0.284	0.116	0.759
Carbaryl	8	0.039	0.013	0.000	0.059
Polar metabolites	8	0.155	0.056	0.067	0.409
Nonpolar metabolites	8	0.430	0.215	0.049	0.291
Unextractable ^{14}C	8	6.261	0.246	0.379	1.113

found at any pH. However, BTI values suggest that the ability to metabolize carbaryl is species-specific. Snails, for instance, metabolized less carbaryl at pH's 4 and 6 than the other microcosm organisms. If heavy contamination of the water by carbaryl were to occur, snails and perhaps other benthic organisms, may be proportionately more affected by carbaryl.

Hazard Evaluation

The considerable persistence of carbaryl at low pH's under abiotic conditions suggests that carbaryl may be extremely hazardous when conditions of low pH occur in the field. Under natural conditions, however, the hazard associated with carbaryl contamination of the water will be tempered by

Table 4. Biotransformation indices and ecological magnification values for microcosm organisms as a function of pH

Organism	pH	Biotransformation index	Ecological magnification
Midge	4	13.44	3.25
	6	20.77	2.42
	8	11.88	6.69
Snail	4	0.99	5.45
	6	2.69	5.33
	8	—	—
Fish	4	27.43	0.56
	6	—	—
	8	21.15	1.45
Algae	4	12.26	2.87
	6	11.02	3.88
	8	14.75	4.51

factors in addition to pH. In the microcosm studies, breakdown of the parent compound in the water held at pH's 4 and 6 as well as pH 8 was evident. On day 7, when the experiment was terminated, carbaryl accounted for averages of 69.2%, 47.3% and 26.9% of total radioactivity at pH's 4, 6, and 8, respectively. Thus, degradation of carbaryl occurred in the microcosm water even at pH 4 where extreme persistence was observed in the abiotic samples. These data indicate that carbaryl was being broken down by aquatic organisms, by microbes introduced with the organisms or by light. Of the three agents, photolysis probably accounts for most of the transformation (Sharom *et al.* 1980; Wolfe *et al.* 1978). Volatilization of carbaryl from water also occurs in nature (Stanley and Trial 1980) and accounts for a substantial loss of carbaryl, especially in running water.

While the factors mentioned above operate to reduce the hazard associated with carbaryl even at pHs which encourage persistence, other environmental parameters can accentuate hazard. Carbaryl is more persistent at lower than warmer temperatures (Aly and El-Dib 1971) and under anaerobic conditions (Liu *et al.* 1981). When conditions of low temperature, dissolved oxygen, and pH occur in combination, the persistence and, potentially, the hazard of carbaryl will be greatest in aquatic systems. In addition, 1-naphthol is acutely toxic to several species of fish (Rao *et al.* 1984; Tilak *et al.* 1981). Thus, elimination of the parent compound may not reduce hazard to all aquatic organisms. In the present study, the impact of 1-naphthol upon microcosm organisms appears to be minimal, since only trace amounts were detected at pH's 4 and 6 in Day 7 water. However, 1-naphthol may have been present in greater amounts earlier in the study, prior to conversion to more polar metabolites. It is likely that the compound will be of greater toxicity to susceptible species at acidic pH levels since 1-naphthol is an ionizable acid. Thus, the compound will be more lipophilic at lower pH levels.

It should be emphasized that the microcosms were treated with levels of carbaryl which fell well below those that proved acutely toxic in toxicity tests. In the event of heavy contamination of water by carbaryl, the role of pH in dictating the hazard associated with carbaryl persistence will be substantially greater. At pH 8, acute hazard will be transient, since 50% of the chemical will be degraded within 1.4 days. At lower pH levels, however, hazardous conditions can prevail for weeks or months. Under these circumstances, detoxification mechanisms may be overwhelmed by continual high level exposure resulting in cholinergic poisoning of aquatic animals which would not occur (or would be less severe) at higher pH levels. These considerations are not academic as Gibbs *et al.* (1984) have found that the impact of carbaryl on amphipods was more intense and of greater duration (>2 years) in contaminated ponds at pH 4.0–5.9 than those with water having pH values in the range of 5.9–7.7

Physical factors such as pH are most important in determining the stability of carbaryl in water (Wolfe *et al.* 1978; Liu *et al.* 1981). In the current study, the persistence of carbaryl decreased in the order pH 4 < pH 6 < pH 8. While the difference in carbaryl stability were correlated with significant changes in the acute toxicity of carbaryl to *C. riparius,* the differences were relatively minor. Similarly, the fate of carbaryl in aquatic microcosms was altered only slightly by pH. However, when carbaryl is contributed to aquatic systems in heavy amounts, which may result from spills, accidents, direct spraying or heavy run-off, pH may play a major role in determining the acute and chronic hazards of carbaryl.

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