CANADA CENTRE FOR INLAND WATERS

Environment Canada

ENVIRONMENTAL ASPECTS OF PHTHALATE ESTERS

by

CCIW TOXIC SUBSTANCES GROUP

Unpublished Manuscript

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February 1974

Summary

Phthalate esters are produced in large quantities and are widespread in their usage - being present in many plastic formulations. More and more reports are available of their being observed in environmental samples ranging from water and sediment through all levels of biota. It would appear that they are not especially toxic although sub-lethal effects have been little investigated. They also seem to be degraded within reasonable periods of time under natural environmental conditions.

The Toxic Substances Group has previously recommended (March, 1973) several areas of interest with respect to research at C.C.I.W. To reiterate these, they are:

1) Development of analytical facility - both procedures and a "clean" area where samples may be worked up free from contamination;

2) An environmental survey - sediments, water and biota;

3) Physiological studies - particularly in microorganisms, plankton and fish. Degradation, food chain transfer and sublethal effects should be investigated.

Environmental Levels of Phthalates

Data giving levels of phthalates in the environment are very sparse. This section will deal largely with water, sediment, soil and biota levels.

Water analyzed from the Charles River, which runs through Boston, Mass., was found to contain up to 1 ppb of di-2-ethylhexyl-phthalate (DEHP) (Hites and Biemann 1972). A water sample from the mouth of the Mississippi River was found to contain 660 ppb of phthalate (Shea, 1972). However such a high concentration appears to be abnormal as it would account for more phthalate than was produced in the U.S. using mass balance calculations on that river. Samples from the Great Lakes area were analyzed by Mayer et al. (1972). In the Black Bay region of Lake Superior (a rural-industrial area), water was found to contain no detectable di-n-butylphthalate (DNBP) and 300 ppb DEHP. Sediments in the bay contained 100 ppb of DNBP and 200 ppb DEHP. A walleye from the bay was analyzed and contained 800 ppb DEHP (the same fish contained 1300 ppb of polychlorinated biphenyls). Hammond Bay in Lake Huron, a forested area, contained 40 ppt DNBP (no detectable DEHP) Lake Huron water had 5.0 ppb DEHP, and the Missouri River, 0.09 ppb DNBP and 4.9 ppb DEHP.

Soil samples also contain phthalates. Ogner and Schnitzer (1970) studied a PEI podzol and found phthalate - fulvic acid complexes with levels of 13 mg phthalate/100 gm fulvic acid; i.e. up to 0.037% dry weight. Matsuda and Schnitzer (1971) found that such fulvic acid complexes make phthalates more water-soluble.

Crude oil was found to contain a branched-chain dioctyl phthalate 0.15% by weight (Phillips and Breger, 1958). Such phthalates could be either a preserved metabolic product, or formed during oil formation (Breger, 1972).

Phthalates have been found in biota. In terms of freshwater organisms, the most comprehensive study was done by Mayer et al. (1972). Both DNBP and DEHP were analyzed for in various samples from agricultural, industrial and forested areas. Channel catfish from agricultural and industrial areas in Mississippi and Arkansas contained traces of DNBP and 1 - 7.5 ppm of DEHP (40 fish mean was 3.2 ppm). The same species from a fish hatchery in Iowa using Mississippi River water (industrial area) contained 200 ppb DNBP, and 400 ppb DEHP; dragon fly naiads contained 200 ppb DNBP and 20 ppb DEHP, while tadpoles had 500 and 300 ppb of these two phthalates. Of interest here was the fact that PCB levels were higher, but of the same order of magnitude. Walleye from Black Bay, Lake Superior (Ont.) had 800 ppb DEHP; Yellow Perch from an agricultural area of Iowa had no DNBP or DEHP, nor did Brook Trout from a high mountain lake in California. Also of interest was that two commercial fish foods contained 2 and 7 ppm of DEHP respectively (no DNBP). Of the fish food components, bone meal levels of DEHP were higher, roughly double those found in casein, corn starch, gelatin and wheat middlings.

Marine specimens also were found to contain phthalates. Morris (1970) found 0.01% (wet weight basis) phthalic acid in deep sea jellyfish (Atolla) in the North Atlantic. Zitko (1972) reported 10-80 ppm DNBP in double-breasted cormorant eggs, in an Atlantic salmon and 10.6 ppm (lipid-basis) in a common seal. Beef hearts contain 13,500µg DEHP/100g heart muscle, found in mitochondrial fractions (Nazir et al., 1971). Rats, rabbit and dog heart muscle contained roughly 1/100 that level.

Phthalates are insidious as has been indicated by the preceding and by the usages to which they are put (see Production and Uses of Phthalates). Their presence in the environment may often be an artifact of the analytical procedures employed (arising from extraction of plastic material or from airborne sources) or it may indeed be that these compounds are biologically synthesized (Thomas et al, 1971; Sofowora and Hardman, 1973). Particularly in the work carried out by Ogner and Schnitzer and by Phillips and Breger, the quantities obtained would seem to preclude contamination. In many of the other cases cited, however, observed values are at low levels and this possibility therefore exists. Since most environmental samples will likely generate only these trace amounts, it is apparent that procedures and facilities free from the possibility of contamination are required in any such studies.

Biomagnification of Phthalate Esters

A food chain study of DEHP was made by Metcalf et al. (1973). This compound with a ^{14}C label was added at concentrations of 0.1 and 10 µg/ℓ to aquaria containing water fleas (Daphnia magna), mosquito larvae (Culex pipiens quinquefasciatus) finger-nail clams (Sphaerium striatinum), guppies (Lebistes reticulatus) and the aquatic plant Elodea canadensis. At the 10 µg/ℓ concentration, biomagnification factors over a 24 hour period varied from 35 in the guppy to 4108 in the Culex larvae, while at 0.1 µg/ℓ DEHP, factors varied from 92 in the guppy to 692 in a snail (Physa) that was studied. These organisms were found to contain degradation products of DEHP such as polar metabolites, phthalic acid, phthalic anhydride, and two unknown compounds. A model ecosystem was also set up containing Daphnia, Physa, Culex larvae & pupae, Elodea, and the mosquito fish Gambusia. The plants and mosquito larvae were apparently unable to degrade much DEHP, while the fish was fairly active in degradation of DEHP to phthalic anhydride and the snail degraded DEHP to mono-2-ethylhexylphthalate (MEHP) phthalic anhydride, and phthalic acid. After 48 hours, concentration factors found were: algae -53,890X, snails - 21,480X, mosquito larvae - 107,670X, and fish - 130X. The concentration figures for the snail and mosquito larvae are fairly similar to studies done by these authors with DDT.

That accumulation factors are variable is indicated by comparing the above results and those of Mayer & Sanders (1973). After 7 days the latter found factors for DNBP varied from 430 (mayfly) to 1350 in the scud. While DEHP accumulation factors varied from 350 in midge to 3900 in scud.

Microbial Metabolism of Phthalate Esters

Despite the widespread use of phthalic acid and its esters, very limited information is available on their fate in biological systems or the aquatic environment.

Phthalic acid esters have occasionally been found as constituents of bacterial lipids, leading to some speculation as to the origin of these esters; i.e., biological production or artifacts of the experimental procedures. These findings suggest that a substantial concentration of phthalic acid esters may occur in bacterial lipids resulting in a magnification through the food chain. Recently Thiele and Truper (1972) have reported that these esters are not of biological origin but may arise as contaminants in the distilled deionized water used, which was stored in plastic containers.

A few scientists apparently believe that phthalate esters are relatively biodegradable, at least in comparison to DDT and the polychlorinated biphenyls but most consider that the evidence for biodegradability is still inconclusive (Marx, 1972). In one

experiment, described by Paul Graham of Monsanto Chemical Co., an "activated sludge" of microorganisms, similar to those found in sewage treatment plants, eliminated more than 90% of the esters within 48 hours. This experiment was performed under laboratory conditions that may not be comparable to those found in the field.

In terms of biodegradation, di-2-ethylhexylphthalate appears to be relatively stable while di-n-butyl phthalate is readily degradable. The mechanism of degradation is still not known (Stalling et al., 1972).

Preliminary studies at C.C.I.W. indicate that DEHP and DBP are relatively nontoxic to bacteria.

Fish are able to metabolize phthalates (Stalling et al, 1972). Within 24 hours, channel catfish exposed to DEHP had only 14% of this compound, with various derivitives being formed. DNBP was found to be 16 times more degradable. Such degradation is related to the hepatic microsomial system.

Toxicology

In terms of toxicological effects upon freshwater organisms, most work has been done at the Fish-Pesticides Laboratory, Columbia, Missouri, U.S.A. Direct toxicity, i.e. lethal dose studies were performed using DNBP to fathead minnows, bluegill channel catfish, rainbow trout, scud (Gammarus) and crayfish. LC_{50} (24 hour) for bluegill, channel catfish and scud were 1.23, 3.72 and 7.00 ppm

respectively while 96 hour LC_{50} studies showed the bluegill was most sensitive (0.73 ppm) and the crayfish least sensitive (>10.0 ppm) (Mayer et al, 1972, Mayer & Sanders, 1973). By way of comparison, Zitko (1972) found no 96 hour DEHP mortality for Atlantic salmon at 10 ppm with DNBP being more toxic.

Sublethal effects of phthalates, especially upon reproduction, indicate these effects may be of greater ecological significance than the lethal effects. For example, Daphnia were exposed to 3, 10 and 30 ppb of DEHP for their entire 21 day life cycle. Reproduction decreased by 60, 70 and 83% over controls at these three concentrations. In this species, a 96 hour LC_{50} of 1 mg/1 was found (Mayer & Sanders, 1973). Zebra fish and guppies were subjected to 50 and 100 µg/g DEHP in their food (Mayer et al, 1972). In the guppy, higher abortion incidence was noted, and in the zebra fish higher fry death rates (88.5% vs 50% in control fish) occurred. Tetany was also noted in survivors, indicative of a disruption of calcium metabolism.

Phthalates also show definite toxic effects upon higher vertebrates. Bower et al (1970) found teratogenic effects upon the developing chick embryo, including skeletal defects and failure of the cornea to develop. Upon hatching, chicks showed indications of damage to their central nervous system such as tremors, and difficulties in standing or walking. Increased death rates were also noted compared to controls. No other bird studies were found.

Mammals are also affected by phthalates. In mice (Fishbein and Albro, 1972), LD_{50} values vary between 1.58 - 14.19 g/kg. Rats subjected to phthalate esters showed significant falls in body weight and changes in activities of some enzymes (Piekacz, 1971). Dogs subjected to 5 g/kg DEHP showed no definite effects (Fishbein & Albro, 1972).

Phthalates were found to cause teratogenic effects in rats (Singh et al., 1972). Levels below LD_{50} values caused fetal malformations, mainly manifested as skeletal defects including deformed rib structures, absence of tail and abnormal skull bones.

The cause of phthalate toxicity is unknown. However, Williams (1959) attributed it to the release of alcohols from the esters upon hydrolysis since phthalic acid itself is of low toxicity.

Production and Uses of Phthalates

Data of the type implied by the title is not presently available for Canada. Figures for 1972 for the United States are given in Table I for the year 1972 and as a rough indication, 10% of the quantities are probably applicable for Canadian usage. In the same year, some 7×10^7 lbs. were imported into Canada (Statistics Canada, 1973) but Canadian production figures are unavailable. Table II gives the type of usage these compounds are put to, the bulk of it being as plasticizers. The quantities plus the usage together indicate the very widespread distribution of phthalate esters.

Table I: Properties and Production of Phthalate Esters *

Phthalate Ester	Molecular weight	Specific gravity	Bp, °C	Solubility in H_2O, g/100 ml	% of 1972 Production (US)
Dimethyl-	194	1.189(25/25)	282	0.5	
Diethyl-	222	1.123(25/4)	296	0.1	2%
Diallyl-	246	1.120(20/20)	290	0.01	
Diisobutyl-	278	1.040	327	Insol.	
Dibutyl-	278	1.0465(21)	340	0.45(25°C)	2%
Dimethoxyethyl-	282	1.171(20)	190-210	0.85	
Dicyclohexyl-	330	1.20(25/25)	220-228	Insol.	
Butyl octyl-	334	-	340	-	
Dihexyl-	334	0.990	-	Insol.	
Butylphthalyl butyl glycolate	336	1.097(25/25)	219/5mm	0.012%	
Dibutoxyethyl ethyl-	366	1.063	210	0.03	
Di-2-ethylhexyl-	391	0.985(20/20)	386/5mm	0.01	
Diisooctyl-	391	0.981	239/5mm	Insol.	50%
Diisodecyl-	447	-	-	Insol.	16%
Di-n-octyl-	391	0.978	220/5mm	Insol.	
Dinonyl-	419	0.965	413	Insol.	
Octyldecyl-	419	-	-	Insol.	6%
Others	-	-	-	-	24%
Total	-	-	-	-	10^9lb/100%

* Derived from Autian, 1973 and Anonymous, 1971

Table II: Uses of phthalate esters *

1.	Plasticizer Uses	
	Building and Construction	38%
	Home Furnishings	20%
	Transportation	11%
	Apparel	7%
	Food surfaces	2%
	Medical products	3%
	Other	14%
2.	Non Plasticizer Uses	5%
	Pesticide Carriers	
	Cosmetics	
	Fragrances	
	Munitions	
	Industrial oils	
	Insect Repellants	

* Derived from Graham, 1973

Analytical Problems with Phthalate Esters

All analytical considerations must be aware of the strong danger of contamination of environmental samples during the collection, preparation and analysis of the samples. Phthalic esters play an important part as plasticizers in almost any kind of plastic material and there are thus virtually unlimited sources for contamination. It seems therefore impossible to create phthalate free laboratories and samples should be handled exclusively under inert gas atmosphere in glass, ceramic or stainless steel systems. This fact has also been pointed out in a recent investigation of DNBP and DEHP in 21 fish samples from the Great Lakes (Williams, 1973). Maximum values found were 160 ppb with most fish having less than twice the background level of 10 - 15 ppb.

It seems therefore likely that several reports of high phthalate concentrations in remote areas such as the 300 ppb in waters from Black Bay (Zitko, 1972) are due to secondary contamination. Though phthalic esters were found at 0.15% level in crude oils (Phillips and Breger, 1958) there is no definite answer as to their natural occurrence. Similarly, reports of their occurrence in microorganisms and plant material extracts (Fishbein and Albro, 1972) should be checked critically. Further evidence for possible secondary contamination of extracts and samples can be deduced from investigations by Stalling et al. (1973), which showed by means of radioactive

labeled DEHP that after 24 hours exposure of channel catfish to water of 1 ppb DEHP only 14% of the total accumulated DEHP (2.6 ppm) was unchanged DEHP. With such apparently rapid degradation, it seems possible that much of the reported environmental phthalates may be due to contamination.

In general, phthalate esters can easily be extracted from water sediments or biota samples by hexane, ether or other solvents (e.g. Nitromethane, Pastorelli and Chiavari, 1971). The separation from accompanying lipids can be achieved by various methods, principally column and thin layer chromatography on silica, florisil, alumina or fibrous materials (Zitko, 1972; Fishbein and Albro, 1972; Stalling et al, 1973). However, recovery data for these methods are incomplete and available data (Williams, 1973) indicate an average of only 60% recovery.

Phthalate esters can be quantitatively determined by several methods, preferably gas chromatography with flame ionization detection using silicon stationary phases. After saponification to the acid and subsequent conversion to the anhydride they can also be determined spectrophotometrically (Fickentscher, 1970; Rapaport and Titoskaya, 1971). Other analytical techniques applied to analysis of these compounds were liquid/liquid chromatography, alkalifusion-reaction chromatography (Fishbein and Albro, 1972), raman spectroscopy (Nyquist, 1972). Of all these methods, gas chromatography with electron capture detection is the most sensitive, convenient and useful in identification and quantitation of the individual esters.

Preliminary work at CCIW indicated that halogenation (bromination and possibly also chlorination) increases the sensitivity of detecting some phthalate esters five to nine fold. Further work is required to obtain the optimum conditions and check the reproducibility and accuracy.

It is necessary to emphasize Stalling and Albro's statement (1972) that, "analysis of PAE's and their degradation products in biological tissues has not, as yet been reduced to routine analysis". Several areas in the methodology have yet to be investigated:

1) Recovery study on all the available methods mentioned above are not sufficiently investigated to enable these methods to be adopted for routine analysis,

2) Column and thin-layer chromatographic clean-up of fish samples and to a lesser extent sediment and plankton samples needs further studies. The variation of lipid content in fish for example, will modify elution pattern and rf values of these esters (Zitko, 1972). This situation is not only encountered for the alumina column but will also be encountered for florisil column. A better clean-up method is definitely necessary before routine analysis can be performed.

3) Methods for confirmation of identity of these esters at low levels are not available (halogenation may provide some solution). Mass Spectroscopy is only applicable for higher

concentrations and TLC necessitates low lipid content in the sample extracts so that reproducible rf values for these esters can be obtained. Moreover, TLC is not considered to provide "positive proof" of identification.

4) A convenient and quantitative method for the cleavage of "phthalate acid protein conjugates" and the "phthalate glucuronides" to a non conjugate form for its determination is not available for routine analysis.

To conclude, investigation on the methodology for analysis of PAE's appears to be necessary before programs for routine analysis can be initiated.

Chemical Behaviour of Phalates

The phthalate esters, chemically, are likely to be quite stable in the aquatic environment. Few studies exist on the hydrolysis of phthalic acid esters in purely aqueous medium at pH's appropriate to the environment. Studies which have been done in other media (Anantakrishnan and Radhakrishnamurti, 1962) indicate that the first ester is hydrolysed some five times as rapidly as the second group and this effect is liable to be increased in purely aqueous solutions. Using this figure, the one study (Bender et al., 1958) of the hydrolysis of methyl hydrogen phthalate in water at pH7 would indicate half-lives for hours of the ester functions of some hundreds of years at 20°C.

Only a slight increase will occur in progressing to the longer chains of alcohols used in plasticizing esters. A decrease will be observed for reaction with other chemical species such as carbonate, phosphate and hydroxyl but this will be small due to their low concentration. As a consequence of these different effects, it is apparent that any chemical breakdown of phthalate esters will take tens to hundreds of years under environmental conditions. Since esterases are common in all biological species, it is far more probable that the hydrolysis of these esters will be biochemical rather than purely chemical.

Biochemical Behaviour of Phthalates

Similar to the chemical behaviour, the biochemical indications are that the first ester function in dialkyl phthalates is rapidly hydrolysed - presumably to the alcohol moiety (the persistence of which will be related to its structure but for the normal alcohols commonly used, this will be low) and to the mono-ester phthalate. The toxicity of this latter metabolite has not been investigated although the low toxicity for the dialkyl compounds would indicate this too is low. Several workers (Albro et al., 1973; Daniel, 1973; Metcalfe et al., 1973) have observed the mono-ester phthalates in metabolic studies but none have indicated quantities. It would seem that these metabolites are formed in substantial quantities within periods of 48 hours and that they persist for an

undetermined time in the same medium. Saunders et al., 1973 have found, however, that at least invertebrates clear the phthalates and residues after exposure to clean water.

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