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Dibutyl- and Di-(2-ethylhexyl)phthalate in Fish

Low levels of dibutylphthalate (0–78 ppb) and di-(2-ethylhexyl)phthalate (0–160 ppb) were found in 21 samples of fish available to the Canadian consumer.

Dibutylphthalate (DBP) and di-(2-ethylhexyl)phthalate (DEHP) have recently been reported in inland waters (Corcoran, 1973; Hites, 1973) and in fish from North American sources (Mayer *et al.*, 1972; Zitko, 1972). Although these phthalate esters do not appear to present a serious short-term health hazard (Tepper, 1973) it was felt that a preliminary survey should be made to determine the levels of these phthalate esters in fish available to the Canadian consumer.

METHOD

Samples. Fish from Canadian lakes and rivers were supplied by Environment Canada; other samples were purchased at local markets. Canned fish were stored at room temperature; other samples were stored at −10° until analyzed.

Reagents. Solvents were distilled in glass and aliquots were concentrated, and the absence of DBP and DEHP was verified by glc. All glassware was rinsed before use with distilled ethyl ether. Silica gel, 0.2–0.5 mm (E. Merck) for column chromatography, was washed with ethyl ether, air dried, and activated for 16–20 hr at 120°.

Glc Conditions. A Varian Aerograph, Model 2100, gas–liquid chromatograph equipped with flame ionization detectors was used with nitrogen (40 ml/min) as the carrier gas. U-Shaped glass columns were used: (A) 6 ft × 3.5 mm i.d. packed with 3% XE-60 and (B) 7 ft × 2 mm i.d. packed with 5% OV-101, both on 100–120 mesh Chromosorb W (HP). The column temperature was 207°, the injection port 220°, and the detector block 270°. Electrometer setting was normally $10^{-11} \times 16$. The retention time for dibutylphthalate was 2.8 (Column A) and 3.2 min (Column B); for di-(2-ethylhexyl)phthalate it was 13.0 (Column A) and 22.4 min (Column B).

Glc–Mass Spectrometer. An Hitachi Perkin-Elmer (RMS-4) mass spectrometer coupled with a Perkin-Elmer Model 900 gas chromatograph fitted with flame ionization detectors was used. The 6 ft × ⅛ in. stainless steel column was packed with 3% silicone gum XE-60 on 100–120 mesh Chromosorb W (HP) and the carrier gas was helium at 40 ml/min. The operating conditions were detector 270°, injection port 240°, and oven 225°.

Sample Preparation. This was based on the method for the determination of DEHP in soy oil (Williams, 1973) with modifications to allow for the greater volatility and different chromatographic properties of DBP. The frozen or canned fish (100–200 g) was chopped into small pieces, macerated manually and heated, with occasional stirring, for 0.5 hr at 60–65° in an equal weight of hexane. The hexane was removed by decantation and the fish extracted two more times with a similar volume of hot hexane. The hexane extracts were combined and an aliquot was concentrated to dryness and weighed. An appropriate volume of hexane solution, containing up to 5 g of lipid, was then concentrated to 60 ml. This solution was extracted with acetonitrile saturated with hexane (6 × 60 ml) and the acetonitrile extracts were combined and concentrated to 300 ml. *m*-Chloroperbenzoic acid (0.5 g) was dissolved in aqueous sodium hydroxide (50 ml, 0.5 *N*), washed with ethyl ether (30 ml), and added to the acetonitrile solution. Sulfuric acid (50 ml, 0.5 *N*) was immediately added and the solution shaken and left overnight at room temperature. Water (200 ml) was added and the aqueous acetonitrile extracted with petroleum ether (3 × 100 ml). The petroleum ether extracts were combined, washed with sodium hydroxide (50 ml, 0.5 *N*) and water (2 × 100 ml), dried over sodium sulfate, concentrated to 20 ml, and transferred to a silica gel column (20 g) made up with 5% ethyl ether in petroleum ether. The column was eluted with 5% ethyl ether in petroleum ether (100 ml) and then with 15% ethyl ether in petroleum ether (250 ml). The 15% ethyl ether eluate was collected, concentrated to 0.3 ml using a micro-Snyder column, and examined by glc. Quantitation was carried out by comparison of peak height (DBP) or peak area (DEHP) with standard samples. Analysis of samples spiked at different stages in the method indicated that three extractions with hot hexane gave virtually complete extraction of the phthalate diesters. Overall recoveries of phthalate esters in spiked samples (0.1–0.5 ppm) were 60–65% for DBP and 65–70% for DEHP.

RESULTS AND DISCUSSION

Twenty-one samples of fish were analyzed for DEHP and DBP, and the results are given in Table I. Only very low levels of these phthalate esters could be detected in any of the fish. Analysis for low levels of phthalate esters is complicated by the presence of trace amounts of these compounds in solvents and chemicals used in the workup. Rigorous purification fails to remove all of the phthalates

Table I. Phthalate Ester Residues (ppb) in Fish Available to the Canadian Consumer

Sample	DEHP	DBP
Unprocessed fish		
Eel	104[b]	−
Catfish (L. St. Pierre)	+[a]	−
Pickerel (L. Huron)	+	−
Pickerel (L. Ontario)	+	+[a]
Pickerel (L. Ontario)	+	+
Pickerel (L. Ontario)	−	+
Processed canned fish		
Tuna	40	−
Tuna	160[b]	78[b]
Tuna	140[b]	+
Salmon	63	+
Salmon	89	37
Sardine	−	−
Crab	−	−
Shrimp	−	+
Baby clams	−	−
Processed frozen fish		
Rainbow trout	+	−
Ocean perch	+	−
Mackerel	+	−
Sole	−	−
Oyster	−	−
Scallop	−	−

[a] Levels less than twice background. [b] Confirmed by glc–mass spectrometry.

and a background level is always found in analysis of "blank" samples (*Chem. Eng. News*, 1971; Williams, 1973). For a 100-g fish sample analyzed by the above method, the background levels were approximately 15 ppb for DEHP and 10 ppb for DBP. Levels of DEHP and DBP of less than twice background are designated as trace amounts in Table I; other values have been corrected for background. The identification of phthalate esters by glc has been extensively reviewed (Bloom, 1972; Fishbein and Albro, 1972). Confirmation by glc–mass spectrometry is simple at the 1-ppm level but due to background interference is difficult at lower levels (Williams, 1973). Phthalate esters at levels greater than five times the background level were confirmed by glc–mass spectrometry but lower levels of phthalate esters were only confirmed by analysis on two glc columns.

Stalling *et al.* (1973) have recently reported that DEHP and DBP are metabolized by fish and it would appear that high residue levels would be expected only in those fish continuously exposed to phthalate esters. Highest levels of phthalate esters have been reported in fish from waters adjacent to industrial areas and in hatchery fish fed diets contaminated with phthalate esters (Mayer *et al.*, 1972; Zitko, 1972).

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Long-Chain Hydrocarbons of *Cannabis* and Its Smoke

A series of long-chain paraffins has been identified in *Cannabis* and its smoke by gas chromatography and mass spectrometry. The level of hydrocarbons was determined to be about half that found in tobacco and its smoke, although the effect of smoking on the paraffins in the *Cannabis* plant material was comparable to analogous studies of tobacco and its smoke.

Many studies have been published concerning the cannabinoids in *Cannabis* (Gaoni and Mechoulam, 1971) but only one report has been found on those compounds present in the smoke condensate. Recently, Fentiman *et al.* (1973) reported the identification of several noncannabinoid phenols present in the smoke condensate of *Cannabis* using gas chromatography–mass spectrometry (gc–ms) techniques. As an extension of this work, we now wish to report the identification and comparison of the hydrocarbons present in the plant extract and those transferred and/or generated during the smoking process.

EXPERIMENTAL SECTION

The *Cannabis* used in this study (strain MS-13) was cultivated by a standard method (Doorenbos *et al.*, 1971) and known weights were extracted with 95% ethanol (Groce and Jones, 1973). The ethanol was concentrated and diluted with water, and the mixture was exhaustively extracted with hexane. After concentration, the hexane solution was made to volume and aliquots were used for analysis. These were chemically separated into basic, acidic, and neutral fractions and the neutral solution was concentrated to a thick syrup. This was chromatographed on silicic acid and initial waxy fractions were eluted with hexane. Treatment with urea in hot methanol formed an adduct which was washed with hexane and decomposed with water and extraction with hexane gave a clean mixture of long-chain hydrocarbons (Johnston and Jones, 1968). Separation of the straight-chain paraffins from those with branching was affected with Linde 5A molecular seives.

For smoke analysis, 70-mm cigarettes were hand rolled and smoked on a smoking machine taking a 40-ml puff of 2 sec duration every minute. The average weight per ciga-