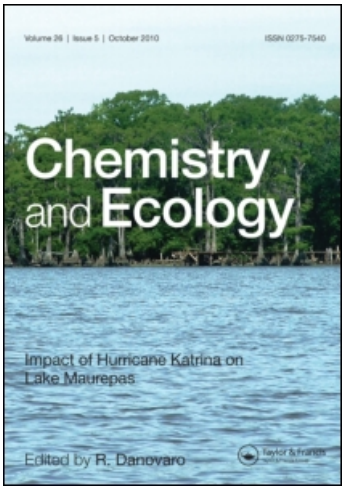

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

Determination of Phthalate Esters in Samples from the Marine Environment Using Gas Chromatography Mass Spectrometry

M. J. Waldock[a]
[a] Ministry of Agriculture, Fisheries and Food, Burnham-on-Crouch

To cite this Article Waldock, M. J.(1983) 'Determination of Phthalate Esters in Samples from the Marine Environment Using Gas Chromatography Mass Spectrometry', Chemistry and Ecology, 1: 4, 261 — 277
To link to this Article: DOI: 10.1080/02757548308070809
URL: http://dx.doi.org/10.1080/02757548308070809

Chemistry in Ecology, 1983, Vol. 1, pp. 261–277
0275–7540/83/0104–0261 $18.50/0

Printed in Great Britain

Determination of Phthalate Esters in Samples from the Marine Environment Using Gas Chromatography Mass Spectrometry

M. J. WALDOCK

Ministry of Agriculture, Fisheries and Food, Burnham-on-Crouch

(*Received October 18, 1982*)

Phthalate esters have been extracted from water, sediment and biota samples using a single solvent system, dichloromethane, and clean up by alumina column chromatography. Analysis for dimethyl, diethyl, di-iso-butyl, di-n-butyl, di-n-heptyl, di-(2-ethylhexyl) and di-n-nonyl phthalates was carried out by gas chromatography and mass spectrometry. Phthalate esters were quantified using multiple ion detection, focussed on m/z 163 and m/z 149.

The procedure was tested on samples of water, sediment, bivalves and fish from the estuary of the River Crouch. The method gave satisfactory sensitivity for the determination of phthalate ester levels above the commonly found blank values of 1 to 20 nanograms per gram of solid or litre of water.

Comparative samples of fish from the heavily industrialised Tees Bay area did not show greatly elevated levels of phthalate esters.

INTRODUCTION

Although few surveys of phthalate esters in samples from the marine environment have been carried out, all authors have reported significant levels of phthalates in water, sediment and biota (see a comprehensive review by Pierce *et al.*, 1980; Ehrhardt and Derenbach, 1980; Musial *et al.*, 1981; Murray *et al.*, 1981). Most researchers recognise that the practical difficulties in precise, accurate determination of phthalate concentrations are enormous, and many pitfalls have been described

(Webster and Nickless, 1976). Some results, particularly the earlier ones, are therefore likely to be inaccurate, and it is difficult to ascertain whether or not phthalates are truly ubiquitous contaminants of the marine envrionment and whether or not the levels are sufficiently high to give cause for concern.

This paper outlines a simple procedure for the determination of phthalates with minimal risks of sample contamination. In order to assess the significance of these compounds a brief survey was made of the background levels of phthalates in sea water, sediments and biota in a non-polluted estuary (that of the River Crouch) using the procedure described below. In addition several species of fish were taken from Tees Bay, an area which is subject to high input of industrial effluent, and is close to one of the major production sites for phthalates in the UK.

MATERIALS AND METHODS

Precautions

Only glass and/or stainless steel laboratory ware was used. Glassware was thoroughly washed with 5% 'Decon 90' in tap water, and soaked in a 10% nitric acid bath. After rinsing with tap water, all items were soaked in dichloromethane:methanol (1:1 v/v) for 24 h, and rinsed three times with dichloromethane before use.

A variety of possibly useful organic solvents was tested for phthalate esters. Quantities less than 1 ng per 200 ml were routinely detected in dichloromethane (glass distilled grade, Rathburn Chemicals Ltd, Scotland). Prolonged exposure to the laboratory atmosphere increased levels of phthalate esters in solvents, and all containers were kept stoppered whenever possible.

Distilled water was found to contain microgram quantities of phthalate esters per 100 ml. Tap water contained lower levels and was used for rinsing glassware prior to solvent washing. For other applications, water for HPLC (BDH Ltd, Poole, Dorset) was extracted with dichloromethane and stored over the solvent.

Commonly used laboratory materials including paper towel, aluminium foil, teflon tape, wooden spatulas, and chemicals such as sodium sulphate, silicic acid and alumina were found to have unacceptable levels of phthalate esters and were subsequently either avoided or extensively extracted with dichloromethane before use.

New GLC septa gave significant additions to the peak areas of di-n-butyl and di-(2(ethylhexyl) phthalates. To overcome this problem septa

were stored in the GLC oven for several weeks, allowing a large proportion of phthalates present to volatilise. The septa were then backed with extracted teflon discs before use.

Extraction

Water Samples of 2.7 l of water were collected in solvent winchesters with teflon-backed lids. One hundred microlitres of mixed standard solution A (3.0 ng per microlitre of dimethyl, diethyl, di-n-butyl, di-iso-butyl, di-n-heptyl, di-2-ethylhexyl, and di-n-nonyl phthalates (BP Ltd)) was added to each sample. The water was decanted into a 3 l separating funnel and the winchester rinsed with 75 ml of dichloromethane, which was subsequently used to extract the water. An empty winchester and separating funnel was provided for the blank. The extraction was repeated with 25 ml of dichloromethane three times and the combined extracts were dried with 5 g of anhydrous sodium sulphate. The sample was reduced in volume on a rotary evaporator at 40°C to approximately 1 ml, prior to subsequent clean up.

Sediment A sample of sediment was collected by scraping a glass jar across the surface of intertidal mud. Approximately 30 g of sediment or 30 g of sediment together with 100 μl of standard A were mixed with the same weight of anhydrous sodium sulphate in a glass jar and left overnight at 4°C. Blanks consisted of anhydrous sodium sulphate in glass jars. The mud/sulphate lumps were broken up with a dissecting pin and soxhlet extracted with 150 ml of dichloromethane for 16 h at 40°C. The extracts were subsequently treated as for water samples.

Molluscs Each sample, consisting of 10–20 *Scrobicularia plana* or *Ostrea edulis*, was washed externally with running tap water. The tissue was then removed from the shells with an oyster knife and placed on glass sheets. Since the gut contents could not be emptied without contaminating the samples, the digestive glands were dissected out and separated from the rest of the tissue. The two samples were then placed in separate glass jars, homogenised for 2 min using an Ultra Turrax homogeniser, mixed with an equal weight of anhydrous sodium sulphate and treated as for sediment. For the blank, 5 ml of water was homogenised in place of the tissues.

Fish As they were landed on deck, samples of dab (*Limanda limanda*), plaice (*Pleuronectes platessa*) and whiting (*Merlangius merlangus*) were

immediately wrapped in aluminium foil and frozen. Each sample consisted of 6 to 10 animals. After thawing, the skin was removed and discarded, and sections of muscle were taken from well below the surface. Liver samples included the gall bladder. All tissues were extracted by the method used for bivalves.

Isolation of phthalate esters from co-extractants

A simple separation of neutral lipids and phthalates from polar lipids was carried out using 3 g alumina (5% deactivated) columns. Columns were packed dry and 50 ml of dichloromethane was used to flush any phthalate contaminants from the alumina. Samples were carefully introduced to the column using a 2 ml glass syringe and eluted with 25 ml of dichloromethane. A high recovery (98–101%) of the standards dissolved in fish oil was achieved with no significant discrimination between various phthalate esters.

The partially purified extracts were then concentrated by rotary evaporation and were stored in 1 ml glass volumetric flasks at 4°C before analysis by GC/MS.

Gas chromatography/mass spectrometry

Dimethyl (DMP), diethyl (DEP), di-n-butyl (DnBP), di-iso-butyl (DiBP), di-n-heptyl (DnHP), di-2-ethylhexyl (DEHP) and di-n-nonyl (DnNP) phthalate standards were analysed individually and together. Calibrations for all standards were linear between 0.03 and 3.00 ng.

Conditions for GC/MS Phthalate esters were separated on a 25 m × 0.3 mm ID fused silica WCOT column of SE-54 (GC^2, Northwich, Cheshire). The column was directly connected to the source of a Finnigan 3200 GC/MS system. The MS was operated in the electron impact mode at 70 eV, and the data were processed by an Incos 2300 data system.

Chromatography conditions: initial temperature 25°C, temperature programme 100 to 250°C at 10°C min^{-1}, carrier helium, flow rate 1.5 ml min^{-1}, Grob type capillary injector operated in the splitless mode.

Samples were quantified using multiple ion detection adjusted at m/z 163 for DMP and m/z 149 for other phthalate esters. The limit of accurate detection of the phthalate esters was 30 pg. Scanning runs were carried out to confirm the identification of phthalate ester peaks.

Quantification A satisfactory internal standard for the quantification of phthalate esters was not available. External standardisation was therefore dependent upon accurately reproducible extraction and injection of samples. In order to test the repeatability of sample injection, 1 μl of the same sample/standard mixture was injected 10 times. Peak area was found to vary by less than $\pm 2\%$.

The loss of sample during extraction and clean up was monitored by comparison of peak areas produced by standard A when added to one of the samples with those obtained from injection of an external standard. Recovery ranged from 85 to 110% and was usually between 90 to 100%.

Since all glassware and reagents had to be kept scrupulously clean it was difficult to run large batches of samples simultaneously for duplicate or triplicate analyses. The heterogeneous nature of most of the samples also made it difficult to produce true replicates, but attempts were made to analyse sea water and sediment in duplicate. The results of duplicates (Table I) varied by less than 25% with the exception of DnBP in sea water. Some of the observed differences between replicates may be due to real small-scale heterogeneity in the samples used.

Quality of results Examples of GC/MS traces are shown in Figures 1 to 5. Figures 1 to 3 are scanning runs of blanks, water and sediment, and show the ease with which the m/z 149 trace isolates phthalate ester peaks from co-extractants, particularly the large peak of elemental sulphur in the sediment sample (peak d in Figure 3). Figures 4 and 5 are MID runs of fish tissue comparing m/z 163 and m/z 149. In muscle tissue (Figure 4), phthalates at (a), (b), (c), (d) and (e) may be distinguished from co-extractant fatty acids since these show peaks in both m/z 163 and m/z 149, whereas the phthalates show peaks only in one trace. Figure 5 is the most difficult trace to interpret, as there are large fatty acid peaks present; phthalate peaks are at (a), (b) and (c).

TABLE I
Duplicate analyses of the phthalate ester content of sea water and sediment

		DMP	DEP	DiBP	DnBP	DnHP	DEHP	DnNP
Sea water	(1)	5.3	39.4	31.6	24.4	6.6	58.5	<1.0
(ng l^{-1})	(2)	7.3	43.9	36.4	58.2	5.3	78.3	<1.0
Blank		<1.0	<1.0	12.2	7.1	<1.0	14.2	<1.0
Sediment	(1)	<1.0	<1.0	11.5	14.5	<1.0	26.2	<1.0
(ng g^{-1})	(2)	<1.0	<1.0	8.5	12.2	<1.0	25.8	<1.0
Blank		<1.0	<1.0	<1.0	1.0	<1.0	1.7	<1.0

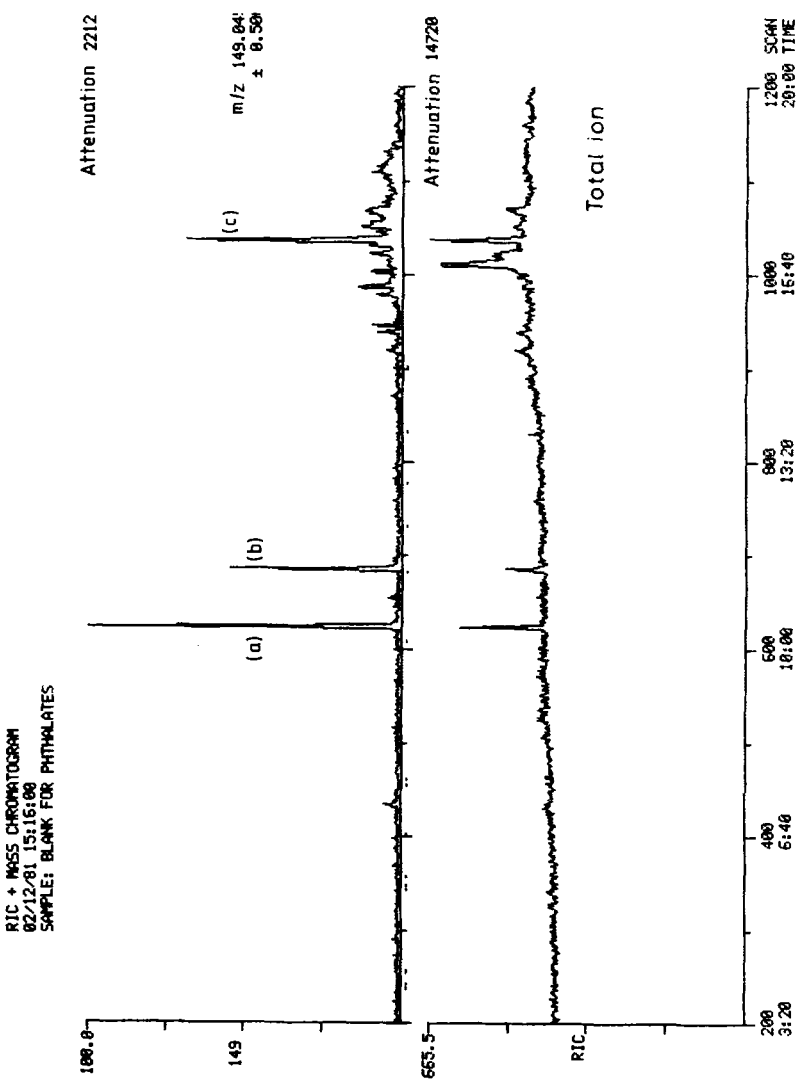

FIGURE 1 Procedural blank for phthalate ester determination. A comparison of total ion current with m/z 149. (a) di-iso-butyl; (b) di-n-butyl; (c) di-(2-ethylhexyl) phthalates.

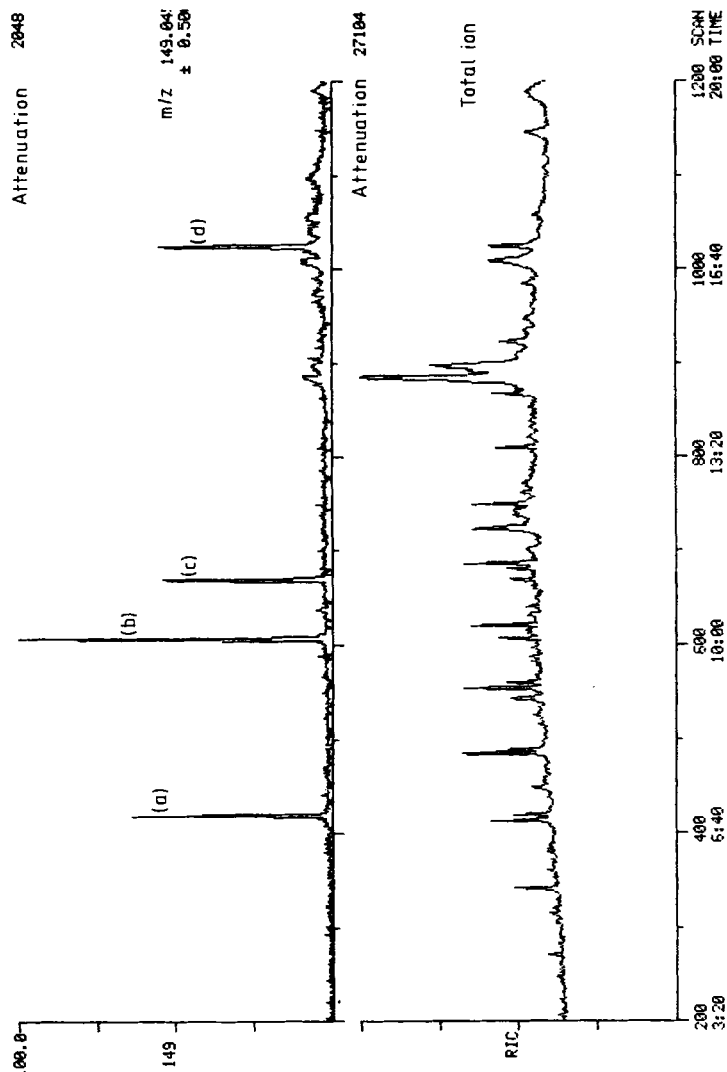

FIGURE 2 Extract of Crouch Estuary water for phthalate ester determination. A comparison of total ion current with m/z 149. (a) di-ethyl; (b) di-iso-butyl; (c) di-n-butyl; (d) di-(2-ethylhexyl) phthalates.

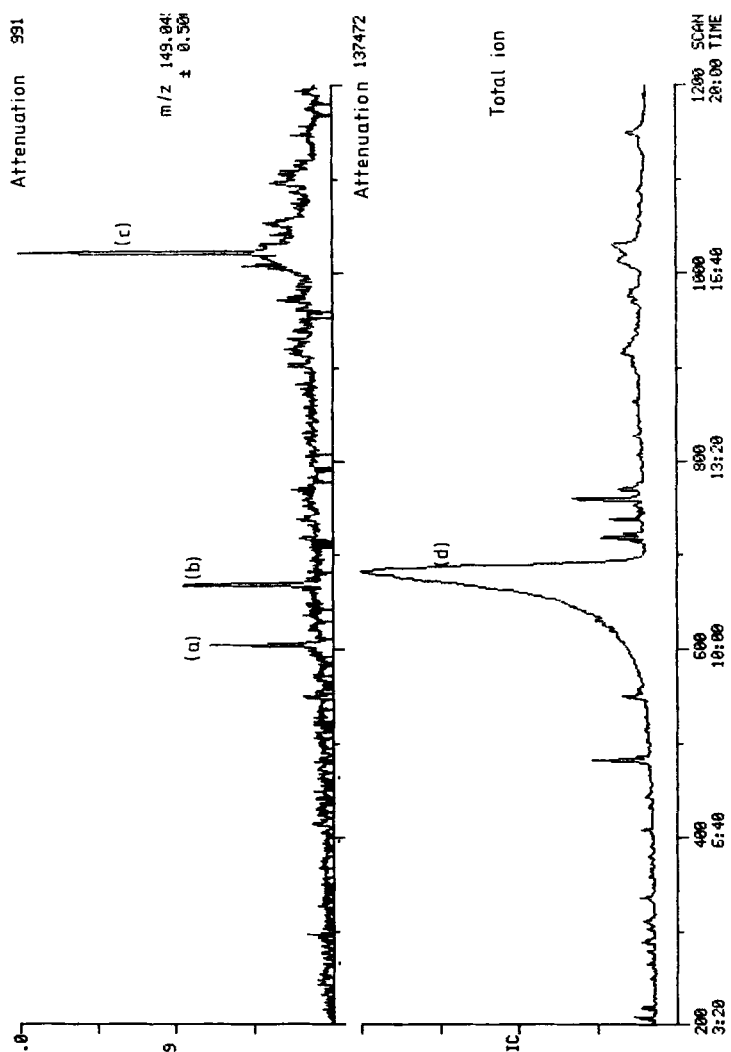

FIGURE 3 Extract of Crouch Estuary sediment for phthalate ester determination. A comparison of total ion current with m/z 149. (a) di-iso-butyl; (b) di-n-butyl; (c) di-(2-ethylhexyl) phthalates; (d) sulphur.

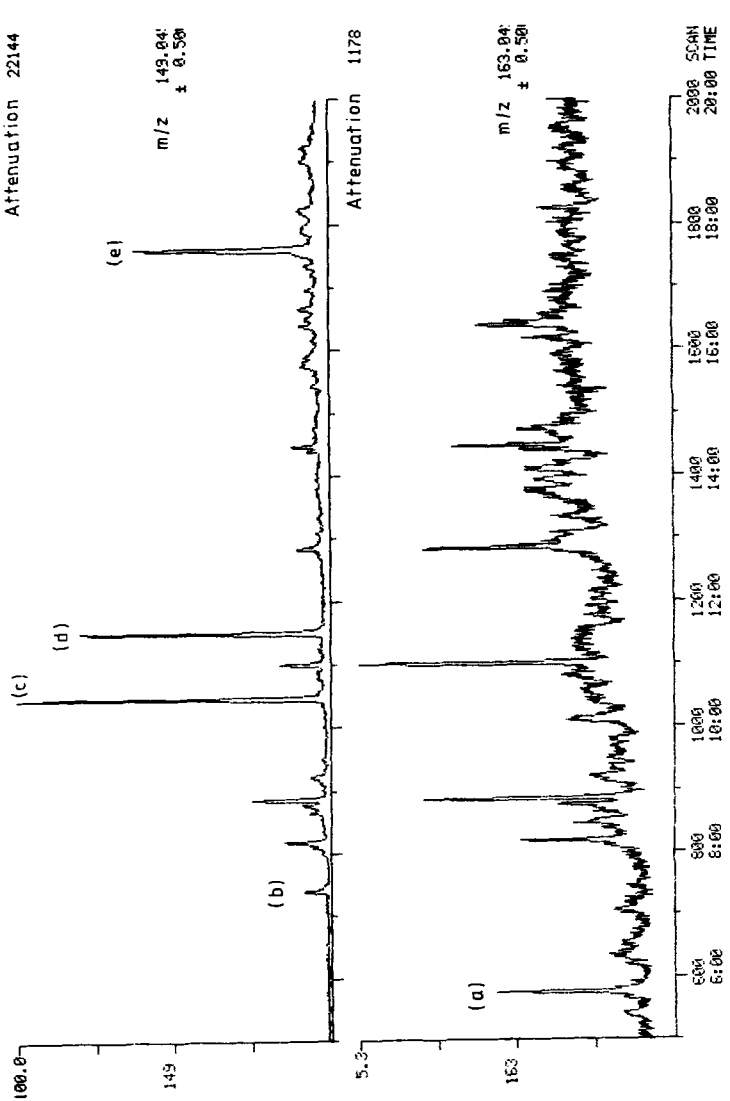

FIGURE 4 Extract of muscle from *Limanda limanda* for phthalate ester determination. M/z 163 and m/z 149. (a) di-methyl; (b) di-ethyl; (c) di-iso-butyl; (d) di-n-butyl; (e) di-(2-ethylhexyl) phthalates.

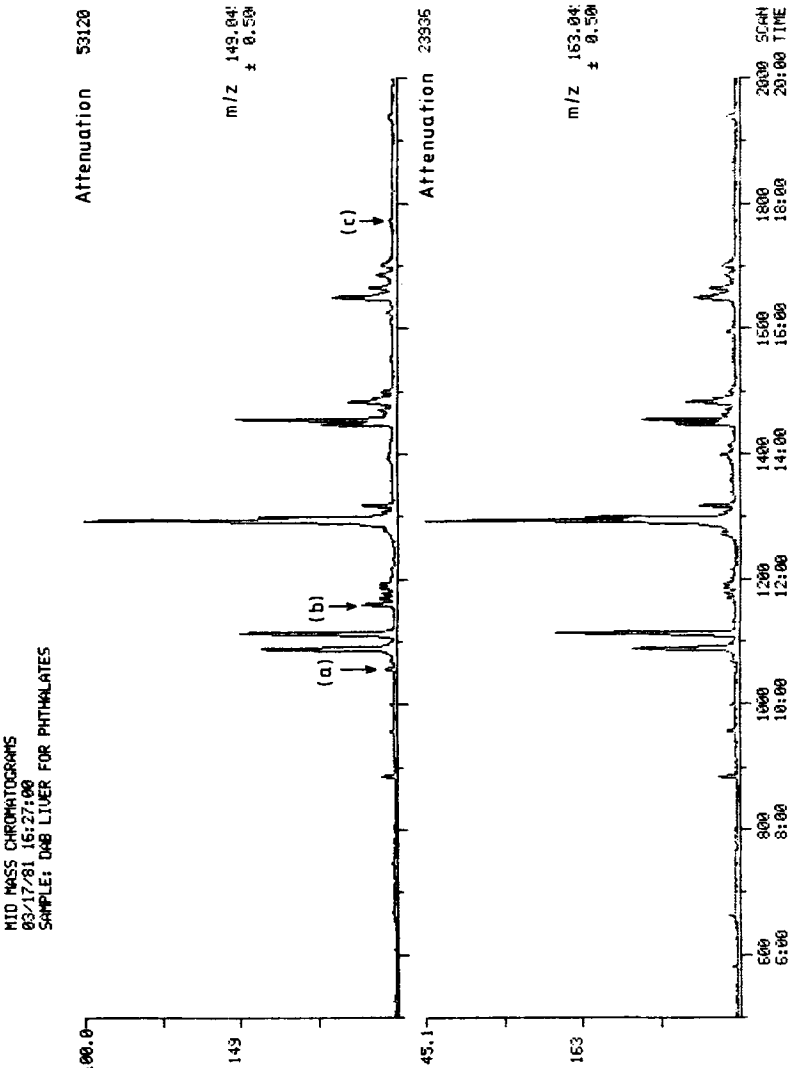

FIGURE 5 Extract of liver from *Limanda limanda* for phthalate ester determination. M/z 163 and m/z 149. (a) di-iso-butyl; (b) di-n-butyl; (c) di-(2-ethylhexyl) phthalates.

RESULTS

Table II shows the level of phthalate esters found in a variety of water, sediment and biota samples. Sample results are uncorrected for blank values. Without a large number of replicate samples it was difficult to obtain a measure of total analytical error. As this was unknown, it is considered that unless the concentration in a sample was more than twice that found in the associated blank, the detected level should not be regarded as significant.

DnNP was not found in any of the samples examined. DMP and DnHP were present in sea water, but only in low concentrations (5 to 10 ng l^{-1}), DMP and DnHP were not found in any of the sediment or biota samples. A small amount (2.9 ng g^{-1}) of a peak of the same retention time as DnHP was found in the body tissues of *Scrobicularia plana*, but this could not be confirmed as DnHP by scanning MS as levels were too low.

Although DEP was present at higher concentrations in the seawater samples, it was virtually absent from sediment and biota samples, occurring only in the digestive glands of the oyster *Ostrea edulis*.

DiBP, DnBP and DEHP were found in nearly all of the samples, but were also found to be very common laboratory contaminants. The concentrations, particularly of DiBP, were usually only just detectable above blank values, although significant concentrations occurred in some tissues, i.e., in dabs from the River Crouch, and in the body tissue of *S. plana*. Significant concentrations of DiBP were more common in water and sediment samples.

DnBP was present in many samples but in no case exceeded 25 ng g^{-1} wet weight in biota or 60 ng l^{-1} in sea water. DEHP was the most common and most abundant phthalate ester, and was found in all samples, with the exception of dabs from the River Crouch. The highest concentrations were found in the digestive glands of *S. plana* (over 200 ng g^{-1}) with lower concentrations in the rest of the body ($\approx$20–40 ng g^{-1}). Digestive glands of the oyster also contained higher concentrations of DEHP than the rest of the body ($\approx$10 ng and $\approx$5 ng g^{-1} respectively), and the fish from Tees Bay had higher concentrations in livers than in muscle ($\approx$50–80 ng and $\approx$10–50 ng g^{-1} respectively).

TABLE II

Phthalate ester concentrations in water, sediment and biota samples and their associated blanks (ng l^{-1} or ng g^{-1} on a wet weight basis)

Sample		DMP		DEP		DiBP		DnBP		DnHP		DEHP		DnNP	
		S	B	S	B	S	B	S	B	S	B	S	B	S	B
CROUCH ESTUARY															
Sea water	(1)			*105.4*	4.4	*44.2*	16.6	*38.5*	6.7			*75.5*	18.3		
	(2)	*5.5*	<1.0	*39.4*	<1.0	*31.6*	12.2	*24.2*	7.1	*6.6*	<1.0	*58.5*	14.2		
	(3)	*7.3*	<1.0	*43.9*	<1.0	*36.4*	12.2	*58.2*	7.1	*5.3*	<1.0	*78.3*	14.2		
Sediment	(1)					1.8	2.1	*3.9*	1.0			*11.2*	1.9		
	(2)					*11.5*	0.9	*14.5*	1.0			*26.2*	1.7		
	(3)					*8.5*	0.9	*12.2*	1.0			*25.8*	1.7		
Scrobicularia plana															
Digestive gland	(1)					1.2	1.0	*5.3*	0.9			*81.5*	1.8		
	(2)					1.3	1.0	*9.2*	1.0			*213.7*	1.7		
Rest of body	(1)							*3.1*	0.9			*42.6*	1.8		
	(2)					*8.1*	1.0	*7.9*	1.0	2.9*	<1.0	*23.0*	1.7		
Ostrea edulis (Oyster)															
Digestive gland				*10.8*	<1.0	2.9	2.9	*3.1*	1.0			*9.2*	2.7		
Rest of body						2.6	2.9	*2.4*	1.0			4.9	2.7		
Limanda limanda (Dab)															
Muscle	(1)					6.2	2.4	*4.1*	1.0			nd	nd		
	(2)					*6.3*	2.4	*4.1*	1.0			13.8	11.5		
Liver	(1)					*15.1*	2.4	*23.0*	1.0			2.0	11.5		
	(2)					2.6	2.4	7.3	1.0			2.4	11.5		

TEES BAY								
Limanda limanda (Dab),								
Muscle			11.3	9.4	7.3	5.5	25.8	17.9
Liver			10.2	9.4	9.2	5.5	*43.0*	17.9
Gonad	2.0	1.4	8.2	9.4	3.6	5.5	12.7	17.9
Pleuronectes platessa (Plaice)								
Muscle			1.9	3.5	1.6	2.2	*13.0*	5.3
Liver			2.5	3.5	*16.1*	2.2	*85.9*	5.3
Merlangius merlangus (Whiting)								
Muscle			2.7	2.9	1.6	1.4	*51.3*	4.1
Liver			2.5	2.9	0.6	1.4	*56.8*	4.1
Gut			1.9	2.9	2.5	1.4	*22.4*	4.1

For simplicity values less than 1 ng are not listed
S = sample; B = blank
* tentative identification
Italic results = concentration considered to be significant (see text).
nd = not determined

DISCUSSION

Pierce *et al.* (1980) have recently tabulated the concentrations of phthalate esters in water, sediment and biota reported by several authors. Seawater phthalate concentrations ranged from 0–300 $\mu g\ l^{-1}$; values found in this study were at the lower end of this range, and are similar to concentrations found in open water in the Kiel Bight (Ehrhardt and Derenbach, 1980) and Gulf of Mexico (Giam *et al.*, 1978) (see Table III).

TABLE III
Approximate mean levels of phthalate esters in sea water

	Phthalate (ng l^{-1})				
	DEP	DiBP	DnBP	niBP	DEHP
Ehrhardt and Derenbach (1980)	2	20	80	50	nd
Giam *et al.* (1978)	nd	nd	90	nd	90
This study	50	30	30	nd	60

nd—not determined.
niBP—n-iso-butyl phthalate.

Concentrations of DEHP and DnBP in sediments from various sources have also been tabulated by Pierce *et al.* (1980). Concentrations of DEHP ranged from 0 ng g^{-1} in Lake Superior to 71 000 ng g^{-1} in the River Rhine, and DnBP was found at concentrations up to 16 000 ng g^{-1} in the River Rhine. Levels of DEHP and DnBP found in sediments in the present study were comparatively low (>30 and >15 ng g^{-1} respectively). Since there is only light industrial and domestic effluent input to the River Crouch and its estuary these results are perhaps not surprising.

No published data on phthalate esters in natural populations of bivalves could be found. In the current study phthalate levels in oyster body were low but DEP, DnBP and DEHP were present in the digestive glands. DEP was not found in the digestive gland of the bivalve *S. plana* and suggests that the different modes of feeding in the two animals may influence the types of phthalate found in the gut. Hence filter feeding oysters contain DEP as does the Crouch estuary water but the deposit feeding *S. plana* ingests surface sediment which contains no DEP (Table II). The digestive gland of *S. plana* had the highest concentration of DEHP found in any of the samples, but exactly how much of the phthalate was in the digestive gland tissue and how much

was due to food debris in the digestive gland was not determined. Interestingly, the proportion of DEHP to DnBP in *S. plana* was higher compared to that of the sediment in which it lives, suggesting that the animals exhibited different rates of uptake or catabolism of the various phthalate esters. The higher concentrations of phthalates in *S. plana* compared with the Crouch estuary fish supports the general thesis that catabolism of phthalates is slow in invertebrate species (Metcalf *et al.*, 1973; Wofford *et al.*, 1981).

Generally, there was little difference between the concentrations of total phthalate esters in the tissue of dabs from the Crouch estuary and those from Tees Bay, although Tees Bay fish contained higher concentrations of DEHP and lower concentrations of DiBP and DBP than dabs from the Crouch estuary. The low concentrations found in Tees Bay samples are perhaps surprising in view of large input of industrial effluent into the River Tees. Concentrations of DEHP were higher in the liver tissue than in muscle and gonad but higher levels might be expected to occur in liver samples as they were dissected with gall bladders; the site of DEHP accumulation and breakdown is in the bile (Melancon and Lech, 1976).

The concentration of individual phthalate esters in fish reviewed by Pierce *et al.* (1980) showed variations from 0 to 3.2 $\mu g\ g^{-1}$. The levels found in Crouch Estuary and Tees Bay fish fall into the lower end of this range, and are similar to those found by Giam *et al.* (1978) for DEHP in fish from the Gulf of Mexico (1–135 $ng\ g^{-1}$).

It was difficult to determine the degree of exposure of Tees Bay fish to phthalate esters because samples of sediment water and food organisms were not examined. A sample of whole gut and contents from five whiting (Table II) was taken to give some indication of levels of phthalates in the semi-digested prey. Bioaccumulation of phthalates in tissue relative to the levels of phthalate in the gut contents was not apparent. The concentrations found in tissue were about 50 $ng\ g^{-1}$, which is approximately 100-fold lower than levels affecting the metabolism of fish noted by Mayer *et al.* (1977).

There is a paucity of information available on possible sub-lethal and synergistic effects involving phthalate esters. Some metabolic interactions have been noted in rats (Seth *et al.*, 1977, 1978; Srivastava *et al.*, 1976) and Melancon *et al.* (1977) have shown that in trout the metabolism of DEHP is inhibited by the insecticide synergist piperonyl butoxide. However in the present study phthalates in fish in Tees Bay were found at low levels although complex mixtures of other pollutants occurred.

It should be noted that this study lists only phthalate esters found in samples for which comparative standards were available in this laboratory. No other large peaks which might possibly be phthalates were consistently detected in samples or blanks (Figures 1–5), e.g. di-n-hexyl phthalate was not detected in any of the present analyses, although it was found in large quantities in herrings and mackerel fillets by Musial *et al.* (1981). Other authors (Ehrhardt and Derenbach, 1980) have found n-iso-butyl phthalate in blanks and in samples at levels similar to that for di-iso-butyl phthalate. One is left with the nagging doubt that no matter how rigorous the cleaning procedures and how carefully the samples are extracted and analysed, the conditions within this and other laboratories may influence the results. Such a concern highlights the need for intercalibration exercises between laboratories before accurate and precise results can be assured and meaningful comparisons of the data can be made.

Acknowledgements

I would like to thank Robin Law and John Portmann for critically reviewing the manuscript and John Tapp (ICI Brixham) for arranging collection of Tees Bay fish samples.

References

Ehrhardt, M. and Derenbach, J. (1980). Phthalate esters in the Kiel Bight. *Marine Chemistry*, **8**, 339–346.

Giam, C. S., Chan, H. S., Neff, G. S. and Atlas, E. L. (1978). Phthalate ester plasticizers: a new class of marine pollutant. *Science, N.Y.*, **199**, 419–421.

Mayer, F. L., Meyrle, P. M. and Schoettger, R. A. (1977). Collagen metabolism in fish exposed to organic chemicals. In *Recent Advances in Fish Technology: a symposium* (Tubb, R. A., ed.) U.S. Environmental Protection Agency, Corvallis, Oregon, EPA EPA Ecological Research Series, EPA 600/3-77-085.

Melancon, M. J. and Lech, J. J. (1976). Distribution and biliary excretion products of di-2-ethylhexyl phthalate in rainbow trout. *Drug Metabolism and Disposition*, **4**, 112–118.

Melancon, M. J., Saybolt, J. and Lech, J. J. (1977). Effect of piperonyl butoxide on disposition of di-2-ethylhexyl phthalate by rainbow trout. *Xenobiotica*, **7**, 633–640.

Metcalf, R. L., Booth, G. M., Schuth, C. K., Hansen, D. J. and Lu, P. V. (1973). Uptake and fate of di-2-ethylhexyl phthalate in aquatic organisms and in a model ecosystem. *Environmental Health Perspectives*, **4**, 27–34.

Murray, H. E., Ray, L. E. and Giam, C. S. (1981). Phthalic acid esters, total DDTs and polychlorinated biphenyls in marine samples from Galveston Bay, Texas. *Bulletin of Environmental Contamination and Toxicology*, **26**, 769–774.

Musial, C. J., Uthe, J. F., Sirota, G. R., Burns, B. G., Gilgan, M. W., Zitko, V. and Matheson, R. A. (1981). Di-n-hexyl phthalate (DHP), a newly identified contaminant in Atlantic herring (*Clupea harengus harengus*) and Atlantic mackerel (*Scomber scombrus*). *Canadian Journal of Fisheries and Aquatic Sciences*, **38**, 856–859.

Pierce, R. C., Mathur, S. P., Williams, D. T. and Boddington, M. J. (1980). *Phthalate esters in the aquatic environment*. Associate Committee on Scientific Criteria for Environmental Quality. National Research Council of Canada, Ottawa, Ontario, No. 17583. 108pp.

Seth, P. K., Agarwal, D. K. and Agarwal, S. (1981). Effect of phthalic acid esters on drug metabolizing enzymes. *Bulletin of Environmental Contamination and Toxicology*, **26**, 764–768.

Seth, P. K., Srivastava, S. P., Agarwal, D. K. and Mushtaq, M. (1977). Interaction of di-(2-ethylhexyl)-phthalate (DEHP) with pentabarbitone and methaqualone. *Bulletin of Environmental Contamination and Toxicology*, **17**, 727–732.

Srivastava, S. P., Agarwal, D. K., Mushtaq, M. and Seth, P. K. (1976). Interaction of di-2-ethylhexyl phthalate (DEHP) with parathion in rats. *Chemosphere*, **3**, 177–181.

Webster, R. D. J. and Nickless, G. (1976). Problems in the environmental analysis of phthalate esters. *Proceedings of the Analytical Division of the Chemical Society*, **13**, 333–335.

Wofford, H. W., Wilsey, C. D., Neff, G. S., Giam, C. S. and Neff, J. M. (1981). Bioaccumulation and metabolism of phthalate esters by oysters, brown shrimp and sheepshead minnows. *Ecotoxicology and Environmental Safety*, **5**, 202–210.