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Environmental Fate and Biodegradability of Benzene Derivatives as Studied in a Model Aquatic Ecosystem

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A model aquatic ecosystem is devised for studying relatively volatile organic compounds and simulating direct discharge of chemical wastes into aquatic ecosystems. Six simple benzene derivatives (aniline, anisole, benzoic acid, chlorobenzene, nitrobenzene, and phthalic anhydride) and other important specialty chemicals: hexachlorobenzene, pentachlorophenol, 2,6-diethylaniline, and 3,5,6-trichloro-2-pyridinol were also chosen for study of environmental behavior and fate in the model aquatic ecosystem. Quantitative relationships of the intrinsic molecular properties of the environmental micropollutants with biological responses are established, e.g., water solubility, partition coefficient, π constant, σ constant, ecological magnification, biodegradability index, and comparative detoxication mechanisms, respectively. Water solubility, π constant, and σ constant are the most significant factors and control the biological responses of the food chain members. Water solubility and π constant control the degree of bioaccumulation, and σ constant limits the metabolism of the xenobiotics via microsomal detoxication enzymes. These highly significant correlations should be useful for predicting environmental fate of organic chemicals.

The discovery of ubiquitous environmental contamination by chlorinated pesticides, polychlorinated biphenyls, and phthalate ester plasticizers has focused attention on the distribution, fate, and possible toxic action of the thousands of synthetic organic chemicals produced commercially.† The accidental entry of these substances into water deserves particular study because of the capacity of many aquatic organisms to biomagnify organic compounds 10^5- to 10^7-fold, from parts per trillion concentrations in water to parts per million levels in their body lipids. This phenomenon is particularly destructive with pollutants such as DDT, DDE, dieldrin, and PCBs in the Great Lakes as biomagnified in lake trout and coho salmon.

Aromatic compounds make up about 46% of the total synthetic organic chemical production in the United States. The simple aromatics are of great importance as the building blocks of the chemical industry and the benzenoid nucleus is incorporated into thousands of specialty chemicals: pesticides, pharmaceuticals, dyestuffs, and detergents. We chose to study the behavior and fate of six simple benzene derivatives in a

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† The Toxic Substances List of the National Institute for Occupational Safety and Health includes 25,043 chemicals (1973).

model aquatic ecosystem with a six element food chain. The compounds studied and their approximate annual U.S. production (1973) are: phthalic anhydride 936 $\times$ 10^6 lb, chlorobenzene 530 $\times$ 10^6 lb, aniline 388 $\times$ 10^6 lb, nitrobenzene 220 $\times$ 10^6 lb, anisole 20 $\times$ 10^6 lb, and benzoic acid 15 $\times$ 10^6 lb (U.S. Tariff Commission). These particular compounds were selected for investigation because of their wide range of physicochemical parameters: reactivity (σ constants), lipid/water partitioning (π constants), and water solubility which could be used to develop quantitative relationships with biomagnification, biodegradability, and comparative detoxication mechanisms. In order to test the predictive properties of these parameters, we have also evaluated several simple benzene derivatives which are important specialty chemicals: hexachlorobenzene and pentachlorophenol which are fungicides and industrial waste pollutants; 2,6-diethylaniline, an intermediate in the production of alachlor herbicide [2-chloro-2′,6′-diethyl-*N*-methoxymethyl) acetanilide], and 3,5,6-trichloro-2-pyridinol, an intermediate in the production of chlorpyrifos insecticide, (*O,O*-diethyl *O*-3,5, 6-trichloro-2-pyridyl phosphorothionate). The model aquatic ecosystem behavior of these specialty chemicals provides confirmation of the accuracy and applicability of generalizations which can be made from the environmental behavior of the simple benzene derivatives. Radiolabeled DDT and aldrin were also evaluated to provide comparisons of the accumulation and degradation of very water-insoluble organic compounds.

Materials and Methods

Radioactive Compounds

Radioactive compounds were obtained from various sources as listed in Table 1. Tritiated anisole was synthesized by the $BF_3 \cdot H_3PO_4$-catalyzed exchange with tritiated water (*1*). The radiochemical purity of all labeled compounds was evaluated by thin-layer chromatography (TLC) and autoradiography and impurities were removed by recrystallization, redistillation, TLC, GC, and silicic acid column chromatography.

Model Aquatic Ecosystem

A model ecosystem was designed for the evaluation of environmental fate of relatively volatile compounds which are directly discharged into the aquatic system. It consists of a 3-liter round-bottomed flask with three necks to which are fitted (a) a microware condenser to reduce the losses of volatile

Table 1. Molecular properties and biological responses of organic chemicals evaluated in model aquatic ecosystem.

Compound	Label (source)[a]	Water solubility, ppb[b] $\times 10^3$	Partition coefficient	Ecological magnification	Biodegradability index
Aldrin	^{14}C-ring-UL (A-S)	0.2	1,030	1,312	0.015
Aniline	^{14}C-ring-UL (A-S)	36,600	7	6	1.784
Anisole	^{3}H-ring-UL (syn)	10,400	119	22	0.250
Benzoic acid	^{14}C-ring-UL (A-S)	4,200	108	21	2.965
Chlorobenzene	^{14}C-ring-UL (A-S)	100	150	650	0.014
DDT	^{14}C-ring-UL (A-S)	0.0012	9,490	16,950	0.012
2,6-Diethylaniline	^{14}C-ring-UL (Mon.)	670	9	124	0.139
Hexachlorobenzene	^{14}C-ring-UL (NIEHS)	0.006	13,560	1,166	0.377
Nitrobenzene	^{14}C-ring-UL (M)	1,780	62	29	0.023
Pentachlorophenol	^{14}C-ring-UL (M)	14	6,405	296	0.338
Phthalic anhydride	^{14}C=0 (NEW)	6,200	0.24	0	11.884
3,5,6-Trichloro-2-pyridinol	^{14}C-2, 6 (Dow)	220	188	16	0.311

[a] Source: A-S = Amersham-Searle, Dow = Dow Chemical, M = Mallinckrodt, Mon. = Monsanto Co., NIEHS = National Institute Environmental Health Science, NEW = New England Nuclear, syn = synthesis.

[b] Data were obtained from Stephen and Stephen (*2*), from manufacturer, and from experimental determinations.

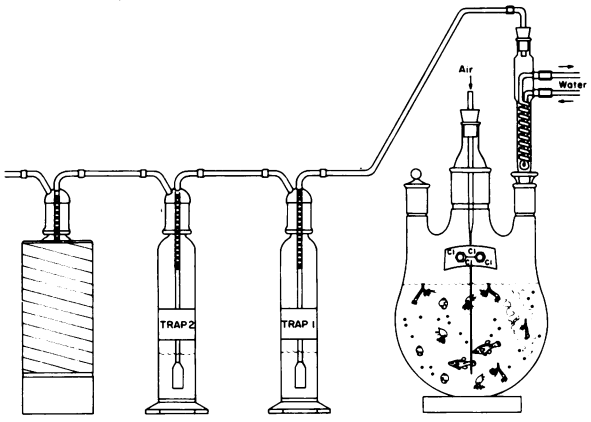

FIGURE 1. Diagram of model aquatic ecosystem for study of volatile environmental pollutants.

compounds and to retain a constant water level in the main flask, (b) a removable filter for a constant air flow and for sampling water in the flask, and (c) two traps for collection of the volatile metabolites and/or parent compound, and radioactive CO_2, respectively. These traps contain 100 ml of acetonitrile and 100 ml of 0.5*N* aqueous sodium hydroxide, respectively. This system contains 2 liters of reference standard water that provides satisfactory minerals for the organisms (*3*). The microenvironment of the food chain members consists of phyto- and zooplanktons, green filamentous algae (*Oedogonium cardiacum*), snails (*Physa*), water flea (*Daphnia magna*), mosquito larvae (fourth instar), (*Culex quinquifasciatus*), and mosquito fish (*Gambusia affinis*). The whole system as shown in Figure 1 was kept in a programmed environmental growth chamber set at 80°F (26.7±2°C) with 12 hr daylight exposure to 750 ft-candles (7500 lux).

Operation of Model Aquatic Ecosystem

The components of the model aquatic ecosystem: 300 daphnia, 200 fourth instar mosquito larvae, 6 snails, strands of alga, and miscellaneous plankton were acclimated in the chamber for 1 day. The water was then treated with 0.01-0.1 ppm of radiolabeled compound to produce the experimental contamination. After 24 hr, 50 mosquito larvae and 100 daphnia were removed for radioassay, 3 fish were added, and after another 24 hr the experiment was terminated. Water and trap samples were taken at zero time for use as blanks and after the first and second days to analyze the distribution of radioactivity. The radioactivity in the water was extracted in diethyl ether and analyzed by TLC on silica gel and autoradiography. The organisms were washed, dried, weighed, homogenized, and extracted three times with acetone. The total radioactivity was measured and the pooled extracts were concentrated and analyzed by TLC and autoradiography. Labeled metabolites and degradation products were scraped from the plates and quantitatively determined by liquid scintillation counting. Wherever possible the identity of the radiolabeled spots was determined by cochromatography with known model compounds. The environmental behavior of the radiolabeled contaminants is expressed in the tables in terms of quantitative distribution of the parent compound and various metabolites and degradation products, and in graphs of the various degradation mechanisms utilized by the various organisms of

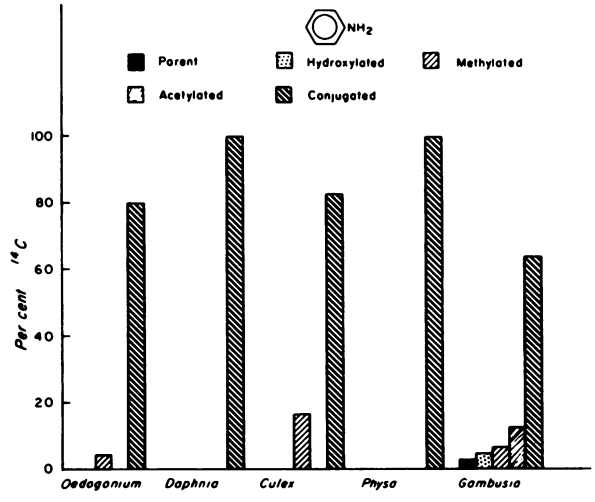

FIGURE 2. Relative detoxication capacities of key organisms of model aquatic ecosystem following treatment with radioactive aniline.

Table 2. Distribution of aniline and degradation products in model aquatic ecosystem.

	R_f[a]	Aniline equivalents, ppm					
		H_2O	*Oedogonium* (alga)	*Daphnia* (daphnia)	*Culex* (mosquito)	*Physa* (snail)	*Gambusia* (fish)
Total ^{14}C		0.15737	1.5935	0.2516	1.1063	1.7939	2.4040
N, *N*-Dimethylaniline	0.75	0.01994	—	—	0.1813	—	0.1048
N-Methylaniline	0.71	0.01005	0.0710	—	—	—	0.0790
Aniline	0.61	0.01620	—	—	—	—	0.0718
Unknown 1	0.55	0.01108	—	—	—	—	—
Phenol	0.49	0.00456	—	—	—	—	—
Acetanilide	0.33	0.04085	—	—	—	—	0.3164
Unknown 2	0.25	0.00376	—	—	—	—	—
o-Hydroxyaniline	0.20	0.00070	—	—	—	—	0.0514
p-Hydroxyaniline	0.17	0.00093	—	—	—	—	0.0480
m-Hydroxyaniline	0.12	0.00110	—	—	—	—	0.0387
Unknown 3	0.07	0.00119	—	—	—	—	0.0541
Unknown 4	0.02	—	0.2458	—	—	—	0.0993
Polar	0.0	0.01491	1.2767	0.2516	0.9250	1.7939	1.5405
Unextractable		0.03210	—	—	—	—	—

[a] TLC with benzene:acetone:Skellysolve B (bp 60–68°C):diethylamine = 65:25:25:5 (v/v).

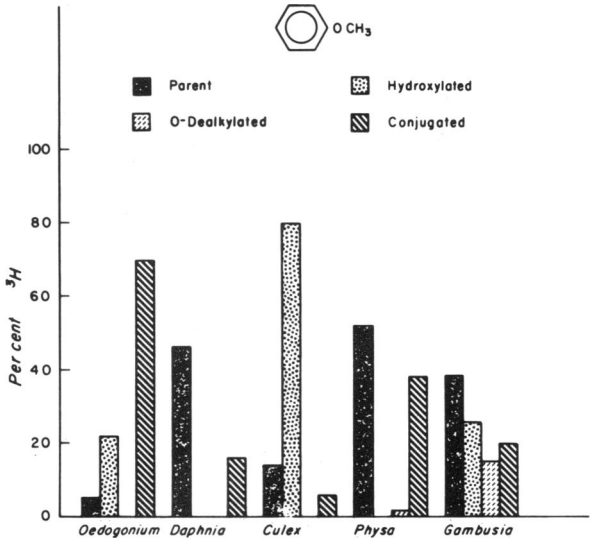

FIGURE 3. Relative detoxication capacities of key organisms of model aquatic ecosystem following treatment with radioactive anisole.

the model aquatic ecosystem. The techniques for measurement of radioactivity, partition coefficients, water solubilities, and for the identification of labeled products have been fully described (*4,5*).

Results and Discussion

The various aquatic contaminants were evaluated by quantitative distribution of the radioactivity in the organisms of the model aquatic ecosystem, in the water, and in the traps. Several of the compounds evaluated were appreciably volatile, and the following percentages were recovered from the traps: anisole, 78.45%, trap I; chlorobenzene, 95.97%, trap I; nitrobenzene, 2.22%, trap I; and phthalic anhydride, 5.02%, trap II. More than 99% of all of the other materials was retained in the water phase and its organisms.

Degradative Pathways

Aniline was rapidly detoxified by methylation, acetylation, hydroxylation and conjugations. Daphnia and snail were able to metabolize aniline completely to polar metabolites as shown in Figure 2. *N*-Methyl and *N*,*N*-dimethylaniline were found in alga and mosquito larva, respectively. The fish was the only species which retained small amounts of aniline with ecological magnification (EM) 6, together with *N*-methylaniline and *N*,*N*-dimethylaniline and almost equal amounts of *o*-, *m*-, and *p*-aminophenols. Acetanilide was found in fish and water extracts and was further metabolized to *p*-acetamidophenol then conjugated (*6*). The quantitative distribution of ^{14}C-aniline equivalents is shown in Table 2.

Table 3. Distribution of anisole and degradation products in model aquatic ecosystem.

	R_f[a]	Anisole equivalents, ppm					
		H_2O	*Oedogonium* (alga)	*Daphnia* (daphnia)	*Culex* (mosquito)	*Physa* (snail)	*Gambusia* (fish)
Total ^3H		0.11801	77.3432	14.2508	1.6380	14.5184	0.4378
Unknown 1	0.88	—	—	2.2321	—	—	—
Unknown 2	0.85	—	—	3.1622	—	—	—
Anisole	0.73	0.00854	4.8107	6.5934	0.2342	7.6787	0.1693
Unknown 3	0.65	0.00318	—	—	—	1.0932	—
o-Hydroxyanisole	0.59	0.00419	17.6806	—	—	—	0.0836
Phenol	0.51	0.00482	—	—	—	0.1669	0.0668
p-Hydroxyanisole	0.41	0.00798	—	—	1.3063	—	0.0285
Unknown 4	0.26	0.00094	—	—	—	—	—
Unknown 5	0.22	0.00112	—	—	—	—	—
Unknown 6	0.14	0.00106	—	—	—	—	—
Polar	0.0	0.01217	54.8519	2.2631	0.0975	5.5796	0.0896
Unextractable		0.07401	—	—	—	—	—

[a] TLC with chloroform:benzene:ethyl acetate = 65: 15:15 (v/v) at 0°C (cold room).

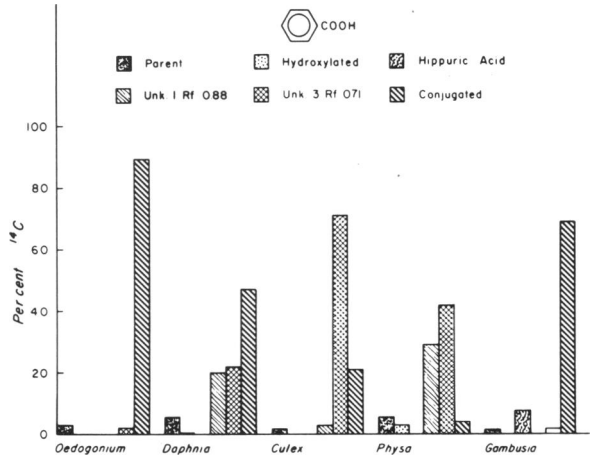

FIGURE 4. Relative detoxication capacities of key organisms of model aquatic ecosystem following treatment with radioactive benzoic acid.

Anisole was stored in various organisms in substantial amounts, EM for the fish, mosquito larva, alga, daphnia, and snail being 22, 27, 563, 771, and 899, respectively. This indicated that anisole is a fairly stable ether, even though it can be degraded by *O*-dealkylation in fish and snail as concluded from the present experiment. Hydroxylation to *o*- and *p*-methoxyphenols occurred in all species except the daphnia. Conjugation was the important detoxification mechanism in alga and snail as shown in Figure 3. The quantitative distribution of ^3H-anisole equivalents is presented in Table 3.

Benzoic acid was not generally stored or ecologically magnified except in snails and daphnia, with EM values of 21 in fish, 102 in alga, 138 in mosquito larva, 1772 in daphnia and 2786 in snail. Conjugation through glycine was an important detoxication mechanism, and substantially higher EM values of hippuric acid were found in daphnia (1317), fish (3973), and snail (4535). Thus these species can detoxify benzoic acid much more effectively by this mechanism than alga and mosquito larva. Hydroxylation to phenolic acids occurred to only limited extent in snail, daphnia, with traces in mosquito larva and none in the fish (Fig. 4). Hydroxybenzoic acids and catechol were found in the water extract. Catechol is the key compound to enter the β-ketoadipate pathway for the dissimilation of aromatic compounds, leading to further complete degradation of benzoic acid (*7*). The quantitative distribution of ^{14}C-benzoic acid equivalents is shown in Table 4.

Chlorobenzene was a very persistent environmental pollutant, as demonstrated by the EM values of 645 in fish, 1292 in mosquito larva, 1313 in snail, 2789 in daphnia, and 4185 in alga. The low biodegradability index (BI) values, ranging from 0.014 to 0.063,

Table 4. Distribution of benzoic acid and degradation products in model aquatic ecosystem.

	R_f[a]	Benzoic acid equivalents, ppm					
		H_2O	*Oedogonium* (alga)	*Daphnia* (daphnia)	*Culex* (mosquito)	*Physa* (snail)	*Gambusia* (fish)
Total ^{14}C		0.06442	6.8322	56.5354	30.4336	87.7822	3.3772
Unknown 1	0.88	—	—	10.9565	0.6232	25.9133	0.0189
Unknown 2	0.78	0.00139	—	—	—	—	—
Unknown 3	0.71	0.00110	0.1920	12.9215	14.6897	37.3425	0.0439
Unknown 4	0.66	—	—	—	—	4.7226	—
Benzoic acid	0.61	0.00178	0.1817	3.1546	0.2472	4.9596	0.0375
o-Hydroxybenzoic acid	0.54	0.00033	—	0.0273	Trace	1.3606	—
Catechol	0.50	0.00173[b]	—	0.0364	Trace	1.4045	—
m- and *p*-Hydroxybenzoic acid	0.44	0.00021	—	0.0395	Trace	0.5003	—
Unknown 5	0.36	—	—	0.4466	0.0817	3.1777	0.0250
Unknown 6	0.33	0.00022	—	—	—	—	—
Unknown 7	0.24	0.00008	0.1032	0.0914	0.1430	0.7988	—
Hippuric acid	0.17	0.00006	—	0.0791	—	0.2721	0.2384
Unknown 8	0.12	—	—	0.2431	—	0.5530	—
Unknown 9	0.07	0.00003	—	0.7349	—	1.0709	0.2296
Unknown 10	0.05	0.00003	0.2699	0.7236	0.1716	1.8873	0.2584
Polar	0.0	0.02155	6.0854	27.0809	4.4772	3.8190	2.5255
Unextractable		0.03764	—	—	—	—	—

[a] TLC with benzene:acetone:Skellysolve B (bp 60–68°C): acetic acid = 65:25:25:5 (v/v).
[b] Found in the hydrolyzed water extract.

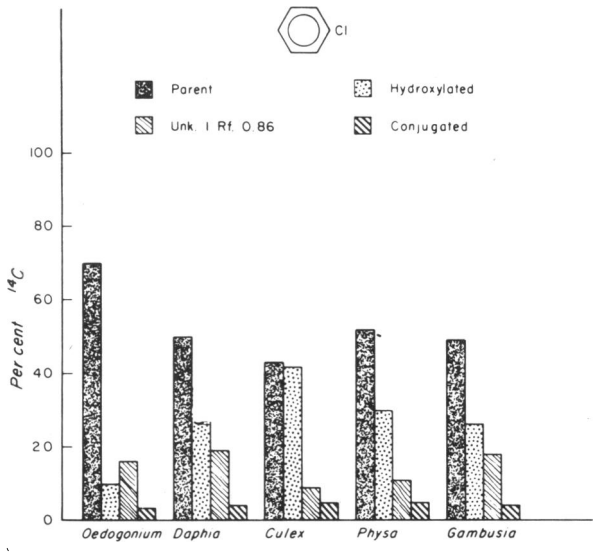

FIGURE 5. Relative detoxication capacities of key organisms of model aquatic ecosystem following treatment with radioactive chlorobenzene.

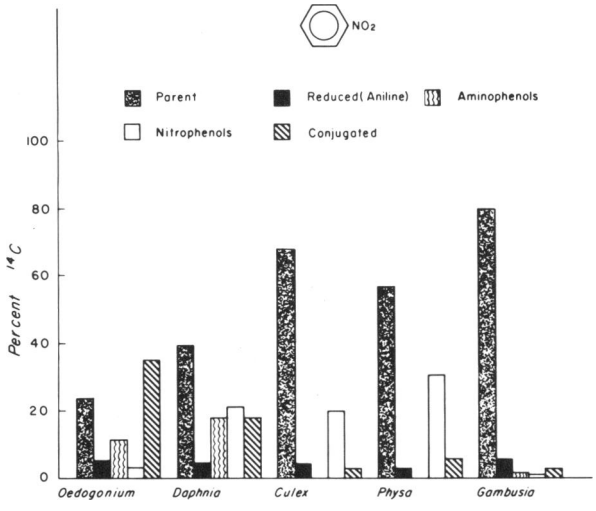

FIGURE 6. Relative detoxication capacities of key organisms of model aquatic ecosystem following treatment with radioactive nitrobenzene.

further explain its undesirable environmental pollutant properties. Hydroxylation of chlorobenzene to *o*- and *p*-chlorophenol and to 4-chlorocatechol was found in mosquito larva and in water extracts. These degradation products accounted for only a very small fraction of the total radioactivity accumulated as compared to that from the parent compound as shown in Figure 5. The quantitative distribution of ^{14}C-chlorobenzene equivalents is shown in Table 5.

Table 5. Distribution of chlorobenzene and degradation products in model aquatic ecosystem.

	R_f[a]	Chlorobenzene equivalents, ppm					
		H_2O	*Oedogonium* (alga)	*Daphnia* (daphnia)	*Culex* (mosquito)	*Physa* (snail)	*Gambusia* (fish)
Total ^{14}C		0.02819	6.0085	5.7088	3.0278	2.5075	1.4191
Unknown 1	0.86	—	0.9926	1.1115	0.2801	0.2871	0.2061
Chlorobenzene	0.81	0.00101	4.2055	2.8173	1.3050	1.3270	0.6521
o-Chlorophenol	0.70	0.00089	0.6246	0.6651	0.3661	—	0.3201
p-Chlorophenol	0.62	0.00327	—	0.8683	0.7881	0.7655	0.1659
4-Chlorocatechol	0.46	0.00207	—	—	0.1075	—	—
Unknown 2	0.40	0.00183	—	—	—	—	—
Unknown 3	0.34	0.00115	—	—	—	—	—
Unknown 4	0.28	0.00108	—	—	—	—	—
Unknown 5	0.20	0.00089	—	—	—	—	—
Unknown 6	0.17	0.00086	—	—	—	—	—
Unknown 7	0.11	0.00038	—	—	—	—	—
Polar	0.0	0.00902	0.2008	0.2466	0.1810	0.1279	0.0209
Unextractable		0.00574	—	—	—	—	—

[a] TLC with chloroform:benezene:ethyl acetate 65:15:15 (v/v) at 0°C (cold room).

Table 6. Distribution of nitrobenzene and degradation products in model aquatic ecosystem.

	R_f[a]	Nitrobenzene equivalents, ppm					
		H_2O	*Oedogonium* (alga)	*Daphnia* (daphnia)	*Culex* (mosquito)	*Physa* (snail)	*Gambusia* (fish)
Total ^{14}C		0.53755	0.0690	0.1812	0.5860	0.6807	4.9541
Nitrobenzene	0.72	0.50681	0.0162	0.0709	0.3952	0.3886	4.0088
Aniline	0.60	0.01262	0.0032	0.0079	0.0272	0.0169	0.2963
Acetanilide	0.35	0.00180	0.0160	—	0.0272	0.0169	0.3527
Aminophenols[b]	0.20	0.00106	0.0080	0.0315	—	—	0.0986
Nitrophenols[b]	0.10	0.00466	0.0016	0.0394	0.1226	0.2190	0.0847
Polar	0.0	0.00896	0.0240	0.0315	0.0138	0.0393	0.1130
Unextractable		0.00164	—	—	—	—	—

[a] TLC with benzene:acetone:Skellysolve B (bp 60–68°C):diethylamine = 65:25:25:5 (v/v).
[b] The isomers could not be separated reliably because of small amounts and similar R_f values.

Nitrobenzene behaved strangely in the model aquatic ecosystem because of its high polarity and was neither stored nor ecologically magnified. However, it is rather resistant to degradation as shown in Figure 6 and the major portion of the radioactivity was retained as parent compound. Nitrobenzene was also reduced to aniline aerobically in all organisms and subsequently acetylated in fish and water extracts. Hydroxylations of nitrobenzene and aniline to the corresponding nitrophenols were found in mosquito larva and snail. The quantitative distribution of ^{14}C-nitrobenzene equivalents is presented in Table 6.

Phthalic anhydride was almost quantitatively converted to phthalic acid and thus underwent further metabolic reactions. Alga was the only ecosystem component that stored the parent compound to high levels, i.e., EM 3169. However, phthalic acid was only magnified to 200. A substantial amount of $^{14}CO_2$, 5.02% of total applied radioactivity, was collected over the 3-day period. Thus decarboxylation occurred as a major degradation pathway and was followed by benzoic acid degradation. Conjugation with the acidic proton was the most important degradation pathway (Fig. 7) as reflected by the high BI values: 1.779 in alga, 2.411 in mosquito

Table 7. Distribution of phthalic anhydride and degradation products in model aquatic ecosystem.

	R_f[a]	Phthalic anhydride equivalents, ppm					
		H_2O	*Oedogonium* (alga)	*Daphnia* (daphnia)	*Culex* (mosquito)	*Physa* (snail)	*Gambusia* (fish)
Total ^{14}C		0.01617	13.1690	0.6381	0.3074	0.4583	1.1622
Unknown 1	0.78	—	0.1356	—	—	—	—
Unknown 2	0.66	—	—	—	0.0260	0.0359	—
Unknown 3	0.56	—	1.2273	—	—	—	—
Phthalic anhydride	0.34	0.00050	2.0267	—	—	—	—
Unknown 4	0.30	—	0.2502	—	—	—	—
Unknown 5	0.25	—	0.3582	—	—	0.0352	—
Unknown 6	0.22	—	0.1000	—	—	—	—
Unknown 7	0.17	—	0.1027	0.0261	—	—	—
Unknown 8	0.12	0.00050	0.1053	—	—	—	—
Phthalic acid	0.10	0.00216	0.4319	0.0126	0.0641	0.0071	0.0902
Polar	0.0	0.00346	8.4311	0.5994	0.2173	0.3801	1.0720
Unextractable		0.00955	—	—	—	—	—

[a] TLC with cyclohexane:toluene:diethyl ether:acetic acid = 50:40:10:7 (v/v).

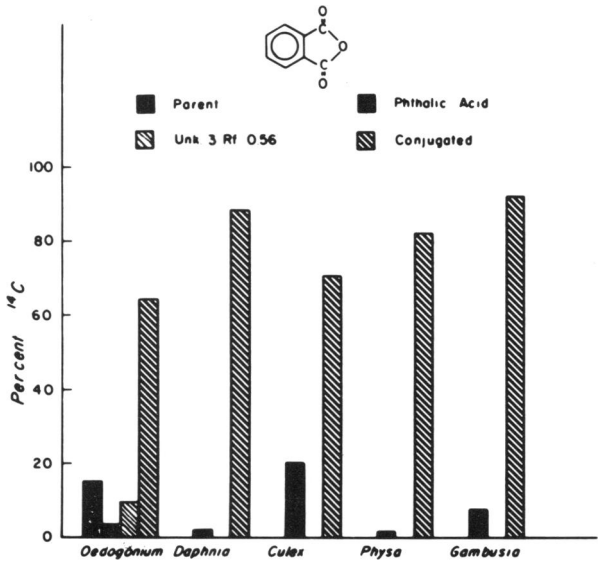

FIGURE 7. Relative detoxication capacities of key organisms of model aquatic ecosystem following treatment with radioactive phthalic anhydride.

larva, 4.869 in snail, 11.844 in fish, and 15.488 in daphnia. The quantitative distribution of ^{14}C-phthalic anhydride equivalents is shown in Table 7.

Hexachlorobenzene was identified as a persistent compound (Fig. 8) that comprised the majority of the total accumulated radioactivity: 84% in snail, 67% in daphnia, 65% in mosquito larva, and 64% in fish. Consequently, HCB was magnified to high levels with EM values: 3969 for alga, 1129 for daphnia, 1166 for fish, 2622 for mosquito larva, and 2672 for snail. Pentachlorophenol was found in alga, mosquito larva, and water extracts as the only identified degradation product. Substantial amount of unknown 1 (R_f 0.72) and several minor degradation products were not identified (Table 8), but presumably were further phenolic degradation products (*8*).

Pentachlorophenol represented 74% of the radioactivity accumulated, with an EM value of 296 in fish. The other organisms had lower quantities as shown by the lower EM values: alga, 1.58; mosquito larva, 16; snail, 121; daphnia, 165. Conjugation at the phenolic OH was the most important means of degradation found among the organisms. As the result of this modification, relatively high BI values were found of the snail 1.06, alga 1.48, daphnia, 1.61 mosquito larva 2.80, and fish 0.338. The water extract and snail gave more complicated degradation products than were found in the others. These minor metabolites, with R_f 0.75, 0.34, 0.26, 0.22, and 0.16, have not yet been identified (Table 9). Photolytic degradation of pentachlorophenol has been studied and certain lower chlorinated degradation products identified (*9*).

2,6-Diethylaniline behaved differently from aniline because the ethyl substituents donate electrons to the ring, thereby changing the

Table 8. Distribution of hexachlorobenzene and degradation products in model aquatic ecosystem.

		Hexachlorobenzene equivalents, ppm					
	R_f[a]	H_2O	*Oedogonium* (alga)	*Daphnia* (daphnia)	*Culex* (mosquito)	*Physa* (snail)	*Gambusia* (fish)
Total ^{14}C		0.02675	43.6962	15.8463	36.9216	27.6085	17.0301
Hexachlorobenzene	0.83	0.00934	37.0718	10.5537	24.4901	24.9609	10.8941
Unknown 1	0.72	0.00031	1.4289	4.9361	1.3495	1.1927	—
Pentachlorophenol	0.42	0.00025	2.3334	—	1.7912	—	—
Unknown 2	0.35	—	1.0924	—	—	—	—
Unknown 3	0.26	0.00008	—	—	0.1524	0.4500	—
Unknown 4	0.10	—	Trace	—	—	—	—
Unknown 5	0.05	0.00022	0.6554	—	1.3319	0.3313	1.4680
Polar	0.0	0.00960	1.1143	9.3565	7.8065	0.6736	4.6680
Unextractable		0.00695	—	—	—	—	—

[a] TLC with acetone:benzene = 50:50 (v/v).

Table 9. Distribution of pentachlorophenol and degradation products in model aquatic ecosystem.

		Pentachlorophenol equivalents, ppm					
	R_f[a]	H_2O	*Oedogonium* (alga)	*Daphnia* (daphnia)	*Culex* (mosquito)	*Physa* (snail)	*Gambusia* (fish)
Total ^{14}C		0.00794	0.0107	1.1725	0.1733	1.1377	1.0764
Unknown 1	0.75	0.00021	—	—	—	—	—
Phentachlorophenol	0.41	0.00271	0.0043	0.4484	0.0456	0.3289	0.8043
Unknown 2	0.34	0.00038	—	—	—	—	—
Unknown 3	0.26	—	—	—	—	0.0272	—
Unknown 4	0.22	0.00004	—	—	—	0.0332	—
Unknown 5	0.16	0.00009	—	—	—	0.1629	—
Polar	0.0	0.00426	0.0064	0.7241	0.1277	0.5855	0.2721
Unextractable		0.00025	—	—	—	—	—

[a] TLC with *n*-hexane:acetone:acetic acid = 80:20:2 (v/v).

Table 10. Distribution of 2,6-diethylaniline and degradation products in model aquatic ecosystem.

		2, 6-Diethylaniline equivalents, ppm					
	R_f[a]	H_2O	*Oedogonium* (alga)	*Daphnia* (daphnia)	*Culex* (mosquito)	*Physa* (snail)	*Gambusia* (fish)
Total ^{14}C		0.06788	0.1434	0.9393	0.0849	0.3033	8.4925
Unknown 1	0.73	—	—	0.0476	0.0022	0.0988	—
2, 6-Diethylaniline	0.68	0.05984	0.0539	0.0899	0.0491	0.1014	7.4564
Unknown 2	0.43	0.00040	—	—	—	—	—
Unknown 3	0.36	0.00024	—	—	—	—	—
Unknown 4	0.30	0.00016	—	—	—	—	—
Unknown 5	0.25	0.00012	—	—	—	—	—
Unknown 6	0.05	0.00013	0.0431	—	—	—	—
Polar	0.0	0.00325	0.0464	0.8018	0.0336	0.1031	1.0361
Unextractable		0.00374	—	—	—	—	—

[a] TLC with benzene:Skellysolve B (bp 60–68°C): acetone:diethylamine = 65:25:25:5 (v/v).

electron distribution by increasing the basicity and creating steric hindrance. Thus the compound becomes a less suitable substrate for MFO enzymes. 2,6-Diethylaniline was not readily degraded by hydroxylation, acetylation and methylation in the various components of the ecosystem. However, 2,6-diethylaniline was not stored except in the fish with an EM of 120, and several degradation products (Table 10) which might be due to the

Table 11. Distribution of 3,5,6-trichloro-2-pyridinol and degradation products in model aquatic ecosystem. [a]

	R_f [a]	3, 5, 6-Trichloro-2-pyridinol equivalents, ppm					
		H_2O	*Oedogonium* (alga)	*Daphnia* (daphnia)	*Culex* (mosquito)	*Physa* (snail)	*Gambusia* (fish)
Total ^{14}C		0.17067	0.3369	1.2176	2.0623	2.7820	0.8891
Unknown 1	0.85	—	—	—	—	0.0867	—
Unknown 2	0.78	—	0.0235	0.0241	0.0717	0.0974	—
3, 5, 6-Trichloro-2-pyridinol	0.71	0.04151	0.0727	0.9037	0.4351	0.5675	0.6781
Unknown 3	0.62	—	—	—	—	0.0815	—
Unknown 4	0.55	—	0.0208	—	—	0.0754	—
Unknown 5	0.51	0.00029	—	—	—	—	—
Unknown 6	0.45	—	0.0257	—	—	—	—
Unknown 7	0.36	0.00030	—	—	—	—	—
Unknown 8	0.32	—	0.0197	0.0191	Trace	—	—
Unknown 9	0.25	—	—	—	—	0.0929	—
Unknown 10	0.18	0.00033	—	—	—	—	—
Unknown 11	0.10	—	0.0519	0.0180	0.0459	0.0748	—
Unknown 12	0.05	—	0.0224	0.0137	0.4471	0.2857	—
Unknown 13	0.03	—	—	—	—	0.3182	—
Polar	0.0	0.10373	0.1002	0.2390	1.0625	1.1019	0.2110
Unextractable		0.02451	—	—	—	—	—

[a] TLC with benzene:*p*-dioxane:acetic acid = 90:30:1 (v/v).

presence of microorganisms in the water were isolated.

3,5,6-Trichloro-2-pyridinol was a fairly persistent compound in all the organisms and especially in fish and daphnia where 74–76% of the total radioactivity was parent compound. However, 3,5,6-trichloro-2-pyridinol, was not accumulated at high levels or magnified through the food chain. Conjugation was not as rapid as with true phenols (*10*) but was very important in mosquito larva, snail, and alga as 51, 40, and 30% of the accumulated radioactivity respectively (Table 11). 3,5,6-Trichloro-2-pyridinol is one of the significant metabolites of chlorpyrifos and methyl-chlorpyrifos in the model ecosystem (*11*) and a metabolite of goldfish which is eliminated into the aquatic environment (*12*). 3,5,6-Trichloro-2-pyridinol is labile to ultraviolet light (*13*), but no $^{14}CO_2$ was monitored over the 3-day period in the present study.

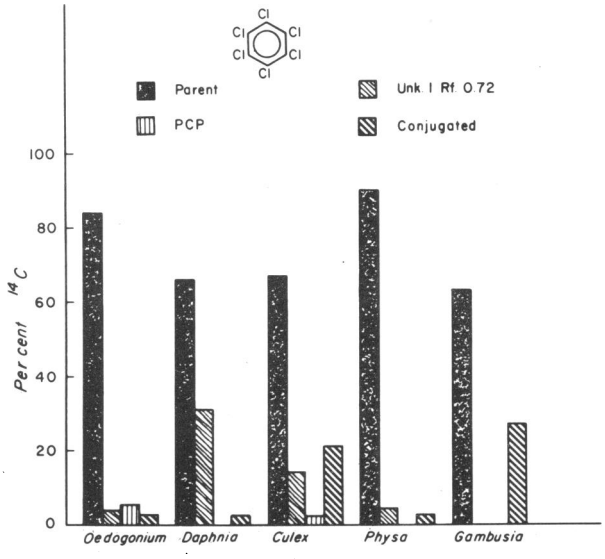

FIGURE 8. Relative detoxication capacities of key organisms of model aquatic ecosystem following treatment with radioactive hexachlorobenzene.

Comparative Metabolism

The organisms in the model aquatic ecosystem represent at least five phyla: Chlorophyta, Protozoa, Mollusca, Arthropoda, and Chordata. Thus the comparative degradative and metabolic pathways for the radio-labeled compounds provide an interesting comparison of evolutionary and phylogenetic processes developed to deal with xenobiotic compounds.

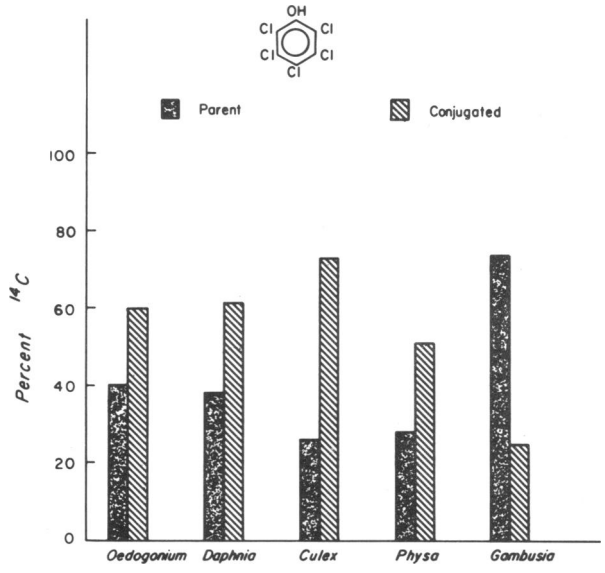

FIGURE 9. Relative detoxication capacities of key organisms of model aquatic ecosystem following treatment with radioactive pentachlorophenol.

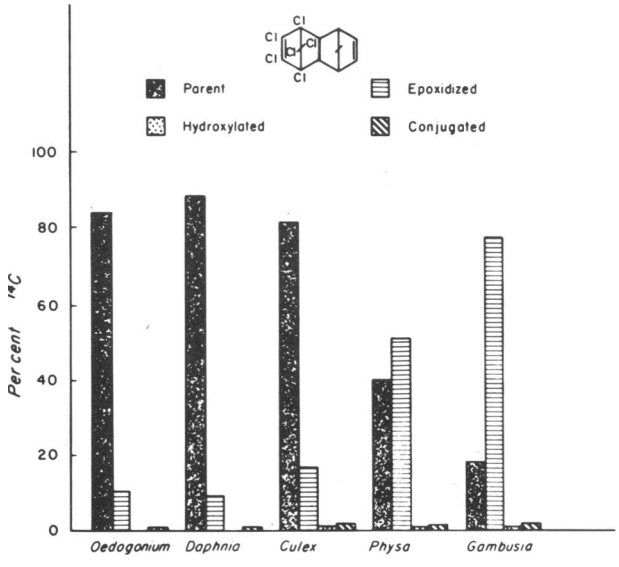

FIGURE 10. Relative detoxication capacities of key organisms of model aquatic ecosystem following treatment with radioactive aldrin.

Five organisms were studied in detail, the filamentous alga *Oedogonium* (Chlorophyta), the water flea *Daphnia* (Cladocera), the snail *Physa* (Mollusca), the mosquito *Culex* (Insecta), and the fish *Gambusia* (Pisces), and Figures 2–12 show the relative amounts of

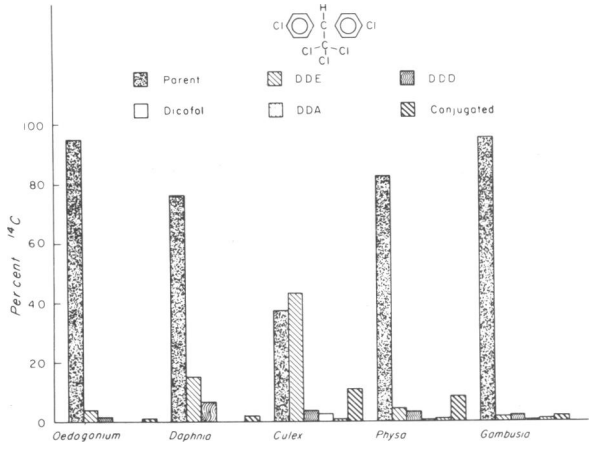

FIGURE 11. Relative detoxication capacities of key organisms of model aquatic ecosystem following treatment with radioactive DDT.

various microsomal and conjugative detoxication products present in these organisms.

Hydroxylation as a detoxication mechanism occurred in the overall order chlorobenezene > anisole > nitrobenzene > aniline > benzoic acid > hexachlorobenzene. The *Culex* larva appears to utilize this mechanism most effectively (e.g., chlorobenzene, anisole) and was the only animal to hydroxylate hexachlorobenzene. Hydroxylation of benzenoid compounds with the strongly electron-withdrawing groups Cl and NO_2 appeared to take place more uniformly than with the electron-donating CH_3O and NH_2.

O-Dealkylation of anisole was appreciable only in *Gambusia* and appears to be of recent evolutionary development as this process is considerably more efficient in mammals than in insects (*14*).

Reduction of nitrobenzene to aniline was remarkably consistent for all the organisms but was of low total importance presumably because of the aerobic nature of the ecosystem environment.

Methylation of aniline was detected only in alga, mosquito larva, and fish and was of minor importance.

Phenolic conjugation of pentachlorophenol was a highly efficient process in all the organisms but *Gambusia*, which stored large quantities of free pentachlorophenol. Conjugation of the phenols with electron-withdraw-

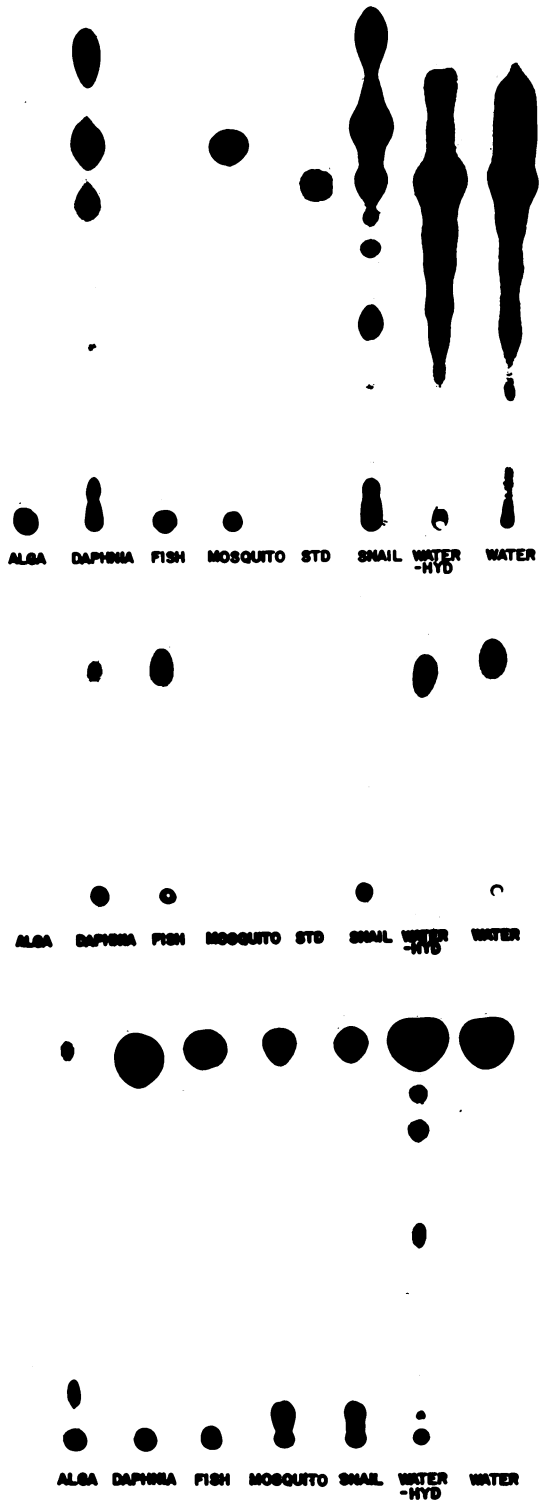

FIGURE 12. Autoradiograph from TLC plates of extracts from components of model aquatic ecosystem: (*a*) benzoic acid; (*b*) pentachlorophenol; (*c*) 3,5,6-trichloro-2-pyridinol.

ing groups Cl (Fig. 5), and NO_2 (Fig. 6) was relatively and uniformly inefficient compared to conjugation of phenols with electron-donating NH_2 (Fig. 2) and CH_3O (Fig. 3). 3,5,6-Trichloro-2-pyridinol was also very uniformly subject to conjugation for all the organisms but to a lesser extent in fish.

Carboxyl conjugation was a highly efficient process in all the organisms except *Physa* (Fig. 4). Hippuric acid, the glycine conjugation product was identified in daphnia, snail, and fish.

Epoxidation was determined in a model aquatic ecosystem using aldrin as the substrate for conversion to dieldrin. This process occurred in almost perfect phylogenetic order from *Daphnia* (8% of total ^{14}C), *Culex* (16%), *Physa* (50%) to *Gambusia* (80%) (Fig. 10).

Dehydrochlorination of DDT to DDE, a unique detoxication process, was responsible for 42% of the total radioactivity in *Culex*, about 15% in *Daphnia* but much smaller amounts in the other organisms. The very high rate in *Culex* explains this mosquito's well known natural immunity to DDT (Fig. 11).

Reductive dechlorination of DDT to DDD occurred in a relatively small amount throughout all the organisms (Fig. 11).

Molecular Properties and Environmental Responses

A major goal of this study was to establish the role of intrinsic molecular properties such as water solubility, polarity, and lipid/water partitioning in determining the environmental behavior in such quantitative terms as ecological magnification (concentration of parent compound in organism/concentration in water) and biodegradability index (concentration of polar products/concentration of nonpolar products) (*15*). If suitable correlations can be established, these molecular properties could be useful in predicting the pollutant potentialities of new or proposed environmental contaminants (*16,17*).

Water solubility was correlated with eco-

logical magnification through a simple two variable linear regression using log values from Table 1 and the graph was plotted as Figure 13. The equation was determined as:

$$y = 3.9950 - 0.3891x \qquad (1)$$

for $n = 11$ and $r = -0.9228$, where $y = \log$ EM of fish and $x = \log$ water solubility, (ppb). The excellent correlation indicates the fundamental importance of water insolubility in causing organic compounds to partition into the lipid tissue of aquatic organisms (*16*).

Partition coefficient in octanol/H_2O was also correlated with EM. The values used in the two-step linear regression analysis were log partition coefficient (π) as determined experimentally (Table 1). For the simple benzene derivatives, these agreed well with those of Fujita et al. (*18*). The graph was plotted as Figure 14 and the regression equation was determined as:

$$y = 0.7285 + 0.6335x \qquad (2)$$

for $n = 11$ and $r = 0.7879$, where $y = \log$ EM of fish, and $x = \pi$ constant. The correlation is good, but the lower correlation coefficient compared to eq. (1) is probably due to the uncertainty in the measurement of partition coefficient values.

The constant was correlated with the percentage of the remaining parent compound of four benzene derivatives (aniline, anisole, chlorobenzene, and nitrobenzene). The plot

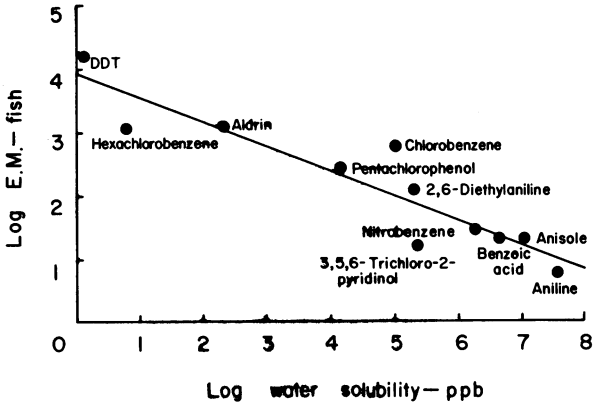

FIGURE 13. Plot of ecological magnification (EM) in fish from model aquatic ecosystem vs. water solubility. The regression line was computed by the method of least squares.

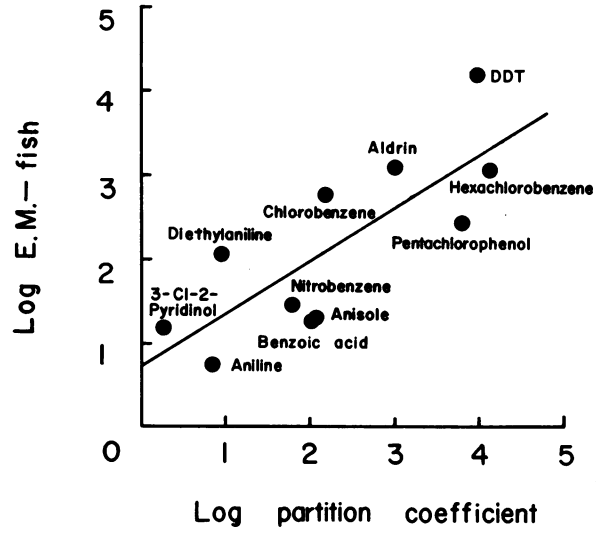

FIGURE 14. Plot of ecological magnification (EM) in fish from model aquatic ecosystem vs. log partition coefficient (π constant). The regression line was computed by the method of least squares.

Table 12. Hammett σ-constant of benzene derivatives and biological responses of the average of the five key organisms and fish in model aquatic ecosystem.

Compound	σ-constant[a]	Parent compound, %		Hydroxylated and conjugated compound, %	
		Average	Fish	Average	Fish
Aniline	−0.66	2.99	2.99	86.69	70.82
Anisole	−0.268	31.69	38.69	60.16	61.31
Chlorobenzene	0.227	52.24	45.95	32.40	35.72
Nitrobenzene	0.778	53.69	80.92	34.36	5.96

[a] Data were obtained from the compilation of McDaniel and Brown (*19*).

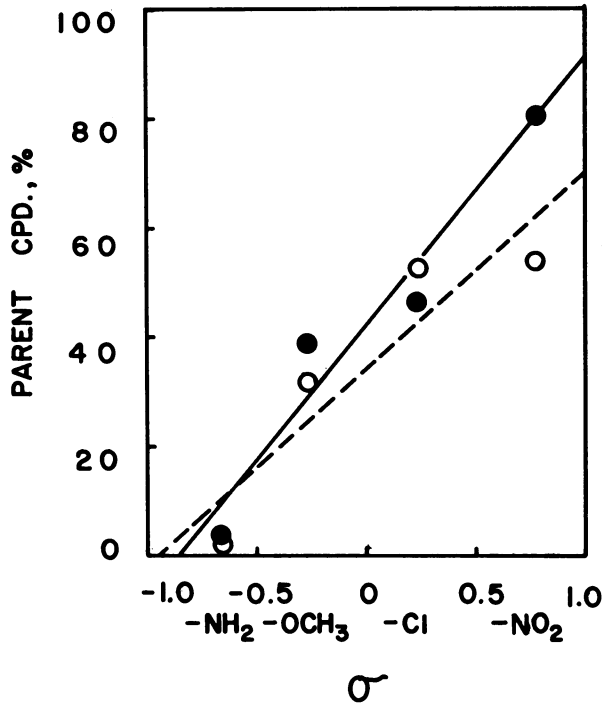

FIGURE 15. Plot of the average percentage of the parent compound for (O) five key organisms and (●) for fish vs. σ constant. The regression lines were computed by the method of least squares.

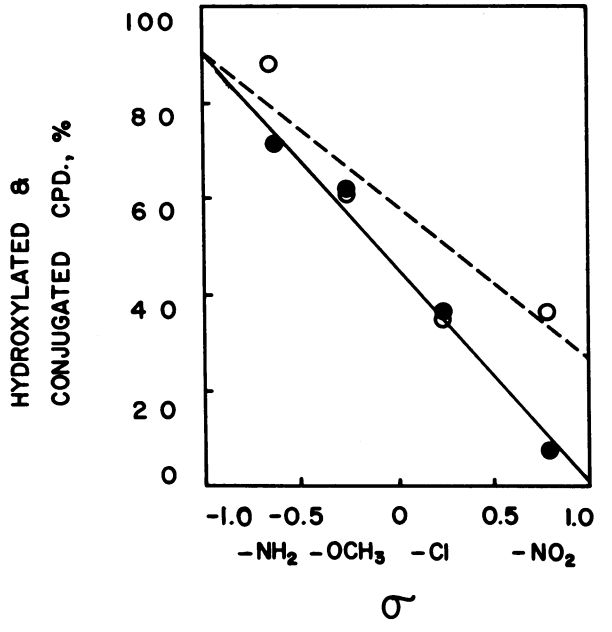

FIGURE 16. Plot of the average of the total percentage of hydroxylated and conjugated compounds (O) for five key organisms and (●) for fish vs. σ constant. The regression lines were computed by the method of least squares.

of the graph from Table 12 in Figure 15 was determined as:

$$y = 33.8559 + 36.1989x \qquad (3)$$

for $n = 4$ and $r = 0.9098$, where $y =$ the average of five key organisms (alga, daphnia, fish, mosquito larva, and snail), and $x = \sigma$ constant. For fish, the top member of the food chain, the correlation showed much better as eq. (4):

$$y = 41.1086 + 40.7901x \qquad (4)$$

for $n = 4$ and $r = 0.9685$. It was clearly indicated that the Hammett σ constant is one of the important intrinsic molecular properties, not only in controlling the disappearance of the parent compound through hydroxylation but also in limiting the formation of the conjugated degradation products. Equations (5) and (6) summarize the data from Table 12 for the correlation of the average of five key organisms and of fishes versus σ-constant, respectively (Fig. 16):

$$y = 54.1393 + 37.2628x \qquad (5)$$

for $n = 4$ and $r = -0.9089$, where $y =$ average of the total percentage of hydroxylated and conjugated products of five key organisms and $x = \sigma$ constant; and

$$y = 44.3475 + 46.2330x \qquad (6)$$

for $n = 4$ and $r = -0.9908$, where $y =$ total percentage of hydroxylated and conjugated products of fish and $x = \sigma$ constant.

Efforts were made to correlate the biodegradability index for the various organisms with various molecular properties. The biodegradability index describes the stability of xenobiotics in living organisms and is strongly influenced by susceptability of the compound to attack by microsomal oxidase enzymes, by aliesterases, by conjugating enzymes, etc. Thus the biodegradability of molecules can be influenced by appropriately chosen substituents or degradaphores. For example, with the simple benzene derivatives, the values for the fish, *Gambusia*, were —COOH, 2.965; —HN_2, 1.784; —NO_2, 0.023; and —Cl, 0.014. As might be expected, there

was lower correlation between BI and π constant ($r = -0.6763$) than EM and π constant. Little correlation was found between BI and σ constant ($r = -0.5654$) and BI and water solubility ($r = 0.3617$).

Lipid Content of Organisms

Clearly, lipid-partitioning micropollutants are stored mostly in the tissue lipids of organisms. The lipid content of the key organisms of the model aquatic ecosystem was determined by extraction in methanol: alga, 2.32%; daphnia, 1.28%; mosquito larva, 1.46%; snail, 1.60%; fish, 6.15%. The mean values of EM of the six benzene derivatives are: fish, 116; mosquito larva, 242; daphnia, 593; snail, 833; and alga, 1407. Regression analysis gave eq. (7):

$$y = 3.0171 - 0.1397x \qquad (7)$$

for $n = 5$ and $r = -0.6618$, where y = mean value of log EM of the six benzene derivatives and x = lipid content (%). The substantial negative correlation suggests that degradation by microsomal oxidase enzymes is more important than passive lipid partitioning in determining bioaccumulation.

Conclusions

The data presented demonstrate that the environmental behavior of a series of simple aromatic compounds, e.g. absorption, bioaccumulation, and biodegradation in a simple food chain system with a wide range of organisms, can be predicted by the basic molecular properties of water solubility, partition coefficient for lipid/water, and reactivity as determined by electron density. Thus, for a large variety of aromatic derivatives it should be possible to estimate potential environmental contamination from such fundamental linear free energy values as Hansch's π and Hammett's σ in a manner analgous to estimation of distribution and reaction of these compounds in a single chemical or biochemical system. This information and its predictive possibilities should simplify the evaluation of new and potentially toxic substances by emphasizing types of compounds to which major investigational efforts should be directed.

Overall environmental degradative pathways for these simple aromatic compounds are generally qualitatively similar in organisms as diverse as alga, clodoceran, snail, mosquito larva, and fish. However, the quantitative aspects of degradation reflect the generally higher levels of microsomal oxidase enzymes (MFO) present in higher evolutionary forms.

Acknowledgments

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