were found to interfere in the analysis. Some peak broadening and tailing were observed after a number of cherry extracts had been run through the column. This was produced by a build-up of co-extractives on the glass wool end plug of the column at the injection port. Replacement of this plug with a new one whenever the problem developed returned the column to its former efficiency.

Studies performed on samples fortified prior to extraction with both dimethoate and dimethoxon showed good recoveries, as reported in Table II. As dimethoate and dimethoxon were applied and allowed to dry on the surface of the fruit prior to extraction, these results should approximate the recoveries to be expected from the surface of cherries.

The results indicate that residues in excess of accepted tolerances will not be found in cherries treated with dimethoate if recommended treatment rates and application practices are followed.

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Distribution of Carbaryl and 3,5-Xylyl Methylcarbamate in an Aquatic Model Ecosystem

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The distribution and metabolism of ^{14}C-1-naphthyl-labeled methylcarbamate (carbaryl) and ^{14}C-*N*-methyl-labeled 3,5-xylyl methylcarbamate (XMC) were studied in an aquatic model ecosystem containing 10 kg of Matapeake soil, 80 l. of water, and catfish, crayfish, daphnids, snails, algae, and duckweed. Maximum carbaryl concentration (based on total radioactivity) in water was 16 ppb after 7 days, and decreased to 9.5 ppb after 22 days. XMC increased gradually to 35.8 ppb after 34 days. Bioaccumulation ratios were relatively large (2000–4000) for algae and duckweed, but those for snails, catfish, and crayfish were low (100–500). Soil was the major repository for ^{14}C in these microecosystems; the biomass contained between 0.11 and 1.59% of the ^{14}C. Forty-five percent of residual ^{14}C from carbaryl and 38% residual ^{14}C from XMC could not be extracted from soils and appeared to be bound. Bound insecticide residues in soil were nontoxic to daphnids. Only α-naphthol, carbaryl, and XMC were identified in soils.

Organochlorine insecticides have been used intensively worldwide for more than 20 years. Some of these insecticides are very persistent in the environment and have resulted in widespread contamination. Moreover, certain of these insecticides are bioconcentrated in aquatic and terrestial food chains. Organochlorines are being increasingly supplanted by organophosphorus and carbamate insecticides. Since these insecticides are esters, they are likely to be degraded in the environment, metabolized in organisms, or excreted after conversion to water-soluble metabolites. Therefore, significant bioaccumulation of these insecticides would not be expected, but they should be investigated because of their extensive use.

The metabolic pathways of 1-naphthyl methylcarbamate (carbaryl) in animals (Dorough, 1970), plants and insects (Kuhr, 1970), and a soil fungus (Liu and Bollag, 1971) have been studied extensively. However, the fate and metabolism of methylcarbamate insecticides in the aquatic environment and in aquatic organisms have not been investigated extensively. Eichelberger and Lichtenberg (1971) examined the persistence of carbaryl in Ohio river water and showed only 5% remained after 1 week and nondetectable levels by 2 weeks. Johnson (1968) reported that the 48-hr LD_{50} of carbaryl for goldfish was 1.75 ppm. It is more toxic to crustacea than to fish and mollusks, but the reverse is true for its metabolites. Koren (1973) studied the uptake and persistence of carbaryl in channel catfish (*Ictalurus punctatus*). Kazano et al. (1972) found that hydrolysis was the main degradation pathway of carbaryl and 3,5-xylyl methylcarbamate (XMC) in five Japanese paddy field soils. They reported that ring opening of ^{14}C-1,4,5,8-ring-labeled naphthol was relatively slow, and more than 70% of the radioactivity was bound to soil humic substances. In Japan, more than ten methylcarbamate insecticides, including carbaryl and substituted phenyl methylcarbamates, have been used successfully for control of leaf hoppers on rice plants. These insecticides are applied directly to water near streams. When applied in upland fields, they adsorb on soil particles which may be transported into streams by runoff.

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Therefore, research is needed on the fate of these insecticides in the aquatic environment and on aquatic organisms.

This paper reports on the distribution and metabolism of ^{14}C-labeled carbaryl and XMC in an aquatic microecosystem that included soil, water, daphnids, algae, fingerling catfish, and crayfish.

MATERIALS AND METHODS

Methylcarbamate Insecticides. ^{14}C-1-Naphthyl-labeled carbaryl, specific activity 1.17 μCi/mg, and unlabeled carbaryl, 99.85% analytical grade, were furnished by the Union Carbide Corporation. ^{14}C-*N*-Methyl labeled XMC was synthesized from 3,5-xylol and methyl isocyanate and used after recrystallization from ethanol, mp 99.5–100.5° (Krishna et al., 1962). Its specific activity was 6.53 μCi/μg. Purity was checked by silica gel TLC developed with ethyl ether–*n*-hexane (2:1), and detected by ultraviolet light or autoradiography. ^{14}C-1-Naphthyl-labeled carbaryl (16 mg) and unlabeled carbaryl (44 mg) were dissolved in benzene. [^{14}C]XMC (20 mg) and unlabeled XMC (40 mg) were dissolved in acetone.

Soil. The carbamates were applied to air-dried Matapeake silt loam soil (pH 5.3, organic matter 1.5%, and sand, silt, and clay contents of 38.4, 49.4, and 12.2%, respectively) at the rate of 3 ppm (the approximate concentration that would result if recommended quantities of these insecticides were leached or incorporated into the surface 2.5 cm of soil). Fifty milliliters of solution (containing 30 mg of carbamate) was applied to duplicate 1-kg soil samples. After the solvent evaporated, the soil was mixed thoroughly in a V-type mixer for 30 min, then combined with 9 kg of untreated soil and mixed for 1 hr by rolling in a 20-l. glass carboy.

Operation of Microecosystems. After mixing, the soil was uniformly spread in the bottom of glass aquarium tanks (75 × 30 × 46 cm) and covered with a 1-cm layer of pea-gravel and aluminum window screen (to prevent catfish and crayfish from disturbing the soil and thus clouding the water with silt). Tanks were further partitioned with screen into two equal compartments to protect catfish from the predaceous crayfish. Each tank was filled with 80 l. of tap water and aerated with compressed air. The sides of each tank were covered with black vinyl plastic to reduce the proliferation of algae. Control tanks, containing all components except carbaryl or XMC, were also prepared. All treatments were replicated twice, carried out in the greenhouse, and the tanks were placed in a water bath maintained at about 23°.

After 2 days, five fingerling catfish (*Ictalurus punctatus*), three crayfish (*Procambaru* sp.), five snails (*Physa* sp.), about 30 daphnids (*Daphnia magna*), some algae (*Oedogonium cardiacum*), and several duckweed (*Lemna minor*) were added to each tank.

Measurement and Analytical Methods. Two 1-ml water samples were taken from each tank at 2-day intervals, starting 1 day after the water was added, and the radioactivity was determined by standard liquid scintillation methods. All values were corrected for background and controls. After 20 days' exposure to carbaryl and 32 days' exposure to XMC, all organisms were harvested from each tank. The pH of the water at harvest was 8.7. The algae and duckweed were washed in distilled water, dried at 60°, and ground; a portion was then weighed into a ceramic combustion boat covered with Al_2O_3–CuO mixture (5:1), and combusted at about 1000° in a stream of oxygen. The $^{14}CO_2$ was dried by passing through a column of anhydrous $CaSO_4$ and trapped in 10 ml of monoethanolamine–2-methoxyethanol (1:7). A 5-ml aliquot of the trapping solution was assayed for radioactivity. Fish and snails were homogenized in methanol and then filtered. The filtrate was concentrated to 15 ml and the radioactivity was determined. The extracted tissue was dried at 60° overnight, weighed, and combusted. All 80 l. of water, after filtering, was extracted with 3 vol of a solution of *n*-hexane–ethyl acetate (3:1) to 1 vol of water; the extract was concentrated to 500 or 1000 ml, and radioactivity measured. Moist soil was extracted in a blendor for 30 min with *n*-hexane–ethyl acetate (3:1) and then re-extracted with methanol. Each extract was concentrated to a suitable volume and radioactivity measured. Residual soil was dried and combusted, and radioactivity was determined.

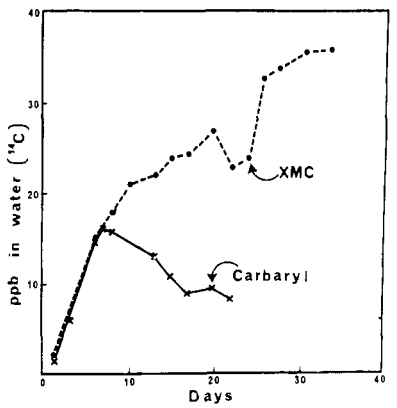

Figure 1. Change in the concentration of carbaryl and XMC (based on ^{14}C) in water.

Aliquots taken from extracts of water, soil, fish, and snails were concentrated to 0.1–0.2 ml and examined for metabolites by preparative silica gel TLC (20 × 20 cm, 2 mm thick), developing with ethyl ether–*n*-hexane (2:1), and detecting spots with ultraviolet light and autoradiography.

RESULTS AND DISCUSSION

All catfish and crayfish in both carbamate-treated tanks survived during the exposure. However, some snails were not recovered in treated tanks, although we could not confirm whether losses were due to natural causes or carbamate insecticides. No daphnids were recovered in carbamate-treated tanks. Daphnids in the control tank grew normally. A bioassay was done to ascertain whether the insecticides were toxic to daphnids. Concentrations of 3 and 30 ppb of carbaryl and XMC were established in small 4-l. aquaria and toxicity observed after 3 hr. Mortality was observed at both 3 and 30 ppb of XMC and at 30 ppb of carbaryl, but the number of organisms surviving the 3-ppb carbaryl treatment was the same as for the controls. Before daphnids died, their movement was extremely erratic and then dead daphnids were detected on the water surface. Although not examined in detail, the growth of algae and duckweed was less in treated tanks than in control tanks.

The change in concentration of carbaryl and XMC (based on ^{14}C) in water with time is shown in Figure 1. Carbaryl mixed in soil slowly desorbed into the water. The concentration of ^{14}C from carbaryl in water reached a peak of 16 ppb after 7 days and then decreased to 9.5 ppb after 22 days. Carbaryl desorbed into water from soil is relatively unstable and is eventually hydrolyzed (Aly and El-Bib, 1971). The XMC concentration in water increased gradually to 35.8 ppb after 34 days. This result suggests that XMC is more stable in water than carbaryl. Eichelberger and Lichtenberg (1971) found that 2-isopropoxyphenyl *N*-methylcarbamate (propoxur) was considerably more persistent than carbaryl in river water. The apparent instability of carbaryl in water (Figure 1) prompted us to end this part of the experiment early.

Distribution of radioactivity among various components in the ecosystems and the percentage of radioactivity in each component (based on the total ^{14}C added to each

Table I. Distribution of Carbaryl and XMC in an Aquatic Model Ecosystem

Component	Carbaryl[a] ecosystem		XMC[a] ecosystem	
	Sum of cpm × 10	%	Sum of cpm × 10	%
Total cpm initially added to each tank	77.659	100.00	105.988	100.00
		Soil		
Hexane–ethyl acetate (3:1) soluble	13.919	17.92	21.688	20.46
Methanol–water soluble	4.033	5.19	8.215	7.75
Remaining in soil	34.746	44.74	40.365	38.09
Subtotal		67.85		66.30
		Water		
Hexane–ethyl acetate (3:1) soluble	0.007	0.01	5.261	4.96
Nonextractable	0.921	1.18	3.870	3.66
Subtotal		1.19		8.62
		Organisms		
Algae	0.024	0.031	1.039	0.981
Duckweed	0.028	0.035	0.452	0.426
Snail	0.002	0.003	0.020	0.019
Catfish	0.004	0.005	0.100	0.095
Crayfish	0.026	0.033	0.062	0.058
Subtotal		0.110		1.59
Total recovery	53.709	69.157	81.069	76.510

[a] Twenty days exposure to carbaryl and 32 days exposure for XMC.

tank) are shown in Table I. Total percent recovery of radioactivity from all ecosystem components was 69% for carbaryl and 76% for XMC, respectively. The loss of radioactivity may be due to gaseous exhaust to air as $^{14}CO_2$, volatilization of the intact insecticide or metabolite, adsorption by some algae that were not recovered, and errors in measurements. The relatively low recovery of radioactivity from carbaryl as compared with XMC suggests that carbaryl is more readily lost in the aquatic environment than XMC. Sixty-eight percent of the added radioactivity remained in carbaryl-treated soil, and only 18% of this residual radioactivity could be extracted with hexane and ethyl acetate (3:1), and 5% with aqueous methanol. About 45% of the original ^{14}C from carbaryl remained as an unextracted residue in soil. This strongly suggests that carbaryl (or degradation products) are tightly bound to soil particles and humic substances. In the XMC-treated tank, 66% of the ^{14}C was found in the soil. Of this, 20% was extracted with the hexane–ethyl acetate solution (3:1), and 8% was extracted with aqueous methanol, leaving about 38% unextracted and apparently tightly bound in soil. Kazano et al. (1972) investigated the degradation of ^{14}C-1,4,5,8-ring labeled naphthol in flooded soils, and reported that more than 70% of the ^{14}C was linked to humic substances. As shown by these results, soil is the major repository for carbamate insecticides in the aquatic microecosystem.

The extreme sensitivity of daphnia to these two insecticides suggested a method for assaying the toxicity of the bound residues. After extraction, the soils were dried for about 2 weeks to remove any solvent residues. One hundred grams of each soil was placed at the bottom of a 1000-ml beaker, covered with filter paper, and carefully flooded with 800 ml of water so as not to disturb the sediment. The water was aerated for 3 days, and then approximately 100 daphnids were added to each beaker and to a control beaker. Although it was difficult to quantitate the bioassay, the number of organisms surviving in the beakers containing bound soil residues did not seem to differ from that in the controls after 4 and 14 days. Soil particles hold XMC residues so tightly that insecticide levels toxic to daphnids would not be attained.

When the experiments were terminated, the ^{14}C in water, based on the concentration originally added to soil, amounted to about 1% for carbaryl and 8.6% for XMC, respectively (Table I). This difference may be due to a greater persistence of XMC than carbaryl in water. Differences in the water solubility [40 ppm at 30° for carbaryl (David et al., 1960) and 182 ppm at 25° for XMC (Uegi and Kanazawa, 1972)] may partially account for the observed ^{14}C content in water.

Although only a very small portion of the initially added radioactivity was distributed in biomass organisms, nearly 15 times as much was found in tissue from XMC treatment as from carbaryl treatment. Unequal stability of the two carbamates in water and organisms and different ^{14}C recoveries may account for this difference. More of the ^{14}C from both carbamates were recovered from algae and duckweed than from fish and snails.

The soil carbamate concentration (based on ^{14}C) decreased, for carbaryl, from 2.43 ppm initially to 1.55 ppm after 22 days and, for XMC, from 2.78 ppm initially to 1.79 ppm after 34 days. However, this decrease was about the same order of magnitude, i.e., 36.2% for carbaryl and 35.6% for XMC. The concentration of both carbamates in each organism was calculated as parts per million on a dried tissue weight basis. Bioaccumulation ratios were calculated by dividing parts per million of dried tissue by parts per million in the final test water. As shown in Table II, the concentration and bioaccumulation ratios of both carbamates were much higher in algae and duckweed (2000 to 4000) than those in catfish, crayfish, and snails (100 to 500). As pointed out by Kenaga (1972), these differences may be partly due to the greater adsorption and absorption rates of organisms that have high surface area to mass ratios. Furthermore, plants and animals may metabolize carbamates at different rates. With the organochlorine insecticides, concentration by algae and bacteria of 500 to 1000 times occurs within a few hours after application (Hannon et al.,

Table II. Accumulation of [^{14}C]Carbaryl and XMC by Various Aquatic Organisms

	Carbaryl		XMC	
Concn in water, ppb	1.66 (1 day) 9.45 (22 days)		2.04 (1 day) 35.8 (34 days)	
Concn in soil, ppm	2.43 (start) 1.55 (22 days)		2.78 (start) 1.79 (34 days)	
Organisms	ppm	BAR[a]	ppm	BAR[a]
Algae	37.9	4000	109.0	3050
Duckweed	34.2	3600	81.0	2300
Snail	2.81	300	9.41	260
Catfish	1.33	140	19.8	550
Crayfish	2.48	260	3.73	104

[a] Bioaccumulation ratio calculated by dividing parts per million of dried tissue by parts per million in water at harvest.

1970; Warnick et al., 1966). It is speculated that algae and bacteria concentrate pesticides by absorption through membranes. However, whether this represents active transport or passive diffusion is not known.

In the hexane–ethyl acetate extract from soil, 9.6% of the recovered activity was 1-naphthol; the remaining 90% (or 16% of that originally added) was carbaryl. This ratio was obtained by eluting the corresponding spots from TLC plates and then counting. Unfortunately, ^{14}C-*N*-methyl-labeled XMC was used, so the ratio of 3,5-xylol to XMC was not easily obtainable. However, the parent XMC was identified by TLC from both soil and water. Unfortunately, the radioactivity was too low in the biomass to detect the parent insecticides or their metabolites.

Although carbamate insecticides have been assumed to degrade rapidly in the environment, our results suggest that some carbamates are relatively persistent in the aquatic environment. Bioaccumulation ratios, especially for algae and duckweed, were relatively high. Therefore, more detailed research on the effect of other carbamate insecticides on aquatic organisms, especially daphnids, at lower concentrations and for longer exposures is needed.

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Binding of 2,4-Dichloro- and 2,4,5-Trichlorophenoxyacetic Acids to Bovine Serum Albumin. A Proton Magnetic Resonance Study

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The binding of two important herbicides, 2,4-dichloro- and 2,4,5-trichlorophenoxyacetic acids (2,4-D and 2,4,5-T), with the protein bovine serum albumin has been studied. The use of the proton magnetic resonance technique to study protein–herbicide binding has been demonstrated. The line widths of methylene and ring protons of 2,4-D and 2,4,5-T showed an increase upon the addition of bovine serum albumin (BSA). These changes have been interpreted in terms of binding to BSA. These studies suggest that the methylene protons are closer to the binding site than the ring protons. The equilibrium constant for the binding process has been determined. The binding shows a decrease with increasing pH.

Once a chemical finds its way into a living system, it may interact with many biological polymers. The interactions will depend upon such factors as the water solubility, lipid/water partition coefficient, and the molecular structure of the chemical. Chlorinated hydrocarbons usually bind to phospholipids (Haque et al., 1973), whereas protein binding may be more important for chemicals possessing increased water solubility. The use of nuclear magnetic resonance technique to study pesticide binding has recently been introduced (Haque, 1974).

2,4-Dichloro- and 2,4,5-trichlorophenoxyacetic acids, commonly known as 2,4-D and 2,4,5-T are two well-known herbicides. These chemicals have been used for some time on a large scale. Although 2,4-D is considered relatively safe to the environment, 2,4,5-T has been linked with toxic dioxin impurities. The mechanism and the mode of action of these chemicals are not very well understood. A qualitative indication of the binding of phenoxy herbicides with proteins and nucleic acids has been reported by monolayer studies (Brian and Rideal, 1952) and equilibrium dialysis measurements (Venis, 1968). However, very little is known about the structural parameters involved in the binding of

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