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

by Robert L. Metcalf,* Gary M. Booth,† Carter K. Schuth,* Dale J. Hansen,† and Po-Yung Lu*

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). DEHP 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 ubiquitous 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).

Experimental Procedures

¹⁴C Carbonyl-labeled DEHP was prepared from 10 mg phthalic 7–¹⁴C-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/m mole or 31,400 dpm/µg. An additional sample of 14C-labeled DEHP was purchased (from New England Nuclear) with a specific activity of 1.64 mCi/m mole.

The degradation products of 14C-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.

Results and Discussion

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 striatun, 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 14C-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. 14C-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.
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 | Fish 24 hr | Fish 48 hr | Fish 168 hr | FFDa 24 hr | FFDa 48 hr | FFDa 168 hr | Snail 24 hr | Snail 48 hr | Snail 7 day | Clam 24 hr | Clam 48 hr | Daphnia 24 hr | Daphnia 48 hr | Daphnia 168 hr | Elodea 24 hr | Elodea 48 hr |
|-------------------|-------------|------------|------------|-------------|------------|------------|-------------|-------------|-------------|-------------|------------|-------------|----------------|----------------|----------------|--------------|--------------|
| DEHP              | K           | 88.5       | 37.1       | 16.8        | 60.7       | 99.5       | 99.6        | 86.6        | 99.6        | 97.8        | 99.9       | 98.8        | 99.9           | 98.8           | 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         | 0.5        | 1.2         | 0.03           | 0.6            | 0.03           | 0.6         |
| Phthalic acid     | D           | –          | 23.8       | 4.8         | 16.0       | –          | –           | –           | –           | –           | 0.8        | 1.0         | 0.08           | 0.6            | 1.2            | 0.08        |
| Unknown I         | E           | –          | –          | 15.3        | –          | –          | –           | –           | –           | –           | –          | –          | –               | –              | –              | –           |
| Unknown II        | F-I         | –          | –          | –           | –          | –          | 9.9         | –           | 1.2         | –           | –          | –          | –               | –              | –              | –           |
| Phthalic anhydride| J           | –          | 4.9        | 2.1         | –          | –          | 3.2         | –           | 1.6         | –           | –          | –          | –               | –              | –              | –           |

*aFish 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 | Gambusia | Physa | Daphnia | Culex larvae | Culex pupae | Elodea |
|----------------------------|--------------|----------|-------|---------|--------------|-------------|--------|
| 10                         | 1            | 152      | 3586  | 592     | 596          | 2272        | 37     |
| 10                         | 6            | 1033     | 4020a | 532     | 2634         | 2578        | 293    |
| 10                         | 12           | 1294a    | 2834  | 893a    | 5978         | 3144        | 1338   |
| 10                         | 24           | 145      | 2350  | 306     | 11,873a      | 3962        | 1138   |
| 10                         | 48           | 469a     | 487   | 1551a   | 3657         | 4346a       | 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   | 132.02a      | 0.97        | 15.46  |
| 0.1                        | 24           | 8.53     | 64.35 | 17.62   | 31.80        | 2.03a       | 27.48a |
| 0.1                        | 48           | 26.53a   | 85.75a| 18.26a  | 16.37        | —           | 23.24  |

*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.

|                        | $R_f$ | $H_2O$ | Oedogonium (algae) | Physa (snail) | Culex (mosquito) | Gambusia (fish) |
|------------------------|-------|--------|--------------------|---------------|-----------------|-----------------|
| Total $^{14}$C         | 0.79  | 0.0078 | 19.105             | 20.325        | 36.609          | 0.206           |
| DEHP                   | 0.70  | 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           |

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

![Figure 4](image-url)

**FIGURE 4.** Time course study of uptake of DEHP by aquatic organisms from water containing 0.1 ppm DEHP.
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 14C found in their bodies was a compound, Rf 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 mono-ethylhexyl phthalate, phthelic 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.
REFERENCES

1. Anonymous. For plasticizers: an unfilled promise. Chem. Eng. News 49: 14 (Nov. 29, 1971).

2. Faith, W. L., Keys, D. B., and Clark, R. L. In: Industrial Chemicals, 3rd ed., John Wiley, New York, 1965, p. 312.

3. Cerbulis, J., and Ard, J. S. Methods for the isolation of di-octylphthalate from milk lipids. J. Assoc. Offic. Anal. Chem. 50: 646 (1967).

4. Perkins, E. G. Characterization of the non-volatile compounds formed during the thermal oxidation of corn oil. II. Phthalate esters. J. Amer. Oil Chemists Soc. 44: 197 (1967).

5. Jaeger, R. J., and Rubin, R. J. Plasticizers from plastic devices: extraction, metabolism, and accumulation by biological systems. Science 170: 460 (1970).

6. Taborysky, R. G. Isolation studies on a lipoidal portion of the bovine pineal gland. J. Agr. Food Chem. 15: 1073 (1967).

7. Nazir, D. J., et al. Isolation, identification, and specific localization of di-2-ethylhexyl phthalate in bovine heart muscle mitochondria. Biochemistry 10: 4228 (1971).

8. Singh, A. R., Lawrence, W. H., and Autian, J. Teratogenicity of phthalate esters in rats. J. Pharm. Sci. 61: 51 (1972).

9. Warner, R. E. Bioassays for microchemical environmental contaminants with special reference to water supplies. Bull. World Health Org. 36: 181 (1967).

10. Metcalf, R. L., Sangha, G. K., and Kapoor, I. P. Model ecosystem for the evaluation of pesticide biodegradability and ecological magnification. Environ. Sci. Tech. 5: 709 (1971).

11. Freeman, L. A Standardized method for determining the toxicity of pure compounds to fish. Sewage Ind. Wastes 25:845, 1131 (1953).