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STUDIES ON PHTHALATE ESTERS IN WESTERN ATLANTIC FINFISH

by
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Abstract

A new simple and rapid method for the determination of phthalate esters in marine fish has been developed. The method is based upon chromatography on small alumina:sulfuric acid impregnated alumina columns, following initial clean-up by gel permeation chromatography. Di-2-ethylhexyl phthalate levels in plaice, eel, redfish, herring and mackerel muscle tissues ranged from traces to about 10 µg/g tissue on a wet weight basis. Dibutyl phthalate levels in herring and mackerel muscle were 1-2 orders of magnitude lower than di-2-ethylhexyl phthalate levels. A previously unidentified phthalate ester was isolated from herring and mackerel but not cod, plaice or redfish and identified as di-n-hexyl phthalate by gas chromatography-mass spectrometry and proton nuclear magnetic resonance spectroscopy. Levels of this phthalate far exceeded those of di-n-butyl and di-2-ethylhexyl phthalate, two common industrial phthalates. Di-n-hexyl phthalate is not produced in significant industrial quantities and accumulation of this phthalate in mackerel and herring may reflect either the inability of these species to metabolize phthalate esters to excretory products or differences in exposure of these species to phthalates in their food supply.

Résumè

On a mis au point une nouvelle méthode, simple et rapide, pour déterminer la présence d'esters d'acide phthlique dans les poissons de mer. La méthode est fondée sur la chromatographie de petites dolonnes d'alumine et d'alumine imprégnées d'acide sulfurique après un premier nettoyage par chromatographie de l'infiltration du gèle. Les niveaux de phtalate de di-2-éthyl-hexyl présent dans les tissus musculaires de la plie canadienne, de l'anguille, du sébaste, du hareng et du maquereau variaient de traces à environ 10 µg par g de tissu sur une base de poids frais. Les niveaux de phtalate de butyle présent dans les muscles du hareng et du maquereau étaient inférieurs de 1 à 2 ordres de grandeur aux niveaux de phtalate de di-2-éthyl-hexyl. On a isolé, chez le hareng et le maquereau, mais non chez la morue, la plie canadienne et le sébaste, un phtalate jusque-là non identifié et on l'a identifié comme phtalate de di-n-hexyle par chromatographie gazeuse-spectrométrie de masse et par spectroscopie de résonance megnétique nucléaire à proton. Les niveaux de ce phtalate dépassaient de beaucoup ceux du phtalate de di-n-butyle et du phtalate de di-2-éthyl-hexyl, deux phtalates industriels courants. Le phtalate de di-n-hexyle n'est pas produit en quantités importantes en industrie et son accumulation chez le maquereau et le hareng peut indiquer soit que ces expèces sont incapables de transformer les esters d'acide phtalique en excrétions, soit que ces expèces sont exposées à des concentrations différentes de phtalates dans leur diète.

Introduction

Phthalate acid esters are a group of anthropogenic organic compounds which are widespread in the environment. Although their presence in fish has been known for some time (Peakall, 1975; Zitko, 1973), these compounds have not been included in the group of compounds being studied within the coordinated monitoring activity carried out by the Working Group on Marine Pollution Baseline and Monitoring Studies in the North Atlantic but were mentioned by Holden at last year's Statutory Meeting in paper C.M.1979/E:65 presented to the Marine Environmental Quality Committee. We wish to describe our investigations of the nature and occurrence of phthalate esters in marine species from the Northwest Atlantic.

Analytical Methodology

Acetone-hexane (1:1; v/v) extracts of fish tissues were taken just to dryness and made up in methylene chloride:cyclohexane (1:1; v/v) for gel-permeation chromatography on Biobeads SX-3 (32 cm x 2.5 cm I.D. Pharmacia column connected to an Analytical Biochemistry Laboratories Autoprep Model 1001). The 80-120 ml fraction was collected, taken to dryness, made up in 2.0 ml hexane and applied to a small chromatography column (0.61 cm ID glass) prepared by plugging the column with glass wool and filling with the following: bottom layer - 2.0 g sulfuric acid - impregnated alumina prepared by activating Fisher A540 alumina at 800°/4 hr, cooling to room temperature, deactivating with 5% H_2O w/w and after 2 hr equilibration, with occasional shaking, mixing carefully and rapidly with 4.0 g conc. H_2SO_4 (A.C.S.) per 100 g deactivated alumina in a mortar in such a way as to avoid breaking the alumina. Middle layer - 2.0 g alumina activated and water-deactivated as above. Top layer - 1-2 cm anhydrous sodium sulfate. Following elution with 20 ml 2% diethyl ether in hexane the phthlate esters were eluted with 10% diethyl ether in hexane. It is necessary to standardize each batch of alumina because the phthalate esters can begin to elute in 2% ether/ hexane.

Gas chromatographic analysis was carried out using a Hewlett Packard model 5700A fitted with a ^{63}Ni electron capture detector and 182 cm x 4 mm ID glass column packed with 3% OV-101 on 80/100 mesh Supelcoport. Argon: methane (95:5) was used as the carrier gas. The operating conditions were: Injection port 250°C; column oven 210°C and detector 300°C. Confirmation was carried out by gas chromatography on a 185 cm x 2 mm ID glass column packed from the injection port end to the 170 cm mark with 1% SP-2401 and from 170 to 185 cm with 3% SP-2100/3% OV-210. All stationary phases were coated separately on 80-100 mesh Supelcoport and mechanically mixed before being packed in the column (see Burns et al., 1980, for all details). Recoveries of di-2-ethyl-hexyl phthalate (DEHP) from fish liver and fish muscle through the whole procedure averaged 82.6 $\pm$ 3.4%. Recoveries of di-n-butyl phthalate (DBP) were 80.6 $\pm$ 4.7%. Recovery of di-n-hexyl phthalate (DHP) was not determined; however, it is expected that DHP would behave like DEHP and DBP in this respect and similar recovery can be expected. Recoveries of DEHP from fish liver and fish muscle averaged 82.6 $\pm$ 3.4%.

Studies on Di-2-ethylhexyl Phthalate (DEHP) and Di-n-butyl Phthalate (DBP)

DEHP levels were determined in fillets obtained from a number of different commercial species (Table 1). In each case, a composite sample of fillets from at least ten individual animals was prepared for analysis.

Results are reported on the basis of both wet and fat weights. A sample of cod liver and two samples of eel muscle (composites of 4 individuals) are included for comparison. The following observations were made.

Both herring (Clupea harengus harengus) and mackerel (Scomber scombrus) muscle had much higher DEHP levels than plaice (Hippoglossoides platessoides), redfish (Sebastes marinus), or eel muscle (Anguilla rostrata) and approximately the same level as cod (Gadus morhua) liver. Herring from the Bay of Fundy had a higher level of DEHP (7.24 µg/g wet weight) than herring from the Gulf of St. Lawrence (4.71 µg/g wet weight) but it is difficult to judge the significance of these differences since only single composite samples were studied. The single mackerel composite sample from the Gulf had higher muscle levels of DEHP than herring from the same location but it is difficult to assess the significance of these differences.

Eel muscle levels of DEHP also were considerably lower than cod liver or herring or mackerel muscle levels even when expressed on a fat weight basis. This suggests that either marked metabolic differences exist between this catadromous species and the marine species or that the coastal areas of the oceans are more polluted with DEHP than the freshwater lakes. The low levels found in redfish and plaice from the deeper parts of the Gulf suggest we are indeed looking at a pelagic coastal problem.

DBP levels were studied in fillet composites of herring, mackerel and plaice and levels of 0.20, 0.02 and 0.01 µg/g respectively were found. This indicates that DBP is not a significant contaminant in marine finfish.

Di-n-hexyl Phthalate (DHP)

During the above investigation a peak was noted in the gas chromatograms (Fig. 1), the chemistry and chromatography of which indicated a phthalate ester, the concentration of which was greater than that of DEHP in herring and mackerel. The compound was isolated from the gel permeation phthalate ester fraction by a combination of high pressure liquid chromatography and selective partitioning and precipitation of impurities. Details of the isolation and characterization are being published (Musial et al., 1980). This phthalate was identified as di-n-hexyl phthalate by gas chromatography - mass spectrometry and proton nuclear magnetic resonance spectroscopy.

Initial quantitative determinations have demonstrated that herring and mackerel fillets contain from 7-25 µg DHP/g wet weight and the compound is not detectable in plaice or redfish fillets nor in cod liver. It is interesting to compare these levels to the levels of DEHP found in these samples of herring and mackerel, i.e. 4-7 µg/g wet weight. The higher levels of DHP as compared to DEHP are surprising as DEHP is the major industrial phthalate produced. No report of industrial production of DHP as a primary product has been found but it is possible that DHP occurs as a contaminant in technical grades of the common industrial phthalate esters.

Since herring and mackerel have demonstrated the presence of DHP, it is possible that its occurrence reflects some remarkable biochemistry being carried out either within these two species or within their food supply. They are both pelagic feeders whereas the other species examined are benthic (bottom feeders). Examination of other pelagic species remains to be done.

The high levels of both DHP and DEHP suggest that both of these species cannot metabolize phthalate esters to the degree observed in other species such as freshwater minnows, carp (Cyprinidae) (Mayer and Sanders, 1973; Mayer, 1976) trout (Salmonidae) (Melanscon and Lech, 1976) and catfish (Ictaluridae) (Stalling, Hogan and Johnson, 1973).

Conclusion

This study led us to make the following recommendations:

(1) Phthalate esters should be included in the ICES Coordinated Monitoring Program.

(2) The source of DHP should be established as well as its potential toxicity to man and marine species, especially herring and mackerel.

(3) The metabolism of phthalate esters in mackerel and herring should be investigated.

References

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Table 1

Concentrations of Phthalate Acid Esters Present in Fillets of Commercial Species

Species	Location	DEHP µg/g		DBP µg/g	
		Wet Wt.	Fat Wt.	Wet Wt.	Fat Wt.
Plaice	Gulf of St. Lawrence	<0.001	<0.001	0.01	2.10
Redfish	Gulf of St. Lawrence	<0.001	<0.001	-	-
Herring	Bay of Fundy	7.24	47.1	0.20	1.39
Herring	Gulf of St. Lawrence	4.71	29.1	0.19	1.18
Mackerel	Gulf of St. Lawrence	6.50	51.3	0.02	0.24
Cod liver	Gulf of St. Lawrence	5.19	10.4	-	-
Eel #1	Freshwater, Nova Scotia	0.22	1.1	-	-
Eel #2	Freshwater, Nova Scotia	0.37	6.7	-	-

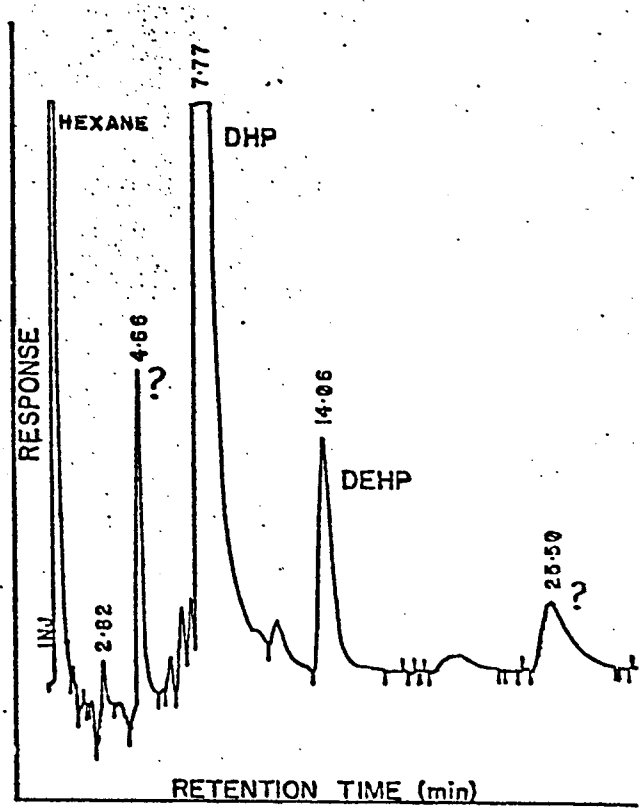

Figure 1: Gas chromatogram of GPC phthalate fraction of Gulf of St. Lawrence mackerel cleaned up by sulfuric acid-alumina chromatography. 3% OV-101 column; for GC conditions see text. ? - unknown.