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Summary: Trichlorobiphenyl degrades much more rapidly than Tetra and Penta. Pentachlorobiphenyl equivalent to DDE rate. DDE not a source of PCBs.

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LABORATORY MODEL ECOSYSTEM STUDIES OF THE DEGRADATION AND FATE OF RADIOLABELED TRI-, TETRA-, AND PENTACHLOROBIPHENYL COMPARED WITH DDE

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Radiolabeled tri-, tetra-, and pentachlorobiphenyls (PCB) and DDE were studied in a laboratory model ecosystem for degradation pathways, and biomagnification in alga, snail, mosquito, and fish. Trichlorobiphenyl was degraded in all the organisms of the model ecosystem much more rapidly than tetrachloro- and pentachlorobiphenyl. Pentachlorobiphenyl was approximately as persistent as DDE. There was a linear relationship between lipid/water partition and ecological magnification and between water solubility and ecological magnification. No evidence of conversion of DDE to PCB was detected.

The laboratory model ecosystem previously described (Metcalf *et al.* 1971) has been employed for the estimation of the environmental fate of DDT and a number of its analogues (Kapoor *et al.* 1970, 1972, 1973) and for study of aldrin, dieldrin, endrin, mirex, lindane, and hexachlorobenzene (Metcalf *et al.* 1973a). The methodology developed has yielded useful information about (1) the degradation pathways of the various xenobiotics, (2) the toxic effects of the compounds and their degradation products, (3) their comparative biomagnification and food chain concentration, and (4) their comparative biodegradability; all in organisms of five phyla linked in several food chains. This information has proved of value in characterizing the potential environmental pollutant effects of candidate insecticides (Kapoor *et al.* 1973, Coats *et al.* 1973) and of plasticizers (Metcalf *et al.* 1973b). In this paper we report the application of these techniques to a better understanding of the comparative environmental properties of trichloro-, tetrachloro-, and pentachlorobiphenyl (PCB's), and of dichlorodiphenyldichloroethylene (DDE) the persistent DDT degradation product.

Methods and materials

The laboratory model ecosystem evaluation was carried out in a small glass aquarium with a sloping terrestrial-aquatic interface of pure white sand exactly as previously described (Metcalf *et al.* 1971). The ^{14}C radio-labeled compounds were applied quantitatively from acetone solution at 5.0 mg (or *ca.* one kg per ha) to *Sorghum vulgare* seedlings grown in the terrestrial portion. The treated leaves were consumed by fourth instar salt

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marsh caterpillar larvae *Estigmene acrea*, whose activities and fecal products contaminated the aquatic portion of the system.

The radiolabeled products were transferred through several food chains, e.g., alga (*Oedogonium cardiacum*) → snail (*Physa*), plankton → water flea (*Daphnia magna*) → mosquito (*Culex pipiens quinquefasciatus*) → fish (*Gambusia affinis*). After 33 days in an environmental chamber at 26°C and a 12-hr photoperiod at 5,000 foot candles simulated daylight, the organisms were extracted with acetonitrile and the ^{14}C-radiolabeled compounds evaluated by TLC on silica gel containing fluorescent marker (E. Merck GF-254) and radioautography on no-screen x-ray film. Liquid scintillation counting of the individual components was done in cocktail D (5 g PPO and 100 g naphthalene in dioxane to make one liter) and counts were corrected to dpm by using channels ratio quenching correction. The residues, after extraction, were counted by total combustion to $^{14}CO_2$ by the Schöniger oxygen flask technique (Kelly *et al.* 1961) to determine the unextractable radioactivity. Whenever possible, the identity of individual components on the TLC plates was determined by cochromatography with known standards and by extraction and mass spectrometry.

Radiolabeled compounds. The individual ^{14}C-labeled PCB's were obtained from Mallinkrodt, St. Louis, Missouri. They were: 2,5,2′-trichlorobiphenyl (2,5-dichlorophenyl-ring-UL-^{14}C), 9.91 mCi per mmole with > 98% radiopurity and 41.5% Cl, and a principal constituent of Aroclor® 1242 (Webb and McCall 1972); 2,5,2′,5′-tetrachlorobiphenyl (ring-UL-^{14}C), 9.87 mCi per mmole with > 98% radiopurity and 48.7% Cl, and a principal constituent of Aroclor® 1248 (Webb and McCall 1972); and 2,4,5,2′,5′-pentachlorobiphenyl (2′,5′-dichlorophenyl-ring-UL-^{14}C), 9.87 mCi per mmole with > 98% radiopurity and 54.4% Cl, a principal constituent of Aroclor® 1254 (Webb and McCall 1972).

^{14}C labeled 2,2-*bis*-(*p*-chlorophenyl)-1,1-dichloroethylene (DDE) was prepared from ^{14}C-ring-UL *p,p′*-DDT obtained from the Radiochemical Centre, Amersham, England, 5.48 mCi per mmole, by dehydrochlorinating with 1.0 *M* alcoholic KOH, and purifying on a silicic acid column with hexane elution to 99% radiopurity.

Results

PCB's. The movement of ^{14}C radioactivity from *Sorghum* plants into the water phase of the model ecosystem is shown in Figure 1. All three chlorinated biphenyls reached a maximum concentration in water at about seven days after treatment and the levels of contamination declined as the PCB's were taken up by the organisms of the system. The levels of the chlorinated biphenyls in the water phase (Table I) were in the ppb range, below the water solubility of the compounds as determined by radiotracer technique (Table II).

Radioautographs of the extracts from the components of the model system after TLC are shown in Figure 2. The data in Table I represent the quantitative distribution of the ^{14}C in the spots on the TLC plates. The results for the three PCB's are also expressed in

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Table II in terms of ecological magnification (E.M.) (ppm in organism/ppm in water) and of biodegradability index (B.I.) (ppm polar degradation products/ppm nonpolar products). The E.M. values for the parent compounds increased substantially with the number of chlorine atoms, from trichlorobiphenyl (41.5% Cl) to tetrachlorobiphenyl (48.7% Cl) to pentachlorobiphenyl (54.4% Cl). Conversely the B.I. values decreased with increasing degree of chlorine. This consistent and regular behavior gives added confidence that these parameters are ecologically significant (see Kapoor *et al.* 1973) and must be a function of the number of C-H bonds available for hydroxylation by microsomal oxidations in the various organisms. The spots of low R_f value (0.02-0.06), Figure 2, are presumably hydroxylated PCB compounds and the polar radioactivity (R_f 0.0) is thought to consist of conjugates of these compounds. Wallnofer *et al.* (1973) have found 4-chloro-4'-hydroxybiphenyl as a metabolite of 4-chlorobiphenyl from soil fungus, *Rhizopus japonicus*. Yoshimura and Yammamoto (1973) have reported the 5-hydroxylated derivative as the major and the 3-hydroxylated derivative as the minor excretion product of 2,4,3',4'-tetrachlorobiphenyl in the rat. Hutzinger *et al.* (1972) have shown that rat and pigeon could hydroxylate 2,5,2',5'-tetrachlorobiphenyl but they could not detect hydroxylated metabolites in brook trout. However, the amounts of polar material in *Gambusia* (Figure 2, Table I) suggest that this fish is able to slowly hydroxylate this tetrachlorobiphenyl.

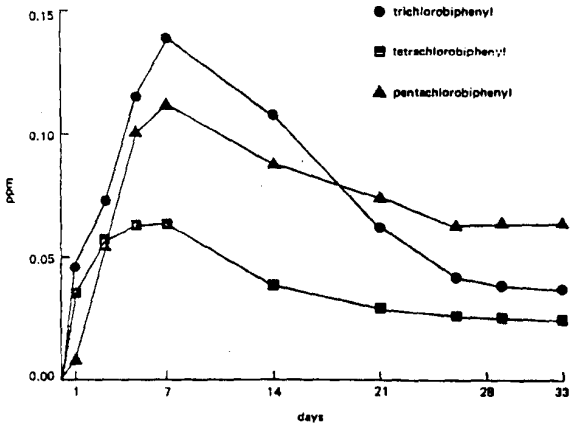


Fig. 1. Movement of total ^{14}C radioactivity from plants into the water phase of the model ecosystem and uptake by organisms.

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Table I. *Distribution of chlorinated biphenyls and their degradation products in the model ecosystem*

	Chlorinated biphenyl equivalents (ppm)				
	H_2O	*Oedogonium* (alga)	*Physa* (snail)	*Culex* (mosquito)	*Gambusia* (fish)
I. 2,5,2′-trichloro-biphenyl total ^{14}C	0.03845	23.2155	31.2015	2.7030	3.2055
Unknown I (R_f 0.66[a])	0.00015	15.9575	18.9720	1.1995	0.2085
trichlorobiphenyl (R_f 0.56)	0.00020	1.4630	1.1590	0.1630	1.2800
Unknown II (R_f 0.23)	0.00005	0.0520	0.6480	+	0.1595
Unknown III (R_f 0.10)	–	–	0.9735	–	–
Unknown IV (R_f 0.06)	0.00055	–	0.5460	–	–
Unknown V (R_f 0.04)	0.00040	–	0.2205	–	–
Unknown VI (R_f 0.03)	0.00040	0.0685	0.4410	–	–
Polar (R_f 0.0)	0.02265	0.5185	3.9315	0.4795	0.9985
Unextractable	0.01405	5.1560	4.3100	0.8610	0.5590
II. 2,5,2′,5′-tetrachloro biphenyl total ^{14}C	0.02065	23.6845	53.7465	14.5335	15.5685
tetrachlorobiphenyl (R_f 0.48[a])	0.00120	21.5975	47.3275	12.6745	14.2360
Unknown I (R_f 0.23)	0.00005	0.3220	0.7560	0.1070	0.0890
Unknown II (R_f 0.04)	0.00155	0.1030	0.4360	–	–
Polar (R_f 0.0)	0.01225	0.3275	3.9850	0.9670	0.8545
Unextractable	0.00560	1.3345	1.2420	0.7850	0.3900
III. 2,5,2′,4′,5′-penta-chlorobiphenyl total ^{14}C	0.04340	62.4660	633.0165	181.4565	127.6945
pentachloro-biphenyl (R_f 0.55[a])	0.00985	53.8440	587.3545	170.8480	119.7060
Unknown I (R_f 0.46)	–	0.6850	8.6210	2.4070	2.5380
Unknown II (R_f 0.39)	0.00020	0.5080	2.2490	1.3195	0.5810
Unknown III (R_f 0.21)	0.00015	0.1425	1.9365	1.0520	0.3285
Unknown IV (R_f 0.04)	0.00030	–	0.5000	–	–
Unknown V (R_f 0.02)	0.00385	0.2570	7.4965	–	0.7450
Polar (R_f 0.0)	0.02055	1.6265	16.5550	2.6745	2.3610
Unextractable	0.00850	5.4330	8.3040	3.1555	1.4350

[a]TLC with hexane (Skellysolve B,bp 60-68°C).

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Table II. *Ecological magnification (E.M.) and Biodegradability index (B.I.) of PCB's and DDE compared with water solubility and partition coefficient*

Chemical	H_2O solubility (ppb)	Partition coefficient	Ecological magnification (E.M.)				Biodegradability index (B.I.)			
			Alga	Snail	Mosquito	Fish	Alga	Snail	Mosquito	Fish
tri-Cl-PCB	16	7,803	7,315	5,795	815	6,400	0.30	0.17	0.35	0.60
tetra-Cl-PCB	16	8,126	17,997	39,439	10,562	11,863	0.015	0.082	0.076	0.060
penta-Cl-PCB	19	16,037	5,464	59,629	17,345	12,152	0.029	0.027	0.0134	0.019
DDE	1.3	18,893	11,251	36,342	59,390	12,037	0.069	0.049	0.033	0.050

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The pentachlorobiphenyl with B.I. values of 0.019 to 0.027 in fish and snail is very comparable in model ecosystem behavior to DDT, B.I. 0.015 and 0.044 (Kapoor *et al.* 1973) and this suggests that the two compounds should behave similarly in the environment (Risebrough *et al.* 1968). Properties of the tetrachlorobiphenyl were similar to those of the pentachlorobiphenyl (Figure 2) but the trichlorobiphenyl was much more degradable. A prominent degradative product (R_f 0.66) is stored in alga, snail, and mosquito larva in much greater quantities than the parent compound. This compound is less polar (higher R_f) in the hexane solvent than any of the three PCB isomers. As shown in Table I it is magnified to very high values, 106,382X in alga and 126,480X in snail, is stored in lipids, and is highly persistent. Its presence in high amounts in alga and in the snail and mosquito which are alga feeders suggests that it might be formed by photochemical processes during photosynthesis in the alga. This compound forms slowly and no traces of it were visible in three-day uptake studies of trichlorobiphenyl by alga, snail, daphnia, mosquito or fish (Metcalf and Lu 1973) although it appeared in alga in

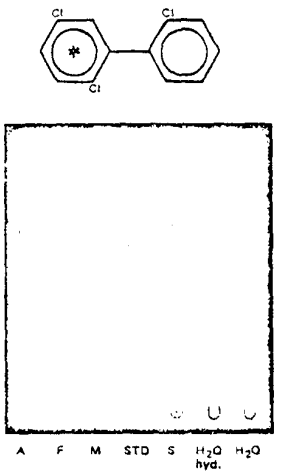


Fig. 2A. Radioautogram of TLC plate containing extracts of water and organisms treated with 2,5,2′-trichlorobiphenyl. A (alga), F (fish), M (mosquito larva), S (snail) and STD (^{14}C-radiolabeled compound).

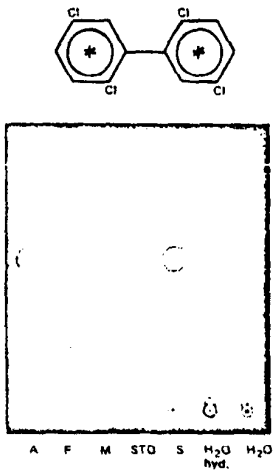


Fig. 2B. Radioautogram of TLC plate containing extracts of water and organisms treated with 2,5,2′,5′-tetrachlorobiphenyl. A (alga), F (fish), M (mosquito larva), S (snail) and STD (^{14}C-radiolabeled compound).

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14-day studies. To data we have been unsuccessful in identifying the unknown by mass spectrometry.

DDE. This compound has been implicated as a possible environmental precursor of PCB isomers through photoxidation reactions involving radical rearrangements to 3,6-dichlorofluorenone intermediates (Plimmer *et al.* 1970, Peakall and Lincer 1970, Moilanen and Crosby 1973). Although such rearrangements could logically produce, 4,4'-dichlorobiphenyl, it is difficult to see how trichloro- and tetrachlorobiphenyls could be formed as suggested by Maugh (1973). Moreover, Kerner *et al.* (1972) could detect only *bis*-(*p*-chlorophenyl)-chloroethylene (DDMU) after ultraviolet irradiation of DDE. Because of the ecological importance of these possible rearrangements we have reinvestigated the behavior of DDE in the model ecosystem (Metcalf *et al.* 1971) to determine if any PCB-like products could be formed under the simulated daylight of the model ecosystem (5000 foot candles) in an environmental chamber. The radioautograph show-

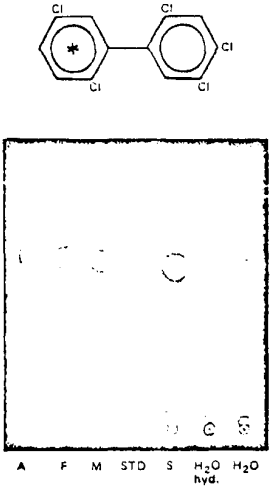


Fig. 2C. Radioautogram of TLC plate containing extracts of water and organisms treated with 2,4,5,2',5'-pentachlorobiphenyl. A (alga), F (fish), M (mosquito larva), S (snail) and STD (^{14}C-radiolabeled compound).

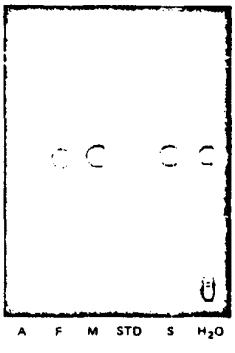


Fig. 2D. Radioautogram of TLC plate containing extracts of water and organisms treated with DDE. A (alga), F (fish), M (mosquito larva), S (snail) and STD (^{14}C-radiolabeled compound).

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ing the fate of pure DDE is presented in Figure 2. When the extracts of water and organisms were developed on TLC plates with Skellysolve B (hexane fraction) there was no trace of any ^{14}C labeled compounds with R_f values between 0.05 and 0.47 (DDE) or of any less polar materials with higher R_f values. Under these conditions, as shown in Figure 2, trichlorobiphenyl has R_f 0.43, tetrachlorobiphenyl R_f 0.50, and pentachlorobiphenyl R_f 0.53. Detection levels with the techniques used are approximately 0.1 ng (*e.g.*, spot at alga origin in DDE, Figure 2) or about 0.00002% of the total ^{14}C applied. Thus under the model ecosystem conditions there is no evidence of formation of PCB isomers from DDE.

DDE is extremely stable in the tissues of the living organisms of the model ecosystem and is stored as approximately 92, 93, 95, and 97% of the total ^{14}C in snail, alga, fish, and mosquito larva. The percent of unextractable ^{14}C in these organisms ranged from 0.10 to 0.93 (Table III). The B.I. value for DDE in fish was 0.049 and the E.M. value 12,037 (compared with 0.032 and 27,358 found by Metcalf *et al* (1971). From these values it is apparent that DDE is a more stable environmental pollutant than 2,4,5,2',5'-pentachlorobiphenyl (Table I) which was stored in the organisms at 86 to 94% of the total radioactivity, with from 1.12 to 8.67% of unextractable ^{14}C, and had a B.I. of 0.019 and an E.M. of 12,152 in fish.

It is of interest that Sodergren (1973) using a model aquatic ecosystem found no major metabolic changes in DDE occurring in passage through a food chain into fish, although similar experiments with a polychlorinated biphenyl mixture (Clophen A) showed that the lower fractions with low chlorine content were degraded when transported through the food chain, as was 2,5,2'-trichlorobiphenyl in our experiments. However, in our studies (Figure 2, Table III) the water phase contained several polar radio

Table III. *Distribution of DDE and degradation products in the model ecosystem*

	DDE equivalents (ppm)				
	H_2O	*Oedogonium* (alga)	*Physa* (snail)	*Culex* (mosquito)	*Gambusia* (fish)
Total ^{14}C	0.00384	7.4720	38.1958	24.8588	7.8653
DDE (R_f 0.49[a])	0.00062	6.9759	22.5325	36.8223	7.4632
Unknown 1 (R_f 0.05)	0.00009	–	0.8035	–	–
Polar (R_f 0.0)	0.00223	0.4881	1.1612	1.2448	0.3746
Unextractable	0.0009	0.0080	0.3616	0.1087	0.0275

[a]TLC with hexane (Skellysolve B, bp 60-68°C).

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labeled degradation products. These were resolved on silica gel into at least 11 distinct compounds using a solvent of benzene:dioxane:acetic acid (90:30:1) and we are presently attempting to identify the pathway of DDE degradation in the environment.

Biomass Recovery. To determine the relative availability of the various organisms of the model ecosystem as reservoirs for the bioaccumulation of the micropollutants studied, the total amounts of ^{14}C-labeled products recovered from the principal organisms of the model ecosystems treated with tri-, tetra-, and pentachloro-PCB's, and DDE were evaluated as shown in Table IV. The evaluations were made on the basis of total recovery of the applied pollutant, recovery of the maximum amount of pollutant in water (Figure 1) for each of the four principal organisms, alga, snail, mosquito, and fish; and biomass recovery (four organisms) of the total amount of pollutant lost from water (Figure 1). The figures of Table IV are very revealing in terms of the biodegradability of the various compounds. The highest recoveries of the ^{14}C lost from solution were obtained from the organisms with DDE, 65.8%, and pentachlorobiphenyl, 57.2%. With tetrachlorobiphenyl recoveries of 8.7% were still substantial, but with trichlorobiphenyl (recovery 0.45%) the compound was nearly completely degraded and excreted.

Table IV. *Biomass recovery of chlorinated biphenyls, and DDE from organisms of model ecosystem*

% Recovery	Alga	Snail	Mosquito	Fish
		trichlorobiphenyl		
^{14}C in solution	0.18	0.015	0.0017	0.12
total ^{14}C	0.033	0.0028	0.00032	0.021
(biomass) of ^{14}C lost from solution ---	0.45			
		tetrachlorobiphenyl		
^{14}C in solution	3.33	1.04	0.23	1.91
total ^{14}C	0.28	0.088	0.019	0.16
(biomass) of ^{14}C lost from solution ---	8.7			
		pentachlorobiphenyl		
^{14}C in solution	4.57	19.0	2.32	11.8
total ^{14}C	0.74	3.06	0.37	1.90
(biomass) of ^{14}C lost from solution ---	57.2			
		DDE		
^{14}C in solution	22.4	4.03	2.25	20.2
total ^{14}C	0.24	0.044	0.055	0.22
(biomass) of ^{14}C lost from solution ---	65.8			

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Degradation in Salt Marsh Caterpillar. This animal was chosen, after considerable study, as the dispersing agent for the model ecosystem because it was able to ingest a large variety of organic compounds without apparent injury (Metcalf *et al.* 1973). The effects of passage of the PCB isomers through the insect are of interest as representing the first stage in the biodegradation of these compounds. Figure 3 shows radioautographs of TLC plates of extracts of feces and body homogenates from larvae feeding on about 30 μg of ^{14}C PCB incorporated in a synthetic diet. Figure 3 and the quantitative

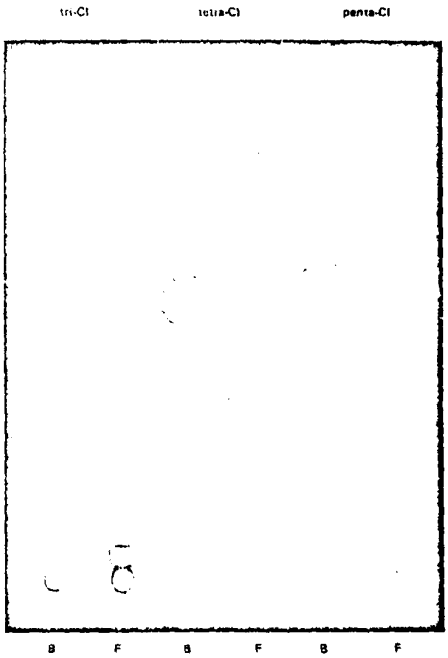


Fig. 3. Radioautogram of TLC plate containing extracts of bodies and feces of salt marsh caterpillar larvae fed ^{14}C-labeled 2,5,2′-tri-, 2,5,2′,5′-tetra-, and 2,4,5,2′,5′-pentachlorobiphenyls. B (body homogenate), and F (fecal excreta).

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evaluation of the radioactivity in the various spots shown in Table V demonstrate conclusively the much greater degradability of the trichlorobiphenyl over the tetrachlorobiphenyl and pentachlorobiphenyl. With the trichloro-compound the caterpillar feces contained 91% of the recovered ^{14}C, with the remainder in the body homogenate, while with the tetrachloro- and pentachlorobiphenyls, the feces contained 21% and 24% of the radioactivity. The unknown (R_f 0.05) found in feces after trichlorobiphenyl is probably the principal hydroxylated degradation product leading to the very large amount of polar radioactivity. Whereas only low levels of trichlorobiphenyl were retained in the salt marsh caterpillar body, with tetrachloro- and pentachlorobiphenyl the major portion of ^{14}C was retained in the insect body.

Table V. *Metabolism of ^{14}C radiolabeled compounds by salt marsh caterpillar*[a]

	Body	Feces
A. 2,5,2′-trichlorobiphenyl total ^{14}C (%)	8.66	91.34
Unknown I (R_f 0.53[a])	0.64	–
trichlorobiphenyl (R_f 0.43)	5.84	8.91
Unknown II (R_f 0.31)	0.27	0.37
Unknown III (R_f 0.13)	0.05	0.12
Unknown IV (R_f 0.05)	0.10	4.67
Unknown V (R_f 0.02)	0.11	0.92
Polar (R_f 0.0)	1.65	76.35
B. 2,5,2′,5′-tetrachlorobiphenyl total ^{14}C (%)	78.68	21.32
tetrachlorobiphenyl (R_f 0.50[a])	75.60	15.08
Unknown I (R_f 0.41)	0.99	1.36
Unknown II (R_f 0.05)	0.13	–
Unknown III (R_f 0.03)	trace	0.20
Polar (R_f 0.0)	1.96	4.64
C. 2,5,2′,4′,5′-pentachlorobiphenyl total ^{14}C (%)	75.86	24.14
pentachlorobiphenyl (R_f 0.53[a])	74.00	20.70
Unknown I (R_f 0.46)	0.74	0.74
Unknown II (R_f 0.39)	0.62	0.56
Unknown III (R_f 0.03)	0.08	0.08
Polar (R_f 0.0)	0.42	2.06
D. 2,2-*bis*-(*p*-chlorophenyl)-1,1-dichloroethylene (DDE) total ^{14}C (%)	80.59	19.41
DDE (R_f 0.49[a])	76.88	19.37
Polar (R_f 0.0)	3.71	0.04

[a]TLC with hexane (Skellysolve B, bp 60-68°C).

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DDE passed through the salt marsh caterpillar largely unchanged with 81% of the total radioactivity recovered retained in the body homogenate and 19% in the fecal excreta (Table V).

Ecological Magnification. The uptake and concentration of organic compounds by living organisms either directly or through food chains appears to be a function of two important factors, their high lipid solubility and low water solubility, *i.e.*, a large lipid/water partition coefficient; and their resistance to degradation by enzymatic processes, especially the multifunction oxidase enzymes (Metcalf *et al.* 1973). Hamelink *et al.* (1971) have suggested that the water insolubility of highly lipid-soluble compounds provides the driving force in producing lipid storage, through a series of simple partitionings from water to lipids. We have correlated the E.M. values for the PCB's and DDE from the fish of the model ecosystems with both water solubility (Table II) in Figure 4, and with the octanol/water partition value (Table II) in Figure 5. Because the values for the PCB's and DDE fall closely together, the relationships have been extended using values for aniline, anisole, benzoic acid, chlorobenzene, and nitrobenzene taken from other model ecosystem studies (Lu and Metcalf 1974). For the limited number of compounds included, the correlation between physical properties and biomagnification is excellent. The regression equation for log water solubility vs log E.M. (Figure 4) was:

$$y = 4.4806 - 0.4732\,X; \qquad n = 9, \qquad r = -0.9677$$

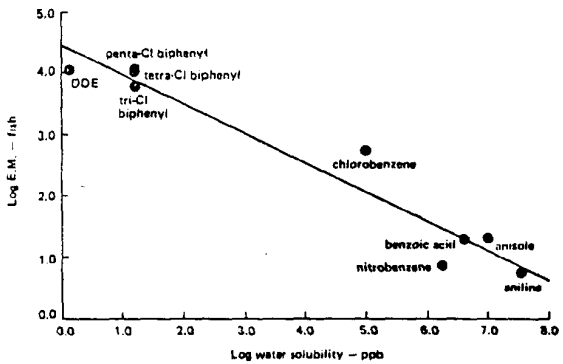


Fig. 4. Plot of log E.M. (ecological magnification) for fish *vs.* log water solubility (ppb).

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The regression equation for log partition coefficient (Hansch's π) vs. log E.M. (Figure 5) was:

$$y = -0.7504 + 1.1587 X; \quad n = 9, \quad r = 0.9771$$

Thus for the organic compounds studied, the properties of water solubility and octanol/water partition coefficient appear to provide a realistic estimate of the biological magnification found in living organisms.

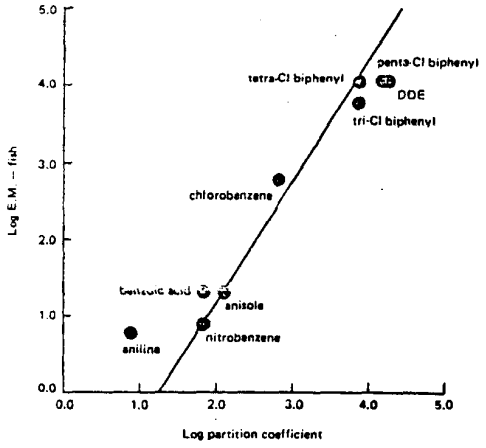


Fig. 5. Plot of log E.M. (ecological magnification) for fish *vs.* log octanol/water partition coefficient.

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