Analysis of Tissues of Mallard Ducks Fed Two Phthalate Esters

by ANDRE A. BELISLE, WILLIAM L. REICHEL, and JAMES W. SPANN
U. S. Fish and Wildlife Service
Patuxent Wildlife Research Center
Laurel, Md. 20811

Phthalic ester plasticizers are widely used and have become environmental contaminants. About 1 billion pounds were produced in 1972 alone, usually as plasticizers in polyvinyl chloride plastics (GRAHAM 1973). Dibutyl phthalate (DBP) also is used as an insect repellent and diethylhexyl phthalate (DEHP) as a pesticide (FARM CHEMICALS 1971). These latter compounds have been found in soil (OGNER AND SCHNITZER 1970) and in aquatic organisms and water (ANONYMOUS 1971, MAYER et al 1972, HITES 1973). NAZIR et al (1973) isolated and identified DEHP in beef heart muscle mitochondria and in much smaller amounts from the heart muscle of the rat, rabbit, and dog.

This paper reports the development of an analytical procedure for the analysis of DBP and DEHP in animal tissue and application of the method to the analysis of tissues of mallard ducks fed diets containing DBP or DEHP. Tissues from untreated control ducks also were analyzed.

EXPERIMENTAL

Dosage

Birds were fed commercial duck mash diets containing 10 ppm of DBP or DEHP. The chemicals were dissolved in corn oil and mixed with the mash in the proportion of 1 part of oil solution to 99 parts (by weight) of feed. An equal amount of corn oil was added to the diet of the control birds.

Treated food was fed ad libitum to two pens of yearling mallards for a period of 5 months; each pen contained five females and two males. Untreated food was fed to control birds in four pens. Dosage began January 1972 and continued through June 1972, when treated and untreated birds were sacrificed.

Bird tissues to be analyzed were randomly selected from each treatment group. Samples of heart muscle, lung, subcutaneous fat, and breast muscle were stored frozen at -25°C until analysis.

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ANALYTICAL PROCEDURES

Reagents

Solvents - hexane (Fisher pesticide grade, cat # H300), anhydrous ethyl ether (Burdick & Jackson, "Distilled in Glass").

Florisil - (Fisher, cat # F-100, 60-100 mesh). Treat as follows: Wash and recalcine at 675°C according to the method of HALL (1971); partially deactivate with water (1-1.5%).

Sodium sulfate - (Baker ACS grade). Heat in porcelain casseroles at 675°C for 3 hours or more before use.

Apparatus

(a) Chromatographic column - 250 ml reservoir on plain column, 225 x 32 mm o.d. with ℨ 19/22 outer joint; inner joint ℨ 19/22 adapter with coarse-fritted disk.

(b) Porcelain casseroles - Coors 1800 ml (no. 7), 175 x 130 mm.

(c) Fritted glass thimbles - 150 x 57 mm, extra coarse.

Extraction Procedures

A 10-g aliquot of heart, lung, or breast muscle or 2 g of fat was ground in a Virtis homogenizer with stainless steel cup, mixed with sodium sulfate, and extracted 7 hours in a Soxhlet apparatus with hexane, using glass thimbles. The extract was evaporated to 15 ml and partitioned with four 30-ml portions of acetonitrile. The acetonitrile extracts were combined in a 1-l separator containing 700 ml 2% NaCl and re-extracted with 2 x 100 ml hexane. The hexane extract was evaporated to 5 ml and separated into two fractions by the following procedure: A chromatographic column containing 21 g of treated Florisil topped with ½ inch of sodium sulfate was prewashed with 50 ml hexane. The sample was added to the column; chlorinated pesticides and polychlorinated biphenyls (PCB's) were eluted with 200 ml of 6% ethyl ether in hexane. The phthalate esters then were eluted with 200 ml of 15% ethyl ether in hexane. The fat samples were not completely cleaned up by these steps, and it was necessary to repartition the samples with acetonitrile. The phthalate fraction was evaporated to a suitable volume and quantitated on a gas chromatograph with an electron capture detector using nitrogen carrier gas at 80 ml/min. The GC columns were a 4/6% SE-30/QF-1 at 190°C for both DBP and DEHP and a 2% Pentasil 350 at 230°C for DEHP. Recoveries from spiked tissues (corrected for procedural blanks) averaged 87% except for fat, which averaged 70%. The minimum measurable quantity was about 5 ng of DBP and 10 ng of DEHP. The lower limit of sensitivity for a 2-g sample was 0.1 ppm of DBP and 0.2 ppm of DEHP. Frequent cleaning of the GC detector was required to maintain adequate sensitivity.

TABLE 1

Residues (ppm wet weight) in tissues from mallard ducks fed DBP or DEHP

Tissue	Treatment	DBP	DEHP
Fat	Control	ND[1]	ND
	DBP	ND	ND
	DEHP	ND	ND
Procedural blank, µg		ND	ND
Heart	Control	ND	0.2
	DBP	ND	0[2]
	DBP	ND	0
	DEHP	ND	0
	DEHP	ND	0
Procedural blank, µg		ND	2.3
Lung	Control	ND	0.06
	DBP	ND	0
	DBP	ND	0
	DEHP	ND	0.05
	DEHP	ND	0.15
	DEHP	ND	0.15
Procedural blank, µg		ND	2.4
Breast	Control	0	0
	DBP	0	0
	DEHP	0	0.1
Procedural blank, µg		0.4	1.6

[1] ND = none detected
[2] 0 = value obtained when corrected for procedural blank

Background levels of DBP and DEHP on glassware and in reagents were a constant problem. When control samples (10 g) were run without special precautions, levels of DBP and DEHP were as high as 30 and 20 μg/g.

Various procedures were adopted to minimize background levels of DBP and DEHP. Sodium sulfate was heated before use to remove phthalates. Aliquots of solvents were concentrated and analyzed by GC to assure that DBP and DEHP were absent or at acceptably low levels. All glassware was rinsed with acetone or ethyl ether until GC profiles showed that the rinses were free of interfering substances. The fritted glass thimbles were pre-extracted with ethyl ether, and the extracts were checked for contaminants by GC. Latex rubber tubing with a molecular sieve and a Drierite trap was used in the air lines for solvent evaporation. These procedures reduced the background level of DBP to $\leq$ 0.4 μg and DEHP to $\leq$ 2.5 μg. Procedural blanks were run with each set of samples analyzed; the quantitation of phthalates was corrected for these background levels. Variability associated with the analytical procedure is estimated to be $\pm$ 0.4 μg DBP and $\pm$ 0.9 μg DEHP.

RESULTS

Results of analysis of mallard tissues are presented in Table 1. The levels of DBP and DEHP found in the procedural blanks were subtracted from each set of samples. DBP was not detected in any of the samples. Low levels of DEHP ($\leq$ 0.15 ppm) were found in the lung and breast muscle of birds fed DEHP, but these levels were within the range of variability of the procedural blanks. DBP and DEHP apparently did not accumulate above our limit of sensitivity in tissues of mallard ducks when fed dietary dosages of 10 ppm for 5 months.

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