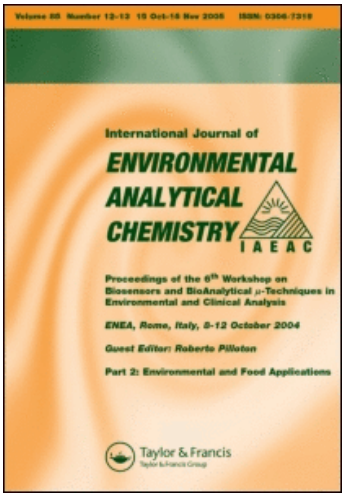

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Liquid Chromatographic Analysis of Phthalate Esters in Dutch River Water

M. J. Schouten[a]; J. W. Copius Peereboom[a]; U. A. Th. Brinkman[a]
[a] Department of Analytical, Chemistry and Institute of Environmental Problems, Free University, Amsterdam, the Netherlands

To cite this Article Schouten, M. J. , Peereboom, J. W. Copius and Brinkman, U. A. Th.(1979) 'Liquid Chromatographic Analysis of Phthalate Esters in Dutch River Water', International Journal of Environmental Analytical Chemistry, 7: 1, 13 — 23
To link to this Article: DOI: 10.1080/03067317908071475
URL: http://dx.doi.org/10.1080/03067317908071475

Intern. J. Environ. Anal. Chem., 1979, Vol. 7, pp. 13–23
0306-7319/79/0701-0013 $04.50/0

Printed in Great Britain

Liquid Chromatographic Analysis of Phthalate Esters in Dutch River Water

M. J. SCHOUTEN, J. W. COPIUS PEEREBOOM and U. A. TH. BRINKMAN

Department of Analytical Chemistry and Institute of Environmental Problems, Free University, De Boelelaan 1083, 1081 HV Amsterdam, the Netherlands

(*Received December 6, 1978*)

Analysis of the phthalate esters di-(2-ethylhexyl) and di-*n*-butyl phthalate—DEHP and DBP, respectively—has been carried out by means of (a) high-performance liquid chromatography (HPLC) in the system silica gel/hexane-dichloromethane (1:2, v/v) containing 0.1–0.2% of ethanol, and (b) gas chromatography (GC) on 4% OV-101 on Chromosorb W (HP) at a temperature of 240°C. Detection was done by U.V. absorption spectrometry at 233 nm and flame ionization, respectively.

The procedure has been used to analyse series of water samples of the Dutch rivers Rhine, IJssel and Meuse. Since the phthalate levels of the samples rapidly decrease with time, extraction invariably was performed without delay at the sampling station. The DEHP values on an average are approx. 1 ppb for all three rivers; for DBP, values of 0.3–0.5 ppb are found for the rivers Rhine and Meuse, while a somewhat high value of 2 ppb is reported for the river IJssel. The results obtained by HPLC and GC show good agreement; the former technique is less time-consuming.

KEY WORDS: Phthalate esters, river water, HPLC and GC analysis

INTRODUCTION

The phthalates, diesters of phthalic acid, are widely used, mainly as plasticisers in polyvinylchloride;[1] minor applications which, however, can add much to pollution, are the use of phthalates as pesticide carriers and insect repellents or in oils.[2,3] Analysis of phthalates in environmental samples is usually limited to the determination of di-(2-ethylhexyl) phthalate (DEHP)—sometimes confusingly called di-octyl phthalate—and di-*n*-butyl phthalate (DBP); in e.g. Western Europe, they nowadays account

for 88 and 7%, respectively, of the total phthalate production. The experimental data on the environmental movement of phthalates have been thoroughly discussed by Peakall,[2] who stresses that the major reservoir of plastics is dumps. However, he also states that data do not appear to exist to make a reasonable estimate of the rate of escape of phthalates from dumps. Actually, the same conclusion appears to hold for all further inputs into the environment considered by Peakall, such as losses from PVC-manufacturing or -processing plants and migration of phthalates into the environment from e.g. tubing, containers, flooring and furniture (also see ref. 4).

Generally, phthalates are considered hazardous contaminants of the environment,[5-8] and a number of papers has been published on the determination of phthalates in environmental and biota samples and on the acute and chronical toxicity of these compounds in e.g. mammals.[2-4] At present, relatively little information is available on the effects of phthalates on the aquatic flora and fauna. It has been reported[9,10] that at a concentration of 3 ppb, DEHP diminishes the amount of offspring of Daphnia by 60%; besides, it is known[4,11,12] that water insects and several types of fish can accumulate DEHP 90-4000-fold, the actual value of the accumulation factor depending on the concentration of the ester in the water (62-0.01 ppb). The literature data[4,10,13-15] seem to suggest that water organisms are more susceptible to phthalates than are mammals such as rats and mice.

As a continuation of our earlier work[16] on the phthalate content of the sediments of Dutch rivers, in the present communication we report on the determination of DEHP and DBP in water samples taken from the rivers Rhine, IJssel and Meuse. Analysis was performed by means of high-performance liquid chromatography (HPLC), the results being compared with those obtained by means of a more conventional technique, i.e., gas chromatography (GC).

EXPERIMENTAL

Glassware

In order to avoid contamination of the samples and extracts, which will generally contain extremely low concentrations of phthalates, all glassware was soaked for 16 h and/or washed thoroughly with 1% Extran (Alkalisch; Merck, Darmstadt, G.F.R.) in tapwater and then rinsed 3 times with hot tapwater. Drying was done by rinsing 2 times with acetone (Baker grade; Baker, Phillipsburg, N.J., U.S.A.). Immediately prior to use, all glassware was rinsed 3 times with hexane (Nanograde; Mallinckrodt, St. Louis, Mo., U.S.A.), used as received from the suppliers. For the rest,

all direct and indirect contact of samples and extracts with plastics other than Teflon-type materials was avoided.

Sampling and extraction procedure

In the laboratory, 1–1 glass bottles having screw-caps with a Teflon inlay, were filled with 50 ml of hexane, tightly closed and transported to the stations shown in Figure 1. Here, 500-ml samples of river water were taken close to the river bank, sampling being done in an upstream direction as indicated by the numbering in the figure. The water samples were immediately poured into the glass bottles and the water–hexane mixture was shaken thoroughly for about 5 min. After arrival at the laboratory, the contents of the bottles were transferred to a 1–1 glass separatory funnel provided with a Teflon stopper and cock. The bottles were rinsed with 2×20 ml hexane, which were added to the separatory funnel. After phase separation, the organic layer was collected, the funnel rinsed with 2×20 ml hexane and the total extract evaporated to a volume of about 1 ml in an all-glass still heated to a temperature of approx. 90°C. The concentrated extract was carefully weighed and aliquot portions were used for analysis by means of HPLC and/or GC.

Analysis

HPLC analysis was carried out on a Siemens S 100 liquid chromatograph equipped with a Siemens F10-34 six-port injection valve (100-μl loop), and a Zeiss PM2 DLC UV detector. The stainless-steel separation column (25 cm $\times$ 3 mm i.d.), obtained from Chrompack (Middelburg, the Netherlands) was pre-packed with 5-μm LiChrosorb SI 60 silica gel. Hexane-dichloromethane (1 : 2, v/v) containing 0.1–0.2% of ethanol, at a flow-rate of about 0.5 ml . min^{-1}, was used as mobile phase (cf. ref. 16); detection was done at 233 nm, which is close to the wavelength of maximum absorption of DEHP and DBP (224 nm). Phthalate concentrations were determined by measuring peak heights at 0.04 AUFS, the detection limits for both compounds being 5–10 ng. Chromatograms were run at a temperature of 25 ± 2°C.

GC analysis was performed on a Pye Unicam gas chromatograph equipped with an FID detector, using a 7 ft $\times$ 2 mm i.d. glass column packed with 4% OV-101 on Chromosorb W (HP) (Chrompack). 4-μl Injections were made using an all-glass solid injector (Chrompack). The injector, column and detector temperature were 240, 240 and 300°C, respectively. The flow-rate of nitrogen, hydrogen and air were 25, 30 and 300 ml . min^{-1}, respectively. Quantitative analysis was done by means of

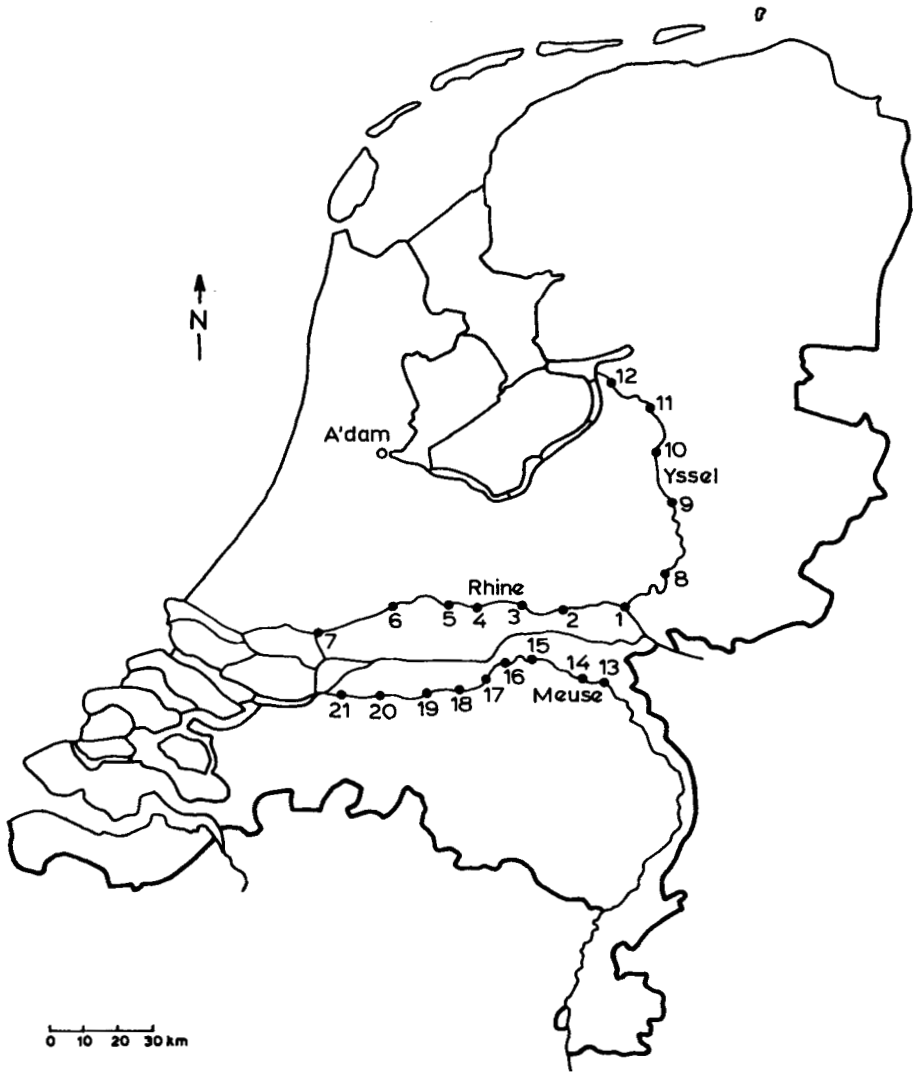

FIGURE 1 Location of sampling stations along the rivers Rhine, IJssel and Meuse.

peak-height measurements. The detection limits for DEHP and DBP were about 0.2 and 0.02 ng, respectively.

Biodegradation

21 of a fresh river water sample kept in a tightly closed 3-l glass infusion bottle were spiked with 40 μl of a 0.25% solution of DEHP and DBP

(reagent grade; Merck) dissolved in ethanol (analytical grade; Baker) to obtain a concentration of 50 ppb for each phthalate. The ethanol solution was added slowly and under stirring using a syringe, the needle of which was kept below the surface of the water in order to prevent adsorption of the phthalates to the wall of the glass bottle. The spiked samples were stored in the dark.[17] In some experiments, microbial inhibitors, in their solid form, were added simultaneously with the phthalates.

Sterilization of spiked water samples was done by autoclaving them for 20 min at 120°C. When taking samples, the top of the sampling bottles was regularly flamed with a Bunsen flame.

RESULTS AND DISCUSSION

Biodegradation

In a preliminary series of experiments it was observed (Figure 2A) that the DEHP, and even more so, the DBP content of the river water samples rapidly decreased with time. At the 50-ppb level, a 50% loss of DEHP typically occurred within 5 days, while over 90% of DBP was lost in about 3 days—it being demonstrated by us that these losses were *not* caused by adsorption of the phthalates to the glass wall of the container. The absence of degradation in a sample spiked with phthalates and sterilized immediately afterwards (less than 10% in 2 weeks; Figure 2B), may suggest that breakdown was caused by biological factors. This is in agreement with conclusions previously reached by Saeger and Tucker.[17] However, in quantitative respect, the present results substantially differ from those reported by these authors, and by Hattori *et al.*,[18] as is evident from Figure 2A. They report a 50% breakdown of 1 ppm of DEHP in about 4 weeks, and of 25 ppm in some 8 days, respectively. No doubt the mutual differences may be partly due to different microbial populations in the various rivers—Dutch rivers, Mississippi, Okawa river. Probably, however, they are mainly caused by the large discrepancies in the phthalate content of the spiked samples and the different methods of analysis used.

As for the former aspect, the quoted authors spike their samples with 20–500 times the amount of phthalates used in the present study. Obviously, a comparable amount of micro-organisms will need substantially more time to break down a certain percentage of 1–25 ppm of phthalates than they do to break down 50 ppb. As for the procedure used, our method (cf. above) was copied from that adopted by Saeger and Tucker.[17] However, Hattori *et al.*[18] continuously and vigorously stir the water sample during their experiments, which moreover were carried out in vessels open to the laboratory air. Since it is known[19–21] that the

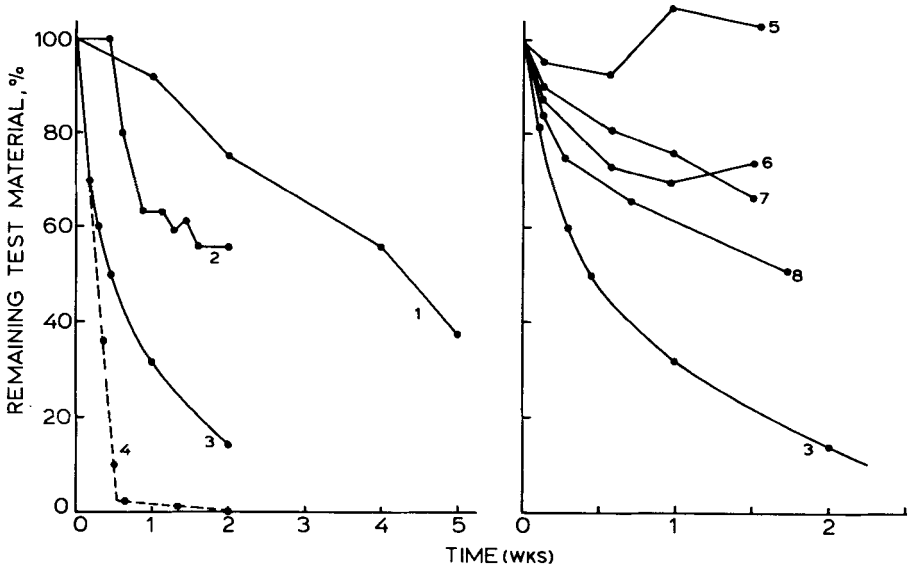

FIGURE 2 (A) Breakdown of DEHP (—) and DBP (– – –) in river water according to (1) Saeger and Tucker[17] (1 ppm; Mississippi), (2) Hattori *et al.*[18] (25 ppm; Okawa), and (3 and 4) the present study (50 ppb; Rhine).

(B) Breakdown of DEHP (50 ppb; Rhine) in the absence of microbial inhibitors (3), after sterilization (5), or in the presence of 3000 ppm Halamid (6), 1500 ppm NaN_3 (7) or 500 ppm $HgCl_2$ (8).

micro-organisms which effect degradation of phthalates are of the aerobic type, the Hattori procedure will inevitably yield high breakdown rates as compared with the method of Saeger and Tucker. We prefer the latter procedure, which ensures that the degradation actually occurring in the river water will at least be as rapid as that measured for the spiked samples.

It is noteworthy that our attempts to prevent biodegradation of the phthalates by adding microbial inhibitors such as $HgCl_2$, NaN_3 and Halamid were only partly successful. Figure 2B demonstrates that, despite the large excess of inhibitor used, degradation—although at a slower rate—continued. No further attempts were made to solve this problem, less so since the use of inhibitors may introduce another source of contamination and/or interference in the analysis step, as has been proven by us in the case of Halamid. As a convenient alternative, in all subsequent work, extraction of the phthalates was performed without delay at the sampling station, as described in the Experimental section. The extraction efficiency of this procedure was shown to be over 99.5% for both phthalates.

Analysis

The results of HPLC analysis of series of water samples taken from the rivers Rhine, IJssel and Meuse are listed in Tables I, II and III, respectively. For reasons of comparison, in Table III data on analysis by GC are included. In all cases blank values (cf. below) have already been subtracted. Figure 3 shows an HPLC chromatogram of a hexane extract of a water sample taken from the river IJssel. When evaluating our results, the following aspects merit further attention.

Blank values were determined by subjecting 50 ml of hexane to the procedure described in the Experimental section, calculating the ultimate result as $(x/500)$ ppb, x being the number of nanograms of phthalates found. For DBP, blank values invariably were below the detection limit (0.1 ppb), while for DEHP blank values were from 0.3 to 1.0 ppb (av., 0.5 ppb); analysis by HPLC and GC yielded identical results. Subsequent

TABLE I

DEHP and DBP content (ppb) of water samples taken from the river Rhine (date, 03-10-1977)

Sampling area	DEHP	DBP
01	1.4	1.7
02	0.6	0.3
03	1.1	0.6
04	0.7	0.2
05	0.3	0.1
06	1.7	<0.1
07	1.1	<0.1

For experimental details, see Analysis section.

TABLE II

DEHP and DBP content (ppb) of water samples taken from the river IJssel (date, 03-10-1977)

Sampling area	DEHP	DBP
08	2.6	2.0
09	0.6	1.1
10	0.9	1.2
11	1.3	2.8
12	0.6	2.4

For experimental details, see Analysis section.

TABLE III

DEHP and DBP content (ppb) of water samples taken from the river Meuse (date, 31-10-1977)

Sampling area	DEHP		DBP	
	HPLC	GC	HPLC	GC
13	0.7	0.5	0.4	0.3
14	0.7	0.5	0.2	0.1
15	1.6	1.5	0.4	0.2
16	0.4	0.2	0.1	0.1
17	0.5	0.4	0.2	0.1
18	1.1	0.9	0.2	0.1
19	1.1	0.8	1.0	0.9
20	4.0	4.2	0.4	0.2
21	0.8	0.8	0.1	<0.1

For experimental details, see Analysis section.

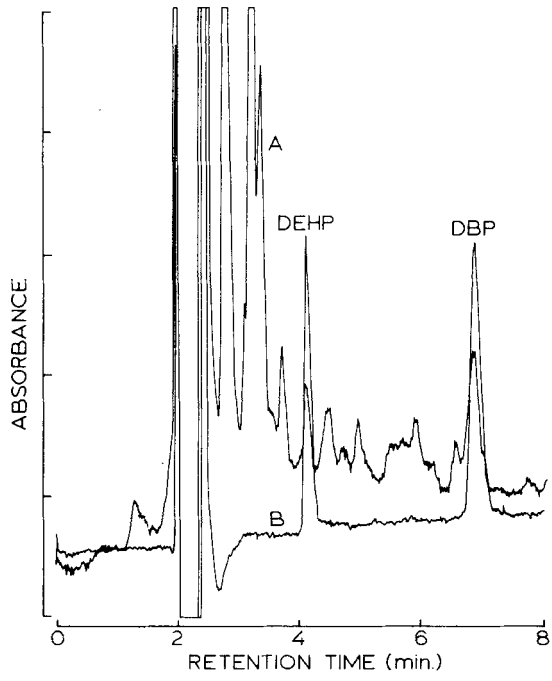

FIGURE 3 HPLC chromatogram of (A) hexane extract of a river water sample taken at Station 12, and (B) a standard solution of DEHP and DBP; detection at 0.04 AUFS. For experimental details, see text.

experiments showed that the Teflon stopper and cock of the separatory funnel and the Teflon inlay of the sample bottle did not significantly contribute to the blank value—i.e., less than 0.1 ppb DEHP—and that the contribution of the hexane used amounted to 0.2–0.4 ppb DEHP.

In summary, in the present series of experiments, the DEHP values on an average are approx. 1 ppb for all three rivers. For DBP, slightly lower values of 0.3–0.5 ppb are determined for the rivers Rhine and Meuse, while a somewhat high value of 2 ppb is found for the river IJssel.

A summary of literature data[22-30] on phthalate contents of river and lake waters is shown in Table IV. Next to a series of relatively low values of less than 0.01 ppb, extremely high values of from 10 to 600 ppb show up. Concerning some of the older data, high results may have been caused by contamination during analysis—a suggestion made by one of the authors himself.[22] On the other hand, the absence of biodegradation studies in almost all papers quoted in Table IV may well imply that some (of the low) values actually are too low. In other words, one can only

TABLE IV

Literature data on the phthalate content (ppb) of river and lake water

Sampling area	Year	DEHP†	DBP†	Reference
Charles and Merrimack R.	1970–71	600	—	22
Lakes in U.S.	1971	total :	0.97–1.9 (1.2)	23
Lakes and river in U.S.	1973	0–300	0–0.09	24
Tama R.	1973 (Jan.)	0.9–44 (2.6)	0.71–3.14 (1.7)	25
Tama R.	1973 (June)	0.5–6.8 (2.3)	0.38–5.61 (2.2)	25
L. Superior	1974	0.1–55 (7.0)		26
Mississippi	1975	0.063	0.014	27
Rivers in Miyashiro	1975	total:	≤ 7.6	28
Ottawa tapwater	1975	0.078	0.029	29
Japanese river	1976	10	45	30

†Values between brackets are mean values calculated by us; data from ref. 27 are mean values reported by the author.

conclude that the DEHP and DBP content of surface waters in North America, Japan and Western Europe often are in the 0–5 ppb range, DEHP generally being more abundantly present than is DBP. This implies that for water fauna and flora acute phthalate intoxication is highly unlikely.[4,9,10,12,14,15] For the rest, the present investigation—which demonstrates the presence of phthalates, at the ppb level, in the water of rivers, the sediments of which contain these compounds at the ppm level[16]—can be regarded as a first attempt to assess the relative importance of the 3 main processes causing the disappearance of phthalates from the water, *viz.* accumulation in sediments, biodegradation, and uptake by aquatic animals and plants (cf. ref. 25).

Comparison of the HPLC and GC data in Table III shows that the mutual agreement is excellent. For the rest, we slightly prefer the former technique—which is less time-consuming (15 *vs.* 45 min)—despite the lower detection limits of GC quoted above. In practice, however, this advantage is largely offset by the much larger injection volumes that can be used in HPLC compared with GC (here, 100 *vs.* 4 μl). Besides, in the analysis of the river water samples, the baseline in GC turned out to be rather noisy, especially in the neighbourhood of the DBP peak. This caused a substantial increase of the GC detection limits compared with those for academic solutions. In summary, under the conditions stated in the Analysis section, the detection limits of DEHP and DBP in the river water samples are about 0.1 ppb in HPLC, and 0.2 and 0.1 ppb, respectively, in GC. [Recent experiments in our laboratory indicate that with the Perkin-Elmer LC55 or Pye-Unicam LC3 U.V. detector, about 4-fold lower detection limits can be obtained in HPLC.]

Future work will be directed at improving the accuracy and precision of our analyses by substantially decreasing the blank values. Obviously, to achieve this end it will be necessary to adopt a procedure which circumvents the use of sample pre-treatment. Currently, we are successfully using a trace-enrichment technique, in which 500-ml samples are pumped through a short pre-column filled with 5-μm LiChrosorb RP-18 at high flow-rates. During this step, the phthalates are concentrated into a small zone on the pre-column, from which they are subsequently eluted using a simple methanol—water gradient. With this technique, blank values for DEHP and DBP of typically less than 0.1 and 0.05 ppb have been obtained.

References

1. P. R. Graham, *Environ. Health Perspect.* **3**, 3 (1973).
2. D. B. Peakall, *Residue Rev.* **54**, 1 (1975).
3. L. Fishbein and P. W. Albro, *J. Chromatogr.* **70**, 365 (1972).

4. B. T. Johnson, D. L. Stalling, J. W. Hogan and R. A. Schoeltger, *Adv. Environ. Sci. Technol.* **8,** 283 (1977).
5. J. Autian, *Environ. Health Perspect.* **4,** 3 (1973).
6. S. P. Mathur, *J. Environ. Quality* **3,** 189 (1974).
7. L. B. Tepper, *Environ. Health Perspect.* **3** (1973).
8. C. S. Giam, H. S. Chan, G. S. Neff and E. L. Atlas, *Science* **199,** 419 (1978).
9. H. O. Sanders, F. L. Mayer Jr. and D. F. Walsh, *Environ. Res.* **6,** 84 (1973).
10. F. L. Mayer Jr. and H. O. Sanders, *Environ. Health Perspect.* **3,** 153 (1973).
11. F. L. Mayer Jr., D. L. Stalling and J. L. Johnson, *Nature* **238,** 411 (1972).
12. F. L. Mayer, *J. Fish. Res. Board Can.* **33,** 2610 (1976).
13. A. A. Belisk, W. K. Reichel and J. W. Spann, *Bull. Environ. Contam. Toxicol.* **13,** 129 (1975).
14. P. Pfuderer and A. A. Francis, *Bull. Environ. Contam. Toxicol.* **13,** 275 (1975).
15. N. Sugawara, *Bull. Environ. Contam. Toxicol.* **12,** 421 (1974).
16. H. E. Schwartz, C. J. M. Anzion, H. P. M. van Vliet, J. W. Copius Peereboom and U. A. Th. Brinkman, *Intern. J. Environ. Anal. Chem.* **6,** 133 (1979).
17. V. W. Saeger and E. S. Tucker, *Appl. Environ. Microbiol.* **31,** 29 (1976).
18. Y. Hattori, Y. Kuge and S. Nakagawa, *Miru Shori Gijutsu,* **16,** 951 (1975).
19. R. Kurane, T. Suzuki, Y. Takahara and K. Komagata, *Agric. Biol. Chem.* **41,** 1031 (1977).
20. G. Engelhardt, P. R. Wallnöfer and H. G. Rast, *Arch. Microbiol.* **109,** 109 (1976).
21. S. P. Mathur and J. W. Rouatt, *J. Environ. Quality* **4,** 273 (1975).
22. E. F. Concoran, *Environ. Health Perspect.* **3,** 13 (1973).
23. R. A. Hites, *Environ. Health Perspect.* **3,** 17 (1973).
24. D. L. Stalling, J. W. Hogan and J. L. Johnson, *Environ. Health Perspect.* **3,** 159 (1973).
25. M. Morita, H. Nakamura and S. Mimura, *Water Res.* **8,** 781 (1974).
26. B. Brownlee and W. M. J. Strachan, *Identif. Anal. Org. Pollut. Water,* p. 661 (1976), Ed. L. H. Keith, Ann Arbor, Michigan (U.S.A.).
27. H. S. Chan, *Diss. Abstr. Inst. B.* **37,** 727 (1976).
28. M. Takahashi, J. Ohgane, H. Sato and M. Sone. *Miyagi-ken Kogai Gijutsu Senta Hokuku* **4,** 39 (1976).
29. E. E. McNeil, R. Otson, W. F. Miles and F. J. M. Rajabalee, *J. Chromatogr.* **132,** 277 (1977).
30. S. Mori, *J. Chromatogr.* **129,** 53 (1976).