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PESTICIDE DEGRADATION BY MARINE ALGAE

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Wisconsin University

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FINAL REPORT

Pesticide Degradation by Marine Algae

by

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13. ABSTRACT

Various algae species are tested for their susceptibilities towards chlorinated hydrocarbon insecticides. Deildrin, which is the most frequently found pesticidal contaminant in the US, and its analogs were found to inhibit the growth of certain of algae species. Anacystis nidulans in particular showed marked susceptibility to endrin, dieldrin, ketoendrin and photodieldrin. This species was also susceptible towards dieldrin metabolites such as metabolite F and G. Among DDT metabolites DDD (TDE) was found to be the most toxic material; followed by DDE, DDT and FW-152. It has not been known that DDT should be more toxic to algae. In terms of acute toxicity phenylmercuric acetate was by far the most algicidal agent among all pesticidal chemicals tested. This pesticide is toxic to both A. nidulans and A. quadruplicatum at the concentration of 1 ppb.

Algae, along with other plankton, are known to bioaccumulate pesticides and thereby play a vital role in the process of food-chain accumulation of these micropollutants. Our studies indicate that the rates of pick-up of pesticides are very rapid. To study the feasibility of constructing a model ecosystem we used algae as a key food chain organism. By this way we could demonstrate that TCDD, the most toxic contaminant of 2,4,5-T does not really accumulate in the aquatic organisms as compared to DDT.

Algae as a whole are not very active in degrading pesticidal chemicals in vivo. They were found to play, however, a key role in the process of environmental alteration of pesticidal residues. The way they participate in such processes was found to be through synergistic actions on photochemical reactions. Algal products, when tested in the form of aqueous extract from dead algal cells, were found to be excellent photosensitizers for DDT and mexacarbate degradation by the sun-light (simulated sun lamp).

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SUMMARY OF RESEARCH ACCOMPLISHED

I. Studies relating to the effects of pesticides on plankton.

It has been suggested that the varying resistance of marine phytoplankton to effects of chlorinated hydrocarbons could have far-reaching effects in terms of phytoplankton population balance. Studies in this laboratory have also shown varied growth inhibition of planktonic blue-green algae by chlorinated hydrocarbons. TABLE 1 shows the effects of aldrin, dieldrin, and endrin on growth rates (generations per 24 hr.) of Anacystis nidulans (freshwater species) and Agmenellum quadruplicatum (marine species). It is noticeable that generally the marine isolate is more tolerant than the freshwater isolate. This may be due to the influence of the growth medium on the insecticide. The toxicity of a pesticide in aquatic environments may vary according to the physical characteristics of water.

Although much variation is noted in the data, the general trend indicates both algae are tolerant to these insecticides except at concentrations higher than reported in natural waters. Also notable is the sensitivity of A. nidulans to dieldrin, an isomer of aldrin.

TABLE 1

Growth Response of Agmenellum quadruplicatum and Anacystis nidulans to Aldrin[a], Dieldrin, and Endrin[b]

	Aldrin		Dieldrin		Endrin	
ppb	A. quadruplicatum[c]	A. nidulans[c]	A. quadruplicatum	A. nidulans	A. quadruplicatum	A. nidulans
950	6.2 ± 0.7	6.4 ± 0.4	5.8 ± 0.9	3.2 ± 0.8	3.5 ± 0.9	2.2 ± 0.7
475	7.1 ± 0.5	6.7 ± 0.2	6.0 ± 0.8	3.9 ± 1.5	4.8 ± 1.5	3.2 ± 1.0
95	6.6 ± 1.2	6.8 ± 0.5	6.0 ± 1.0	6.9 ± 0.6	4.9 ± 2.2	6.3 ± 0.3
19	6.8 ± 0.3	7.1 ± 0.4	6.5 ± 0.7	7.2 ± 0.9	5.6 ± 1.3	6.6 ± 0.4
0.2	6.4 ± 1.0	7.2 ± 0.3	5.3 ± 1.2	6.7 ± 1.3	4.8 ± 0.3	7.0 ± 0.5
Control	6.6 ± 0.5	6.6 ± 0.4	6.2 ± 0.9	6.9 ± 0.6	6.6 ± 0.5	6.6 ± 0.4

[a] Concentrations for aldrin 910, 455, 91, 18 and 0.2 ppb.
[b] Values reported as number of generations per 24 hours, represents mean of 3 to 5 replicate cultures
[c] Agmenellum quadruplicatum (strain PR-6), Anacystic nidulans (strain TX20)

In preliminary experiments the growth response of these two algae was also tested against phenylmercuric acetate (PMA), an algicide and fungicide once used extensively in industry. The results are summarized in TABLE 2.

TABLE 2

Susceptibility of Two Species of Blue-Green Algae
Against Phenylmercuric Acetate

	Phenylmercuric acetate (ppb)					
	0.10	0.25	0.50	0.75	1.00	10.00
A. nidulans	109	100	112[b]	87[c]	68[d]	0
A. quadruplicatum	109	-	100	-	112	0

[a] Expressed in % relative growth against controls as 100.
[b] Only 3 of 4 replicates grew during the experiment.
[c] Only 2 of 4 replicates grew.
[d] Only 3 of 6 replicates grew.

Thus results showed A. nidulans to be affected by as little as 0.50 ppb PMA. At this and higher concentrations growth was irregular and was preceded by lag phases. In view of mercury contamination reported in oceanic environments it was of interest to also consider the toxicity of PMA to A. quadruplicatum. Duplicate cultures in two experiments yielded the growth values as compared to controls (TABLE 2). Thus it is evident that A. quadruplicatum is more tolerant to PMA than A. nidulans; however, neither organism showed any growth at 10 ppb PMA.

Much research has shown that in addition to growth, beneficial activities of microorganisms can be affected by pesticides as well. Bacteria in soil which convert organic matter to ammonia, and several herbicides have been seen to influence soil nitrification.

II. Effect of degradation products.

Pesticides, as they may adversely affect microorganisms, involve not only the parent compound, but the intermediate and terminal residues of these compounds as well. Recent investigations have pointed out the potential of certain "terminal" residues to be as toxic as the original pesticides. Data from this laboratory also support this obeservation. Anacystis nidulans and Agmenellum quadruplicatum were grown in media containing microbial degradation products of aldrin, dieldrin, and endrin. The data in TABLE 3 show that A. nidulans continues to be sensitive to photoaldrin and ketoendrin, two metabolites of aldrin and endrin, respectively. Agmenellum quadruplicatum appears resistant to both compounds.

However, both organisms show continued sensitivity to metabolites of dieldrin, as shown in TABLE 4. While metabolites F and G are only formed microbially, photodieldrin is also known to form on plant surfaces by the action of UV or sunlight. Hence, it was of interest to assay the

TABLE 3

Growth Response[a] of Agmenellum quadruplicatum and Anacystis nidulans to effects of Metabolites of Aldrin and Endrin, 408-411

ppb	Photoaldrin		Ketoendrin	
	A. quadruplicatum	A. nidulans	A. quadruplicatum	A. nidulans
950	6.2 ± 1.0	5.3 ± 0.8	7.4 ± 0.4	4.5 ± 1.1
475	6.4 ± 0.4	6.3 ± 0.7	6.8 ± 0.2	3.5 ± 0.1
95	6.5 ± 0.7	6.4 ± 0.7	7.3 ± 0.3	6.0 ± 0.8
19	5.9 ± 0.7	7.0 ± 0.6	7.1 ± 0.8	6.6 ± 0.2
0.2	6.0 ± 0.6	7.0 ± 0.6	7.3 ± 0.6	6.3 ± 0.1
Control	6.6 ± 0.5	6.8 ± 0.4	6.6 ± 0.5	6.8 ± 0.4

[a] Conditions as in TABLE 1.

TABLE 4

Growth Response of Agmenellum quadruplicatum and Anacystis nidulans to Metabolites of Dieldrin

ppb	Metabolite F		Metabolite G		Photodieldrin	
	A. quadruplicatum	A. nidulans	A. quadruplicatum	A. nidulans	A. quadruplicatum	A. nidulans
950	5.4 ± 0.7	3.5 ± 0.4	4.9 ± 1.0	4.2 ± 1.4	5.6 ± 1.0	4.0 ± 0.4
475	5.9 ± 0.9	4.8 ± 0.3	6.4 ± 0.8	5.9 ± 0.6	6.9 ± 0.5	5.3 ± 0.8
95	4.6 ± 0.7	6.5 ± 0.5	6.4 ± 0.9	6.7 ± 0.1	6.6 ± 0.4	7.2 ± 0.1
19	6.4 ± 1.2	7.2 ± 0.5	6.4 ± 1.2	6.7 ± 0.5	6.4 ± 0.6	7.1 ± 0.9
0.2	6.8 ± 0.8	6.7 ± 0.3	6.5 ± 1.1	6.4 ± 0.1	7.1 ± 0.2	7.1 ± 0.8
Control	6.2 ± 0.9	6.9 ± 0.6	6.2 ± 0.9	6.9 ± 0.6	6.2 ± 0.9	6.9 ± 0.6

response of other algal species to photodieldrin. TABLE 5 shows that of the algae tested, Nostoc sp. and the green alga Chlorella soporensis appeared only slightly affected by photodieldrin at high concentration, while A. nidulans was most inhibited. Continued growth of A. nidulans in successive cultures in medium plus various levels of photodieldrin did not improve initial growth rates. Other attempts to improve the organisms' tolerance by varying growth conditions and dark incubation intervals were unsuccessful.

TABLE 5

Growth Response of Several Blue-Green Algae[a] to Photodieldrin[b,c]

Alga*	Control	0.2 ppb	19 ppb	95 ppb	475 ppb	950 ppb
I	2.6 ± 0.1 (5)	2.7 ± 0.1 (3)	2.8 ± 0.2 (3)	2.7 ± 0.2 (6)	2.9 ± 0.3 (6)	2.7 ± 0.2 (6)
II	3.5 ± 0.2 (2)	3.6 ± 0.1 (2)	3.4 ± 0.3 (3)	3.6 ± 0.0 (3)	3.3 ± 0.2 (3)	2.6 ± 0.6 (3)
III	7.0 ± 0.7 (6)	6.7 ± 0.1 (3)	-	7.1 ± 0.2 (3)	4.7 ± 0.2 (5)	3.1 ± 0.3 (8)
IV[d]	6.2 ± 0.6 (4)	6.9 ± 0.3 (6)	6.5 ± 0.5 (6)	5.0 ± 0.3 (5)	5.2 ± 0.2 (5)	5.2 ± 0.4 (6)
V[d]	3.3 ± 0.3 (4)	3.3 ± 0.4 (4)	3.3 ± 1.0 (5)	3.1 ± 0.6 (4)	3.2 ± 0.8 (5)	3.4 ± 0.3 (6)
VI[d]	6.5 ± 0.2 (7)	7.1 ± 0.5 (5)	-	7.2 ± 0.3 (3)	7.1 ± 0.5 (4)	6.8 ± 0.2 (7)
VII[d]	3.6 ± 0.5 (6)	3.7 ± 0.7 (6)	3.5 ± 0.5 (6)	4.0 ± 0.6 (5)	3.4 ± 0.1 (5)	3.5 ± 0.3 (6)

* I = Anabaena variabilis; II = Nostoc sp.; III = Anacystis nidulans; IV = Chlorella sopronensis; V = Agmenellum quadruplicatum (strain BG-1); VI = Agmenellum quadruplicatum (train PR-6); VII = Coccochloris elabans.

[a] All algae tested here are blue-green species, except C. sopronensis, a gre[illegible]

[b] Values reported as number generations per 24 hours.

[c] Numbers in parentheses indicate replicate cultures.

[d] Indicates marine isolate; others are freshwater isolates.

TABLE 6

Growth Response of Agmenellum quadruplicatum and Anacystis nidulans to DDT and its Metabolites[a,b]

	ppb	A. quadruplicatum	A. nidulans
DDT	885	5.4 ± 1.1 (13)	5.8 ± 1.1 (16)
	442	5.4 ± 1.3 (12)	5.8 ± 0.8 (5)
	88	6.3 ± 0.6 (4)	6.5 ± 0.6 (4)
	18	6.5 ± 0.4 (4)	6.2 ± 0.2 (4)
	0.2	6.1 ± 0.6 (4)	6.8 ± 0.7 (4)
	0.0	6.1 ± 0.7 (16)	6.8 ± 0.5 (16)
DDE	791	5.3 ± 1.0 (6)	4.6 ± 0.0 (2)[c]
	395	5.4 ± 0.9 (5)	5.4 ± 2.0 (3)
	79	6.2 ± 0.4 (3)	6.5 ± 1.5 (4)
	8	6.2 ± 0.3 (4)	6.5 ± 0.4 (2)
	0.8	6.7 ± 0.4 (4)	7.2 ± 0.0 (2)
	0.0	6.3 ± 0.6 (8)	6.8 ± 0.5 (7)
DDD	791	3.4 ± 0.0 (2)[c]	4.2 ± 0.3 (1)[c]
	395	5.3 ± 0.8 (4)	5.3 ± 0.3 (4)
	79	6.7 ± 0.1 (2)	6.2 ± 0.3 (6)
	8	6.8 ± 0.3 (2)	6.8 ± 0.3 (2)
	0.8	7.0 ± 0.4 (2)	6.4 ± 0.3 (2)
	0.0	6.4 ± 0.7 (6)	7.0 ± 0.3 (7)
DDA	700	6.9 ± 0.2 (2)	6.2 ± 0.1 (2)
	350	6.7 ± 0.0 (2)	5.9 ± 0.2 (2)
	70	7.4 ± 0.2 (2)	6.5 ± 0.4 (2)
	7	7.1 ± 0.0 (2)	6.6 ± 0.6 (2)
	0.7	7.4 ± 0.2 (2)	6.8 ± 0.0 (2)
	0.0	6.9 ± 0.2 (3)	6.0 ± 0.3 (3)
DBP	720	6.5 ± 0.3 (2)	6.4 ± 0.1 (2)
	360	6.9 ± 0.3 (2)	6.2 ± 0.0 (2)
	72	6.6 ± 0.1 (2)	6.8 ± 0.2 (2)
	7	6.8 ± 0.4 (2)	7.1 ± 0.4 (2)
	0.7	6.6 ± 0.1 (2)	6.6 ± 0.1 (2)
	0.0	6.9 ± 0.2 (3)	6.0 ± 0.3 (3)
FW 152	821	6.1 ± 0.1 (4)	6.3 ± 0.4 (2)
	410	6.8 ± 0.3 (3)	7.2 ± 0.2 (2)
	82	6.6 ± 0.4 (3)	7.4 ± 0.2 (2)
	8	6.3 ± 0.4 (4)	7.2 ± 0.1 (2)
	0.8	6.8 ± 0.4 (3)	7.4 ± 0.2 (2)
	0.0	6.6 ± 0.3 (4)	7.0 ± 0.3 (3)

[a] Values expressed as number of generations/24 hours.
[b] Numbers in parentheses represent numbers of replicates.
[c] Growth occurred in only one or two of several replicates.

TABLE 6 incorporates data of several growth-response experiments of A. quadruplicatum and A. nidulans to DDT and five DDT-analogs. Again, although much variation occurred, the data show little growth-rate depression of either alga at concentrations below 100 ppb insecticide. Both species show continued, perhaps greater, sensitivity to the two analogs DDE and DDD, as well as DDT. However, the more polar compounds DDA, DBP, and FW 152 apparently have little effect as seen in these experiments.

III. Microbial uptake and accumulation.

Toxicants, one taken up by primary producers such as marine algae, can be passed up the food chain to higher trophic levels. In addition, many toxicants can, depending upon the environmental conditions and species involved, be accumulated within the cell to levels many-fold higher than ambient. From the few studies available, accumulation appears to be primarily by inactive surface adsorption. However, Glooschenko found that dividing marine diatom cells in light accumulated ^{203}Hg longer than did non-dividing cells, thus indicating the possibility of some active uptake mechanism. The exotic and demanding minor-element nutritional requirements of many organisms would tend to support active uptake in some instances.

We have found that yeast cells of Rhodotorula gracilis rapidly accumulated 97% of the DDT in a 2-ppm aqueous solution. Likewise, another yeast, Torulopsis utilis, took up 94% of the DDT in 3 minutes.

In TABLE 7, showing the percent radioactivity of the ^{14}C-DDT in the cellular fraction, the control values showed a random distribution of DDT between the medium and the cellular fractions, ranging from 21 to 56%. These results were obtained by centrifuging, decanting, and filtering the aqueous medium, and the distribution of ^{14}C-DDT between the medium and cellular fractions was determined by liquid scintillation counting.

In contrast to the erratic control values, the cellular fractions accumulated DDT at a constant rate over the 90% level after 3 minutes.

An extract of R. gracilis was prepared by sonicating the cells before the addition of the ^{14}C-DDT. The sonicated pellet was found to accumulate an average of 96% of the DDT.

We also attempted to correlate cellular lipid content with the uptake of DDT. Rhodotorula gracilis when grown on a medium rich in carbohydrates and deficient in nitrogen and phosphorus will produce approximately 60% lipids, whereas when not deprived of N and P, lipid production is reduced to approximately 40%. When cultures were grown under both circumstances, no differences in DDT pick-up were noted.

It is apparent that the complexities of pesticidal-microbial in-

terrelationships warrants continued study. It has been amply demonstrated that members of the microbial world vary widely in their response to pesticides and that several factors may influence the toxicity of pesticides. Likewise, the microbial tolerance of pesticides may be affected by growth conditions, physiological condition of the cells, and various stress factors which might exist in natural populations (e.g., temperature, limited nutrients, competition). For example, growth experiments with A. nidulans established separate tolerances of 1% NaCl and 800 ppb DDT (Batterton, Boush and Matsumura, 1972). However, growth of this alga is severely inhibited in media containing both 1% NaCl and 800 ppb DDT. Figure 1 illustrates relative growth of A. nidulans in various concentrations of DDT and NaCl. The resulting growth pattern indicates the combined stresses of NaCl and DDT significantly changes the tolerance of A. nidulans to either substance. However, in similar experiments with test-tube cultures, growth inhibition was contraindicated when the calcium concentration of the growth medium was increased five-fold.

It is particularly interesting to note the similarities in nearly all of the uptake-accumulation studies. First, pick-up of the toxicant is extremely rapid--varying from a matter of seconds to a few minutes. And secondly, removal of the toxicant from the medium is quite high--usually more than 90% of the total being removed by the cells (dead or alive), even when ambient levels were many-fold higher than those usually encountered in nature. However, one factor should not be ignored. Few, if any, studies have included competitive adsorptive substrates. Might not DDT, for example, readily adsorb to organic matter, silica, etc., if available? The apolarity, affinity for lipids, and low water solubility of virtually all of the persistent insecticides make studies in aqueous substrates difficult. Of even greater importance, can we extrapolate from our work, even to a limited degree, to conjecture as to what occurs in nature?

It is doubtful if we can overstress the important of microbial accumulation. After more than 25 years of world-wide use and study, the real threat from persistent pesticides is in their unfortunate ability to concentrate with food chains. This would not occur were the toxicants not picked up from low background levels, concentrated in the cell, and finally, stable for considerable periods of time.

The results shown in Figure 2 indicate the general susceptibilities of brine shrimp, Artemia salina, to various terminal residues and analogs of DDT. It can be seen that these analogs, though many of them have been regarded as non-insecticidal, are indeed toxic to this species.

IV. Effects of chlorinated insecticides on NaCl-tolerance mechanisms.

Since Na^+, K^+-ATPases have been known to serve as the enzyme re-

sponsible for Na^+ and K^+ exchanging across many biological membranes, we have decided to study first the effect of DDT on the salinity regulatory mechanism of a blue-green algae (Batterton et al., 1972). The initial experimental results indicate that the susceptibility of a blue-green alga, Anacystis nidulans, against DDT varies greatly under different salt concentrations. At high NaCl concentrations the blue-green alga becomes extremely sensitive to DDT. It is clear from the result that this fresh water species loses its NaCl-tolerance capability in the presence of a low level of DDT: the level normally would not affect the specie. . A spearate experiment in vitro showed also that DDT was indeed an inhibitor of Na^+, K^+-ATPases of A. nidulans, blocking all ouabain sensitive ATPase activities. The most important indication that the ATPase is related to the NaCl-tolerance mechanism comes from the in vivo finding that Ca^{++}, when added externally to the medium, can antagonize the effects of DDT.

TABLE 7

Percent Radioactivity of ^{14}C-DDT in Yeast Cells

Culture	Time (minutes) 2.5	7.5	12.5	17.5	32.5	Average
Control	21	42	56	38	--	39
Torulopsis utilis	92	96	91	95	--	94
Rhodotorula gracilis	97	98	97	98	96	97
Extract-R. gracilis	98	--	--	--	94	96
Protein producing medium-R. gracilis	97	--	--	--	96	97
Lipid producing medium-R. gracilis	95	--	--	--	98	97

In another set of experiments, the brine shrimp, A. salina, was subjected to various chlorinated hydrocarbon insecticides under different salt concentrations (Figure 10). The results clearly indicate that the effects of these chlorinated hydrocarbons are strongest at either extremely low or high salt concentrations. The brine shrimp is noted for its great capabilities of tolerance on different salt concentrations. It is often found in abundance in inland salt lakes (e.g., salt ponds and lakes in Utah) where the salt concentration is so high that no other organism can survive. The loss of salt tolerance mechanisms for this species by the presence of these insecticides is, therefore, quite a surprising phenomenon.

The examples illustrate only one aspect of pesticidal pollution. It is important to note, however, that such a finding comes from fundamental knowledge of the chemical interactions with biological materials.

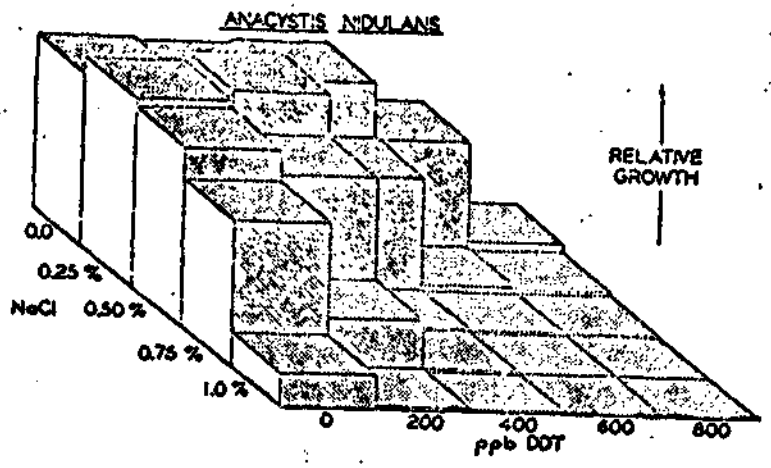

FIGURE 1. Relative growth of Anacystis nidulans in response to varied DDT and NaCl concentrations. Liquid culture (15 ml) in 60 mm petri dishes inoculated (120,000 cells/ml) and incubated 72 hr. under 200 ft-c fluorescent lamps at 37° C. After correction for evaporation growth was measured as optical density at 660 nm. All O.D. values normalized to 1.0.

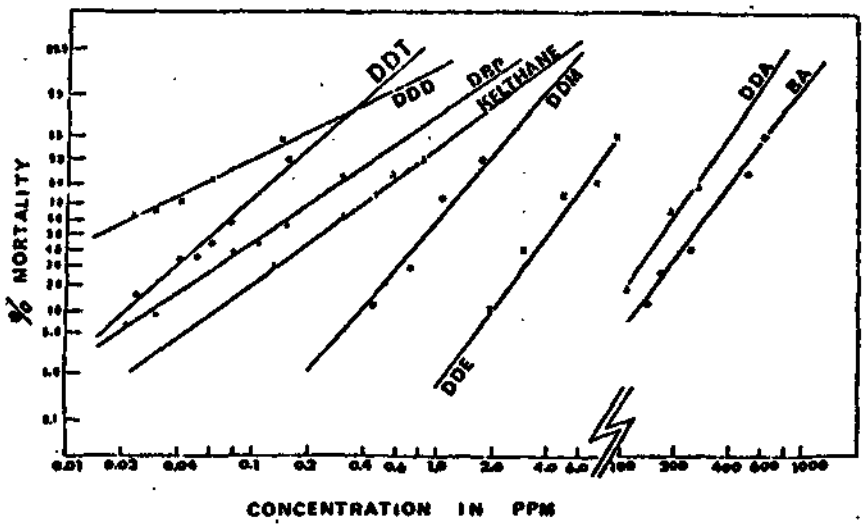

FIGURE 2. Differential toxicities of DDT analogs and metabolites on brine shrimp, Artemia salina; 24 hours exposure at 24° C.

It is also necessary to stress that those stable terminal residues and contaminants would not have been detected from the environments if not for the specific knowledge accumulated through basic researches in the laboratory as to their chemical characteristics and behavior. Factors involved in the interactions of pesticides with various ecosystems are numerous and complicated, but it certainly is hoped that there are a number of rate-limiting, key factors that can be analyzed through controlled laboratory experiments.

V. Effects of pesticide micro-contaminants: Model ecosystem study.

While the problem of pesticidal contamination of the environment is far from being solved, considerable useful information has emerged from the research efforts made by many scientists in recent years.

First, we now know by experience that the chemicals that cause environmental problems are the ones which are extremely persistent in nature, biologically active, and easily concentrated in biological systems. Compounds which lack any of the above qualifications usually do not play any significant role in pesticidal pollution no matter how acutely toxic they are. The above analysis becomes more important, when one considers other aspects of pesticidal pollution. For instance, we are concerned about only biological effects in considering pollution, with particular emphasis on the effects on non-target organisms.

In the case of polychlorinated dibenzo-p-dioxins, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the question of bioactivity is indisputable, as it is one of the most toxic compounds known to occur as a pesticidal impurity. Its chemical stability is also questionable. Thus the central question of its hazard to the environment must be studied from the viewpoint of bioconcentration in various ecosystems.

Published data on environmental fate of chlorodibenzo-p-dioxins are scarce at present. For instance, residues of dioxins were not found in several aquatic animals at detection limits of 0.01-0.04 μg/g.

In the study reported herein we have made efforts to measure the degree of bioaccumulation of TCDD in relation to well established pesticides by using several model ecosystems. The data are still preliminary, in that several model ecosystems are still being compared for their relative merits in assessing the actual impact of pesticides in nature. The data obtained have been, however, useful in assessing the relative tendency of a pesticide in comparison with other pesticides.

Materials and Methods--Approximately 100 microbial strains which have previously shown the ability to degrade persistent pesticides were screened for their ability to degrade TCDD. Screening was carried out and the metabolic products were examined by thin-layer chromatography (TLC) by the method of Matsumura and Boush. The pesticides (0.1 μmole each) were deposited on 1 g of clean sea sand, which was placed on a

column of sandy loam type soil. Water was then slowly dropped onto the surface of the sand at a rate of approximately 2 ml/hr. The water and sections of soil were extracted with chloroform. Three groups of invertebrates were used for the pesticide accumulation study: Ostracoda species, Artemia salina, and Aedes aegypti larvae, and one fish species northern brook silverside, Laludesthes sicculus sicculus. Four pesticides were selected from representative groups of important compounds: dioxin (TCDD), DDT, γ-BHC, and zectran. All compounds were ^{14}C-labeled in the benzene rings. Three model ecosystems were used to study bioaccumulation.

In model I, the pesticides (5 and 10 pmole) dissolved in a solvent were added directly to water along with the primary food organism, such as algae and yeast, and this mixture was then added to the aquarium containing the invertebrate test organisms.

In model II, the pesticides (20 pmole) were deposited on the inner surface of the glass container by evaporating the solvent to form a thin film. The primary food organism were grown in the container for 24 hr. and then transferred along with the culture media to the aquarium containing the test invertebrate organism.

In model III, the pesticides (5 and 10 pmole) were deposited on 1 g of sand and the solvent evaporated to form a thin film on the surface of the sand particles. The sand was added to the test aquarium containing invertebrates and/or fish.

In all cases the test organisms were maintained in the aquarium at room temperature (24° C), except for the fish cultures which were maintained at 12° C. Test organisms were either homogenized in counting solution or carbonized (Model 300 Packard Tri-Carb Oxidizer), and the amount of $^{14}CO_2$ measured. Measurements of the amount of labeled material in the water, primary food organism, and on sand and glass surfaces were made by extracting with chloroform. All studies were short-term (4-7 days), in small volume containers (200 ml).

As shown in Table 8, the extent of translocation of TCDD from the sand to the organic soil layer is extremely small. Virtually no TCDD was found to leach out from the column. The mobility of TCDD in soil, therefore, must be considered much less than that of DDT. Thus, the mode of translocation of TCDD in the environment would be limited to movement of soil particles or dust-carried dispersion and biological transfer (but not plant-mediated transfer), particularly in aquatic environments.

As for the microbially mediated degradation of TCDD, our current survey indicates that such capabilities are rather rare in nature. Approximately 100 microbial strains in which the ability to degrade persistent pesticides has been previously demonstrated were screened for this purpose. Among them, only five strains showed some ability to de-

grade this compound. We ha e not been able to manipulate cultural conditions to increase the rate of degradation of TCDD in any of the microorganisms so far.

In studying the extent of biological transfer of TCDD, three different model systems were devised. In model system I, pesticides in acetone were introduced directly into water along with the primary food organisms. In model system II, pesticides were applied to the inner surface of a glass container, and the primary food organisms were grown in the container for 24 hr. and were transferred to the aquarium. In model III, pesticide-coated sands were placed directly in the aquarium containing the test organisms.

In the model I experiment (Table 9), DDT behaved quite differently from other pesticides, showing high degrees of affinity to each test organism, in close agreement with the phenomenon actually observed in nature. Although this model system is simple and appears to offer a quick straight-forward answer to the general tendency of pesticidal accumulation by biological systems, it has one weakness, i.e., that one is forced to work above the limit of water solubility of some of the compounds. TCDD for instance was measured at a level 100 times its water solubility. Also the extent of direct pick-up due to partitioning and food intake is uncertain. In the model II experiment, where only the portion of pesticide picked up by the primary food organisms and the media were introduced into the test aquarium, the levels of total pick-up were further reduced in the case of TCDD (but not DDT) (Table 10).

To circumvent the problem of solubility, the model III system was devised. In this way, only that portion of pesticide that is soluble should be present in water at any time. The results shown in Table 11 indicate that the rate of TCDD pick-up is extremely low in brine shrimp and fish under the experimental conditions. Mosquito larvae, which are bottom feeders, showed a surprising rate of TCDD pick up. The reaction is not at its maximal rate, since further increase in the level of the pesticide apparently increases the pick up by the larvae. Also noted is the difference between the bioconcentration pattern in fish as compared to other invertebrates. γ-BHC, in particular, shows high degree of concentration in fish. To study the effects of food consumption, the same test was repeated in the presence of mosquito larvae.

As expected, the level of TCDD (Table 12) in the fish increased in the presence of mosquito larvae, which are the best concentrators of TCDD among the organisms tested. On the other hand, the levels of other pesticides did not significantly change, indicating that the route through ingestion of mosquito larvae does not represent the major source of uptake in these pesticides.

It is apparent from these data that the reaction of biological concentration is greatly influenced by the external conditions and the design of the experiment, the physical and biological nature of the

organisms, and by chemical characteristics of the pesticides. To facilitate understanding of the role of chemical nature of pesticides in determining the rate of bioconcentration, a comprehensive list has been prepared to illustrate their important properties (Table 13).

It can be seen here that general tendencies of bioaccumulation in invertebrate species follow closely the trend of the partition coefficients. In model II experiments, however, the values for TCDD come much lower than expected from this rule. Thus it is likely that water solubility (and solvent solubility) must play an important role where the initial pick-up is the rate-limiting factor.

It is apparent that species-specific factors play a much more important role than once suspected. For instance, the pattern of bioaccumulation and concentration in fish is quite different from those in other organisms studies, in that both γ-BHC and zectran show higher degrees of affinity than DDT and TCDD, respectively. Although the data are not sufficient to permit a definite conclusion, they suggest the possibility that water-soluble pesticides tend to accumulate in fish.

TABLE 8

Vertical translocation of pesticides from sand to organic soil.[a]

	Pesticide content, %		
	Dioxin[b]	DDT[b]	Zectran[b]
Top sand	90.41	65.01	0.07
0-0.5 cm	7.32	30.75	0.06
0.5-1.0 cm	1.04	3.51	0.08
1.0-1.5 cm	0.50	0.55	0.05
1.5-2.0 cm	0.26	0.26	0.06
2.0-2.5 cm	0.18	0.19	0.06
Water eluate[c]			
1st 50 ml	0.12	0.06	49.4
2nd 50 ml	0.08	0.04	17.6
3rd 50 ml	0.09	0.02	29.1

[a] 10 x 1.5 cm glass column
[b] Pesticide introduced: 0.1 µmole each (33.8 µg for dioxin, 35.5 µg for DDT, and 22.2 µg for Zectran)
[c] Water eluted per day, 50 ml

TABLE 9

Bioaccumulation of pesticides by aquatic invertebrates for model I (pesticides introduced directly into ambient water with the primary food organisms).

Test organisms (primary food)	Pesticide	Original concentration in water, ppb	Final concentration found in test organisms, ppb	Concentration factor
Daphnia (algae)	Dioxin	32.4	1,592	49
	DDT	35.8	44,164	1234
	Zectran	22.2	1,969	89
Ostracod (algae)	Dioxin	32.4	7,069	218
	DDT	35.8	50,771	1418
	Zectran	22.2	7,265	327
Brine shrimp (yeast)	Dioxin	16.2	1,956	121
	DDT	17.9	12,336	689
	γ-BHC	14.7	2,688	183
	Zectran	11.1	155	14

TABLE 10

Bioconcentration of pesticides by aquatic invertebrates for model II (primary food organisms allowed to pick up pesticide from glass surface and then given to the test organisms).

Test organism (primary food)	Pesticide	Original amount, µg (theoretical concentration, ppb)	Final concentration found, ppb		Concentration factor[a]
			Water aquarium	Test organisms	
Daphnia (algae)	Dioxin	6.48 (162)	0.4	879	2,198
	DDT	3.58 (179)	22.9	43,123	1,883
	Zectran	2.22 (111)	15.1	37,499	2,488
Ostracod (algae)	Dioxin	6.48 (162)	2.6	279	107
	DDT	3.58 (179)	50.8	36,391	716
	Zectran	2.22 (111)	43.5	6,177	142

[a] Measured against the final pesticide concentrations acutally found at the end of the test.

TABLE 11

Bioconcentration of pesticides by aquatic organisms for model III (pesticides introduced into system in the form of residues on sand).

Test organism	Pesticide	Amount of pesticide μg	Concentration found, ppb		Concentration factor
			Water (including food)	Test organisms	
Brine shrimp	Dioxin	1.62	0.1	157	1,570
	DDT	1.79	0.5	3,092	6,184
	γ-BHC	1.47	5.2	495	95
	Zectran	1.11	5.0	89	18
Mosquito larvae	Dioxin	1.62	0.45	4,150	9,222
		3.24	2.40	12,000	5,000
	DDT	1.79	0.85	14,250	16,765
		3.58	1.40	30,200	21,571
	γ-BHC	1.47	6.6	1,450	220
		2.94	13.1	2,900	221
	Zectran	1.11	5.45	0	0
		2.22	10.8	89	8
Fish (silverside)	Dioxin	1.62	0	2	-
	DDT	1.79	2.1	458	218
	γ-BHC	1.47	1.8	2,904	1,613
	Zectran	1.11	4.7	213	45

TABLE 12

Two-step bioconcentration of pesticide by mosquito larvae, and northern brook silverside (model III).

Pesticide	Amount of pesticide µg	Water (including food)	Concentration found, ppb		Concentration factor	
			Mosquito larvae	Fish	Mosquito larvae	Fish
Dioxin	1.62	1.3	3,700	708	2,846	54
DDT	1.79	1.1	17,900	337	16,273	306
√-BHC	1.47	1.9	690	1080	383	600
Zectran	1.11	5	0	76	0	15

TABLE 13

Physiocochemical characteristics of dioxin in comparison with other insecticides.

	Water solubility	Solvent solubility / Water solubility	Partition coefficient (vs. hexane)	Benzene solubility, g/100 g
Dioxin	0.2 ppb	10^6	1,000[a]	0.047
DDT	1.2 ppb	10^{10}	100,000	80
Zectran	100 ppm	10^4	100[a]	-
√-BHC	10 ppm	10^5	1,700	80

[a] Estimates

The data indicate that TCDD is not likely to accumulate in as many biological systems as DDT. This is likely because of TCDD's low solubility in water and lipids as well as its low partition coefficient in lipids. Since microbial degradation is not expected to be a major factor, the predominant mode of elimination of this compound in the environment is photodecomposition by sunlight.

VI. Degradation of pesticides by algae.

Three salt water algae, Porphyridium sp., Dunaliella tertiolecta, and Coccochloris elabans strain Di, were selected and studied for their ability to degrade a number of environmentally important pesticides (2,4-D, 2,4,5-T, mexacarbate, and DDT) and a pesticide contaminant

(tetrachlorodibenzo dioxin). Pure algae cultures were grown on a definitive media under laboratory conditions. The compounds were studied under the following conditions: (1) growing algae under 24 hour light, (2) heat killed algae under 24 hour light, (3) growing algae under total dark (standard media amended with glucose) and (4) controls (the medium alone but no algae) under 24 hour light. The above studies were conducted for 7 days.

Three compounds, 2,4-D, 2,4,5-T and TCDD were resistent to breakdown under these conditions. Mexacarbate, and DDT were readily broken down. Although mexacarbate was degraded in the presence of light alone, in the presence of algae (living and dead), over 40% of the compound was converted to water soluble materials not found in controls. These compounds became solvent extractable only after acid hydrolysis. A chloroform soluble metabolite was also detected which was not found in controls. This material had an Rf (ethyl ether, hexane, ethanol; 77:20:3) between methyl formamido and formamido mexacarbate derivatives.

The degradation of DDT under the above light conditions seem to give a small amount of DEA. In the presence of algae (living and dead), under light conditions, two other compounds were formed. One compound has tentatively been identified as DDE. The other compound using three TLC systems has been identified as DDOH. Due to the fact that dead algae also forms the metabolites of mexacarbate and DDT it is postulated that a compound is formed by the algae which causes photo-decomposition. The identification of the metabolites and the nature of the photosensitizers are proposed for future study.

INDEX OF TECHNICAL REPORTS

1. No Technical Reports have been issued.

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MAJOR ACCOMPLISHMENTS

Various algae species are tested for their susceptibilities towards chlorinated hydrocarbon insecticides. Dieldrin, which is the most frequently found pesticidal contaminant in the US, and its analogs were found to inhibit the growth of certain of algae species. Anacystis nidulans in particular showed marked susceptibility to endrin, dieldrin, ketoendrin and photodieldrin. This species was also susceptible towards dieldrin metabolites such as metabolite F and G. Among DDT metabolites DDD (TDE) was found to be the most toxic material; followed by DDE, DDT and FW-152. It has not been known that DDD should be more toxic to algae. In terms of acute toxicity phenylmercuric acetate was by far the most algicidal agent among all pesticidal chemicals tested. This pesticide is toxic to both A. nidulans and A. quadruplicatum at the concentration of 1 ppb.

Algae, along with other plankton, are known to bioaccumulate pesticides and thereby play a vital role in the process of food-chain accumulation of these micropollutants. Our studies indicate that the rates of pick-up of pesticides are very rapid. To study the feasibility of constructing a model ecosystem we used algae as a key food chain organism. By this way we could demonstrate that TCDD, the most toxic contaminant of 2,4,5-T does not really accumulate in the aquatic organisms as compared to DDT.

Algae as a whole are not very active in degrading pesticidal chemicals in vivo. They were found to play, however, a key role in the process of enviromental alteration of pesticidal residues. The way they participate in such processes was found to be through synergistic actions on photochemical reactions. Algal products, when tested in the form of aqueous extract from dead algal cells, were found to be excellent photosensitizers for DDT and mexacarbate degradation by the sun-light (simulated sun lamp).