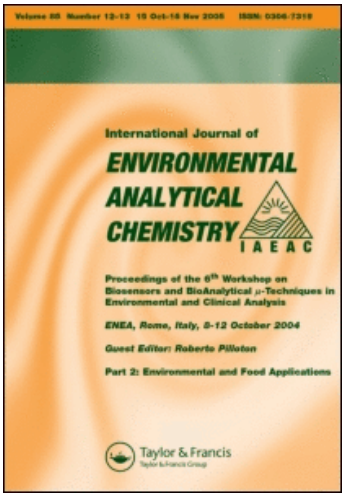

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Analysis of Phthalate Esters in Sediments from Dutch Rivers by means of High Performance Liquid Chromatography†

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Analysis of the phthalate esters di-(2-ethylhexyl) and di-*n*-butyl phthalate—DEHP and DBP, respectively—can conveniently be carried out by means of high-performance liquid chromatography in the system silica gel/*n*-hexane—dichloromethane (containing approx. 0.2% of ethanol) (1:2, v/v). Detection is done by UV absorption spectrometry at 233 nm; the detection limit for both phthalates is approx. 10 ng.

The procedure has been used for a survey of the sediments of three main rivers in The Netherlands. The sediments of the rivers Rhine and IJssel contain 1–70 ppm DEHP and 0–15 ppm DBP. For the river Meuse, slightly lower levels of 1–17 ppm DEHP and 0–2 ppm DBP are measured. In several sediments of a nature-reserve, DEHP and DBP contents are below the detection limit of approx. 0.5 ppm. The combined data suggest that the phthalate pollution of the river sediments is mainly due to transport of these pollutants via the river, and not via the atmosphere.

KEY WORDS: Phthalate esters, river sediments, HPLC analysis.

INTRODUCTION

The phthalates, diesters of phthalic acid, find widespread application as plasticizers (1974: 2,300,000 tons in U.S.A. and Western Europe[1]), especially in polyvinyl chloride. This has led to their increasing occurrence

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in the environment and several reports have been published on the determination of these compounds, e.g. in water,[2] soil,[3] air[4] and biota samples.[4-6] However, only little information is known concerning the occurrence of phthalates in the aquatic environment in Western Europe.

Although the acute toxicity of phthalates is relatively low,[7,8] mutagenic and teratogenic activity has been established.[9] Furthermore, phthalates can influence enzyme activity; e.g., in some organs of the rat, di-(2-ethylhexyl) phthalate inhibits the activity of succinate dehydrogenase and ATP-ase.[10] There is also evidence that the phthalates can disturb ecosystems[5,11] and can provide a hazard to aquatic organisms like daphnia's already in ppb concentrations.[6] As a consequence, phthalates should be considered hazardous environmental pollutants.

Several reviews concerning analytical procedures for the determination of phthalates have been published.[12,13] Generally, analysis is done by means of gas-liquid chromatography (GLC), the various procedures sharing the disadvantage that an extensive clean-up of the sample extracts is usually necessary prior to measurement. This increases the danger of high phthalate background levels, since contamination of solvents, further chemicals and laboratory equipment by these esters is a well-known phenomenon.[4,5,14] In the present study, a rapid and sensitive high-performance liquid chromatographic (HPLC) method is presented for the determination of two widely used plasticizers, di-(2-ethylhexyl) and di-*n*-butyl phthalate (DEHP and DBP, respectively) in sediments from three main rivers in The Netherlands, viz. Rhine, IJssel and Meuse. The procedure requires no clean-up of the sample and consists of a single extraction step followed by quantitative analysis by means of HPLC in an adsorption system.

EXPERIMENTAL

Apparatus

Sediment samples were taken with a Hydro-Bios dredge. A Leybold–Hereus Model GT-2 freeze dryer was employed to dry the samples. A Siemens Model S100 liquid chromatograph equipped with an Orlita Model Mk 00 pump and a Zeiss Model PM2 DLC multiple-wavelength detector was used to quantitate the phthalates. The injection port was a Siemens Model F 10–34 six-port valve to which a 100-μl loop was attached. The Chrompack separation column was a stainless-steel tube (25 cm × 3 mm I.D.) pre-packed with 5-μm LiChrosorb SI 60 silica gel. Mixtures of *n*-hexane—dichloromethane (containing approx. 0.2% of ethanol) (1:2, v/v)—which were thoroughly mixed before use—were used

as mobile phase. All chromatograms were run at a temperature of approx. 25°C.

Gas chromatography was carried out on a Pye Unicam Model 104 gas chromatograph equipped with an electron-capture and a flame ionization detector, using a 7 ft × 2 mm I.D. glass column packed with 4% OV-101 on Chromosorb WHP (80–100 mesh). The injector, detector and column temperatures were 250, 300 and 220°C, respectively. Nitrogen was used as carrier gas at a flow-rate of 30 ml · min^{-1}.

Reagents and materials

Reagent-grade DEHP and DBP were obtained from Merck (Darmstadt, G.F.R.). Nanograde *n*-hexane (Mallinckrodt, St. Louis, Mo., USA), reagent-grade dichloromethane (Merck), analysed-grade acetone, methanol and diethyl ether (Baker, Phillipsburg, N.J., USA) were used as solvents without further purification.

Folding filters, cellulose Soxhlet thimbles (Whatman, Maidstone, England) and Florisil adsorbent (60–100 mesh; Sigma, St. Louis, Mo., USA) were washed by means of extraction in a Soxhlet apparatus for at least 12 h. The filters were stored under *n*-hexane. Prior to use, all glassware was washed with a detergent solution and thoroughly rinsed with hot tap water, acetone and *n*-hexane. Soxhlet and distillation apparatus were rinsed with *n*-hexane.

Procedure

During 1977, river sediment samples were collected from 32 stations along the rivers Rhine, IJssel and Meuse (cf. Figure 1). Preferably, homogeneous fine-grained samples from muddy sites were taken. However, occasionally the river sediment contained coarser material (sand). A further series of samples was collected in a nature-reserve, Polder Kortenhoef (cf. Figure 1). Approximately 1 l of sediment was put into a 2.5 l glass jar.

After arrival in the laboratory, 100–200 g samples were immediately frozen at a temperature of −30°C and freeze-dried. Subsequently, the samples were homogenized and 10 g were placed in a Soxhlet thimble. Extraction was carried out for 17 h, using 200 ml of a *n*-hexane-acetone-methanol (8:1:1, v/v) mixture. The extract was carefully evaporated to a volume of 1–2 ml, dissolved in 30 ml of *n*-hexane and filtrated over a paper filter. 100-μl aliquots from a known weight of the sample solution were injected into the HPLC system, which was run at a flow-rate of approx. 0.5 ml. min^{-1}. Analysis was done at a wavelength of 233 nm, DEHP and DBP contents being determined by measuring peak heights. The calibration curve was shown to be linear over the concentration range 0.1–

FIGURE 1 Location of sampling stations (1–32) along the rivers Rhine, IJssel and Meuse. Stations 33–44, nature-reserve Kortenhoef; Station 45, sanitary landfill Zeldam.

15 μg of phthalates per ml of *n*-hexane solution. Recovery was found to be between 90 and 110% for both DEHP and DBP.

The fraction of particles having a diameter smaller than 16 μm was determined by the method of Hofstee and Fien.[15]

For analysis by GLC samples were pre-cleaned by applying an aliquot of the hexane extract to a 0.9×20 cm column filled with 7 g of Florisil. After elution of the column with 100 ml of *n*-hexane-diethyl ether (50:3, v/v) (eluate discarded), percolation of the column was continued with *n*-hexane-diethyl ether (17:3, v/v) to yield two further 100-ml fractions, which contained DEHP and DBP, respectively.[14,16] These fractions were carefully evaporated to dryness, dissolved in *n*-hexane and subjected to GLC analysis.

RESULTS AND DISCUSSION

Pretreatment. In a preliminary series of experiments, it was observed that traces of phthalates were present in the extraction thimbles, filter paper and Florisil used. Therefore, these materials were thoroughly cleaned by means of Soxhlet extraction. Further, all glassware was invariably cleaned and rinsed as described in the Experimental section immediately prior to use. These precautions turned out to eliminate noticeable background contamination; that is, blank values always were below the minimum detectable quantity of approx. 10 ng of phthalate (cf. below).

Composition of the mobile phase. In our experience, HPLC with silica gel as stationary phase and a mixture of *n*-hexane and dichloromethane as mobile phase is well suited for the analysis of a large number of phthalates; this is in accordance with the data recently published by Persiani and Cukor.[17] However, it should be noted that initially, the retention times of DEHP and DBP occasionally showed unexpectedly large variations. This turned out to be effected by the presence of varying amounts of ethanol in different brands, or even lots, of dichloromethane. With decreasing percentage of ethanol, the retention times of the phthalates sharply increase. The relationship between percentage of ethanol or *n*-butanol added to the mobile phase and the value of the capacity ratio, k', of the phthalates is shown in Figure 2. In the present study, good results—i.e., fairly small capacity ratios and sufficient resolution—were obtained by using *n*-hexane-dichloromethane (1:2, v/v) containing approx. 0.1% of ethanol (or 0.15% of *n*-butanol) as mobile phase. Under these conditions, reproducibility of the chromatographic system was excellent; column life-time was 4–6 months.

Detection and identification. Since the U.V. cut-off of the present mobile phase system lies around 228 nm, U.V. detection was carried out at

233 nm, which is near the wavelength of maximum absorption of DEHP and DBP (224 nm). Using 0.04 AUFS and 100-μl injections, the minimum detectable quantity of both DEHP and DBP was approx. 10 ng. Consequently, following the procedure outlined in the Experimental section one can detect DEHP and DBP levels down to 0.5 ppm in river

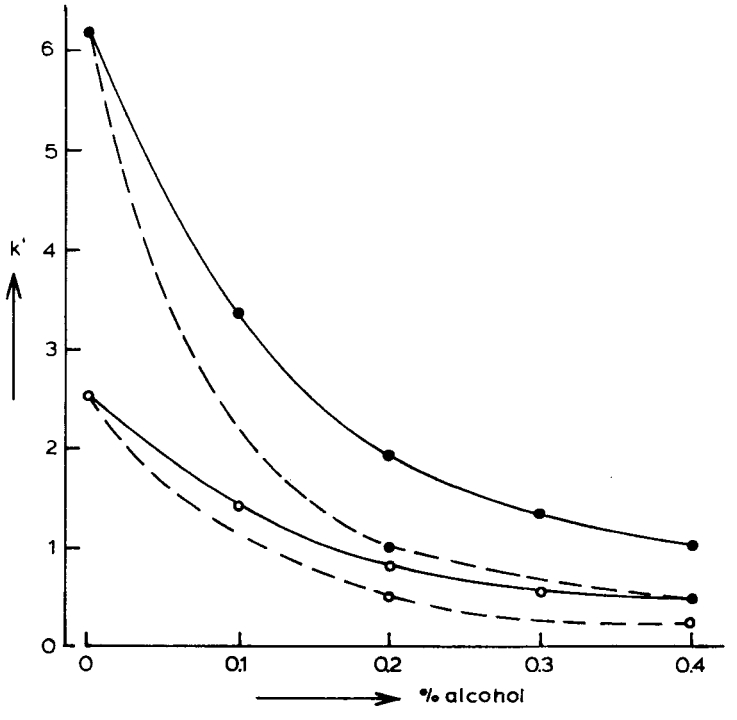

FIGURE 2 Dependence of k' values of DEHP (○) and DBP (●) on the amount of ethanol (——) or *n*-butanol (———) present in the mobile phase (*n*-hexane-dichloromethane, 1:2, v/v).

sediments. Figure 3 shows a typical chromatogram of a hexane extract of a sediment taken from the river Rhine.

For a few samples, the presence of DEHP and DBP was confirmed by means of GLC. In this case, clean-up on a Florisil column prior to analysis was necessary in order to eliminate interfering peaks situated close to the DBP peaks.

Biodegradation. Repeated analysis of a representative sediment sample over a period of time of 2 weeks after actual sampling was carried out in

order to study biodegradation of phthalates. In the literature, biodegradation has been demonstrated in activated sludge,[18] whereas it appears to be virtually absent under anaerobic conditions.[19] The DEHP content of the selected sample turned out to remain essentially constant (s.d., 5%; $n = 10$), irrespective of the absence or presence of a microbial inhibitor (500 ppm of NaN_3 or $HgCl_2$ added immediately after sampling); i.e., no marked biodegradation did occur.

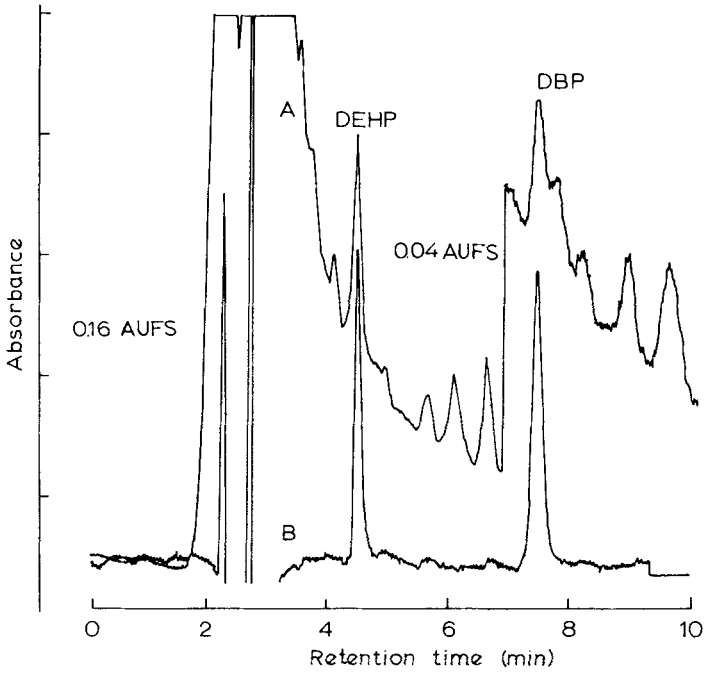

FIGURE 3 HPLC chromatogram of (A) hexane extract of a sediment taken at Station 2 (cf. Table I, 22–08) and (B) a standard solution of DEHP and DBP. Retention times: DEHP 4.5 min; DBP, 7.5 min.

River sediments. The results of analyses of sediment samples taken from the rivers Rhine, IJssel and Meuse are listed in Tables I, II and III, respectively. All data in these and subsequent tables are reported as ppm (μg of phthalate per g dry weight of sediment). As for samples taken at the same station, but at different dates, no special care was taken to sample at exactly the same location. This is clearly illustrated by the data for e.g. Station 12, where large variations in both DEHP content and percentage dry weight are noticed. Except for Stations 8 and 19, all sample stations were chosen at places where the movement of the river water was sluggish.

Here, deposition of suspended matter takes place and the samples mainly consist of sludge. Stagnant water was especially encountered with some of the samples at Stations 16–19 in the mouth of the river IJssel. As for Stations 8 and 19, here the sediment largely consisted of sand and relatively high percentages dry weight were found. At station 20, parti-

TABLE I

DEHP and DBP content (ppm) and composition of sediments taken from the river Rhine in 1977

Sample station	Date	DEHP	DBP	% dry weight	fraction with $d_p < 16\,\mu m$
01	10–05	15.0	5.0	—	—
	22–08	70.5	15.5	34	—
	03–10	9.5	n.d.	57	0.12
02	10–05	20.5	7.5	—	—
	22–08	25.0	4.5	43	—
	03–10	19.0	n.d.	45	0.40
03	22–08	26.0		34	—
	03–10	27.0	n.d.	33	0.40
04	22–08	10.0	n.d.	58	—
	03–10	17.0	n.d.	48	0.36
05	10–05	13.0	4.0	—	—
	22–08	21.0	3.0	37	—
	03–10	27.0	n.d.	24	0.51
07	22–08	10.5	n.d.	46	—
	03–10	6.5	n.d.	59	0.11
08	10–05	0.1†	n.d.	77	—
09	22–08	14.5	n.d.	45	—
10	10–05	9.0	2.0	—	—
	22–08	28.5	0.5	32	—
	03–10	25.0	n.d.	25	—
11	22–08	45.0	0.5	34	—

n.d., not detectable.
†Procedure: 25 instead of 10 g dry sample; 5 instead of 30 ml *n*-hexane.

culate matter from the river Meuse precipitates in a precipitation tank used by RIZA, the governmental institute for the purification of waste water.

From Tables I and II one reads that for the sediments of the rivers Rhine and IJssel DEHP and DBP concentrations generally are between 2–50 and 0–7 ppm, respectively. For the river Meuse, distinctly lower values are observed, i.e. 1–17 and 0–2 ppm, respectively. These high phthalate levels are comparable with those previously reported for sedi-

TABLE II

DEHP and DBP content (ppm) and composition of sediments taken from the river IJssel in 1977

Sample station	Date	DEHP	DBP	% dry weight	fraction with $d_p < 16\,\mu m$
12	21–06	21.5	2.0	—	—
	15–08	24.0	n.d.	28	—
	03–10	8.0	n.d.	56	0.12
13	21–06	12.0	0.5	—	—
	15–08	11.5	n.d.	45	—
	03–10	4.0	n.d.	60	0.16
14	21–06	27.0	1.0	—	—
	15–08	12.0	n.d.	52	—
	03–10	6.5	n.d.	56	0.18
15	21–06	14.5	1.0	—	—
	15–08	23.0	n.d.	40	—
	03–10	36.0	n.d.	35	0.42
16	21–06	28.0	n.d.	—	—
	15–08	25.5	n.d.	23	—
17	22–08	52.5	7.5	29	—
18	22–08	34.0	6.5	35	—
19	22–08	2.5	0.5	73	—

n.d., not detectable.

TABLE III

DEHP and DBP content (ppm) and composition of sediments taken from the river Meuse in 1977

Sample station	Date	DEHP	DBP	% dry weight	fraction with $d_p < 16\,\mu m$
20	31–08	13.0	1.5	38	0.37
21	31–08	3.5	0.5	67	0.11
22	31–08	11.5	1.5	42	0.32
23	31–08	9.5	1.5	44	0.32
24	31–08	16.0	1.0	46	0.29
25	31–08	2.5	1.0	50	0.45
26	31–08	17.0	0.5	47	0.27
	31–10	11.0	n.d.	52	0.26
27	31–10	7.0	n.d.	50	0.32
28	31–10	6.0	n.d.	52	0.30
29	31–10	4.5	n.d.	50	0.37
30	31–10	1.0	n.d.	59	0.38
31	31–10	1.0	n.d.	79	0.07
32	31–10	11.5	n.d.	32	0.48

n.d., not detectable.

TABLE IV

Variation of DEHP content (ppm) as a function of distance d between sampling locations

$d = 0.1$ m		$d = 2$ m	
Station 6	Station 8′	Station 6	Station 8′
23.0	21.5	23.0	21.5
23.0	23.5	24.0	22.0
23.0	21.0	35.5	32.5
23.0	20.5	26.0	21.0
21.0	38.0	17.0	22.0

TABLE V

Temporal variation in DEHP content (ppm) of sediments taken at Station 6

Date	DEHP	% dry weight
28–11–77	22.5	35
05–12–77	19.5	37
19–12–77	20.5	34
03–01–78	22.5	32
16–01–78	22.5	34
30–01–78	19.0	35

TABLE VI

DEHP content (ppm), copper content (ppm) and percentage dry weight of sediments from the nature-reserve Polder Kortenhoef

Sample station	DEHP†	Cu	% dry weight†
33	n.d.	0	11
34	n.d.	1	6
35	n.d.	1	27
36	3	3	8
37	2	1	6
38	2	1	7
39	n.d.	1	4
40	34	62	6
41	11	127	10
42	7	133	17
43	n.d.	6	10
44	7	3	11

†Sampling for DEHP and dry-weight determination done in January 1978; n.d., not detectable.

ments of the rivers Usk[20] and Mersey.[14] In the present study, in all cases the DEHP content is considerably higher than that of DBP; this agrees with results reported for North American[16,21] and British[14,20] rivers, but is in marked contrast with data published for the Tama river in Japan.[2] This divergence is partly caused by different production ratios DEHP/DBP in the various countries: for Western Europe, a value of 12.5 has been reported for 1977, whereas Morita *et al.*[2] quote a value of only 4 for Japan.

The phthalate levels of the sediment samples should actually be regarded as minimum values, since only the amount of extractable phthalates has been determined. Eglinton *et al.*[20] report that some organic pollutants in sediments may be converted into insoluble complexes, such as humates. On the other hand, data by Cifrulak[22] suggest that the use of a methanol-containing solvent mixture, rather similar to the one employed in the present study, effectively removes all phthalates from soil samples.

DEHP content and nature of sediment. Generally, low phthalate levels were found when the percentage dry weight of the sediment was high; this was especially true with samples containing coarse particles, i.e., sand. Similarly, in soil samples collected at a sanitary landfill (Zeldam, Station 45; cf. Figure 1), which displayed high dry weight values of 70–80%, low levels of DEHP (0.2–0.8 ppm) and DBP (0.1–0.4 ppm) were determined.

In the literature,[23,24] it has been observed that a relationship exists between the heavy metal level of sediments in polluted areas and the particle size of the sediment, measured as the fraction of particles having a diameter d_p smaller than 16 μm. In the present study, for 25 samples the DEHP content, the percentage dry weight and the fraction having $d_p < 16\,\mu$m were determined. The correlation between the DEHP content and the percentage dry weight displayed a correlation coefficient, r_s, of -0.77 and -0.91 (no significant difference) for the river Meuse and the rivers Rhine and IJssel, respectively, while the correlation coefficients between the DEHP level and the fraction having $d_p < 16\,\mu$m were $r_s = 0.03$ and 0.85, respectively. That is, with the rivers Rhine and IJssel more sorption of phthalates occurs onto particles having $d_p < 16\,\mu$m than onto larger particles; in the case of the river Meuse, no such difference is observed.

In order to study the variation of DEHP contents in the sampling area, at Stations 6 and 8′ (Station 8 was located in the middle of the river—Station 8′ near the bank) series of samples were taken at mutual distances of 0.1 and 2 m. The data reported in Table IV demonstrate that the variation in DEHP content was small in all cases. In another experiment, for a single location (Station 6), the DEHP content was determined biweekly over a period of time of 9 weeks. As is shown by the data in Table V, only insignificant changes were observed, the standard deviation being calculated as $\pm 7\%$.

Table VI presents the DEHP content of sediment samples from the nature-reserve Polder Kortenhoef. The sampling stations were selected on the basis of a copper gradient reported by the Provincial Department of Public Works (Provinciale Waterstaat) of North-Holland. The copper gradient can be regarded as an indication of the pollution gradient in this area. The data show that generally in areas with high copper contents relatively high DEHP levels are found, whereas DEHP levels are low—and occasionally even below the detection limit—in areas with a low copper pollution. Here, one should consider that neither the sampling stations nor the sampling dates for DEHP and copper were (exactly) the same.

The combined data suggest that for the present sampling areas, phthalate pollution is mainly due to transport of these esters via the river—transport via the atmosphere playing only an unimportant role. Current research is directed at the determination of phthalate levels in surface waters in The Netherlands. Preliminary experiments indicate DEHP and DBP levels of 0.5–4 and 0–1.5 ppb, respectively.

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