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Effects of Carbaryl, Diazinon and Malathion on Native Aquatic Populations of Microorganisms

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Insecticides can enter surface water by both direct and indirect routes (BOCKRIS 1977, LEH & LAK 1974). Direct insecticide additions to water for purposes of pest control, and discharges as industrial wastes, are in most cases carefully regulated; however, in our geographical area two types of operations, rice farming and mosquito control, make it likely that insecticides will reach surface waters in repeated doses during relatively long periods of time each year. In mass applications of insecticide, as in mosquito control, the chemical may reach surface water directly, or adsorb on particulate matter from which it may move to surface waters in agricultural or urban run-off. After aerial application of malathion, CONTE & PARKER (1971) found residues in surface water ranging from 0.8 to 3.2 mg/L. In light of reports of the biological degradation of insecticides and of inhibition of a number of algae by some of these chemicals, it seems apparent that these effects may alter the natural balance of indigenous aquatic populations, and that significant effects on water quality may result (LEH & LAK 1974, CONTE & PARKER 1971, BOOKHART & MONROE 1977).

Our objectives in this study were: [1] to determine whether application of carbaryl, malathion, and diazinon will affect indigenous aquatic microorganisms in Lake Houston water, [2] to determine if some major metabolites formed during biodegradation of carbaryl and malathion cause effects similar to the parent compound. Three insecticides frequently used in both agricultural and urban settings for pest control were chosen for initial study: carbaryl, a carbamate, malathion and diazinon, organophosphates. Experiments were designed to determine whether each of these chemicals would affect the total numbers and growth of indigenous bacterial and algal populations in surface water.

MATERIALS AND METHODS

Water for this study was obtained from Lake Houston, the major surface water supply reservoir for Houston, Texas. It was collected in 20-liter polyethylene carboys for transport to the laboratory, placed in four 19-liter glass aquaria and allowed to

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stabilize at room temperature (21±2°C) for one week. Each aquarium had water circulated from end to end through Tygon tubing by a continuous flow pump (1 liter/min), and was illuminated on a 12-h diurnal light-dark cycle by 750 ft-C, 20W cool white fluorescent lights. Temperature and dissolved oxygen were monitored daily with a YSI temperature-dissolved oxygen meter. Evaporation was reduced by covering with foil, and loss was replaced one time during an experiment with autoclaved water from the same source. Samples were collected at daily intervals for bacterial culture, and at weekly intervals for phytoplankton counts. Bacteria were cultured from appropriate dilutions on ¼ strength standard plate count agar. The spread plate method was used and all cultures were in duplicate, incubated for 72 h at 21±2°C. Following counting, isolated colonies were picked for identification with API 20E strips (Analytab Products, Plainview, N.Y.). Warburg respirometry by the methods of UMBRIET (1972) was done using increased bacterial biomass obtained by sub-culture.

Phytoplankton counts and identification to genus were done with concentrated samples. Chlorophyll determinations, total carbon analyses, BOD, ATP, light-dark bottle analyses and algal biomass determinations were made by Standard Methods (AMERICAN PUBLIC HEALTH ASSOCIATION 1975) at different intervals.

Preliminary testing indicated that in concentrations less than 1 mg/L of insecticide, microbial populations were little affected, and between 1 mg/L and 50 mg/L consistent effects were observed. A standard dosage of 5 mg/L was chosen for this study.

All insecticide tests were done in triplicate (i.e., carbaryl added to each of three aquaria, etc.) and were run with one control aquarium. All bacterial plate counts, direct algal counts, Warburg tests, BOD determinations, ATP determinations, carbon analyses, biomass accumulations, and chlorophyll-a determinations were done in triplicate. Results reported are means of these tests.

Data were statistically analyzed for significant differences between control and test counts of bacteria and algae and Warburg results were tested by linear regression and comparison of slopes and intercepts for significant differences (SNEDECOR & COCHRAN 1967).

RESULTS AND DISCUSSION

In all aquaria, following the period of stabilization, culturable bacterial counts were approximately $1x10^4$ in three aquaria, and $1x10^5$ ^{5} in one aquarium which was randomly chosen for treatment with diazinon (Fig. 1). In the aquarium randomly chosen as the untreated control culturable bacteria counts increased gradually, but not significantly, over a period of three days to about $1x10^5$, and following a brief drop were at about $1x10^5$ ^{5} on Days 5 and 7. On succeeding days, the counts returned to about $1x10^4$ (the beginning count) and remained relatively stationary during the remainder of the 14-day test period.

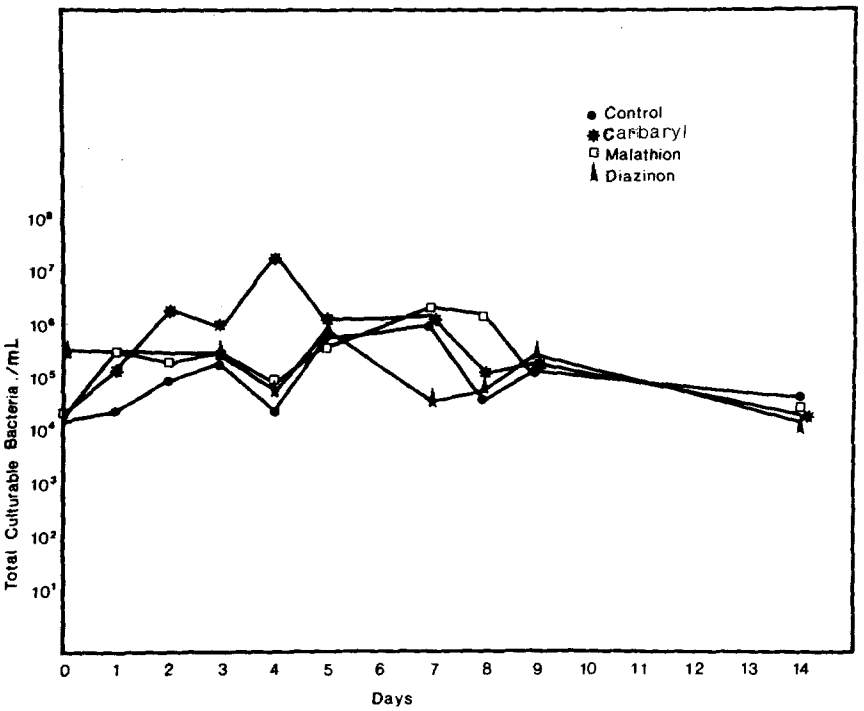

Figure 1. The effects of carbaryl, malathion, and diazinon on total culturable bacteria in Lake Houston water. Data plotted as mean of triplicate determinations.

When 5 mg/L of carbaryl was added to the Lake Houston water the total plate counts of aerobic heterotrophic bacteria increased significantly during the first 3-5 day period, and after five days these counts had returned to about the same level as the control for the remainder of the two-week test (Fig. 1). During this period dissolved oxygen decreased in the carbaryl treated aquaria from 7.8 to 5.2 after 24 h and to a low of 3.8 at two days. After three days DO had returned to 5.6 in these tests, and to the level of the control (which remained essentially constant at 7.8) by the seventh day. Direct counts revealed that there was an initial decrease in total algal numbers in all aquaria including controls which continued for the first 3-5 days (Fig. 2). The only significant decrease in numbers ($p<0.05$) occurred in the Cyanophyta with least change in the Crysophyta. Following this period, algal numbers in all aquaria began to increase, and this increase was greatest in the control aquaria throughout the test period of two weeks. With the overall increase Cyanophyta numbers never reached initial control levels

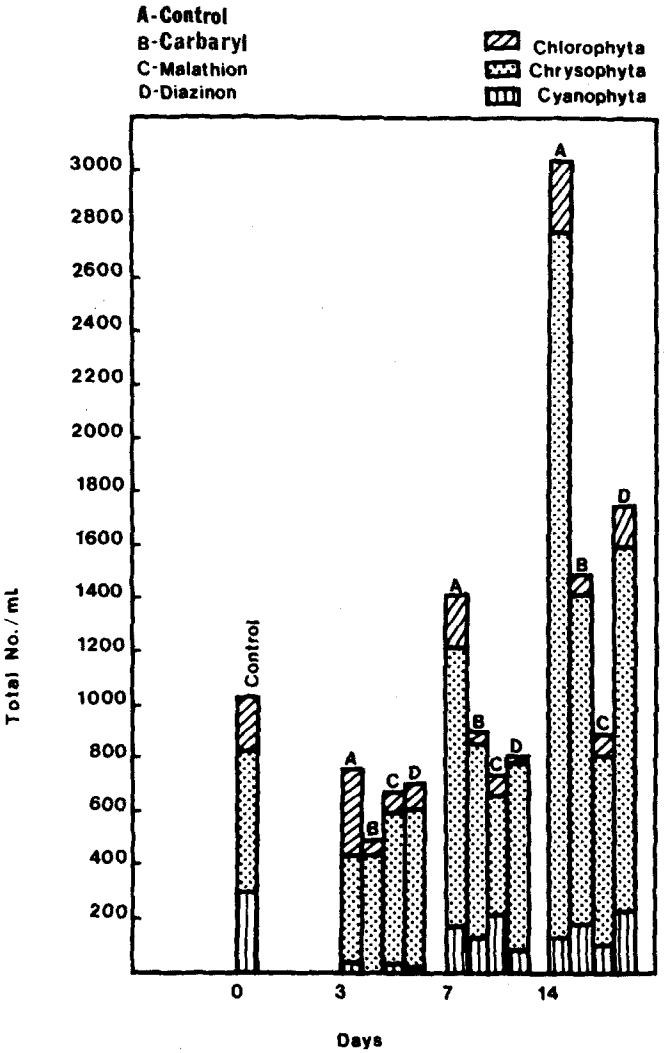

Figure 2. The effects of carbaryl, malathion and diazinon on algal populations in Lake Houston water. Data plotted as means of triplicate counts.

during the two-week period, nor did the Chlorophyta in any treated test system.

Chrysophyta numbers were greater in malathion and diazinon systems by Day 3 than in the initial control, and were significantly greater ($p<0.05$) than that number in carbaryl and diazinon tests by Day 14. Crysophyta, however, were significantly greater in control aquaria by Day 7 than in the initial control counts

(Fig. 2). Since bacterial plate counts and direct cell counts of all types hold considerable margins for error even when done repeatedly from multiple samples additional methods were used for study of the observed population changes.

Metabolic rates of bacterial populations were increased by the addition of 5 mg/L carbaryl at water temperatures of 23, 28, and 33°C, although the greatest difference between insecticide treated and control occurred at 28°C (Fig. 3) ($p < .05$). This result might be expected since this temperature is more nearly the optimum observed for the mesophilic aquatic bacterial populations of our area. Similar results were observed with 1-naphthol.

When malathion was added to Lake Houston water at a concentration of 5 mg/L, a slight increase in BOD ensued; however, bacterial plate counts (Fig. 1) and direct algal counts were not significantly changed ($p>0.05$) as compared to the control. Dissolved oxygen remained essentially unchanged and both Warburg respirometry and living biomass as calculated from ATP measurements were slightly reduced (Table 1). ATP biomass increased to a level above control by Day 7 of the test, and changed little thereafter in the presence of malathion. Malathion-monocarboxylic acid and malathion-dicarboxylic acid, principal degradation products reported by BOURQUIN (1977) in tests resulted in essentially the same changes in bacterial and algal counts as were observed with the parent compound. The addition of 5 mg/L of diazinon, on the other hand, did not result in any consistent effect on the bacteria present.

Light-dark bottle tests on algal populations resulted in increased photosynthesis and respiration in the presence of sevin as compared to the control (1.7±0.6 vs. 1.0±0.6 photosynthesis and 2.7±0.3 vs. 1.4±0.5 respiration) (Table 1). There was little effect from malathion presence when the standard deviations are taken into account (0.7±0.6 vs. 0.4±0.1 photosynthesis and 1.1± 1.2 vs. 0.2±0.3 respiration). ATP was not consistently altered in the presence of either insecticide. Total carbon remained essentially constant in tests and controls after pesticide addition. Chlorophyll-a increased in both test and control to day 14 after carbaryl addition (test 0.34±0.01 to 0.11±0; control 0.03± 0.01 to 0.13±0.01). At day 21 these values had returned to 0.13±0.01 for the test and 0.47±0.01 for the control (Table 1). Biomass accumulation as reflected by dry weight from diatometers most closely correlated with the direct counts of algae in that both test and control counts increased from 1.1±0.5 to 2.8±1.6 at 14 days and to 4.3±0. 2at days for test; from 0.4±0.1 to 0.6±0.2 and 1.2±0.1 for control. Biomass accumulation following carbaryl addition was significantly greater than control ($p<0.05$), but there was no significant difference between test and control following the addition of malathion.

When aquaria were dosed for a second time with carbaryl, after the two-week test the events observed after initial dosing were repeated. The magnitude of changes was altered in some cases

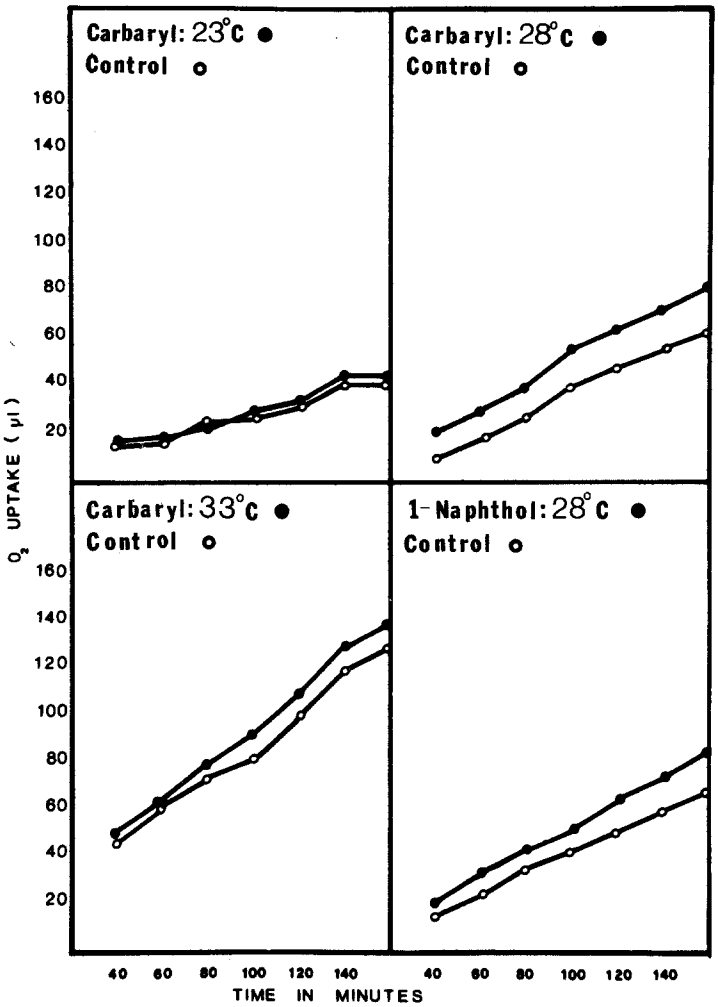

Figure 3. The effects of carbaryl on respiration of indigenous bacterial populations from Lake Houston at 23, 28 and 33°C, and 1-naphthol at 28°C. Data plotted as means of triplicate determinations.

perhaps because of the selection of populations of microorganisms during the first two weeks of test; however, increased counts, and BOD were observed in the first 3-5 days, followed by a tailing off to return to control level.

These results do not disagree with the findings of PARIS (1975) & BORQUIN (1977) that malathion was degraded by bacteria, and of

TABLE 1. Effects of Insecticides on Indigenous Algal Populations in Lake Houston
(All data reported as mean of triplicate determinations.)

	Time in Days							
	0		7		14		21	
	Carbaryl (Malathion)	Control (Control)	Carbaryl (Malathion)	Control (Control)	Carbaryl (Malathion)	Control (Control)	Carbaryl (Malathion)	Control (Control)
Light-Dark Bottle (g O_2/m^3/day)								
Photosynthesis, net	1.60 (0.72)	1.04 (0.40)	ND[a] (ND)	ND (ND)	ND (ND)	ND (ND)	ND (ND)	ND (ND)
Respiration	2.72 (1.12)	1.44 (0.16)	ND (ND)	ND (ND)	ND (ND)	ND (ND)	ND (ND)	ND (ND)
Adenosine Triphosphate (μg/mL)	0.247 (0.141)	0.191 (0.191)	0.175 (0.274)	0.183 (0.183)	0.347 (0.333)	0.439 (0.439)	ND (ND)	ND (ND)
Carbon (mg/L)								
Total	15 (17)	15 (16)	15 (18)	15 (17)	15 (20)	15 (16)	16 (17)	16 (17)
Chlorophyll-a (mg/L)	.0335 (0.0268)	.0335 (0.0268)	ND (0.0094)	ND (0.0268)	0.1072 (0.0040)	0.1273 (0.201)	.1257 (0.0134)	.0469 (0.0134)
Biomass Accumulation (g/m^2)								
Dry weight	ND (ND)	ND (ND)	1.0800 (0.1734)	0.3867 (0.1333)	2.8267 (0.2000)	0.6267 (0.2934)	4.2934 (0.7200)	1.2400 (0.7200)
Ash free weight	ND (ND)	ND (ND)	0.5467 (0.400)	0.3067 (0.0533)	1.2267 (0.0534)	0.6134 (0.0934)	1.9067 (0.5467)	0.6000 (0.6000)

[a] Test not done.

BUTLER (1975) that both malathion and diazinon were inhibitory to some algal growth. In addition this study has shown that although carbaryl, malathion and diazinon appear initially to inhibit algal growth, this inhibition is short-lived and the eventual result is that algal populations approach or exceed those of controls by some measures. Although direct effects on algae by insecticides have been reported (BOURQUIN 1977), the effects observed in this work, and indirect effects as reported by KUENTZEL (1969) and SILVEY & ROACH (1964) must also be considered.

Whether delayed algal growth observed simply follows bacterial growth or results following bacterial degradation of the inhibitory chemical is not known. However, SILVEY & ROACH (1964) described the seasonal microbial cycles occurring in eutrophying reservoirs in the Southwest which consisted of increased bacterial numbers in the warmer summer months, followed by increased algal growth. This cyclic pattern has been duplicated in this study in a period of two weeks following the addition of 5 mg/L carbaryl to the water. Using the Trophic State Index (TSI) of CARLSON (1977) and calculating from chlorophyll-a values, within

a two-week period, algal biomass was found to have doubled (initial TSI=65, two-week TSI=78) following addition of 5 mg/L carbaryl. In the malathion tests, the biomass was twice reduced by half (initial TSI=65, two-week TSI=44). If dry weight biomass were used, differences would be greater than these calculated using chlorophyll-a data. If one estimates the TSI in the SILVEY & ROACH (1964) report, using the ASU as a measure of biomass, algal biomass doubled six times (initial TSI=68, October TSI=126) in a period of 10 months. This study has shown that when addition is repeated, the same eutrophying cycle is repeated. The pest control seasons along the Texas Gulf Coast may well extend over a ten-month period. Thus eutrophication processes which would require a complete season for completion with no artificial stimulation, may be repeated when this pesticide is added to surface water.

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