TOXICITY AND BIOLOGICAL EFFECTS OF PHTHALATE ESTERS ON MIDGES *(CHIRONOMUS PLUMOSUS)*[1]

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ABSTRACT: The acute toxicity (48-h LC50 and EC50) of di-2-ethylhexyl phthalate (DEHP) to midge larvae (*Chironomus plumosus*) was greater than 18 mg/L and greater than 72 mg/L for its degradation products (mono-2-ethylhexyl phthalate and phthalic acid). The toxicities of di-n-butyl phthalate was 0.76 mg/L and 2-ethylhexanol was 34 mg/L. Chronic life cycle toxicity tests showed no effect up to 0.36 mg/L DEHP on midge emergence, egg production, or egg hatchability. Larvae accumulated ^{14}C-DEHP to 292 times the water exposure concentration of 0.2 μg/L in two days. After 3.4 days the residues in the larvae declined by 50%. The water concentration of DEHP in exposure chambers with hydrosoil substrate were lower than values from tests conducted with sand substrates. This did not effect midge emergence from either substrate, however, hydrosoil-reared midges had lower total body residues of ^{14}C-DEHP than sand-reared midges.

KEY WORDS: Phthalate esters, toxicity, midge, bioaccumulation.

Di-2-ethyhexyl phthalate (DEHP) is one of the phthalic acid esters used extensively as a plasticizer, particularly in polyvinyl chloride plastics (U.S. Tariff Commission 1974). Plasticizers are chemicals added to synthetic resins to impart flexibility to the original resin. Concentrations used in plastics can be up to 60 parts per hundred. Some esters are also used as orchard acaricides and insect repellents (Farm Chemicals Handbook 1977). Although DEHP is not intentionally applied to natural waters, phthalate esters have been identified as environmental contaminants in both freshwater and marine ecosystems (Mayer et al. 1972, Morris 1970). Municipal and industrial effluents are probably the major source of phthalate contamination of aquatic habitats (Hites 1973).

Past studies have indicated that the acute toxicity of DEHP to freshwater invertebrates is low (Mayer and Sanders 1973, Laughlin et al. 1978). The 96-h LC50's ranged from 2.1 mg/L for amphipods (*Gammarus pseudolimnaeus*) to more than 10 mg/L for crayfish (*Orconectes nais*). Chronic toxicity studies, however, have indicated that DEHP may be detrimental to some aquatic organisms at low water concentrations. Sanders et al. (1973) reported that the exposure of *Daphnia magna* to a DEHP concentration of 0.003 mg/L for 21 days caused a 60 percent decrease in the number of young produced; and Mehrle and Mayer (1976) reported that a DEHP concentration of 0.01 mg/L significantly increased mortality in sac fry of rainbow trout (*Salmo gairdneri*) within 5 days after they hatched.

Because immature midges are a major food source for many fishes (Johnson and Munger 1930), it is important that such widespread aquatic contaminants as phthalate esters be examined for their toxicity to these aquatic organisms. Our

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study was designed to determine the effects of DEHP on emergence of midges (*Chironomus plumosus*), hatchability of midge eggs, and the uptake and elimination of the chemical in midge larvae. We also determined acute toxicities of four phthalate acid esters and DDT to midge larvae. In the environment these larvae are found in substrates varying widely in cation exchange capacities, organic matter content and other parameters. Because of this, our studies of DEHP were conducted using both sand and hydrosoil substrates to determine possible effects of these different substrates in laboratory tests.

Materials and Methods. Laboratory populations of the midges were reared at the Columbia National Fisheries Research Laboratory, Columbia, Missouri, according to the techniques described by Biever (1965). Well water with pH of 7.4 and a total hardness of 270 mg/L as $CaCO_3$ was used for cultures and all experiments. All tests were conducted at 22 ± 1 C in a 16–h light, 8–h dark photoperiod. During the chronic exposure, the dissolved oxygen concentration and water temperature in test containers were measured daily. The pH and hardness were measured at the beginning and end of each test. The actual concentration of toxicant in each container was measured at the beginning of each test. Methods used for water residue analysis were described by Stalling et al. (1973).

Di–2–ethylhexyl phthalate and three of its degradation products, the mono–2–ethylhexyl phthalate (MEHP), phthalic acid (PA), and 2–ethylhexanol (2–EH) were provided by Monsanto Chemical Company, St. Louis, Missouri. The di–n–butyl phthalate (DBP) was obtained from Aldrich Company, Milwaukee, Wisconsin. DDT, which was used as a reference chemical for both the acute and chronic toxicity tests, came from City Chemical Corporation, New York, New York. The ^{14}C–ring–labeled DEHP used for the uptake study was from Pathfinder Laboratories, Inc., St. Louis, Missouri, and had a specific activity of 10.52 mCi/mM.

We conducted acute toxicity tests with late third and early fourth–instar midge larvae, using standard methods for static toxicity tests (Committee on Methods of Toxicity Tests with Aquatic Organisms 1975). Acute toxicity was measured as the 48–h EC50 (effective concentration causing immobilization of 50 percent of the test organisms). The method of Litchfield and Wilcoxon (1949) was used to calculate EC50's and LC50's and their 95 percent confidence limits. All concentrations reported in the text and tables were based on active ingredients. Ethanol was used as a solvent in all tests but the accumulation studies; its concentration never exceeded 0.1 m l/L in test solutions on controls.

Chronic toxicity tests were conducted with proportional diluters modeled after those of Mount and Brungs (1967) and Chandler et al. (1974). The hydrosoil substrate used was taken from Little Dixie Reservoir, Callaway County, Missouri. Samples of the top three cm of sediment were oven–dried at 57 C, ground to a powder, and thoroughly mixed. Soil chemistry tests were conducted on the hydrosoil according to methods described by Brown et al. (1977) (Table 1). The fine quartzite sand used was obtained locally, and was washed of all debris. The studies were started with first–instar larvae (1.5 mm long and up to 24 h old).

Table 1. Chemical characteristics of hydrosoil from Little Dixie Reservoir, Missouri.

Extractable[1] Phosphorus kg P_2O_5/ha P-I[2]	P-II[3]	Organic Matter (dry wt)	pH_w	me H^+ /100g	me Ca^{++} /100g	me Mg^{++} /100g	me K^+ /100g	me Na^+ /100g
18.6	178	1.6	7.3	0.2	11.3	1.9	0.54	0.15
(4.14)	(21)	(0.14)	(0.04)	(0.14)	(0.74)	(0.14)	(0.03)	(0.01)

[1]Mean and standard error of the eight samples in parenthesis

[2]Extracting solution of 0.03 N NH_4F + 0.025 N HCl

[3]Extracting solution of 0.03 N NH_4F + 0.1 N HCl

One hundred larvae, counted with the aid of a 10x lens, were placed in exposure containers that had been previously prepared by adding 200 g of sand or hydrosoil. Larvae were fed 0.12 g of a commercial dog candy (Biever 1965) every 4 days until they transformed into the pupal stage. The larvae were exposed to duplicate DEHP concentrations of 0.14, 0.20, and 0.36 mg/L (plus a control). Cast pupal skins at the water surface in exposure containers were counted and removed daily to determine emergence. The test was terminated when no cast pupal skins were present in exposure containers for 2 consecutive days after the onset of emergence. We determined the effects of DEHP on midge emergence by analysis of variance, using the arcsin transformation for portions (angle = arcsin $\sqrt{}$percentage) followed by a least significance difference test (Snedecor and Cochran 1974). In the egg hatchability studies we placed screens over the exposure chambers to confine emerging adults. Egg masses produced by mating pairs in the sand substrate were removed daily and placed in individual exposure chambers. The effects of DEHP on the hatchability of midge eggs were determined by counting the number of eggs that hatched from each mass produced in exposure and control chambers. The numbers of egg mass produced were also recorded.

Uptake and elimination experiments were conducted in water only using an intermittent–flow system modified from that of Mount and Brungs (1967). Stock solutions of ^{14}C–DEHP were prepared in water and further diluted in the flow system to a concentration of 0.2 ± 0.02 μg/L. This concentration was selected on the basis of the results from the acute toxicity tests, as was recommended for uptake studies by Johnson and Schoettger (1975).

Results and Discussion. *Acute toxicity.* In static tests, 48–h EC50's of the phthalic acid esters to midge larvae ranged from 0.76 mg/L for di–n–butyl phthalate to more than 72 mg/L for both the mono–2–ethylhexyl phthalate and phthalic acid (Table 2). The 48–h EC50 for midge larvae exposed to 2–ethylhexanol was 34 mg/L. Di–2–ethylhexyl phthalate was not toxic to larvae in 48–h at a concentration of 18 mg/L. The reference chemical DDT was considerably more toxic to midge larvae (48–h EC50, 0.023 mg/L) than phthalate acid esters.

Table 2. Acute toxicities of phthalic acid esters and DDT (water exposure) to midge larvae (*Chironomus plumosus*).

Compound	48-h EC50 and 95% confidence interval (mg/l)	Slope
DBP	0.76 (0.52-1.10)	1.46
2-EH	34.0 (28-41)	1.25
DEHP	>18	--
MEHP	>72	--
PA	>72	--
DDT	0.023 (0.019-0.028)	1.37

Chronic toxicity. In flow–through chronic toxicity tests of DEHP, with both sand and hydrosoil, concentrations as high as 0.36 mg/L had no significant ($P<0.05$) effect on growth and development of midge larvae when compared to control groups (Table 3). The continuous exposure of first generation midge eggs in sand substrate to mean measured DEHP concentrations between 0.14 and 0.36 mg/L had no significant effect ($P<0.05$) on hatchability (Table 4). All exposure concentrations in the chronic toxicity studies were similar to those reported to be found in the aquatic environment (Great Lakes Water Quality Board 1975).

Uptake and elimination. Third–instar midge larvae exposed continuously to 0.2 ± 0.02 μg/L of ^{14}C–DEHP accumulated total body residues in 2 days that were 292 times (wet weight) the water concentration (Figure 1). After this initial rapid uptake the rate of accumulation of DEHP slowed until an apparent plateau was reached at 7 days. The total body residues were then 82 ng/g, or a concentration 408 times that in water. The 2 and 7 day accumulation factors determined for midges are similar to those reported by Mayer and Sanders (1973).

To determine the time required for biological elimination of phthalate residues by midge larvae, we exposed the larvae to 0.2 μg/L of ^{14}C–DEHP for 4 days. This exposure resulted in a residue accumulation of 56 ng/g (Figure 1). When the larvae were transferred to phthalate–free flowing water, 30 percent of the total radioactivity was lost after 1 day, 50 percent after 3.4 days and 70 percent after 5 days. The metabolic fate of DEHP in aquatic invertebrates is incompletely known. However, Laughlin et al. (1978) found evidence of active metabolism of phthalates by grass shrimp (*Palaemonetes pugio*) larvae. Mayer

Table 3. Percent emergence of midges (*Chironomus plumosus*) from sand and hydrosoil after exposure of lots of 100 larvae each to different concentrations of di–2–ethylhexyl phthalate (DEHP) at 22 ± 1 C.

Exposure Concentration (mg/l)	Days of Exposure			
	20	25	30	35
		Sand Substrate		
0.0	48	68	77	79
0.14	49	72	81	82
0.20	49	72	83	83
0.36	44	74	80	80
		Hydrosoil Substrate		
0.0	16	41	60	72
0.11	4	25	50	63
0.20	13	36	57	70
0.24	13	45	53	65

Table 4. Mean egg production and hatchability (SE in parentheses) of midges (*Chironomus plumosus*) exposed in a sand substrate to di–2–ethylhexyl phthalate.

Exposure concentration (mg/l)	Total egg masses produced (no.)	Eggs per egg mass (no.)	Egg hatchability (%)
0 (control)	35	198 (11)	89.7 (1.9)
0.14	26	237 (18)	81.1 (3.8)
0.20	45	241 (16)	86.6 (2.8)
0.36	28	211 (20)	88.2 (3.7)

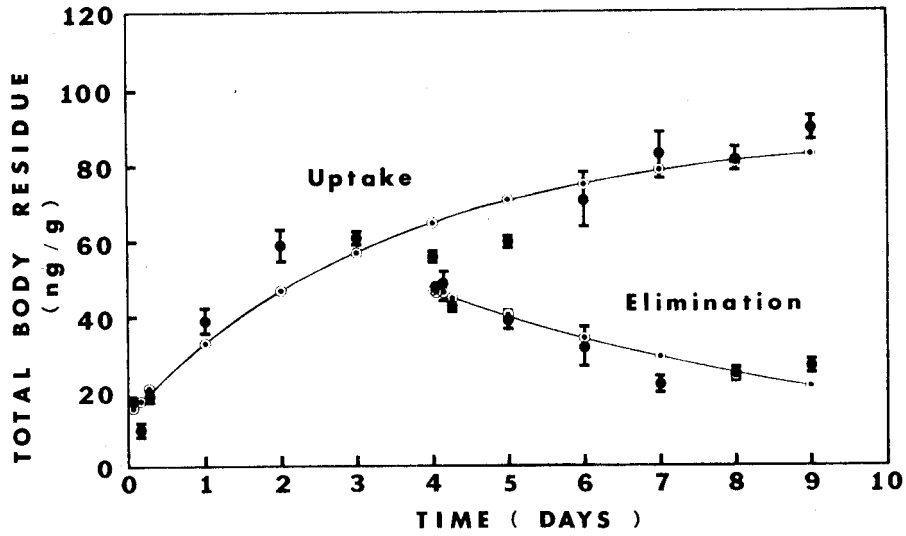

Figure 1. Uptake of ^{14}C residue (ng/g wet weight) by *Chironomus plumosus* larvae from water only that contained 0.20 μg/L of ^{14}C–di–2–ethylhexyl phthalate (upper curve), and elimination of residue in midges that had accumulated 56 mg/g during the 5 days after transfer to toxicant–free water (lower curve). Solid dots with bars represent mean and SE of three samples. Circles with dots in the center represent theoretical values.

(1976) reported that rainbow trout and fathead minnows (*Pimephales promelas*) readily degraded DEHP to 2–ethylhexyl phthalate and phthalic acid. Saeger and Tucker (1976) reported that phthalic acid ester plasticizers and intermediate degradation products at concentrations of 1 to 83 mg/L readily undergo ultimate degradation in different microbial systems. A similar metabolic process could occur in aquatic invertebrates.

Substrate effects. Both sand and hydrosoil reduced the concentration of DEHP in solution. In flow–through toxicity tests, a measured concentration of DEHP in water was reduced 28% by sand and 62% by hydrosoil. In one test, hydrosoil exposed to 0.30 mg DEHP/L for 35 days accumulated 29.2 μg/g; a 97-fold increase. This same hydrosoil decreased DDT concentrations to 3.1% of the delivered values during a flow-through exposure. These results were supported by a three day static test in which the initial DEHP concentrations were reduced 35% in containers containing water only, 53% in containers containing sand and 64% in containers with hydrosoil. The reason for this difference is that hydrosoil particles have a greater surface area and organic content, thus a greater capacity for chemical absorption per unit weight than sand (Edwards 1966, Pionke and Chesters 1973). Phthalates absorb strongly to surfaces and this adsorption can rapidly reduce water concentrations (Giam et al. 1975).

Despite the difference in DEHP adsorption between sand and hydrosoil, there was no significant ($P<0.05$) difference in midge emergence from either substrate (Table 3). However, hydrosoil–reared midges laid egg masses averaging twice as

many eggs (396) as those reared in sand (198). This difference was significant ($P<0.05$). Also the larvae developing in hydrosoil seemed to be a brighter red and more robust than sand–reared larvae. This difference may be related to the differences in organic content between sand and hydrosoil.

The uptake of ^{14}C–DEHP by midge larvae exposed in a flow–through system was affected by the type of substrate. In a residue dynamics study, larvae in hydrosoil exposed continuously to 0.27 μg/L DEHP (water concentration) had average total body residues 26% lower than larvae in sand exposed to the same water concentration. Substrate particles likely compete with the larvae for DEHP and the hydrosoil particles because their adsorption capacity would reduce the availability of the compound to the larvae more than sand. Weber and Coble (1968) have demonstrated clay mineral adsorption of organic compounds reduces the availability to microbes and our data indicate this may be extended to benthic invertebrates.

Conclusions. We were unable to determine a no–effect concentration of DEHP for midges because emergence, egg production, and egg hatchability were not affected at 0.36 mg/L, the highest concentration tested. This concentration is 120 times greater than the highest level causing reproductive impairment in daphnids (Sanders et al. 1973). Therefore, the recommended freshwater criterion of 0.003 mg/L (U.S. Environmental Protection Agency 1973) should protect this valuable freshwater fish food organism.

Because of chemical adsorption the effect of an aquatic contaminant on benthic organisms may decrease as a function of the surface area and organic content of the substrate. The water concentration of DEHP and the uptake of DEHP by midge larvae in exposure chambers with hydrosoils were lower than values obtained from similar tests conducted with sand substrates. The difference in water concetration and toxicity is attributed to the increased potential for adsorption of the test chemical by the hydrosoil.

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