Toxicology of Phthalic Acid Esters in Aquatic Organisms

by Foster L. Mayer Jr.,* and Herman O. Sanders*

Phthalic acid esters are presently being used in amounts and products that can easily, although inadvertently, contribute to environmental pollution. As plasticizers, phthalic acid esters are added to synthetic plastic resins to impart flexibility to the finished product, improve workability during fabrication, and extend or modify properties not present in the original resins *(1)*. Plastic formulations may contain up to 60% of plasticizers *(2)*. Phthalic acid esters are the most widely used plasticizers, particularly in poly(vinyl chloride) plastics. More than 800 million pounds of these plasticizers were produced in 1969 *(1)*. Di-*n*-butyl and di-2-ethylhexyl phthalates are used as plasticizers and also as an insect repellant and orchard acaricide, respectively *(3)*.

Phthalic acid esters have been identified as environmental contaminants because of their discovery in soil *(4)*, a deep sea jellyfish *(5)*, aquatic organisms and water *(6)*, and bovine tissues *(7,8)*. The acute toxicities of various phthalic acid esters have been determined in mammals and birds and the compounds were found to have a very low order of toxicity *(3,9–12)*. Teratogenic effects of phthalic acid esters have been demonstrated in rats *(13)*. Problems of phthalic acid esters leaching from plastics used in human medical practices have also been reported *(14–17)*. The biological significance of phthalic acid ester residues in aquatic organisms is unknown at present, and this report describes the toxicology of di-*n*-butyl and di-2-ethylhexyl phthalates in some fish and aquatic invertebrates.

*Fish-Pesticide Research Laboratory, Bureau of Sport Fisheries and Wildlife, United States Department of the Interior, Columbia, Missouri 65201.

Toxicity

Standard static and flow-through bioassay procedures *(18)* were followed to evaluate the toxicity of di-*n*-butyl and di-2-ethylhexyl phthalates. Acute 96-hr bioassays indicate that the toxicity of di-*n*-butyl phthalate to fish is relatively low (Table 1) as compared with DDT (7–19 μg/l.). The 96-hr LC_{50} to scuds *(Gammarus pseudolimnaeus)* and crayfish *(Orconectes nais)* was 2.1 mg/l. and >10.0 mg/l., respectively. Di-2-ethylhexyl phthalate toxicity was difficult to determine

Table 1. Acute Toxicity of di-*n*-butyl phthalate to aquatic organisms.

Species	LC_{50} value, mg/l.		
	24 hr	48 hr	96 hr
Fathead minnow *(Pimephales promelas)*	—	1.49	1.30
Bluegill *(Lepomis macrochirus)*	1.23	0.73	0.73
Channel catfish *(Ictalurus punctatus)*	3.72	2.91	2.91
Rainbow trout *(Salmo gairdneri)*	—	—	6.47
Scud *(Gammarus pseudolimnaeus)*	7.00	—	2.10
Crayfish *(Orconectes nais)*	—	—	>10.00

due to its insolubility. The 96-hr LC_{50} value of di-2-ethylhexyl phthalate was greater than 10 mg/l. for both fish and invertebrates. However, fulvic acid, which occurs widely in soils and waters, has been shown to solubilize di-2-ethylhexyl phthalate *(4)* and could possibly produce a greater availability of this compound to aquatic organisms in their natural habitat.

In vitro studies with channel catfish *(Ictalurus punctatus)* liver indicate that di-*n*-butyl phthalate is degraded sixteen times more rapidly than di-2-ethylhexyl phthalate *(19)*. The large difference in toxicity between the two phthalic acid esters may be due to the rate of degradation of the parent compound to a more toxic substance such as phthalic acid.

Accumulation and Excretion

All invertebrates continuously exposed to ^{14}C-di-*n*-butyl and di-2-ethylhexyl phthalates showed an initial rapid uptake and accumulation of radioactive residues several hundred times greater than the concentration in water (Tables 2 and 3). With the exception of some species of invertebrates, the accumulation of di-2-ethylhexyl phthalate was greater than that of di-*n*-butyl phthalate. Di-2-ethylhexyl phthalate residues were accumulated and stored by scuds during a 14-day exposure at levels 3600 times greater than the 0.1 μg/l. concentrations in the surrounding water, whereas scuds exposed for 14 days to 0.1 μg/l. of di-*n*-butyl phthalate accumulated total body concentrations 1400

Table 2. Accumulation of ^{14}C-di-*n*-butyl phthalate by aquatic invertebrates.

Organism	Water concentration, μg/l.	Accumulation factor after			
		1 day	3 days	7 days	14 days
Waterflea *(Daphnia magna)*	0.08	170	280	400	400
Scud *(Gammarus pseudolimnaeus)*	0.10	360	780	1,350	1,400
Midge *(Chironomus plumosus)*	0.18	380	420	720	—
Mayfly *(Hexagenia bilineata)*	0.08	130	230	430	—

Table 3. Accumulation of ^{14}C-di-2-ethylhexyl phthalate by aquatic organisms.

Organism	Water concentration, μg/l.	Accumulation factor after			
		1 day	3 days	7 days	14 days
Waterflea *(Daphnia magna)*	0.3	93	250	420	—
Scud *(Gammarus pseudolimnaeus)*	0.1	720	1,380	3,900	3,600
Midge *(Chironomus plumosus)*	0.3	270	330	350	—
Mayfly *(Hexagenia bilineata)*	0.1	210	250	575	—
Fathead minnow *(Pimephales promelas)*	1.9	135	245	369	458

times the phthalate concentrations in water. Phthalic acid esters were accumulated in invertebrates to a similar degree as that found with the same species of invertebrates exposed to organochlorine insecticides *(20)*.

Waterfleas *(Daphnia magna)* were exposed to 0.1 μg/l. of di-*n*-butyl phthalate for 7 days and then transferred to fresh flowing water to determine the time required for elimination of phthalate residues. After 3 days, 50% of the total radioactivity remained; 25% of the activity was still present after 7 days in fresh water. In similar experiments, scuds were exposed to 0.1 μg/l. of di-2-ethylhexyl phthalate for 7 days and transferred to fresh water. Residual radioactivity decreased rapidly during 4 days in fresh water to 20% of the beginning activity. After 10 days, only 6% of the total activity remained. Invertebrate metabolism of phthalic acid esters was not determined, and the loss in radioactive residues may have been due to metabolism and/or excretion of the parent compound.

Fathead minnows *(Pimephales promelas)* were continuously exposed to 1.9 μg/l. ^{14}C-di-2-ethylhexyl phthalate for 56 days and then transferred to fresh water for 28 days. The accumulation of di-2-ethylhexyl phthalate by fathead minnows was 458 times that of the water after 14 days exposure (Table 3). An equilibrium was reached after 28 days exposure with an accumulation factor of 1380. Once this equilibrium was reached within the fish, no further residue accumulation was observed after an additional 28 days of exposure. After placing the fish in fresh water, the time required for 50% elimination of di-2-ethylhexyl phthalate and its degradation products was 7 days. The amount of phthalate accumulated was comparable to or exceeded that of the organochlorine insecticides DDT, heptachlor, and methoxychlor.

The radioactive residues consisted mainly of di-2-ethylhexyl phthalate and the monoester (Table 4). The other degradation products isolated were free phthalic acid, the conjugated monoester, and conjugated phthalic acid. Phthalic acid and the two conjugates represented from 5 to 26% of the total radioactivity.

Reproduction

Waterfleas were continuously exposed to 3, 10, and 30 μg/l. of di-2-ethylhexyl phthalate for a complete life cycle (21 days). All three treatment levels significantly ($P < 0.01$) reduced waterflea reproduction (Table 5), and total production of offspring was inhibited 60, 70, and 83% in the respective treatment levels. The degree of reproductive impairment was relatively constant during the 21-day period. The observed toxicity values (LC_{50}) for aquatic invertebrates are 700 to 3300 times that which inhibited reproduction in waterfleas. For comparison, a 50% inhibition of waterflea reproduction occurs at 0.13 and 2.5 μg/l. for DDT and di-2-ethylhexyl phthalate, respectively. The 96-hr LC_{50} of DDT to waterfleas is 1.0 μg/l., which is only eight times the amount necessary for reproductive impairment.

Table 4. Composition of ^{14}C-labeled residues in fathead minnows *(Pimephales promelas)* exposed to ^{14}C carbonyl-labeled di-2-ethylhexyl phthalate.

Exposure, days	Composition				
	Di-2-ethylhexyl phthalate, %	Monoester, %	Phthalic acid, %	Conjugated monoester, %	Conjugated phthalic acid, %
28	49.6	37.1	5.2	0.7	3.0
56 [a]	60.3	28.7	4.9	1.4	3.7
63	71.0	23.8	2.9	1.0	1.2
70	73.0	12.8	3.1	7.0	4.7
84	63.7	9.6	9.8	7.2	9.1

[a] Exposure terminated.

Table 5. Waterflea *(Daphnia magna)* reproduction as affected by continuous exposure to di-2-ethylhexyl phthalate for 21 days.

Concentration, μg/l.	Offspring produced per ten adults			
	2-week sample		3-week sample	
	$\bar{X}$ ± S. D. [a]	Inhibition, %	$\bar{X}$ ± S. D. [a]	Inhibition, %
Control	71.5 ± 13.4	—	114.0 ± 8.5	—
3	26.5 ± 7.8	62.9	45.5 ± 14.8	60.1
10	30.0 ± 8.5	58.1	34.0 ± 8.5	70.2
30	12.5 ± 3.5	82.5	19.5 ± 3.5	82.9

[a] Mean ± standard deviation.

The effects of di-2-ethylhexyl phthalate on reproduction of zebra fish *(Brachydanio rerio)* and guppies *(Poecilia reticulatus)* were determined with 90-day dietary exposures of the phthalate. Zebra fish were fed diets containing 50 and 100 μg di-2-ethylhexyl phthalate per gram of food, and guppies were fed 100 μg/g. Although the number of spawns were greater in the treated zebra fish, the control fish produced more eggs per spawn than those fish exposed to di-2-ethylhexyl phthalate (Table 6). Fry survival was significantly reduced ($P < 0.05$) by phthalate exposure. The least number of guppy fry were born to parents fed di-2-ethylhexyl phthalate, and an 8% incidence of abortions was noted.

All of the dying fry exposed to di-2-ethylhexyl phthalate died in tetany; however, tetany did not occur in dying controls. Coho salmon *(Oncorhynchus kisutch)* injected intraperitoneally with 3 μg di-2-ethylhexyl phthalate/kg of fish demonstrated increased serum calcium *(21)*. The tetany observed in zebra fish and the increased serum calcium found in coho salmon suggest that di-2-ethylhexyl phthalate may alter normal calcium metabolism in fish.

Summary

The low degree of toxicity and the high excretion rate of di-*n*-butyl and di-2-ethylhexyl phthalates might suggest that these compounds would be relatively safe as far as aquatic organisms are concerned. However, the present data indicate that these compounds can be detrimental to the reproduction of aquatic organisms at low chronic concentrations. The concentrations of phthalic acid esters presently found in waters of the United States are, in some cases, detrimental to aquatic invertebrates in view of laboratory results. Phthalic acid esters are produced in large amounts, they are in wide use as plasticizers, and they are entering aquatic ecosystems. Thus, these compounds should be considered as environmental pollutants, and a more detailed evaluation of toxicological effects of phthalic acid esters is needed to elucidate their impact on aquatic ecosystems. Also, more research is

Table 6. Reproduction in zebra fish *(Brachydamio rerio)* and guppies *(Poecilia reticulatus)* fed di-2-ethylhexyl phthalate.

Species	Reproductive variable	Dietary di-2-ethylhexyl phthalate concentration, μg/g		
		0	50	100
Zebra fish				
	Number of spawns	6	8	14
	Eggs per spawn	20:3	15.2	10.1
	Percent fry survival	51.1	31.7	11.5
Guppies				
	Fry per female	33	—	29
	Percent abortions	0	—	8

warranted in developing and/or reducing the potential for these compounds to enter aquatic environments.

Research in Progress

In response to the results of preliminary investigations, we are presently evaluating the effect of dietary di-2-ethylhexyl phthalate (0.3 to 10.0 μg/g of food) on calcium, amino acid, and steroid metabolism in rainbow trout *(Salmo gairdneri)*. Continuous exposure of di-*n*-butyl and di-2-ethylhexyl phthalates in water at concentrations ranging from 0.8 to 50 μg/l. are being tested for reproductive effects in fathead minnows *(Pimephales promelas)*.

REFERENCES

1. U. S. Tariff Commission Plasticizers Rept. 1971. GPO, Washington, D.C.
2. Nematollahi, J., Guess, W. L., and Autian, J. 1967. Plasticizers in medical application. I. Analysis and toxicity evaluation of dialkyl benzenedicarboxylates. J. Pharm. Sci. 56: 1446.
3. Farm Chemicals. 1971. Dictionary of pesticides. Meister Publishing Co., Willoughby, Ohio.
4. Ogner, G., and Schnitzer, M. 1970. Humic substances: Fulvic acid–dialkyl phthalate complexes and their role in pollution. Science 170: 317.
5. Morris, R. J. 1970. Phthalic acid in the deep sea jellyfish *Atolla*. Nature 227: 1264.
6. Mayer, F. L., Jr., Stalling, D. L., and Johnson, J. L. 1972. Phthalate esters: An environmental contaminant. Nature 238:411.
7. Nazir, D. J., Beroza, M., and Nair, P. P. 1967. Isolation and identification of di(2-ethylhexyl) phthalate — a component of heart muscle mitochondria. Fed. Proc. 26: 412.
8. Taborsky, R. G. 1967. Isolation studies on a lipoidal portion of the bovine pineal gland. J. Agr. Food Chem. 15: 1073.
9. Hodge, H. C. 1943. Acute toxicity for rats and mice of 2-ethylhexanol and 2-ethylhexyl phthalate. Proc. Soc. Exp. Biol. Med. 53: 20.
10. Shaffer, G. B., Carpenter, C. P., and Smyth, H. F., Jr. 1945. Acute and subacute toxicity of di(2-ethylhexyl) phthalate with note upon its metabolism. J. Ind. Hyg. Toxicol. 27: 130.
11. Calley, D., Autian, J., and Guess, W. L. 1966. Toxicology of a series of phthalate esters. J. Pharm. Sci. 55: 158.
12. Guess, W. L. 1968. Toxicity profiles of vinyl and polyolefinic plastics and their additives. J. Biomed. Mater. Res. 2: 313.
13. Singh, A. R., Lawrence, W. H., and Autian, J. 1971. Teratogenicity of a group of phthalate esters in rats. Toxicol. Appl. Pharmacol. 19: 372.
14. Guess, W. L., Jacob, J., and Autian, J. 1967. A study of polyvinyl chloride blood bag assemblies. I. Alteration or contamination of ACD solutions. Drug Intell. 1: 120.
15. Jaeger, R. J., and Rubin, R. J. 1970. Plasticizers from plastic devices: extraction, metabolism, and accumulation by biological systems. Science 170: 460.
16. Marcel, Y. L., and Noel, S. P. 1970. Contamination of blood stored in plastic packs. Lancet 1: 35.
17. Neergaard, J., et al. 1971. Plasticizers in PVC and the occurrence of hepatitis in a haemodialysis unit. Scand. J. Urol. Nephrol. 5: 141.
18. American Public Health Association. 1971. Standard methods for the examination of water and wastewater. Amer. Publ. Health Assn., Inc., New York.
19. Hogan, J. W. 1972. Unpublished data, Fish-Pesticide Research Laboratory, Columbia, Missouri.
20. Johnson, B. T., et al. 1971. Biological magnification and degradation of DDT and aldrin by freshwater invertebrates. J. Fish. Res. Board Can. 28: 705.
21. Grant. B. F. 1970. Pesticide effects on fish endocrine functions. In: Progress in Sport Fishery Research, Bureau of Sport Fisheries and Wildlife, Resource Pub. 106. p. 22.