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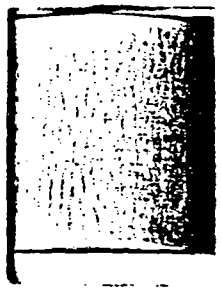
Phthalate Esters as Environmental Contaminants

WE have found traces of phthalate esters in fish caught in various parts of North America. In a routine monitoring programme, gas-liquid chromatographic (GLC) analyses revealed two unidentified peaks in many fish extracts and in procedural blanks examined for insecticide residues. The two compounds have now been identified with gas chromatograph-mass spectrometer techniques[1] as di-*n*-butyl and di-2-ethylhexyl phthalates. These compounds, widely used as plasticizers, proved to be of low toxicity, but our data suggest that they could be detrimental to some aquatic organisms.

Plasticizers 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[2]. Plastic formulations may contain up to 60 parts per hundred of plasticizers[3]. Phthalic acid esters are the most widely used plasticizers, particularly in polyvinyl chloride plastics. More than 800 million pounds of these plasticizers were produced in 1969[3]. Di-2-ethylhexyl and di-*n*-butyl phthalates are also used as an orchard acaricide and an insect repellent, respectively[4]. There is no well documented information on the fate of phthalate compounds in aquatic environments, nor have water quality criteria been established for them.

Phthalate esters were recovered from a 'Florisil' column in the 15% diethyl ether-petroleum ether eluate. The percentage recoveries for di-*n*-butyl and di-2-ethylhexyl phthalates were 90 and 50 respectively and the residue data in Table 1 are corrected values. The GLC retention time of the phthalate esters does not pose a significant interference problem in pesticide analyses. Retention times relative to aldrin were 1.24 and 14.9 for di-*n*-butyl and di-2-ethylhexyl phthalate esters, respectively. The GLC column used was a 1.8 m×2 mm inner diameter packed with 80–100 mesh Corning GLC-110 glass beads coated with 0.3% OV-7. The retention time of methoxychlor relative to aldrin on this column was 11.9. A ^{63}Ni-electron capture detector served to establish residue levels after sample clean-up. The GLC column temperature was 155° C and the nitrogen carrier flow was 20 ml./min. The electron capture detector peak area response relative to an equal amount of DDT was 0.12 for di-*n*-butyl phthalate and 0.09 for di-2-ethylhexyl phthalate. The minimum detection limits for di-*n*-butyl and di-2-ethylhexyl phthalates were 0.1 and 0.5 μg/g, respectively. Various components of laboratory equipment, such as blender seals, disposable plastic gloves and plastic tubing, were found to be sources of phthalate ester contamination in residue samples. Reagent grade anhydrous sodium sulphate also contained some di-*n*-butyl phthalate and prewashing with petroleum ether was required before use.



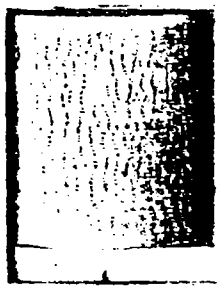


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Table 1 Phthalate Ester, Polychlorinated Biphenyl and Insecticide Residues found in Selected Samples from North America

Source	Sample type	Residue (ng/g) DNBP	DEHP	PCB*	Insecticide
Mississippi and Arkansas (agricultural and industrial areas)	Channel catfish †	Trace	3,200	400	DDT-300, dieldrin-40, endrin-30, toxaphene-2200
Fairport National Fish Hatchery, Iowa (water supply from industrial area of Mississippi River)	Channel catfish	200	400	700	—
	Dragonfly naiads	200	200	200	—
	Tadpoles	500	300	1,000	Dieldrin-10
Black Bay, Lake Superior, Ontario (rural and industrial area)	Walleye	—	800	1,300	DDT-120
	Water	—	300	—	—
	Sediment	100	200	40	—
Hammond Bay, Lake Huron, Michigan (forested area)	Water	0.040	—	0.003	—
Lake Huron, Michigan	Water	—	5.0	0.20	—
Spirit Lake, Iowa (agricultural area)	Yellow perch	—	—	—	DDT-160, dieldrin-120, chlordane-30
Clover Leaf Lake, California (10,300 feet elevation)	Brook trout	—	—	—	DDT-110
Missouri River, McBaine, Missouri	Water (turbid)	0.09	4.9	0.20	—
Fish food and components	Commercial fish food	—	2,000	700	—
		—	7,000	60	DDT-30, dieldrin-60
	Casein	20	190	—	—
	Corn starch	20	170	80	—
	Gelatin	20	140	—	—
	Bone meal	30	400	—	—
	Wheat middlings	30	200	20	—
	Carboxymethyl cellulose	—	—	—	Chlordane-720

DNBP, di-*n*-butyl phthalate; DEHP, di-2-ethylhexyl phthalate.
*Residues approximated by 'Aroclor' 1254 (80%), 1248, and traces of 1232.
†Residues presented for this group are the means of values for forty fish. Di-2-ethylhexyl phthalate residues ranged from 1,000 to 7,500 ng/g.

Table 2 Act

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Table 2 Acute Toxicity of Di-*n*-butyl Phthalate to Four Species o Fish

Species	Temperature (°C)	TL_{50}* (μg/l.) 24 h	48 h	96 h
Fathead minnow (*Pimephales promelas*)	17	—	1,490	1,300
Bluegill (*Lepomis macrochirus*)	17	1,230	731	731
Channel catfish (*Ictalurus punctatus*)	17	3,720	2,910	2,910
Rainbow trout (*Salmo gairdneri*)	12	—	—	6,470

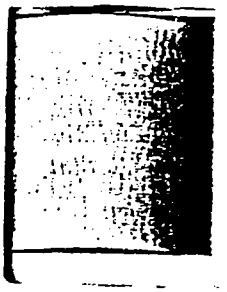
Toxicity was measured by standard static bioassay.

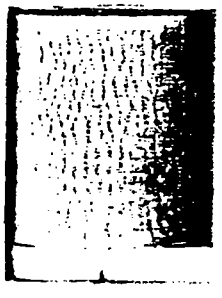
*The tolerance limit is the concentration in which 50% of fish survive for a specified time.

Standard static bioassay procedures were followed[5] and acetone (0.7 ml./l.) was used as the solvent in evaluating phthalate ester toxicity. Mortality data were analysed by probit analysis[6]. Acute 96-h bioassays indicate that the toxicity of di-*n*-butyl phthalate to fish is relatively low (Table 2) compared with DDT (7–19 μg/l.). The 96-h TL_{50} of di-*n*-butyl phthalate to scud (*Gammarus pseudolimnaeus*) was 2.1 mg/l. (DDT = 1.0 μg/l.). The insolubility of di-2-ethylhexyl phthalate makes it difficult to evaluate the acute toxicity of this compound. However, fulvic acid, which occurs widely in soils and waters, has been shown to solubilize di-2-ethylhexyl phthalate[7], and could possibly result in a greater availability of this compound to aquatic organisms.

Preliminary investigations indicate di-2-ethylhexyl phthalate is readily accumulated by fathead minnows (*Pimephales promelas*) continuously exposed to water containing 2.5 μg/l. phthalate, and residue concentrations in fish were eight hundred times that in the water after a 28-day exposure. However, we have found that scud will accumulate di-*n*-butyl and di-2-ethylhexyl phthalates 6,700 and 13,400 times the water concentration (0.1 μg/l.), respectively, within 14 days. Only 6% of residual di-2-ethylhexyl phthalate remained in the scud after 10 days in fresh water.

Di-2-ethylhexyl phthalate was examined for reproductive effects in zebra fish (*Brachydanio rerio*) and guppies (*Poecilia reticulata*) by dietary exposure. Zebra fish were fed diets containing 50 and 100 μg/g of food and guppies were fed 100 μg/g. Up to 88.5% of the zebra fish fry died before foraging began as compared with a 50% mortality in control fish. All the dying fry exposed to di-2-ethylhexyl phthalate died in tetany, which suggests that this compound may alter normal calcium metabolism. However, tetany did not occur in the dying controls. Intraperitoneal injections of di-2-ethylhexyl phthalate (3 μg/kg) increased serum calcium, decreased serum potassium, but did not affect serum sodium and chloride in coho salmon (*Oncorhynchus kisutch*). All of the adult guppies fed di-2-ethylhexyl phthalate became lethargic after 2 months of exposure and an





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8% incidence of abortions was noted in this group. Continuous exposure of waterfleas (*Daphnia magna*) to 3 μg/l. of di-2-ethylhexyl phthalate significantly ($P<0.05$) reduced reproduction by 60%. Details of these studies will be published separately.

The incidence of phthalate esters in fish seemed to be greater in aquatic areas associated with industrial and heavily populated regions, although hatchery and farmed fish fed diets contaminated with the esters also contained residues. Dietary contamination was probably a consequence of the use of contaminated fish products in feeds. Residues of phthalate esters previously reported in milk[8] and bovine tissues[9,10] may have resulted from dietary intake of phthalate esters. Phthalate esters have also been found in deep sea jellyfish[11] and soil[12]. The actual amounts and distribution of these pollutants in the environment have not been fully investigated, but a recent report of 100 mg/l. phthalate esters in a water sample from the Ohio River, West Virginia, was not anticipated (R. Sandridge, personal communication).

The acute toxicity of phthalate esters seems relatively insignificant, but there are indications that these compounds can be detrimental to aquatic organisms at low chronic concentrations. They are produced in large amounts, they are in wide use as plasticizers, and, by some means, they are entering aquatic ecosystems. Thus, these compounds should be considered as environmental pollutants and a more detailed evaluation of toxicological effects of phthalate esters is essential to elucidate their impact on these systems.

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