Uptake and Fate of Di-2-ethylhexyl Phthalate in Aquatic Organisms and in a Model Ecosystem

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Di-2-ethylhexyl phthalate (DEHP), often referred to as dioctyl phthalate or DOP, is the most widely used plasticizer for vinyl plastics, and approximately 350 million pounds were produced in the United States during 1970. Altogether the production of phthalate ester plasticizers was 855 million pounds *(1)*. The cumulative production of DEHP and related phthalate plasticizers in the United States since 1943 is in excess of 8000 million pounds *(2)*. DEPH is an external plasticizer which softens resins without reacting with them chemically, and it may be present in concentrations up to 40% of the weight of the plastic as in the familiar laboratory tubing. As a result of the large production and wide distribution and destruction of plastics, DEHP has become uniquitous and has been found in milk *(3)*, deep frying fat *(4)*, and human blood plasma *(5)*. DEHP has also begun to appear as a micropollutant in the tissues of a variety of organisms. Taborsky *(6)* isolated it from bovine pineal glands, and Nazir et al. *(7)* found it in mitochondria from the hearts of cattle, dogs, rabbit, and rat. DEHP has been found in spleen, liver, lung, and human abdominal fat in quantities ranging from 25 ppm (dry weight) in spleen to 270 ppm in abdominal fat *(5)*. This distribution of DEHP is not surprising, in view of the enormous quantities produced and their widespread use in a variety of plastic containers, tubing, and oils in vacuum pumps, air conditioners, etc. DEHP is exceptionally stable and high-boiling (bp 386°C) and is of high fat solubility and low water solubility (0.01 g/100 g). Recently, DEHP and other phthalate esters have been shown to be teratogenic in rats *(8)*. Therefore DEHP has all the requisite properties to be classified as an environmental micropollutant *(9)*. Our investigation was undertaken to study its metabolism and possible biomagnification in a variety of aquatic organisms and its ecological behavior in food chains of a laboratory model ecosystem *(10)*.

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Experimental Procedures

^{14}C Carbonyl-labeled DEHP was prepared from 10 mg phthalic $\overline{7}-^{14}C$-anhydride, 9.2 mCi/mmole (New England Nuclear), by heating it in a sealed tube for 25 hr at 130°C with 100 μl of 2-ethylhexyl alcohol and a trace of phthalic acid. The product consisted of 78% DEHP and was purified by column chromatography on silica gel and by subse-

quent preparative thin-layer chromatography (TLC) on silicic acid with a solvent system of benzene, Skellysolve B, acetone, and acetic acid in proportions of 65:25:25:5 by volume. The final product used in these experiments had a radiopurity of more than 99% and was diluted to a specific activity of 5.56 mCi/mmole or 31,400 dpm/μg. An additional sample of ^{14}C-labeled DEHP was purchased (from New England Nuclear) with a specific activity of 1.64 mCi/mmole.

The degradation products of ^{14}C-DEHP in the various animal and plant tissues were separated by TLC and autoradiographs were made on Eastman no-screen x-ray film. The identities of the various metabolites were determined by cochromatography with known standards; radioactive spots were removed from the TLC plates by elution in scintillation fluid and determined quantitatively by liquid scintillation counting.

Uptake Studies

These were carried out to determine the uptake of DEHP directly from water by a variety of aquatic organisms and to study the metabolic transformations occurring over a short period. The organisms chosen were the water flea *Daphnia magna*, the mosquito larva *Culex pipiens quinquefasciatus*, the "fingernail" clam *Sphaerium striatinum*, the guppy *Lebistes reticulatus*, and the aquatic plant *Elodea canadensis*. Standard reference water *(11)* was made from glass-distilled water, and 2.5-liter portions were placed in 5-liter battery jars. The ^{14}C-labeled DEHP was dissolved in 1 ml of acetone and added quantitatively to the battery jars at concentrations of 0.1 and 10 ppm. Groups of organisms were exposed to the DEHP for various intervals as indicated in the tables.

Model Ecosystem Study

A laboratory model ecosystem with a terrestrial-aquatic interface and a seven-element food chain has been found very useful in estimating the potential environmental effects of DDT and other pesticides *(10)*, particularly in regard to ecological magnification and biodegradability. ^{14}C-DEHP was evaluated in this system by applying 5 mg of the carbonyl-labeled compound to *Sorghum* plants on the terrestrial end of the system. After 33 days, the various organisms in the system were homogenized in water, extracted with diethyl ether, and the concentrated extracts resolved by TLC on silicic acid by use of benzene, Skellysolve B, acetone, and acetic acid in the proportions 65:25:25:5 by volume. The radioactive spots were located by autoradiography and the parent compound and metabolites determined quantitatively by liquid scintillation counting.

Results and Discussion

Uptake Studies

Autoradiograms of the homogenized extracts of the organisms exposed to ^{14}C-DEHP at 10 ppm for 1, 2, and 7 days are shown in Figures 1 and 2; a quantitative estimation of the distribution of the radioactivity in the various spots is given in Table 1. The data demonstrate the steady decrease of DEHP in the guppy from 88.5% of total radioactivity after 1 day, to 37.1% after 2 days and 16.8% after 7 days. There was a commensurate increase in the polar metabolites at the bottom of the chromatograms, from 11.5% after 1 day to 34.2% after 2 days and to 80.6% after 7 days. One of the identified metabolites was phthalic acid, which comprised 23.8% of the radioactivity after 2 days and then decreased to 4.8% after 7 days. Small amounts of a metabolite which is apparently phthalic anhydride appeared after 2 and 7 days. Uncontaminated guppies fed *Daphnia* with a 1-day uptake of ^{14}C-labeled DEHP showed the same degradation pattern (Table 1).

The other organisms used in the uptake studies accumulated large amounts of ^{14}C-labeled DEHP but degraded it very slowly. The snail contained 86.6% DEHP after 7 days together with small amounts of phthalic anhydride and unknown II. The clam, *Daphnia*, and the plant *Elodea* behaved in similar fashion, showing low degradative capacity for DEHP.

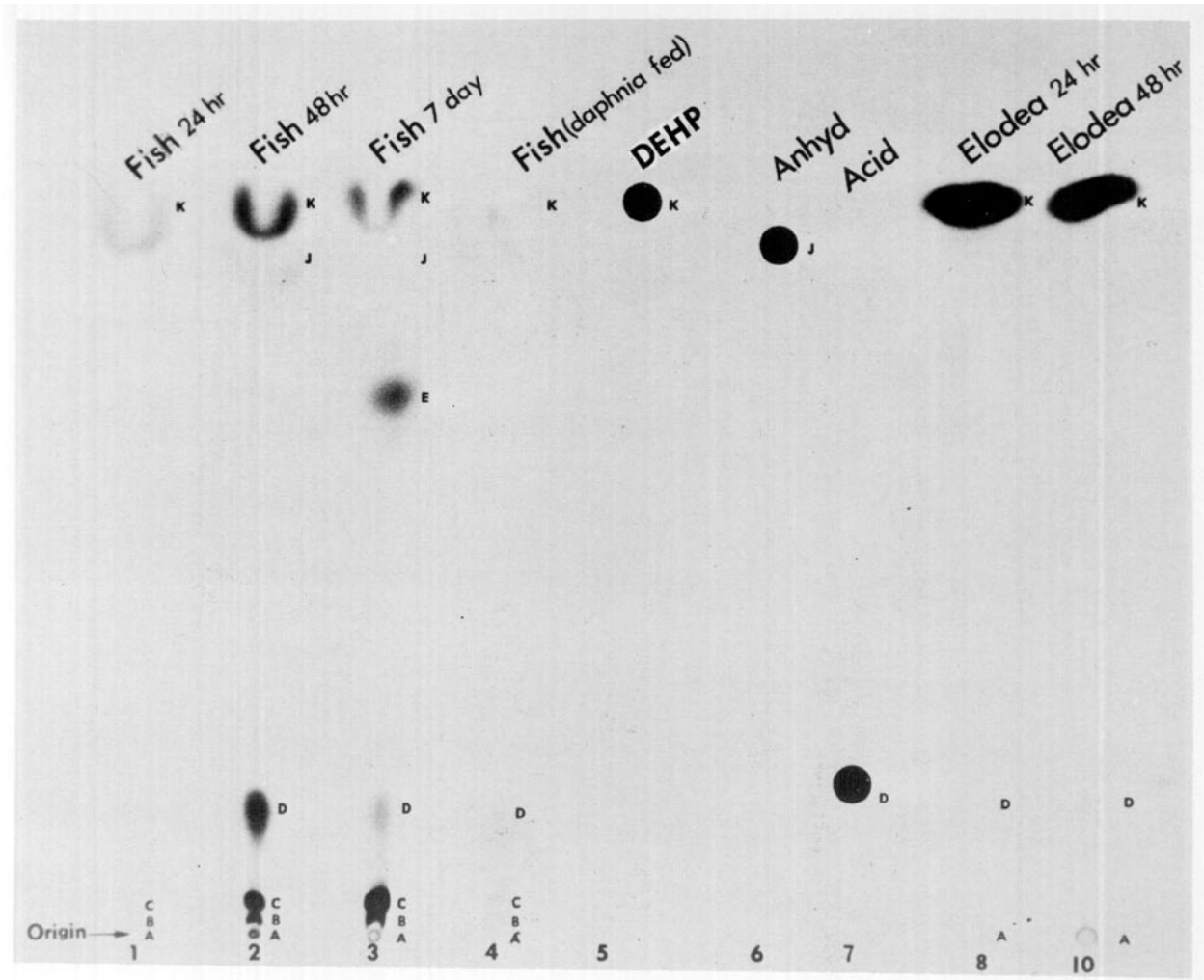

FIGURE 1. Autoradiograph of DEHP uptake by fish and *Elodea*.

A detailed study was made of the rate of uptake of ^{14}C-DEHP by *Culex* larvae, *Daphnia*, snail, *Elodea*, and guppy exposed to 0.1 ppm and 10 ppm over intervals from 1 hr to 48 hr. The distribution of ^{14}C in the organisms at the various intervals is shown in Table 2.

From these uptake studies, the following conclusions can be drawn. (1) At the 10 ppm level, the organisms concentrated DEHP from a low of 37 ppm in *Elodea* to a high of 11,873 ppm in the *Culex* larvae. At the 0.1 ppm level, the concentration varied from a low of 0.85 ppm in the guppy to 85.75 ppm in the snail. (2) *Culex* larvae accumulated DEHP to much higher levels than any other organism investigated as shown in Figures 3 and 4. (3) The biomagnification factors at 10 ppm over a 24-hr period varied from 35 in guppy to 4108 in *Culex* larvae and at 0.1 ppm over a 24-hr period varied from 92 in guppy to 692 in the snail.

Model Ecosystem Study

During the 33-day period of the model ecosystem study, the concentration of ^{14}C in the aquatic phase reached a peak of 0.031 ppm at the fifth day after treatment and declined to 0.0077 ppm at the end of the experiment. This decline was clearly the result of the uptake of DEHP and its degradation products by the organisms of the model ecosystem as shown in Table 3. The distribu-

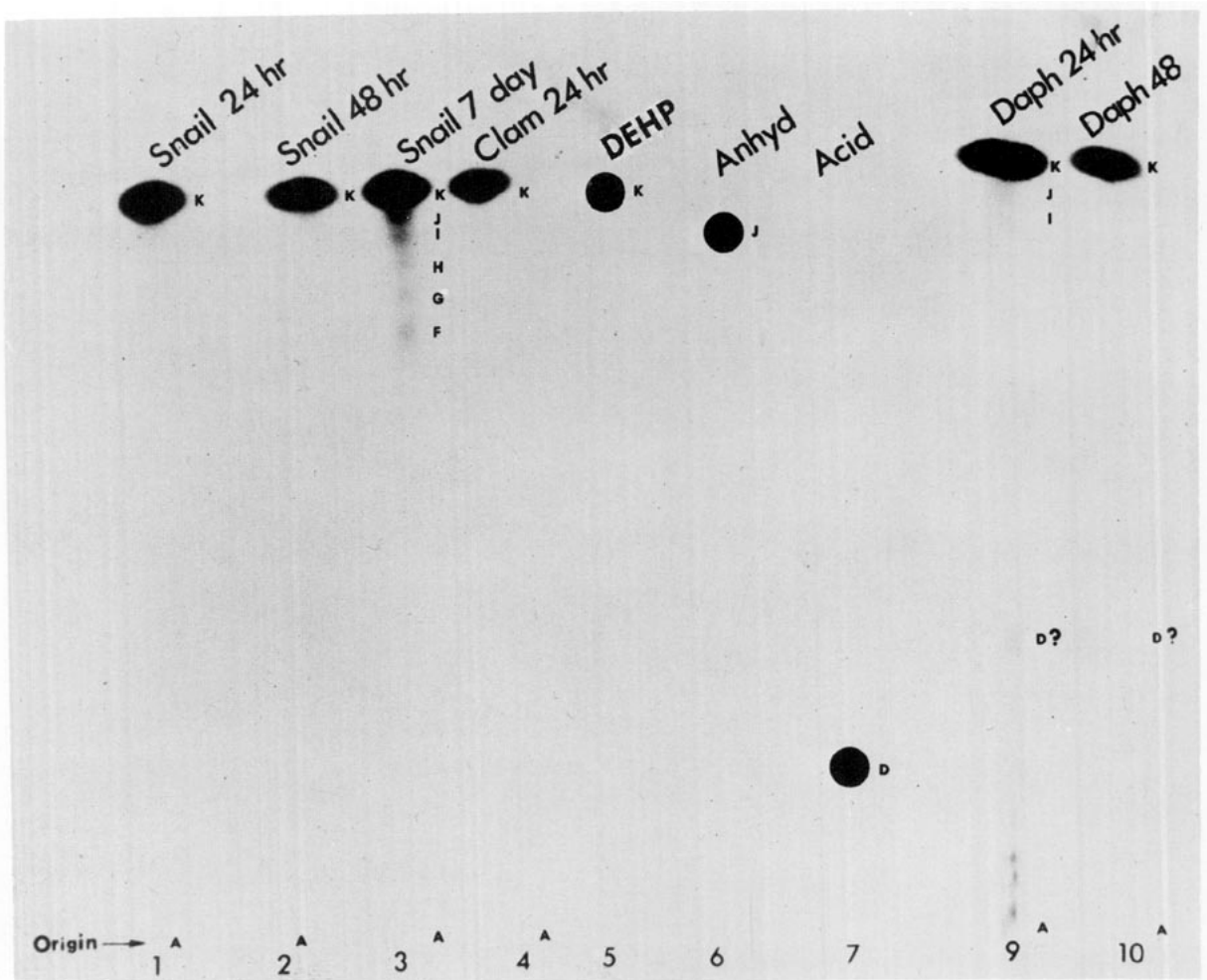

FIGURE 2. Autoradiograph of DEHP uptake in snail, clam and *Daphnia.*

Table 1. ^{14}C Distribution of DEHP and metabolites on radiochromatograms at various time intervals.

Compound	Chrom. spot	Distribution of ^{14}C, %											
		Fish				Snail			Clam	Daphnia		Elodea	
		24 hr	48 hr	168 hr	FFD[a]	24 hr	48 hr	168 hr	24 hr	24 hr	48 hr	24 hr	48 hr
DEHP	K	88.5	37.1	16.8	60.7	99.5	99.6	86.6	99.6	95.9	97.8	99.9	98.8
Polar metabolites	A-C	11.5	34.2	80.6	23.4	0.5	0.4	0.3	0.4	0.5	1.2	0.03	0.6
Phthalic acid	D	—	23.8	4.8	16.0	—	—	—	—	0.8	1.0	0.08	0.6
Unknown I	E	—	—	15.8	—	—	—	—	—	—	—	—	—
Unknown II	F-I	—	—	—	—	—	—	9.9	—	1.2	—	—	—
Phthalic anhydride	J	—	4.9	2.1	—	—	—	3.2	—	1.6	—	—	—

[a]Fish that had been fed contaminated *Daphnia.*

Table 2. Concentration of DEHP in organisms in water containing 10 and 0.1 ppm for various intervals.

Concentration in water, ppm	Exposure, hr	Concentration of DEHP, ppm					
		Gambusia	*Physa*	*Daphnia*	*Culex* larvae	*Culex* pupae	*Elodea*
10	1	152	3586	592	596	2272	37
10	6	1033	4020[a]	532	2634	2578	293
10	12	1294[a]	2834	893[a]	5978	3144	1338
10	24	145	2350	306	11,873[a]	3962	1138
10	48	469[a]	487	1551[a]	3657	4346[a]	290
0.1	1	0.85	12.06	42.1	23.2	0.73	1.98
0.1	6	7.23	45.08	19.61	91.5	1.51	7.72
0.1	12	5.61	45.45	15.54	132.02[a]	0.97	15.46
0.1	24	8.53	64.35	17.62	31.80	2.03[a]	27.48[a]
0.1	48	26.53[a]	85.75[a]	18.26[a]	16.37	—	23.24

[a]Peak value for particular organism

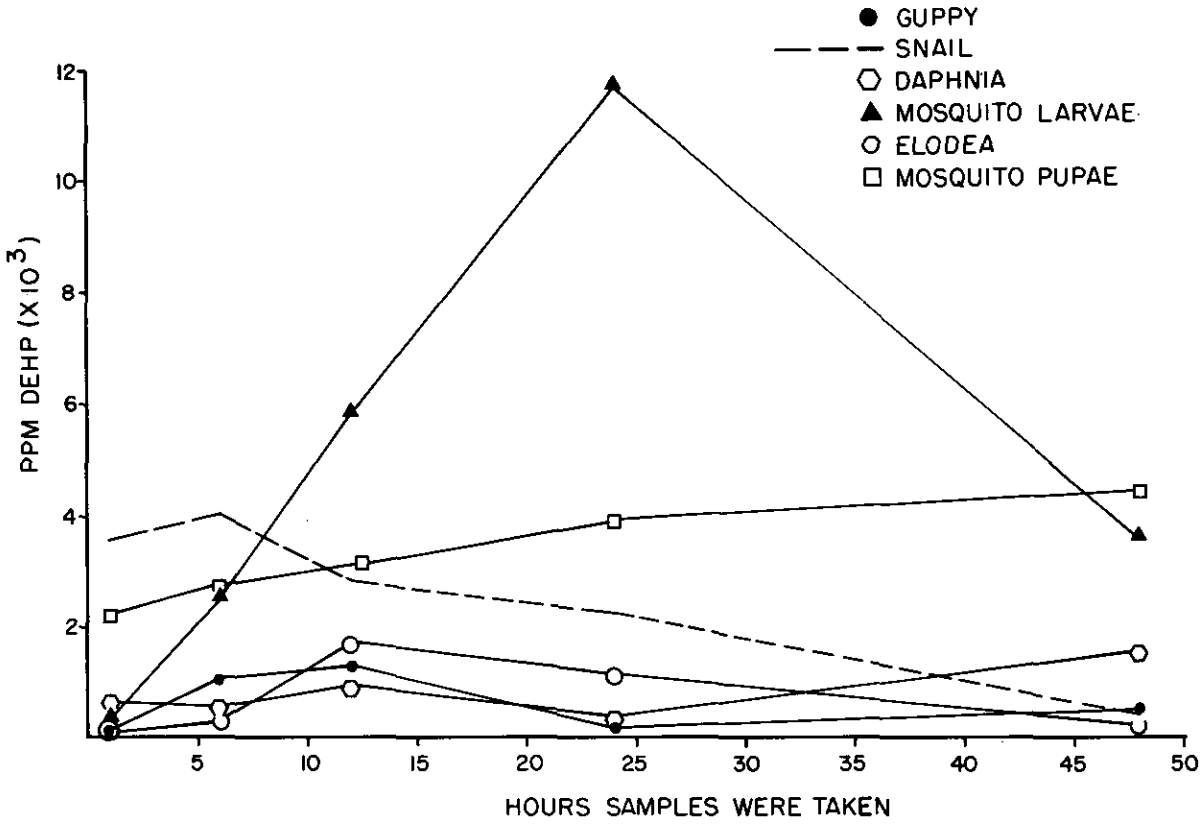

FIGURE 3. Time course study of uptake of DEHP by aquatic organisms from water containing 10 ppm DEHP.

Table 3. Distribution of ^{14}C DEHP and metabolites in laboratory model ecosystem.

	R_f[a]	Concentration, ppm as DEHP equivalents				
		H_2O	*Oedogonium* (algae)	*Physa* (snail)	*Culex* (mosquito)	*Gambusia* (fish)
Total ^{14}C		0.0078	19.105	20.325	36.609	0.206
DEHP	0.79	0.00034	18.322	7.302	36.609	0.044
MEHP	0.70	0.00099	0.325	2.541	—	0.021
Phthalic anhydride	0.65	0.00363	0.108	5.772	—	0.113
Unknown I	0.50	0.00136	—	—	—	—
Phthalic acid	0.35	0.00077	0.094	2.724	—	0.018
Unknown II	0.13	0.00054	0.029	0.768	—	—
Polar metabolites	0.0	0.00016	0.155	1.218	—	0.010

[a]Thin layer chromatography in solvent system of benzene: Skellysolve B: acetone: acetic acid, 65:25:25:5 by volume.

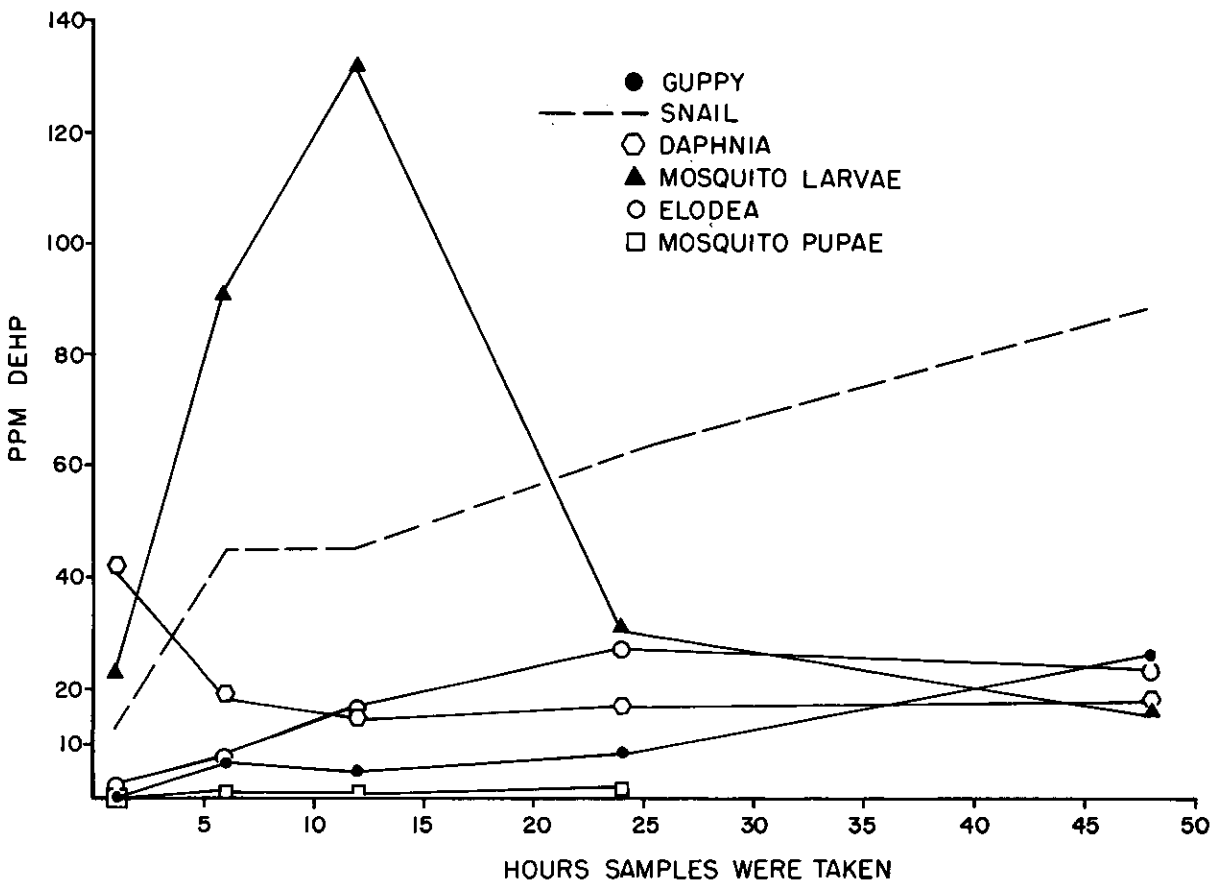

FIGURE 4. Time course study of uptake of DEHP by aquatic organisms from water containing 0.1 ppm DEHP.

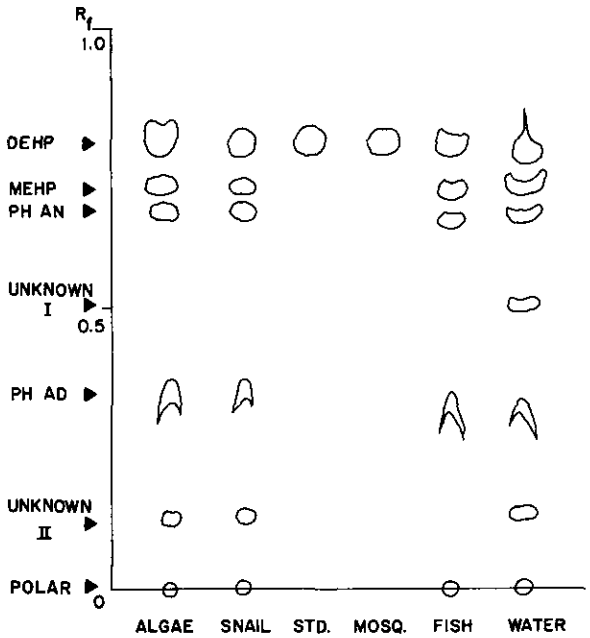

FIGURE 5. Autoradiograph of DEHP and metabolites in organisms of model ecosystem.

tion of radioactivity was clearly indicated by the autoradiograph in Figure 5. At the conclusion of the experiment, the water contained 0.00034 ppm DEHP, the algae 18.32 ppm (53,890X) the snails 7.30 ppm (21,480X), the mosquito larvae 36.61 ppm (107,670X), and the fish 0.044 ppm (130X). Thus the model ecosystem experiment was in good agreement with the uptake studies in showing that the mosquito larvae had the highest bioconcentration factor, and the fish the least.

The autoradiographs (Fig. 5) showed the presence of almost nothing but DEHP in algae and mosquito larvae, indicating that these organisms have little degradative capacity. The snail produced substantial amounts of mono-2-ethylhexyl phthalate (MEHP), phthalic anhydride, and phthalic acid. The fish were more active in metabolism, and about half the total amount of ^{14}C found in their bodies was a compound, R_f 0.65, which cochromatographed with phthalic anhydride and is presumed to be that compound reformed from phthalic acid.

It is interesting to compare the model ecosystem studies of DEHP with that of DDT conducted under identical conditions *(10)*. DDT was biomagnified in the snail 34,500 times, the mosquito larvae 8,200 times, and the fish 84,500 times. It appears that DEHP is biomagnified in snail and mosquito larvae at least as efficiently as the well known pollutant DDT and that only the fish of the organisms examined is able to substantially degrade the DEHP and slowly excrete its metabolites. From the array of degradation products found in the model ecosystem, the major degradative pathways seem to be through hydrolysis of the ester groups to produce MEHP, then phthalic acid, and then phthalic anhydride.

Conclusion

The experiments reported above demonstrate that DEHP is a microchemical environmental pollutant which is rapidly biomagnified by a variety of plants and animals in an aquatic system. DEHP is biodegraded very slowly in algae, *Daphnia*, mosquito larvae, snails, and clams and more rapidly in fish by hydrolysis at the ester bonds to form monoethylhexyl phthalate, phthalic acid, phthalic anhydride, and a variety of polar metabolites and conjugates. However, DEHP closely resembles DDT in rate of uptake and storage, and it obviously partitions strongly in the lipids of plants and animals and is concentrated through food chains. The biomagnification of DEHP together with its teratogenic properties and its enormous rate of production and ubiquitous use indicate the need for much further study of its environmental distribution and fate. Present data suggest the need for restrictions on the use and waste disposal of DEHP.

Acknowledgement

This research was supported by a grant from the Federal Water Quality Administration through the University of Illinois Water Resources Center (B-050 Ill.) to R. L. Metcalf, and by grants from the Illinois Environmental Protection Agency and Illinois State Department of Mental Health to G. M. Booth.

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