

Persistence of Carbaryl in the Marine Estuarine Environment. Chemical and Biological Stability in Aquarium Systems

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Colorimetric and radiometric analyses have been used to study the persistence of carbaryl in estuarine water and mud in laboratory aquaria held at two temperatures. In the absence of mud, the carbaryl concentration decreased approximately 50% in 38 days at 8°C. Most of this decrease was accounted for by the production of 1-naphthol. At 20°C. after 17 days, the carbaryl had almost completely disappeared, with 43% converting to 1-naphthol. When mud was present, both carbaryl and 1-naphthol declined to less than 10% in the sea water in 10 days. Both compounds were adsorbed by the mud, where decomposition continued at a slower rate. Radioactive carbon

dioxide was produced in the aquaria containing ¹⁴C carbonyl-labeled and ¹⁴C ring-labeled carbaryl, indicating decomposition by hydrolysis of the carbamate and oxidation of the naphthyl ring. The total recovery of the ¹⁴C activity was only 40%. It is postulated that much of the remainder was evolved as methane. In a preliminary field experiment in which a portion of a mud flat was treated with carbaryl at rates similar to those used in the control of pests of oyster beds, carbaryl could be detected in the mud for 42 days. 1-Naphthol persisted in significant quantities for only one day.

Pesticides may enter estuaries incidentally through their use in forestry and agriculture, or intentionally through their use in the control of estuarine pests. An example of the latter is the use of carbaryl (1-naphthyl-*N*-methylcarbamate) in the control of pests of the oyster (10). The toxic effect of carbaryl in moist soil has been reported to persist from 3 to 6 months (12); however, in aquatic systems the insecticide has generally been used in conjunction with other toxicants and bonding agents (6, 9), which makes it difficult to ascertain its duration of toxic persistence. Stewart, Millemann, and Breese (16) have studied the toxic effects of carbaryl and have shown that 1-naphthol, the principal hydrolytic product of carbaryl, is as toxic as its precursor to some aquatic species.

In field use against pests of oysters, wettable carbaryl has been sprayed at rates up to 10 pounds per acre in shallow areas of the estuary (15). Other formulations have been designed to carry the toxicant to the mud-water interface (6, 9). The stability of carbaryl and 1-naphthol in sea water and in estuarine mud has received little attention other than the observations of biological effect noted above. The research reported here was done in an effort to gather such information. Laboratory experiments have been conducted in studies of the stability of carbaryl and 1-naphthol in natural sea water alone and in the presence of mud from an estuarine mud flat. In addition, a preliminary study has been made of the persistence of carbaryl in the field.

Methods

Chemicals. ¹⁴C carbonyl-labeled carbaryl, specific activity 2.0 mc. per mmole, was obtained from the

Nuclear Chicago Co. The purity was 98%, as determined by paper and thin-layer chromatography.

¹⁴C ring-labeled carbaryl was prepared in the laboratory in a reaction between 1-naphthol-1-¹⁴C and methyl isocyanate (14). The insecticide was isolated and purified by the method of Krishna, Dorough, and Casida (8). The specific activity was 2.3 mc. per mmole.

The carbaryl products used were Sevin, 80 wettable, a finely ground mixture of carbaryl and inert ingredients, and Sevin, analytical grade, furnished by the Miller Products Co., Portland, Ore., and the Union Carbide Corp., New York, N. Y., respectively. The 1-naphthol used was a recrystallized reagent obtained from the Fisher Scientific Co.

Aquarium Studies. Three aquaria, 12 inches in diameter and 6 inches deep, which contained 4 liters of sea water and 2 inches of bay mud, were placed in a constant temperature room at 8° ± 1.5° C. The mud in each aquarium weighed approximately 1400 grams, dry weight. The pH and salinity of the sea water at the start of the experiment were 8.0 and approximately 30 ‰, respectively. The pH was determined periodically throughout the experiment. After a 60-day equilibration period, three aquaria were treated with an ethanol solution of carbaryl to produce concentrations of 5, 10, and 25 mg. per liter of water, respectively. A fourth aquarium, containing only sea water, was treated with carbaryl at a concentration of 10 mg. per liter. These concentrations did not exceed the solubility limit of carbaryl in sea water, which was found to be 31 mg. per liter at 8° C. in preliminary experiments. All aquaria were aerated continuously and were exposed to fluorescent lighting (warm white) for 10 hours each day. The aquaria were covered with glass plates to reduce evaporation. Mud and sea water controls were also maintained.

When the alcohol solutions of carbaryl were added to

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the tanks, part of the insecticide crystallized from solution. Most of these crystals redissolved within 2 days, but part of those which formed in the 25 mg. per liter aquarium adhered to the walls of the container above the water line and did not redissolve throughout the experiment.

Samples of water from the aquaria were analyzed daily for carbaryl and 1-naphthol for the first week and weekly thereafter for 38 days following treatment. The mud was analyzed at 1- to 4-day intervals during the first 8 days of the experiment and once every 7 days thereafter. Core samples were collected with a glass tube, 13-mm. i.d., which was inserted to a depth of 4 cm. into the mud. Three cores were taken from each aquarium at each sampling period. The wet mud samples weighed 15 to 20 grams.

A second experiment, conducted at $20^{\circ} \pm 1.0^{\circ} \text{C}$., involved the use of ring (1-naphthyl- $1\text{-}^{14}\text{C}$) and carbonyl-labeled carbaryl. Three rectangular aquaria, $5\frac{1}{2} \times 9 \times 7$ inches, which contained 2 inches of bay mud and 3 liters of sea water were used. The dry weight of the mud in each aquarium was approximately 2000 grams. The aquaria were exposed to fluorescent lighting (warm white) for 12 hours of each day. Three aquaria were covered with glass lids and sealed with plastic tape to prevent gas leakage. Two half-inch diameter holes, 8 inches apart, were drilled in the covers. One of the stoppered holes was provided with an air inlet tube extending about 2 inches below the water surface and the other opening, which also served as an access port, was equipped with an exit tube leading to a gas-washing trap. The traps were filled with 50 ml. of 2*N* NaOH. The exit side of the traps was connected to a gentle vacuum. A manometer in each system allowed adjustment of air flow to maintain atmospheric pressure in the aquaria.

One aquarium was treated with 6.8 μc . of carbonyl-labeled carbaryl and sufficient cold carbaryl to bring the total concentration of carbaryl in the water to 15 mg. per liter. The specific activity of the carbaryl in the aquarium was calculated to be 0.034 mc. per mmole. A second aquarium was treated with 8.7 μc . of ring-labeled carbaryl and brought to a total concentration of 25 mg. per liter with cold carbaryl for a calculated specific activity of 0.024 mc. per mmole. The insecticide was added to the sea water by dissolving it in 2 ml. of 2-methoxyethanol and washing this solution into the respective aquaria with an additional 3 ml. of this solvent. A sealed control aquarium which contained sea water and mud received 5 ml. of the solvent only. As observed in the previous experiment, there was some crystallization of carbaryl when the solution was added to the aquaria, but the crystals soon redissolved.

Two additional control aquaria were used; one contained sea water fortified with cold carbaryl at 25 mg. per liter, and the second contained sea water and 5 ml. of 2-methoxyethanol. These two aquaria were covered and aerated but not sealed.

Water samples were removed from the aquaria one hour after the application of the insecticide and at 1, 2, 3, 5, 6, 7, 8, 10, 13, and 17 days. Two 1-ml. samples were collected from each aquarium, one for 1-naphthol

and one for carbaryl plus 1-naphthol determinations. In addition 0.2-ml. samples were removed from the aquaria containing ^{14}C for liquid scintillation measurements of radioactivity.

Mud samples were collected as previously described, 3, 5, 8, 11, and 17 days after treatment, frozen, and stored for later analysis and radioassay.

The carbon dioxide traps were changed 1, 2, 3, 4, 5, 8, 10, 14, and 17 days after the experiment began. The NaOH solutions were set aside for later determination of ^{14}C content.

Stability of Carbaryl in Sea Water. Several experiments were conducted at the Oregon State University Marine Science Laboratory at Newport, Ore., by one of us (N.E.S.), to gather information on the effect of temperature, quality of water, and light on the stability of carbaryl and 1-naphthol dissolved in sea water. Solutions of carbaryl and 1-naphthol ranging from 10 to 40 mg. per liter were prepared with filtered sea water which had been diluted with distilled water to a salinity of 25 ‰. Covered 1-gallon glass jars, containing 3 liters of each solution, were held up to 6 days at temperatures ranging from 3.8° to 28°C . under conditions of natural light, artificial light, or darkness. The water was analyzed daily for carbaryl and 1-naphthol.

The effect of water quality on the stability of the two compounds was studied by preparing solutions in distilled water, autoclaved sea water, filtered natural sea water, and filtered natural sea water plus an inoculum from decomposing clam tissue. The pH of each solution was adjusted to 7.8 with phosphate buffer when necessary. Each test solution (400 ml.) was held in a 500-ml. flask at room temperature.

Persistence of Carbaryl in Mud Flats. In order to obtain data on the persistence of carbaryl under actual field conditions, the boundaries of two 25×25 foot plots were established on a mud flat in Yaquina Bay, at Newport, Ore. One plot was treated in February 1966 with carbaryl, 80 wettable, mixed in sea water, and applied at a rate of 10 pounds of active ingredient per acre. The second plot served as a control. The insecticide was applied during a low tide period. The temperature of the mud at treatment was 12.5°C . and ranged from 7.5° to 14.5°C . during the sampling periods, which covered 6 weeks. The test plot was subdivided into six areas with sampling sites marked so that samples could be taken from the same site at each collection.

Twelve-inch sections of plastic pipe, 2 cm. in diameter, served as both a sampling tool and storage container. Six-inch core samples were taken from each of three subplots and combined into one sample. Samples from the remaining three subplots were combined as a second sample. The cores were collected at 2 hours and at 1, 2, 4, 8, 16, and 42 days after treatment. All samples were frozen and stored for about one month prior to analysis. The mud cores were cut into three sections: the top 1 inch, the next 2 inches, and the remaining 3 inches. These sections were analyzed separately for carbaryl and 1-naphthol. Dry weights were determined on the extracted samples.

Analyses. Carbaryl and 1-naphthol were determined

directly in the sea water samples following a modified spectrophotometric method for 1-naphthol as described by van Asperen (1). Carbaryl was converted to 1-naphthol by alkaline hydrolysis (11). To determine 1-naphthol, 1 ml. of the sample was added to 8 ml. of 0.2M phosphate buffer, pH 7, followed by 1 ml. of the diazo blue reagent described by this author (1). The absorbance was measured at 600 m μ with a Beckman Model B spectrophotometer after 10 minutes. Combined carbaryl and 1-naphthol were determined by treating a 1-ml. sample with 1 ml. of 0.5N NaOH. After 10 minutes, 0.5 ml. of 1N acetic acid was added. The volume was brought to 9 ml. with buffer, followed by 1 ml. of diazo blue reagent, and the absorbance was measured as before. Carbaryl was calculated by difference. The efficiency of the colorimetric reaction in sea water was 100%.

The mud samples were extracted according to procedures of Claborn *et al.* (3), except that dichloromethane (DCM) was used as the extraction solvent. The extracts were concentrated and after passage through Florisil were transferred to a rotary evaporator. One milliliter of 0.5N NaOH in 80% methanol and 0.1 ml. of diethylene glycol were added and the solvent was removed under vacuum at 10° C. When approximately 1 ml. of liquid remained, another 1 ml. of the methanolic NaOH was added. After 10 minutes the mixture was diluted with 1 ml. of 1N acetic acid and 6 ml. of phosphate buffer, pH 7. The color was developed and the absorbance measured as above after the sample was filtered through Whatman No. 1 filter paper. The 1-naphthol content of the DCM extracts was determined by difference after removal of the 1-naphthol from an aliquot by shaking with 0.5N NaOH (3). Recovery of carbaryl averaged 91%.

In the experiments with ¹⁴C-labeled carbaryl, the moist samples were extracted with acetone instead of DCM. A 20-gram sample, obtained after mixing three core samples, was shaken with three successive 100-ml. volumes of acetone. The combined extracts were concentrated to 100 ml., an aliquot was set aside for liquid scintillation counting, and the evaporation was continued to near dryness. The contents were then dissolved in water and extracted with an equal volume of DCM. The colorimetric procedures were followed as above.

Thin-Layer Chromatography. Extracts of water or mud were spotted on silica gel plates and developed in 4 to 1 ethyl ether-*n*-hexane (5). For two-dimensional migrations the second solvent was 4 to 1 DCM-acetonitrile. Spots were detected by radiometric scanning (of 2-inch plates) and by exposure to x-ray film (2- and 8-inch plates).

Results and Discussion

Juvenile clams and mud shrimp, amphipods, at least two species of worms, and microscopic unicellular algae were observed in aquaria of the first experiment during the 60-day pretreatment period. Upon addition of the insecticide, the crustacea showed visible signs of distress

within 5 minutes and paralysis after 10 minutes. All crustacea had died within 5 days. By the second day after fortification, juvenile clams, nemertean worms, and polychaetes appeared on the surface of the mud. Within a few days, these organisms were also paralyzed. The nemertean worms and polychaetes had died by the 20th day, but some clams apparently survived the treatment and burrowed back into the mud. Yellow and red colonies of microorganisms developed on the mud surface and on the walls of the fortified aquaria. cursory microscopic examination of these cultures showed several species of pennate diatoms, unicellular flagellates, motile bacilli, diplobacilli, and cocci occurring in a gelatinous mass.

There were fewer macroorganisms observed in the aquaria of the second experiment during the one-week pretreatment period. Since the bay mud used in the aquaria had not been sterilized, it was probably rich in microscopic life. By the tenth day after addition of carbaryl to the aquaria, colonies of microorganisms, probably predominantly periphytic diatoms, developed on the surface of the mud and on the sides of the aquaria. This growth, similar to that observed in the first experiment, was heavier in the two treated aquaria containing mud than in any of the control aquaria. A rapid change in microflora seemed to take place during the last 6 days of the experiment and there was evidence that anaerobic conditions prevailed at the end of the experiment. It is likely that biological conditions were abnormal beyond the tenth day of the experiment, although the organisms present were typical of those which occur in mud from shallow regions of an estuary.

The range in pH of the sea water in the four aquaria of the first experiment dropped from 7.5 to 8.1 at the beginning of the treatment period to 7.0 to 7.7 at the end of the experiment. The aquaria containing mud were about 0.3 pH lower than the control tank which contained only sea water. Similar patterns of pH change were observed in the second experiment conducted at 20° C. At the end of this experiment the range in pH had dropped from 7.7 to 8.0 to 6.4 to 6.9. These more acid values probably reflect the anaerobic conditions prevailing at the end of this experiment.

Loss of Carbaryl from Sea Water. After an initial increase in carbaryl concentration in the water of the aquaria held at 8° C., there was a sharp decrease (Figure 1). 1-Naphthol production during this period was slight, indicating that adsorption by the mud was the major reason for the decline. The role of the mud was confirmed by the data from the control aquarium (Figure 1D). Approximately 90% of the carbaryl added to the control aquarium without mud was present as unchanged insecticide or as 1-naphthol, on the 38th day.

Both radiometric and spectrophotometric analyses for carbaryl and 1-naphthol were performed on the sea water in the aquaria of the second experiment (Figure 2). Until the fifth day the radiometric curves, showing total ¹⁴C activity, coincide with the colorimetric curves. At that point about half of the carbaryl had left the water and the balance had been hydrolyzed to 1-naphthol.

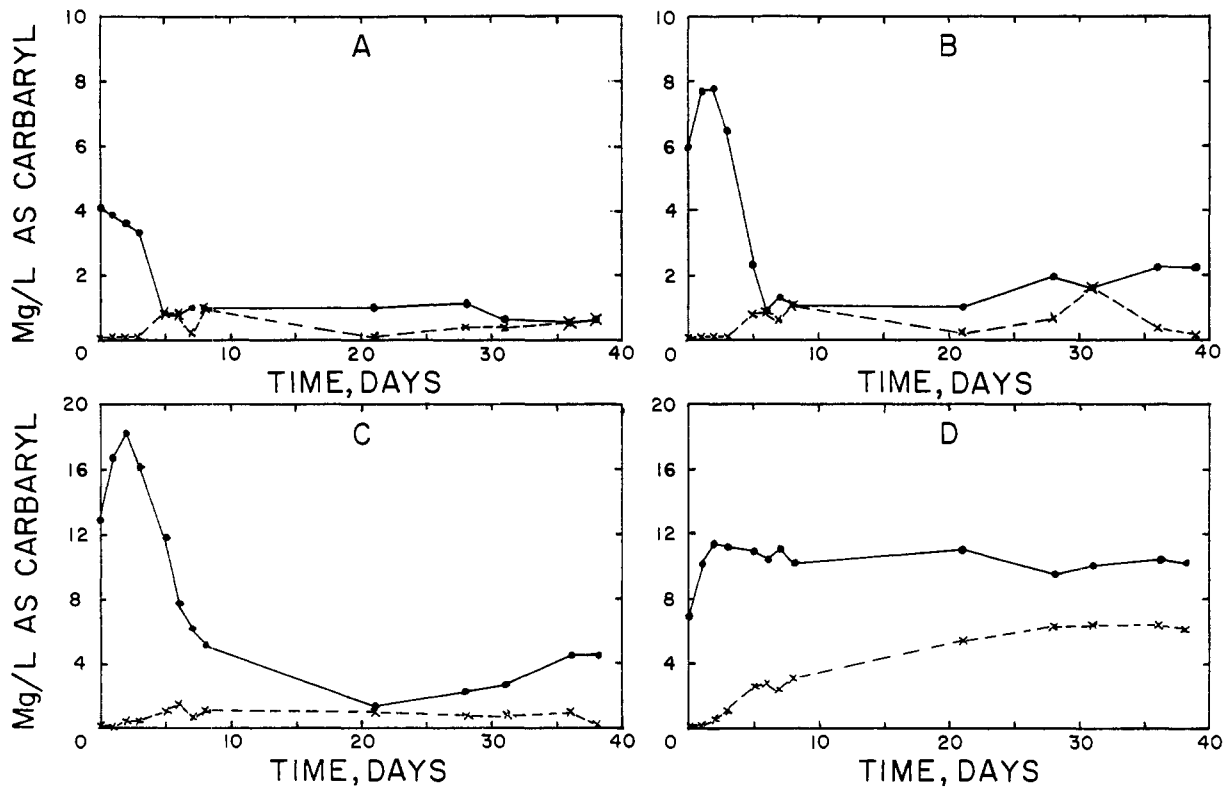


Figure 1. Concentration of carbaryl and 1-naphthol in water of sea water-mud aquaria held at 8° C.

— Carbaryl plus 1-naphthol
 - - - 1-Naphthol
 A. Water treated at 5 mg. per liter
 B. Water treated at 10 mg. per liter
 C. Water treated at 25 mg. per liter
 D. Sea water only, treated at 10 mg. per liter

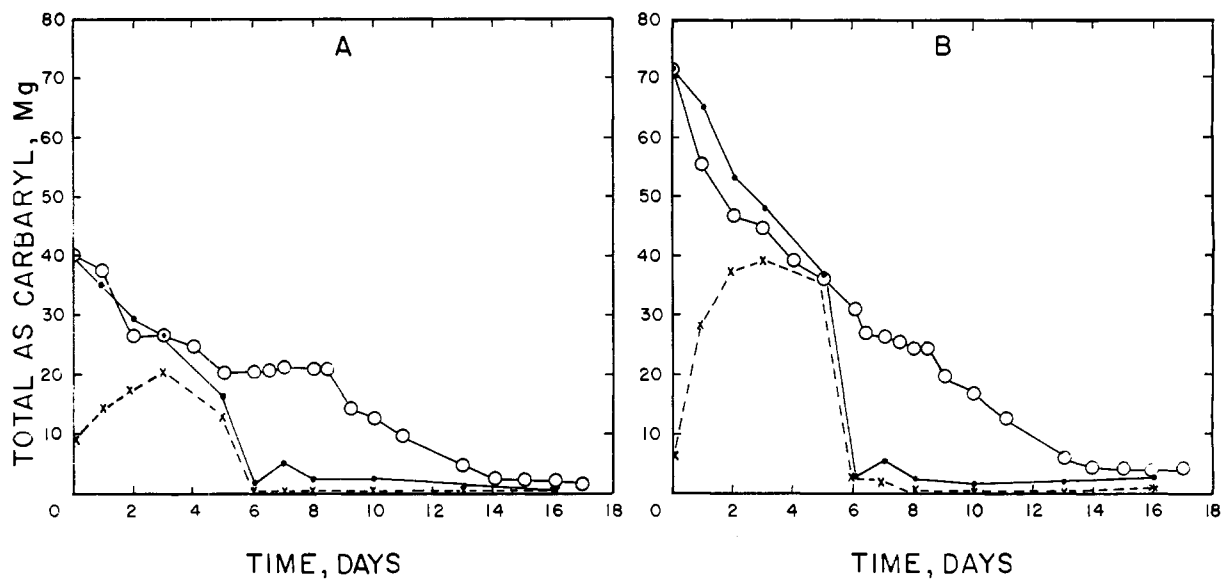


Figure 2. Decline of carbaryl and 1-naphthol in water of sea water-mud aquaria at 20° C. as indicated by colorimetric and radiometric assays

- - - 1-Naphthol colorimetric
 ● Carbaryl plus 1-naphthol colorimetric
 ○ Total ¹⁴C decline
 All values calculated as mg. carbaryl
 A. Water treated at 15 mg. per liter
 B. Water treated at 25 mg. per liter

The 1-naphthol concentration then dropped to a low level; however, traces of unchanged carbaryl remained in the water until the end of the experiment. Other products remained in the water after the 1-naphthol had declined, as indicated by the radiometric curves.

A better comparison of the loss of radioactivity from the water for the carbonyl- and ring-labeled carbaryl is obtained when the loss is expressed as per cent of ^{14}C added (Figure 3A, upper pair of lines). Aside from the short period of time when there is virtually no loss of radioactivity from the water of the aquarium containing carbonyl-labeled carbaryl, the two aquaria show similar ^{14}C loss curves. This indicates that after 6 or 7 days the carbaryl molecule, with carbonyl group intact, has been converted to one more water-soluble compounds. These water-soluble metabolites are lost from the water as the conditions change in the aquaria. The short period of time when there is virtually no loss of radioactivity from the water of the aquarium containing carbonyl-labeled carbaryl may be due to a temporary fixation of $^{14}\text{CO}_2$ by an autotrophic or heterotrophic planktonic organism.

Measurements of carbaryl and 1-naphthol in the water of the control tank of the second experiment gave results similar to those of the earlier experiment (Figure 3B). About 43% of the insecticide could still be accounted for at 17 days, most of it as 1-naphthol.

Uptake of Carbaryl by Mud. The increase in the carbaryl and 1-naphthol levels in the mud of the 8°C . aquaria (Figure 4) corresponds to the decrease in water

concentrations of the two compounds. These mud levels reached a near maximum in about 8 to 12 days and remained near this level for about 30 days. It is apparent that, at 8°C ., hydrolysis of carbaryl proceeds slowly in the mud.

The results of radiometric and colorimetric assays of the mud cores from the experiment with radioactive carbaryl are shown in Figure 5. The radiometric data are for compounds extracted from the mud with acetone. Maximum radioactivity was found in the mud of the tank containing carbonyl-labeled carbaryl on the 3rd day and in the mud exposed to ring-labeled carbaryl on the 5th day. The colorimetric data show that 1-naphthol persists in the mud for only a short time, but that carbaryl can be detected for approximately 3 weeks. Comparison of the radiometric and colorimetric data shows that the naphthol moiety of the pesticide molecule is converted to persistent products.

When the acetone-soluble radioactivity was partitioned between water and dichloromethane, all of the activity from the carbonyl-labeled carbaryl partitioned into the DCM. About 33% of the activity from the ring-labeled carbaryl, however, remained in the water phase. This also indicates that polar degradation products were formed from the naphthyl portion of the carbaryl molecule. Combustion (13) of the acetone-extracted mud from the 17-day sample yielded an additional 8% of the ^{14}C activity initially added to the aquarium. No additional ^{14}C was obtained when mud from the carbonyl-labeled carbaryl treatments was burned.

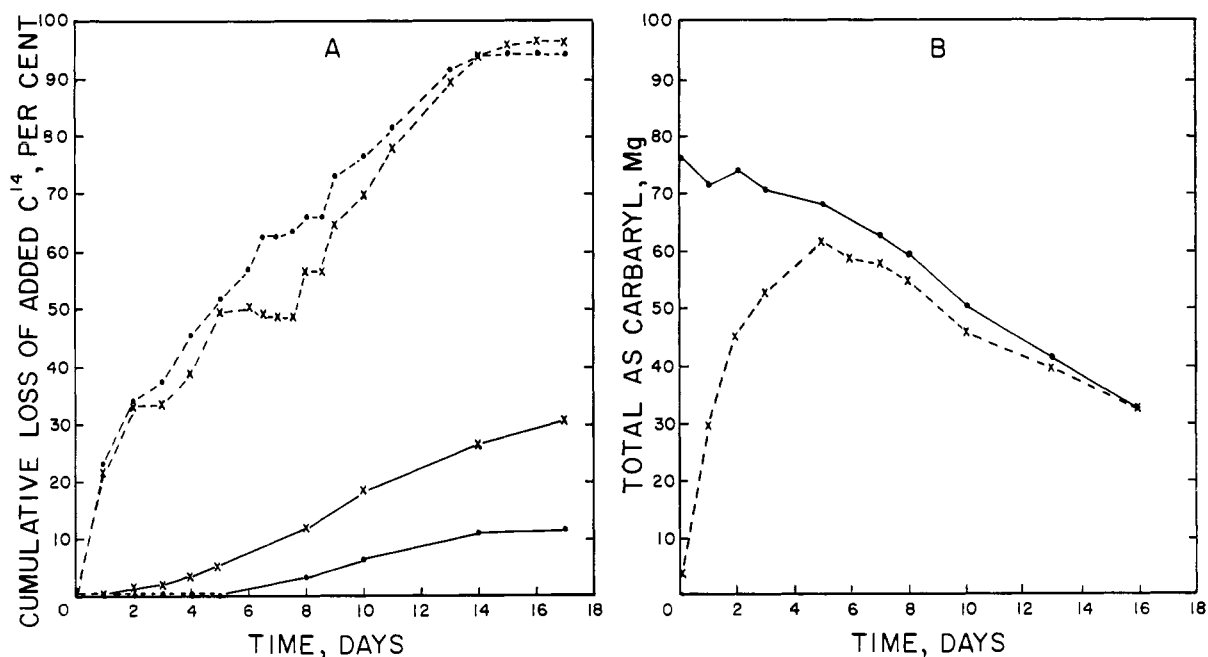


Figure 3. Loss of radioactivity from water

- A. ——— Loss of $^{14}\text{CO}_2$
 - - - - Loss from water
 X ^{14}C -carbonyl carbaryl
 1- ^{14}C -naphthyl carbaryl
 B. ——— Carbaryl plus 1-naphthol vs. time in sea water without bottom mud
 - - - - 1-Naphthol
 Aquarium treated at 25 mg. per liter and held at 20°C

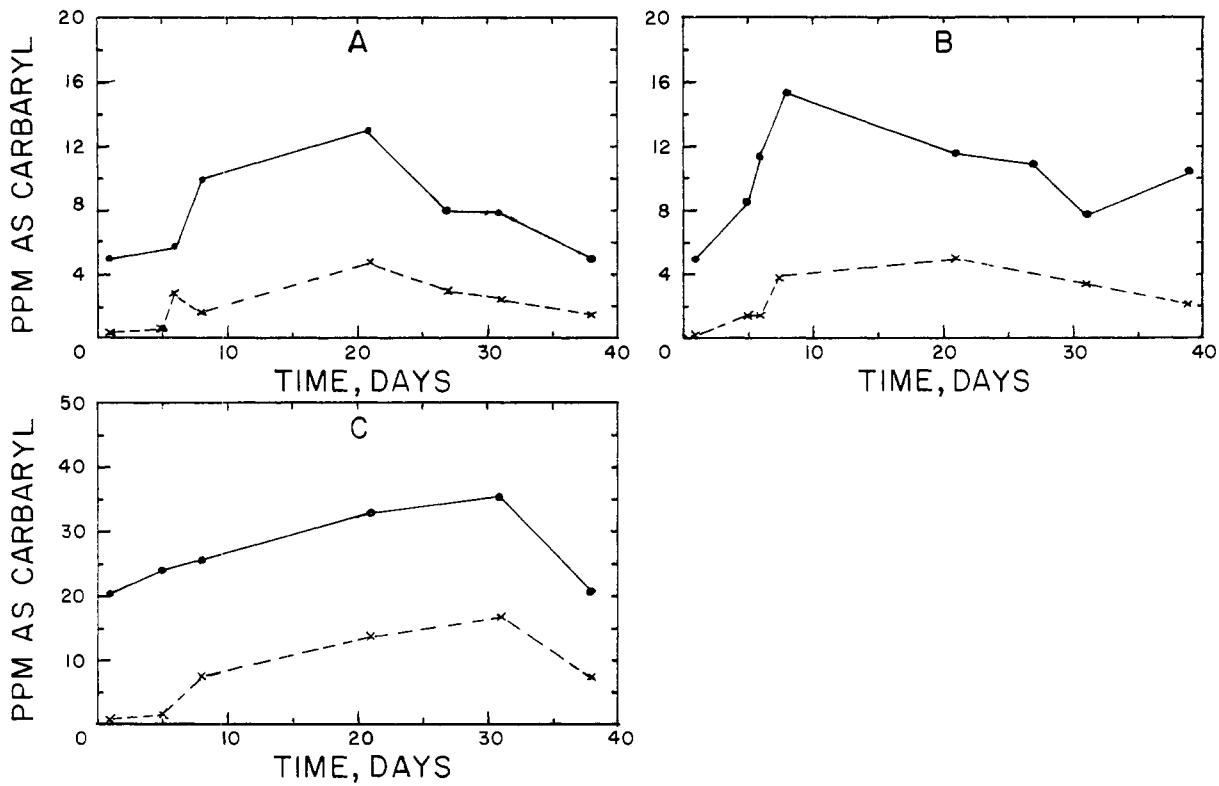


Figure 4. Concentration of carbaryl plus 1-naphthol and 1-naphthol in mud (dry weight basis) of sea water-mud aquaria held at 8° C.

— Carbaryl plus 1-naphthol
 - - - 1-Naphthol
 A. Water treated at 5 mg. per liter
 B. Water treated at 10 mg. per liter
 C. Water treated at 25 mg. per liter

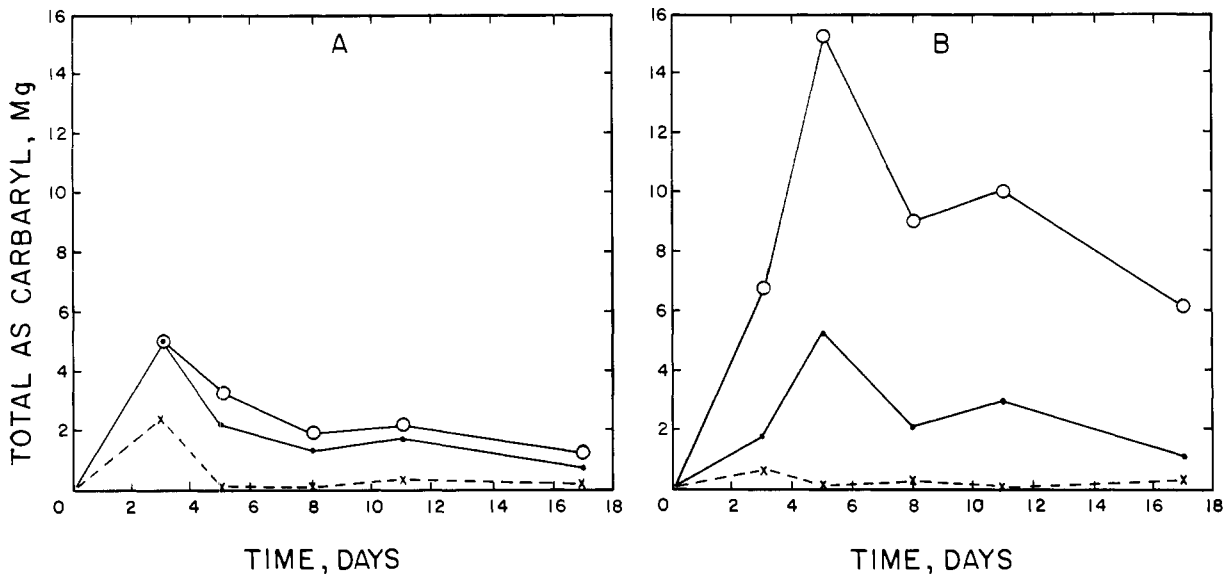


Figure 5. Radiometric and colorimetric assays of mud from aquaria held at 20° C.

- - - Naphthol concentration
 ●● Carbaryl plus naphthol
 ○○○ Acetone-soluble ¹⁴C compounds
 A. From aquaria treated with ¹⁴C-carbonyl carbaryl (15 mg. per liter)
 B. From aquaria treated with ¹⁴C-naphthyl carbaryl (25 mg. per liter)
 Calculation based on dry weight of mud cores

CO₂ Production. A graph of the evolution of ¹⁴CO₂ from the aquaria of the second experiment is shown in Figure 3A. Radioactive CO₂ began evolving from the aquarium containing carbonyl-labeled toxicant during the first day and continued steadily to the end of the experiment to account for approximately 30% of the initial radioactivity. Five days elapsed before ¹⁴CO₂ could be detected in the traps of the aquarium containing ring-labeled carbaryl. From the 5th to the 12th day there was steady evolution of the gas. The curve then flattened, suggesting that this route of degradation had decreased. About 11% of the ¹⁴C activity of the ring-labeled carbaryl was accounted for in this way.

Thin-Layer Chromatography (TLC) Results. At the end of the second experiment the water and mud from the treated aquaria were examined by TLC. Ether extracts of the water from the aquaria containing carbonyl-labeled carbaryl yielded a significant spot which coincided with carbaryl. There was also a slight amount of radioactivity at the origin. Four significant spots were found in the tests with ring-labeled carbaryl: one corresponding to carbaryl, another at the origin, and two of intermediate R_f values. A trace of 1-naphthol was also seen. These results show that most of the breakdown which had occurred by the 17th day involved hydrolysis, either before or after oxidation of the ring. The unknown metabolites show TLC characteristics similar to those discussed by Dorough and Casida (5).

Chromatograms of mud extracted at the end of the experiment showed four major spots in the case of the aquarium containing ring-labeled toxicant, two of these coinciding with 1-naphthol and carbaryl and corresponding closely with those found in the water of the same aquarium. Traces of two additional metabolites were also seen.

The chromatograms of the mud extracts from the aquarium containing carbonyl-labeled insecticide showed a major spot corresponding to carbaryl. On two-dimensional chromatograms two minor regions of

radioactivity separated from the carbaryl region.

Recovery of Added Carbaryl. When the first experiment was completed, all of the mud remaining in the aquaria was removed and weighed, and its carbaryl plus 1-naphthol content estimated from the analyses of the mud core samples. With the levels estimated in the water, the total carbaryl accounted for colorimetrically was approximately 50% of that originally added.

The total radioactivity recovered from aquaria of the second experiment declined steadily from the day of treatment. The summation of radioactivity in CO₂, water, and mud of this experiment is shown in Table I. The final values are based on an extraction of approximately 75% of the mud remaining in each aquarium.

Two explanations can be offered for the failure to account for all of the carbaryl added to the aquaria: Degradation produced a volatile substance not trapped in alkali, or a metabolite not extracted from mud with acetone. As mentioned earlier, combustion of the extracted mud yielded some additional radioactivity in one case, but the amount was far short of that needed to account for all of the insecticide added to the aquaria. Thus the first possibility, production of a nonreactive gas, remains as the most plausible explanation of the loss. Methane-producing bacteria use carbon dioxide, formic acid, and other simple compounds as sources of energy, while producing methane (2). Such organisms are known to be present in mud flats of estuaries.

Stability of Carbaryl in Sea Water. Experiments done in the dark on the stability of carbaryl in sea water showed that at low temperatures (3.5° C.) no hydrolysis of 10 mg. of carbaryl per liter could be detected after 4 days, and at 8 days only 9% of the compound was hydrolyzed. The amount of carbaryl hydrolyzed in 4 days at 17° C. was 44%; at 20° C., 55%; and at 28° C., 93%. Fluorescent light seemed to have a slight accelerating effect on hydrolysis in one experiment at 20° C., with hydrolysis averaging approximately 63% in 4 days in the dark and 72% under fluorescent light. Carbaryl concentrations fort his experi-

Table I. Distribution of ¹⁴C among CO₂, Water, and Mud, and Total Accounted for in Aquaria Treated with ¹⁴C-Carbaryl

Fraction	Carbaryl Label	Days after Treatment					
		1	3	5	8	11	17
		% Distribution of C ¹⁴					
CO ₂	Carbonyl	0.5	2.0	5.4	11.1	20.2	30.9
	1-Naphthyl	0	0	0	3.1	7.3	11.5
Water	Carbonyl	78.6	65.7	51.0	43.6	22.1	4.0
	1-Naphthyl	77.0	62.3	49.8	31.4	18.3	7.9
Mud ^a	Carbonyl	2.6	12.2	7.7	4.5	5.1	4.5
	1-Naphthyl	3.1	9.4	20.4	12.6	14.1	8.4
Mud, residue	Carbonyl	0.0
	1-Naphthyl	8.3
Total	Carbonyl	81.7	79.9	64.1	59.2	47.4	39.4
	1-Naphthyl	80.1	71.7	70.2	47.1	39.7	36.1

^a Acetone-soluble ¹⁴C activity.

ment were 10, 20, and 40 mg. per liter, and there was no apparent difference due to concentration.

Low temperature, 3.7° C., also had a stabilizing effect on the degradation of 1-naphthol with no significant change in 1-naphthol concentration appearing during the 4-day test period. However, at the two higher temperatures, 19.5° and 28° C., complete conversion to nondetectable compounds occurred in 4 days. In these cases most of the conversion occurred after a 2-day lag period. Other tests showed that the decline in concentration of this compound was erratic, usually preceded by a 1- to 4-day lag period. Unlike the results with carbaryl, the dilute solutions (10 mg. per liter) were more rapidly destroyed. Degradation was only slightly accelerated by fluorescent light.

The biological quality of sea water appeared not to influence the rate of hydrolysis of carbaryl, pH being the critical factor. However, 1-naphthol was considerably more unstable in the three sea water solutions than in buffered distilled water, pH 7.8.

Experiments designed to study the effect of sunlight on the degradation of carbaryl and 1-naphthol were complicated by other variables, notably temperature. However, it was possible to note a relationship between the stability of 1-naphthol and sunlight, light having an accelerating effect on decomposition.

The results obtained in these studies are in agreement with those of Crosby, Leitis, and Winterlin (4), who noted that ultraviolet light accelerated the breakdown of carbaryl. According to these authors, who made their studies with ethanol and hexane solutions of carbaryl, several decomposition products result from exposure to ultraviolet light, at least two of which have anticholinesterase activity.

Persistence of Carbaryl in Mud Flats. Analysis of the samples collected from the carbaryl-treated mud flat plot (Table II) showed that the pesticide could be detected up to 42 days. This is in agreement with the results obtained in the aquarium studies conducted at 8° C. and in field studies on the degradation of carbaryl

in agricultural soil (7), where its half life was demonstrated to be approximately 8 days and the residue appeared to be completely degraded within 40 days. A sharp drop in the carbaryl plus 1-naphthol concentration occurred during the first 24 hours of our field study, probably reflecting the action of the tide in removing unadsorbed toxicant. 1-Naphthol levels were low after the first day, a result which supports the previous indication that hydrolysis proceeds slowly in the mud. There is evidence that the toxicant penetrates at least 3 inches.

Summary and Conclusion

Studies in static aquaria cannot be fully compared with the dynamic systems of estuaries. However, the results presented here permit the drawing of certain conclusions. At low temperatures and under conditions where adsorption by mud is prevented, carbaryl will be degraded slowly, persisting for several weeks. One of the products of decomposition under these conditions is 1-naphthol, which is converted to unknown products by the action of light. The above processes will be accelerated at higher temperatures.

Under conditions of actual use, however—e.g., application of carbaryl to shallow mud flats for oyster pest control—the pesticide would probably be rapidly removed from water, by adsorption on bottom mud. Degradation proceeds in this medium, ultimately to the rupture of the naphthyl ring to produce carbon dioxide and, possibly, methane. Intermediate products in the degradation process are polar compounds arising from modifications of the naphthyl portion of the carbaryl molecule. Even with such processes, however, carbaryl and 1-naphthol are likely to persist in mud for 2 to 6 weeks.

Acknowledgment

The authors are indebted to James Barbour, Department of Agricultural Chemistry, for the synthesis and purification of 1-naphthyl-1-¹⁴C-N-methylcarbamate.

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Table II. Carbaryl and 1-Naphthol Concentrations in Mud from Mud Flats Treated with 80 Wettable Carbaryl at 10 Pounds of Active Ingredient per Acre

Days after Treatment	Concentrations of Carbaryl				
	Top 1 Inch		2-3-Inch Level		4-6-Inch Level
	Total, ^a p.p.m.	As carbaryl, p.p.m.	Total ^a p.p.m.	As carbaryl, p.p.m.	Total, ^a p.p.m.
0	10.7	5.4	0.34	0.32	... ^b
1	3.8	3.3	0.46	0.46	... ^b
2	4.1	5.2	0.35	0.27	0.06
4	1.5	1.5	0.18	0.18	... ^b
8	2.1	2.2	0.54	0.38	0.04
16	0.5	0.3	0.13	0.10	0.12
42	0.1	0.1	0.20	0.20	0.08

^a Includes carbaryl and 1-naphthol calculated as carbaryl.

^b Sample not analyzed.

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Received for review July 18, 1966. Accepted October 12, 1966. Technical paper No. 2170, Oregon Agricultural Experiment Station. Investigation supported in part by Public Health Service Research Grant 5 R01 EF 00628-02, from the Division of Environmental Engineering and Food Protection.