Monsanto

FROM (NAME & LOCATION): E. S. Tucker - Technology Department - South Second Street

DATE: August 12, 1971

cc: R. E. Keller - Res. 1
W. B. Papageorge - GO

SUBJECT:

REFERENCE: Monthly Summary Details - June 1971
Specialty Products - Research
Page 71 - H. A. Vodden - "Pond Experiment"

TO: W. R. Richard
General Offices

I recently ran across the attached article in the latest issue of Environmental Science and Technology and it reminded me of Vodden remarks concerning a "pond experiment". Dr. Metcalf's approach, as outlined in his publication, certainly would accomplish what the National Swedish Env. Prot. Board asked Vodden to consider and if the results were positive enough it sure could be used to create some good PR for our cleaned up Aroclors.

As you probably remember, Dr. Metcalf is one of the Monsanto consultants we talked with at Bio-Test about the PCB problem and as such he might consider evaluating Aroclor 1016 versus Aroclor 1254.

If he could do it with unradiolabeled PCB's or with Cl^{37} labeled material (cheaper than ^{14}C radiolabeled PCB's), it sure might be worth a try.

E. S. Tucker

db
Attach.

MONS 206044

IM 10 REV. 11-69

which is now the case, one mu[illegible]ceed in treating more than 75% of the rats in order to eradicate the population.

Literature Cited

Ames, W. F., "Nonlinear Partial Differential Equations in Engineering," Academic Press, New York, N.Y., 1965, p 315.
Ardrey, R., "The Territorial Imperative," Atheneum, New York, N.Y., 1966, p 1.
Asdell, S. A., "Patterns of Mammalian Reproduction," 2nd ed., Comstock, Ithaca, N.Y., 1964, p 324.
Bellman, R., Juncosa, M., Kalaba, R., Rand Corp. Rept. no. P-2200, Santa Monica, Calif., 1961.
Chiang, C. L., "Introduction to Stochastic Processes in Biostatistics," Wiley, New York, N. Y., 1968, p 266.
Crandall, S. H., *Quart. Appl. Math.* 13, 318 (1955).
Crank, J., Nicolson, P., *Proc. Cambridge Phil. Soc.* 43, 50 (1947).
Dahlchakoff, V., *Compt. Rend. Acad. Sci.* 206, 945 (1938a).
Dahlchakoff, V., *Compt. Rend. Soc. Biol.* 127, 1255 (1938b).
Dahlchakoff, V., *ibid.* 141, 114 (1947).
Diamond, M., *Proc. Amer. Soc. Zool. Anat. Rec.* 137, 349 (1960).
Gentry, J. W., [illegible] *J. Chem. Eng.* 48, 451 (1970a).
Gentry, J. W., [illegible] *Eng. Chem. Fundam.* 9, 671 (1970b).
Lee, E. S., *AIChE J.*, 14, 490 (1968a).
Lee, E. S., *ibid.* 14, 908 (1968b).
Phoenix, E. H., Goy, R. W., Gerall, A. A., Young, W. C., *Endocrinology* 65, 369 (1959).
Richards, C. E., *Analog Comput. Ed. Users Group Trans.* 2, 203 (1970).
Tedford, M. D., Young, W. C., *Abstr. Proc. Amer. Ass. Anat. Rec.* 136, 325 (1960).
Wilkes, J. O., *AIChE J.* 16, 501 (1970).
Young, W. C., "The Hormones and Mating Behavior," in "Sex and Internal Secretions," Vol II, Williams and Wilkins, Baltimore, Md., 1964, p 1173.
Young, D. M., Frank, T. G., *Int. Comput. Cent. Bull.* 2 (1963).

Received for review July 7, 1970. Accepted April 5, 197[illegible]. The computer time for this project was supported by the National Aeronautics and Space Administration Grant W's G-398 to the Computer Science Center of the University of Maryland, College Park, Md.

Model Ecosystem for the Evaluation of Pesticide Biodegradability and Ecological Magnification

Robert L. Metcalf, Gurcharan K. Sangha, and Inder P. Kapoor

Departments of Zoology and Entomology, University of Illinois, Urbana-Champaign, Ill. 61801

■ A small laboratory "model ecosystem" has been developed to study the biodegradability of pesticides, and evaluated with radiolabeled DDT, DDE, DDD, and methoxychlor. The model ecosystem has a terrestrial-aquatic interface and a seven-element food chain. It simulates the application of pesticides to crop plants and the eventual contamination of the aquatic environment. When used with 5 mg of ^{14}C-labeled DDT applied at an equivalent rate of about 1 lb per acre, the radiolabeled DDT was accumulated in mosquito larvae, snails, and fish as DDE, DDD, and DDT and concentrated from 10,000- to 100,000-fold. ^{14}C-DDE was concentrated 30,000- to 50,000-fold and stored with little metabolism, while ^{3}H methoxychlor was readily metabolized to mono- and di-OH derivatives and was stored at comparatively low levels. The model ecosystem approach has considerable value for studying the biodegradability and ecological fate not only of new candidate pesticides, but also of industrial chemicals.

Environmental pollution by stable lipid-partitioning substances such as DDT, dieldrin, polychlorbiphenyls, and dimethyl mercury has caused successive waves of concern by ecologists as the extent of their global distribution is revealed. We are slowly learning that such substances may accumulate to almost unbelievable levels—10^7 to 10^9-fold—in the lipids of carnivors at the upper ends of food chains. It appears that the presence of such stable contaminants in reservoirs such as the Great Lakes is virtually irreversible and may cause immense damage to important food chains and to fish and game.

There is an urgent need to develop persistent, yet biodegradable, pesticides that will remain effective for relatively long periods on household surfaces, in plants, animals, and the soil; yet when ingested by all sorts of living organisms from microorganisms to man will be decomposed by enzymatic processes to readily eliminable, water soluble fragments.

The threat of environmental catastrophes has demonstrated that we can no longer afford to learn about environmental behavior of new pesticides through one to two decades of wide-scale use. We need a realistic laboratory method for screening proposed new pesticides for their environmental fate. The use of radiolabeled compounds in such a model ecosystem would provide both qualitative and quantitative information about the metabolism of the compound in various plants and animals and should ideally provide for simulation of a simple environmental situation as a multicomponented laboratory organism.

We report here the evaluation of such a simple "model ecosystem" which has yielded valuable information about the biodegradability of numerous pesticides. The methodology described is capable of almost infinite variation and extrapolation and is essential in the study of new pesticides as a result of recent U.S. Department of Agriculture regulations providing for detailed information of potential effects on environmental pollution, as a registration requirement for new products (USDA, 1969).

MONS 206045

Experimental

Radiolabeled Compounds. The ^{14}C-DDT used in the evaluation of the model ecosystem was supplied by the Radiochemical Centre (Amersham, England). The compound was labeled in the aryl rings and had a specific activity of 5.48 mCi/mmol. The compound had been stored for several years in benzene solution, and thin-layer chromatography (TLC) with silicic acid and petroleum ether bp 60° to 68°C (Skellysolve B) showed it to contain approximately 69.5% DDT; 1.13% DDE, or 2,2-*bis*-(*p*-chlorophenyl)-1,1-dichloroethylene; and 5.8% TDE or DDD, or 2,2-*bis*-(*p*-chlorophenyl)-1,1-dichloroethane, together with 18.7% of more polar compounds such as *p,p*-dichlorobenzophenone. Since these latter impurities were desired as labeled compounds, approximately 50 mg of the labeled DDT was passed through a preparative column of silica gel and eluted with hexane. Pure fractions were collected and contained ^{14}C-labeled DDT, DDE, and DDD, all of which were +99% pure upon TLC in two systems: (*a*) petroleum ether bp 60° to 80°C, and (*b*) ether–petroleum ether, 1:9; and which cochromatographed with authentic samples of the pure compounds using fluorescent silicic acid (E. Merck $G_1$254). The R_f values for DDT and its degradation products are shown in Table I.

^{3}H-ring substituted methoxychlor was prepared by tritiation of methoxychlor as described by Hilton and O'Brien (1964) and purified by column chromatography to a purity of +99% as evaluated by TLC on silicic acid using (*a*) ether–petroleum ether (60° to 80°C), 3:1; or (*b*) petroleum ether, chloroform, methanol, 3:2:1. The ^{3}H-methoxychlor had a specific activity of 4.81 mCi/mmol.

Radioautographs of TLC plates were made using Eastman no-screen x-ray film and the plates were evaluated quantitatively by scraping 1-cm sections or fluorescent spots into scintillation vials. These were counted with use of "cocktail D" (100 g naphthalene and 5 g PPO in dioxane to make 1 liter) or for ^{3}H-labeled compounds in "^{3}H cocktail" (200 g naphthalene, 10 g PPO, and 0.25 g POPOP in dioxane to make 1 liter). Tritium-labeled compounds in biological fluids were assayed by the Schöniger oxygen flask combustion technique (Kelly *et al.*, 1961) as described by Kapoor *et al.* (1970). This method uniformly gave 99 to 100% recoveries.

Model compounds for TLC cochromatography were prepared as described by Kapoor *et al.* (1970) and were detected on TLC plates by (*a*) quenching of fluorescence, (*b*) spraying with 0.5% diphenylamine plus zinc chloride in acetone and heating to 110° for 10 min, followed by exposure to ultraviolet light to give pink spots with ethylene derivatives and black or bluish spots with dichloro- or trichloroethanes, or (*c*) for methoxychlor derivatives by exposure to ultraviolet light which produced yellow to orange spots from *p*-hydroxylated metabolites.

Table I. R_f Values for Model DDT Metabolites on Thin-Layer Chromatography

Compound	mp, °C	R_f on silicic acid with petroleum ether
2,2-*bis*-(*p*-Chlorophenyl) acetic acid	162	0.0
4,4'-Dichlorobenzophenone	146	0.06
2,2-*bis*-(*p*-Chlorophenyl)-2-hydroxy-1,1,1-trichloroethane	79	0.11
2,2-*bis*-(*p*-Chlorophenyl)-1,1-dichloroethane	110	0.17
2,2-*bis*-(*p*-Chlorophenyl)-1-chloroethane	54	0.33
2,2-*bis*-(*p*-Chlorophenyl)-1,1,1-trichloroethane	109	0.34
2,2-*bis*-(*p*-Chlorophenyl)-1,1-dichloroethylene	82	0.53
2,2-*bis*-(*p*-Chlorophenyl)-1-chloroethylene	67	0.50

The general methods for preparation and evaluation of radiolabeled samples have been described in detail by Kapoor *et al.* (1970).

Model Ecosystem Methodology. The basic units are glass aquaria 10 × 12 × 20 in. with 180 in.^{2} of surface. They contain 15 kg of washed white quartz sand molded into a sloping soil/air/water interface (Figure 1). The terrestrial end of the sand shelf is flattened for about 6 to 7 in. in width to accommodate growing plants. The aquaria contain 7 liters of "standard reference water" (Freeman, 1953) containing the following concentrations of salts, in ppm: $MgSO_4$, 36.4; K_2SO_4, 0.135; $CaCl_2$, 14.0; $NaHCO_3$, 25; NH_4NO_3, 3.0; K_2HPO_4, 0.78; $CaCO_3$, 57.5; $NaSiO_3$, 23.6; and $FeCl_3$, 0.81, adjusted to pH 7.9. This standard water has provided satisfactory mineral nutrition for the growth of the *Sorghum halpense* plants in the aerial portion of the chamber and for the *Oedogonium cardiacum* algae in the aquatic portion. It is also satisfactory for the growth and development of plankton, *Physa* snails, cladocera, mosquitoes, and fish. The use of pure sand and water culture is not only much more reproducible than soil but also eliminates a large amount of organic material which had uncertain effects and interfered with extraction and determination of radiolabeled products.

The model ecosystems containing 10 snails, algae, and a few milliliters of old aquarium water are aerated continuously by air bubbles from a Silent Giant aquarium pump. A Plexiglas cover (11 × 12 in.) covers the aquatic portion of the chambers and greatly reduces the rate of evaporation of the water. The individual aquarium units are housed in an environmental plant-growth chamber (Percival P-80) which is operated at 80°F ± 1° on a 12-hr diurnal cycle of 5000 ft candles.

Rearing Plants and Animals. A major part of the technology of the model ecosystems has dealt with the selection of a series of food chains of organisms compatible with the conditions in the aquarium. These, of necessity, must be readily reared and available throughout the year.

Sorghum halpense (Pioneer 846) was selected after many trials, as a thoroughly satisfactory plant for growth in the semiaquatic environment of the terrestrial end of the chamber. It combines uniform and rapid growth, palatability for the salt marsh caterpillar (the second element in the food chain), good germination and growth in the sand culture, and ease of treatment with the radiolabeled pesticide. Fifty seeds are planted in five rows along the flattened terrestrial end of the chamber. The seeds germinate in about three to four days, and, after 20 days, the plants are about 4 in. high and satisfactory for treatment with the radiolabeled pesticide.

Estigmene acrea, the salt marsh caterpillar, was selected as the key terrestrial insect because of its omnivorous and voracious nature and the considerable amount of information available in our laboratory about its ability to metabolize a variety of pesticides (Metcalf *et al.*, 1968; Kapoor *et al.*, 1970). To ensure its year-round availability, a synthetic diet modified from that of Searle and MacFarlane (1968) was used in paper cups with five to ten larvae. The eggs of the caterpillars are placed on top of 15 g of the diet, and the larvae transferred weekly to fresh diet, requiring about four weeks to

MONS 206046

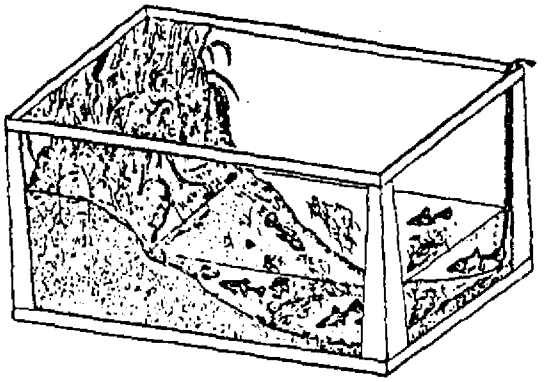

Figure 1. Schematic drawing of model ecosystem for studying pesticide biodegradability and ecological magnification

reach maturity. Ten early fifth instar larvae (27 to 33 days old) are placed on the treated sorghum plants and the 4 in. open end of the aquarium covered with copper screen to confine the insects. The larvae generally consume most of the treated plant surface within three to four days and their feces, leaf frass, and the larvae themselves contaminate the moist sand and water, and begin the distribution of the radiolabeled metabolic products throughout the model ecosystem. If the larvae are killed by the pesticide treatment as has occurred with carbofuran (2,2-dimethyl-2,3-dihydrobenzofuranyl-7 *N*-methylcarbamate) and aldicarb (2-methylthio-2-methylpropionaldehyde oximyl *N*-methylcarbamate) new groups of larvae are added at intervals until the sorghum is consumed. The time interval for consumption is noted as evidence of the rate of environmental degradability.

Daphnia magna, the water flea, was selected as the intermediate plankton organism because of its size and ease of rearing on a 0.5% infusion of powdered sheep manure in the "standard reference water" (Frear and Boyd, 1967). The *Daphnia* are extremely sensitive to pesticides in the ppt to ppb range (Frear and Boyd, 1967) and serve as a valuable indicator organism for the presence of toxic metabolites. Approximately 30 *Daphnia* are added to the model ecosystem at the beginning of the experiment, and if they are killed by the treatment, the aquarium is reinfested at intervals until the culture survives. This again provides useful ecosystem information.

Culex pipiens quinquefasciatus, mosquito larvae, are added to the model ecosystem after 26 days using 300 larvae. This species was chosen because of ease in rearing and a background of 20 years of evaluation in our laboratory of its response to thousands of insecticidal compounds. If the larvae do not survive, reinfestation can be accomplished at intervals as with *Daphnia*. However, none of the 10 insecticides studied to date have killed the larvae in the ecosystem procedure. After the larvae have remained in the model ecosystem for four days, 50 are removed for tissue analysis of their radioactive contaminants as described below.

Fish. The food chains are completed after 30 days by the addition of three *Gambusia affinis* mosquito fish which readily devour all the remaining mosquito larvae and the *Daphnia*. After three days in the system, these are sampled for radioactivity as described below.

Operation of Model Ecosystem. After sand and water are equilibrated for a day, the sorghum seeds are planted and 10 *Physa* snails, about 30 *Daphnia magna*, a few strands of *Oedogonium cardiacum* algae, and a few milliliters of old aquarium water containing a plankton culture are added. At about 20 days, when the *Sorghum* plants are approximately 4 in. high, they are treated quantitatively with 5.0 mg of radiolabeled pesticide (ca. 15 to 25 μmol). This is applied from about 1% acetone solution using a capillary pipet so that the compound contaminates only the leaves. This rate of application for the 6-in. wide plant area is approximately 1 lb per acre, and thus simulates a rate of treatment commonly used in pesticide applications.

The experiment is terminated after 33 days, when weighed samples of the organisms were examined for radioactivity. The samples are homogenized in water and extracted with equal volumes of diethyl ether. Both layers are examined by TLC to determine the qualitative and quantitative nature of the radiolabeled products, with use of radioautography and scintillation counting of serial sections. The water partitioning radioactivity is hydrolyzed by heating with 0.1*M* HCl to free metabolites conjugated as aglycones, phosphates, or sulfates. Samples of sand are counted for radioactivity together with duplicate 1-ml aliquots of water taken from the aquarium at 1, 3, 7, 14, and 28 days after treatment of the sorghum.

Food Chains. The food-chain pathways for the radiolabeled pesticide in the model ecosystem are:

(1) *Sorghum* → *Estigmene* (larva)
(2) *Estigmene* (excreta) → *Oedogonium* (algae)
(3) *Oedogonium* → *Physa* (snail)
(4) *Estigmene* (excreta) → Diatoms
(5) Diatoms → Plankton
(6) Plankton → *Culex* (larva)
(7) *Culex* → *Gambusia* (fish)

Among the diatoms identified are *Navicula*, *Coscinodiscus*, *Diploneis*, and *Diatomella*. The plankton species include *Daphnia*; the protozoans *Nuclearia*, *Caleps*, *Vorticella*, *Paramecium*; and the rotifers *Asplanchnopus*, *Notomatta*, *Euclaris*, and *Scaridium*.

Results and Discussion

During the past two years, more than 10 insecticides radiolabeled with ^{14}C or ^{3}H have been evaluated by the model ecosystem technique as described. Many of these are new insecticidal carbamates and the results obtained will be described elsewhere. The purpose of this paper is to describe and evaluate the proposed methodology and to demonstrate the results obtained with DDT and its derivatives which are persistent and essentially nonbiodegradable and with methoxychlor which is persistent and biodegradable.

Reproducibility. The ultimate purpose of the model ecosystem is to be used as a single living unit for in-depth studies of environmental biodegradability. The question of reproducibility of results is of considerable importance as even this simple ecosystem is substantially more complex than any single experimental animal or groups of animals. This factor has been evaluated by two complete individual evaluations of the behavior of ^{14}C-labeled DDT in the model ecosystem with the results shown in Table II. The two studies gave remarkably similar results, both as to distribution and accumulation of total radioactivity in the components of the systems and as to the quantitative biodegradability of DDT to DDE, DDD, and polar metabolites. Both experiments clearly demonstrate why DDT is an environmentally undesirable compound because of its stability and high lipid/water partitioning and because of its metabolic conversion to DDE and DDD which have

MONS 206047

Table II. Comparison of ^{14}C Distribution in Two Replicates of DDT in Model Ecosystem

	Water		Snail		Fish	
	I	II	I	II	I	II
Total ^{14}C, ppm	0.002	0.004	17.2	22.9	20.8	54.2
DDT, %	5.3	5.4	29.0	33.4	28.3	34.3
DDE, %	7.8	6.5	41.0	52.3	57.5	53.9
DDD, %	13.4	3.0	15.5	7.2	13.2	9.9
Polar compounds, %	68.5	79.8	9.8	4.3	1.0	1.6

environmental behavior very similar to the parent compound. These findings are analyzed more completely in the following discussion.

The data shown in Table II demonstrate that DDT is concentrated and stored in the tissues of the *Gambusia* fish which is at the top of the food chains in the model ecosystem to approximately 10,000-fold in Experiment I and 13,000-fold in Experiment II, over the concentration of ^{14}C in the water phase of 100 to 200 ppt, which is approaching the true solubility of DDT in water (Gunther *et al.*, 1968). The pure *p,p'*-DDT has been substantially converted to *p,p'*-DDE, which is clearly the predominant stable metabolite, accounting for about half of the ^{14}C radioactivity in both the fish and snails. Typical radioautographs from the TLC resolution of these compounds are shown in Figure 2.

Comparative Environmental Behavior of DDT, DDD, and DDE. It has been demonstrated repeatedly that DDT is biologically dehydrochlorinated to form DDE and, more recently, that under anerobic conditions it may undergo reductive dechlorination to form DDD (Peterson and Robison, 1964). However, little is known of the relative environmental stabilities of these three compounds or of their comparative food-chain effects. DDD is well known, from the Clear Lake, Calif., experience, to undergo ecological magnification in food-chain organisms in a manner very similar to DDT (Hunt and Bischoff, 1960). The model ecosystem approach with ^{14}C-labeled compounds provides a convenient method for investigation of this problem. Conversely, if the model ecosystem method is valid, the results of such evaluations should conform closely to data accumulating from environmental studies and should indicate the expected effects on environmental quality from the employment of these compounds.

Table III presents the comparative results of the model

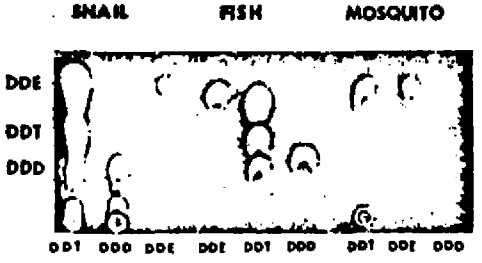

Figure 2. Radioautographs of TLC plates with extracts of fish, snails, and mosquito larvae from model ecosystems containing ^{14}C-DDT, DDE, and DDD

Table III. Distribution and Metabolism of Radiolabeled DDT, DDE, DDD, and Methoxychlor in a Model Ecosystem

	Concentration, ppm			
Pesticide	H_2O	Snail	Mosquito, dry wt	Fish
DDT–total ^{14}C	0.004	22.9	8.9	54.2
DDT	0.00022	7.6	1.8	18.6
DDE	0.00026	12.0	5.2	29.2
DDD	0.00012	1.6	0.4	5.3
Polar metabolites	0.0032	0.98	1.5	0.85
DDE–total ^{14}C	0.008	121.6	168.9	149.8
DDE	0.0053	103.5	159.5	145.0
Polar metabolites	0.0027	18.1	9.4	4.8
DDD–total ^{14}C	0.006	5.6	5.8	39.1
DDD	0.0004	3.3	3.43	33.4
DDD ethylene	...	0.24	...	2.08
Unknown I	...	0.14	...	1.54
Unknown II	...	0.87	...	...
Polar metabolites	0.0056	1.1	...	2.0
Methoxychlor–total ^{3}H	0.0016	15.7	0.48	0.33
Methoxychlor	0.00011	13.2	...	0.17
Methoxychlor ethylene	...	0.7	...	...
Mono-OH-ethane	0.00013	1.0	...	...
Di-OH-ethane	0.00003	...	...	...
Di-OH-ethylene	0.00003	...	...	...
Unknowns	0.00009	...	...	...
Polar metabolites	0.00125	0.8	...	0.16

ecosystem study of pure DDT, DDE, and DDD. The results in Table III show the route of conversion of DDT to DDE which is the predominately stable and persistent contaminant in the ecosystem. One month after the application of ^{14}C-DDT to the sorghum, 52% of the radioactivity in snails, 58% in mosquito larvae, and 54% in fish was DDE. In *Gambusia* fish, DDE was present at a concentration of 110,000 times that in water and DDT at 84,000 times that in the water, although the fish were present in the system for only three days. Substantial amounts of DDD were present in all three organisms reaching approximately 10% of the total ^{14}C in *Gambusia*. This, together with the relatively large percentage of polar metabolites in the water, 80%, shows that DDT is not by any means nonbiodegradable. However, its principal disadvantage environmentally would seem to be its conversion to DDE, which is relatively nonbiodegradable (Table III). In the DDT experiment, the snails contained about 3% of a metabolite R_f 0.05 which is probably *p,p'*-dichlorobenzophenone. The polar metabolite fraction is probably *p,p'*-dichlorodiphenylacetic acid, R_f 0.0.

The conclusions about the environmental fate of DDT are amplified by the experiment summarized in Table III, where ^{14}C-DDE was the starting contaminant in the model ecosystem. Although a substantial proportion of polar metabolites (34%) was found in the water phase after 30 days, these were minimal in the organisms (7% in snail, 6% in mosquito larvae, and 4% in fish), and no ^{14}C-labeled compounds other than DDE were identified in any of the organisms. The environmental stability of DDE is demonstrated by the radioautograph of snail extracts in Figure 2. Thus, it is apparent that DDE is a highly persistent lipid-partitioning metabolite of DDT which is responsible for the major portion of the adverse environ-

MONS 206048

mental effects of concentration storage in animal tissues following the use of DDT.

The data shown in Table III, where ^{14}C-DDD was the starting contaminant of the model ecosystem, show that DDD, like DDT and DDE, is a stable lipid-partitioning compound. However, DDD is more biodegradable than either DDT or DDE as shown by the appreciable amounts of polar metabolites and other compounds present in snail and fish (Figure 2). In *Gambusia*, in addition to about 5% polar metabolites, there were nearly equal quantities of two compounds with solubility characteristics similar to DDE—i.e., R_f 0.53 [probably 2,2-*bis*-(*p*-chlorophenyl)-1-chloroethylene] and R_f 0.47, identity unknown. The snail contained a much higher proportion of polar metabolites (20%), presumably resulting from the much longer exposure to the radiotracer over the 30-day period. In addition, there were traces of the same two metabolites, R_f 0.53 and R_f 0.47, and substantial amounts of two compounds with R_f values less than DDD, R_f 0.11, 11% [possibly 2,2-*bis*-(*p*-chlorophenyl)-2-hydroxy-1,1-dichloroethane] and R_f 0.05, 5% (probably *p,p'*-dichlorobenzophenone). (Figure 2.)

The overall picture of DDT contamination of the terrestrial-aquatic model ecosystem as revealed by the data of Table III is remarkably similar to that now being demonstrated in nature. For example, in Lake Michigan following widespread terrestrial applications of DDT to the watershed for the control of the gypsy moth, bark beetle, Japanese beetle, etc., recent studies show DDT in the water at 0.000002 ppm yet present in lake trout and coho salmon at 3 to 10 ppm (Johnson and Pecor, 1969). Thus, the simple model ecosystem studies described here have given a clear picture of the accumulation of DDT and its stable lipid-partitioning metabolites in food-chain organisms. Some 20 years have been required to demonstrate this phenomenon in the Lake Michigan ecosystem. The model ecosystem studies have also shown rather clearly the metabolic and degradative pathways which have produced the ecosystem phenomena.

Environmental Behavior of Methoxychlor. A comparison study has been made of the model ecosystem behavior of ^{14}C-methoxychlor, or 2,2-*bis*-(*p*-methoxyphenyl)-1,1,1-trichloroethane. This compound is a persistent, biodegradable pesticide because of its ready *o*-demethylation to various phenols as described in detail by Kapoor *et al.* (1970). The results obtained in the model system with methoxychlor are shown in Table III. The relatively large number of methoxychlor degradation products found demonstrates substantial degradation. The amounts of methoxychlor and derivatives found in fish were of the order of 0.01 those found with DDT, and half the radioactivity in the fish was as polar metabolites suggesting a rapid turnover of the xenobiotic. In the snail *Physa*, however, methoxychlor was stored to substantial levels and it appears that this snail [illegible]pable to metabolize methoxychlor rapidly. These results, [illegible]ether with other studies in progress, suggest that methoxychlor is environmentally degradable and will not be stored in high concentrations in most organisms.

The rate of leaching of the radiolabeled compounds into the aquatic phase of the ecosystem was evaluated. Whereas DDT, DDE, and DDD rose to maximum values over the four weeks, methoxychlor reached an earlier peak and declined.

Conclusion

Several years of study of the model ecosystem with a variety of radiolabeled insecticides have shown that this relatively simple method can be used to investigate the biodegradability and ecological fate of candidate new compounds. The results obtained with DDT after one month in the food chains of the model system show remarkable approximation to conditions observed after many years in nature. The model ecosystem technique is precise and permits the observation and study of metabolic transformations of the pesticide in the various elements of the system. The method gives a good estimation of the potential toxicity of the degradation products of the pesticide to a variety of organisms. The system seems to be especially suitable for computer modeling and for study of the effects of small changes in molecular properties upon environmental biodegradability. We believe it has utility in the screening of candidate pesticides for potentially disadvantageous environmental effects.

Literature Cited

Frear, D. E. H., Boyd, J. E., *J. Econ. Entomol.* 60, 1228 (1967).
Freeman, L., *Sewage Ind. Wastes* 25, 845, 1331 (1953).
Gunther, F. A., Westlake, W. E., Jaglan, P. S., *Residue Revs.* 20, 1 (1968).
Hilton, B. D., O'Brien, R. D., *J. Agr. Food Chem.* 12, 236 (1964).
Hunt, G., Bischoff, A. I., *Calif. Fish Game* 46, 91 (1960).
Johnson, H. E., Pecor, C., Trans. 34th N. American Wildlife, Natural Resources Conference, 1969.
Kapoor, I. P., Metcalf, R. L., Nystrom, R. F., Sangha, G. K., *J. Agr. Food Chem.* 18, 1145 (1970).
Kelly, R. G., Peets, E. A., Gordon, S., Buyske, D. A., *Anal. Biochem.* 2, 267 (1961).
Metcalf, R. L., Fukuto, T. R., Borck, K., El-Aziz, S., Munoz, R., Cassil, C. C., *J. Agr. Food Chem.* 16, 300 (1968).
Peterson, J. E., Robison, W. H., *Toxicol. Appl. Pharmacol.* 6, 321 (1964).
Searle, N. W., MacFarlane, J., *Ann. Entomol. Soc. Amer.* 61, 949 (1968).
U.S. Department of Agriculture, C-72, "Data Requirements with Respect to Pesticides in the Environment," 1969.

Received for review August 17, 1970. Accepted January 18, 1971. This research was supported by a grant from the Herman Frasch Foundation administered by the American Chemical Society.