Development of a Method for the Determination of Phthalate Esters in Sewage Sludge Including Chromatographic Separation From Polychlorinated Biphenyls, Pesticides and Polyaromatic Hydrocarbons

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A method was developed that allows phthalate esters (PEs) to be determined in sewage sludge samples by use of gas chromatography (GC) with electron-capture detection. Discrimination of the PEs in the split injector of the GC system was detected and the resulting problems are discussed. The ultrasonic technique was optimised for extraction time and number of extractions and compared with the Soxhlet extraction technique. It was shown that extraction efficiency is slightly better with ultrasonication than using Soxhlet extraction. The Soxhlet extraction also yielded much higher blank levels. The extract clean-up was carried out by dual column liquid chromatography with neutral alumina and Florisil. In this way separation of polychlorinated biphenyls, pesticides and polyaromatic hydrocarbons from the PEs was attained. This procedure was used successfully to determine PEs in several sewage sludge samples.

Keywords: *Phthalate ester; discrimination in split injector; sewage sludge; ultrasonic extraction; liquid chromatography clean-up*

The application of municipal sewage sludge in agriculture and forestry is a good way of fertilising crops and plants and it serves to reduce the amount of sewage sludge which has to be disposed of. However, it may also enrich the soil with a variety of inorganic, *i.e.*, heavy metals, and organic compounds that may be toxic to either plants or animals.

One group of organic compounds identified in sewage sludge samples are phthalate esters (PEs). These compounds are widely used as plasticisers with a total annual production of 20×10^9 kg.[1] Owing to their high rate of production and application PEs are ubiquitous contaminants in the biosphere and have been found in environmental samples.[2–5] Although PEs are considered to be substances of low toxicity their input into the soil and potential uptake into crop plants is of special interest as hepatotoxic,[6] mutagenic[7] and carcinogenic[8] effects have been observed. For this reason it is necessary to know the amount of PEs present in sewage sludges in order to evaluate their potential enrichment in the soil.[9] As there is evidence for the occurrence of polychlorinated biphenyls (PCBs), pesticides[10] and polyaromatic hydrocarbons (PAHs)[11] in sewage sludge the separation of PEs from these compounds and other interfering substances is necessary.

Several publications that deal with the presence of phthalate esters in sewage sludge exist.[11–14] These investigations however are either only semi-quantitative[14] or they are designed for the analysis of a wide spectrum of organic priority pollutants.[11–13] The objective of this research was to develop a method for the determination of PEs in sewage sludge including the separation of interfering compounds for reliable gas chromatography with electron-capture detection (GC-ECD). Five PEs included by the Environmental Protection Agency in a list of priority pollutants were selected for this research: dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), butylbenzyl phthalate (BBP) and di-2-ethylhexyl phthalate (DEHP).

Experimental

Reagents

Solvents. Acetone, hexane and methylene chloride (Merck); all solvents were doubly distilled.

Alumina. Neutral, Brockman activity I, 70–230 mesh (Merck); heated to 200 °C for several hours, cooled in a desiccator and de-activated with 15% (m/m) doubly distilled water.

Florisil. Activated, 60–100 mesh (Merck), heated to 300 °C for at least 8 h, then cooled in a desiccator.

Phthalate esters. DMP, DEP, DBP, BBP and DEHP (Fluka).

Pesticides. 1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane (DDT), α-benzenehexachloride, γ-benzenehexachloride, aldrine and hexachlorobenzene (Baker).

PCBs. PCB 60 (Baker).

PAHs. Naphthalene, pyrene, fluoranthene, phenanthrene, benzo[*a*]pyrene, anthracene and perylene (Polyscience Corporation).

Internal standards. 9-Bromophenanthrene (BrP) and dimethyl isophthalate (DMIP) (Fluka).

Apparatus

Glassware. All items were cleaned with Extran (Merck), rinsed with doubly distilled acetone, heated at 300 °C for at least 10 h and stored in clean aluminium foil. Before use they were rinsed with the solvent to be applied.

Liquid chromatography columns, 300 × 9 mm i.d. These were filled with either 15.0 g of alumina or 7.5 g of Florisil.

Ultra turrax homogeniser.

Freeze-drying apparatus.

Ultrasonic water-bath.

Centrifuge.

Mill. A Retsch mill was used to grind the freeze-dried sewage sludge (<0.2 mm).

Rotary evaporator.

Gas chromatograph. Varian Model 3700 instrument, equipped with a ^{63}Ni electron-capture detector and a split-injector. A DB-5 fused silica capillary column (30 m, 0.25 μm film thickness) was used for the separation of the PEs. The operating conditions were as follows: injection port, 250 °C; electron-capture detector, 300 °C, column temperature, 140 °C for 3 min, increased at 12 °C min^{-1} to 260 °C, then held for 10 min; carrier gas, helium; and make-up gas, nitrogen.

Integrator. A Spectra-Physics Model SP 4290 integrator was used to measure peak areas.

Procedure

Sampling

Samples of anaerobic digested sewage sludge were collected at different sewage treatment plants in and near the town of Bayreuth in the north-east of Bavaria, FRG. The sludge was collected in clean dark glass-stoppered bottles, immediately transported to the laboratory and stored at 4 °C for not more than 4 d.

Extraction of samples

Ultrasonic extraction. Homogenise the sewage sludge with the Ultra turrax homogeniser for about 5 min. Prepare a PE standard solution in methanol. For recovery and extraction efficiency studies spike the sewage sludge with the methanol standard solution. Homogenise the samples a second time. After storage for 24 h at 4 °C for equilibration take sub-samples for freeze-drying. Grind the dried sewage sludge with a mill to obtain particles with a diameter of less than 0.2 mm. Place about 1.0 g of the dried and ground sludge into a centrifuge tube, add 40 ml of methylene chloride and place the sample in an ultrasonic water-bath for 30 min. Settle the suspended particles by centrifugation and filter the supernatant with a glass fibre filter-paper. Repeat the extraction four times with fresh solvent and concentrate the five combined extracts to *ca.* 1–2 ml.

Soxhlet extraction. Pre-extract the Soxhlet paper thimble with methylene chloride for 24 h. Extract about 1 g of the dried and ground sewage sludge for 48 h with 300 ml of methylene chloride. Evaporate the solvent extract to *ca.* 1–2 ml with a rotary evaporator.

Liquid chromatography (LC) clean-up procedure

Transfer the methylene chloride extract into hexane and concentrate the hexane phase to *ca.* 1–2 ml with a rotary evaporator. Fill a glass column with 15 g of de-activated alumina (15% water, m/m). Introduce the hexane concentrate on to the column and elute with three 30-ml fractions: (*i*) hexane, (*ii*) 10% methylene chloride in hexane and (*iii*) 50% methylene chloride in hexane. Fill a second glass column with 7.5 g of activated Florisil and pour the last fraction from the alumina column (which contains the PEs) through the Florisil column. Elute this column with 30 ml of methylene chloride followed by 30 ml of 5% acetone in methylene chloride. This last fraction contains all five of the PEs included in this study. Evaporate the last fraction with a rotary evaporator and finally under a stream of purified nitrogen to dryness. Add hexane with the internal standards DMIP and BrP and analyse by GC-ECD. Find the linear response range of the electron-capture detector and calibrate the measuring system by determining the response factors (R_F values) of the PEs with the internal standards DMIP and BrP.

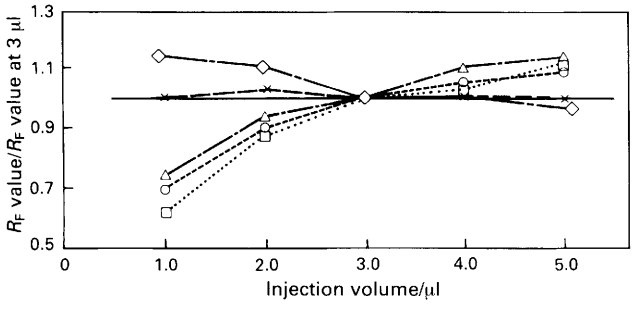

Fig. 1. R_F values with respect to BrP at different injection volumes. □, DMP; ○, DEP; △, DBP; ◇, BBP; and ×, DEHP

Results and Discussion

Discrimination in the Split-injector System

In modern GC analysis it is fairly common to use internal standards (IS) for the calibration of the GC system and for the evaluation of samples. For this purpose a response factor is calculated using the following equation:

$$\text{Response factor } (R_F) = \frac{\text{IS response}}{\text{IS concentration}} \times \frac{\text{analyte concentration}}{\text{analyte response}} \quad (1)$$

The advantage of using R_F values is that after the IS has been added to the sample extract, a knowledge of the solvent volume is no longer necessary; the injection volume should not affect the results of the quantification. To test for the latter assertion, different volumes (1–5 µl) of the same standard solution were injected several times and the R_F values with respect to BrP determined. In Fig. 1 the injection volumes are plotted against the R_F values which are normalised to the R_F value at 3 µl.

If the R_F value is independent of the injection volume, then the plots for all the PEs should give a straight line parallel to the *x*-axis. However, the R_F values, particularly those of DMP, DEP and DBP, show a different behaviour. These deviations are caused by the fact that discrimination effects due to the molecular size and polarity of the different PEs and the IS occurred in the injector port. This discrimination can arise when the more volatile sample components distil from the syringe needle at a greater rate than the less volatile

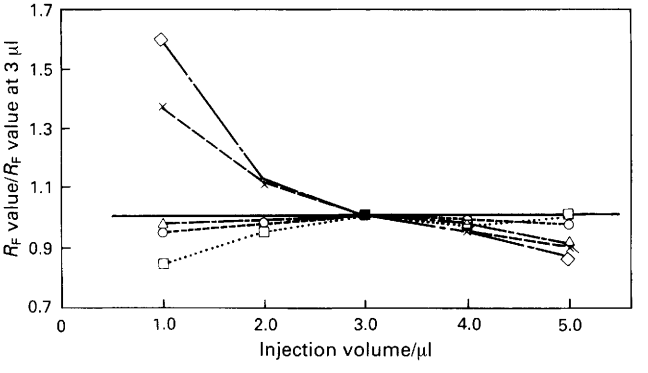

Fig. 2. R_F values with respect to DMIP at different injection volumes. □, DMP; ○, DEP; △, DBP; ◇, BBP; and ×, DEHP

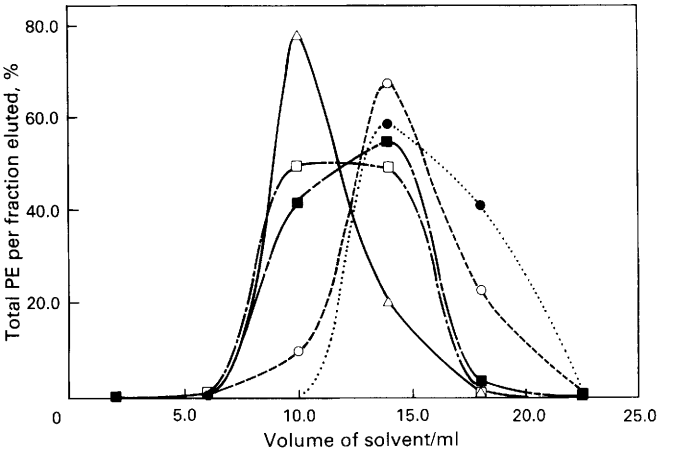

Fig. 3. Elution of PEs from alumina with 50% CH_2Cl_2 in hexane. ●, DMP; ○, DEP; □, DBP; ■, BBP; and △, DEHP

Table 1. Recovery of PEs from the alumina column (n = 5 in all instances)

PE	Recovery, %		
	x*	s†	s/x
DMP	98.0	2.4	2.5
DEP	99.5	1.7	1.7
DBP	98.5	2.7	2.8
BBP	99.0	2.4	2.4
DEHP	103.1	5.8	5.6

* Mean value.
† Standard deviation.

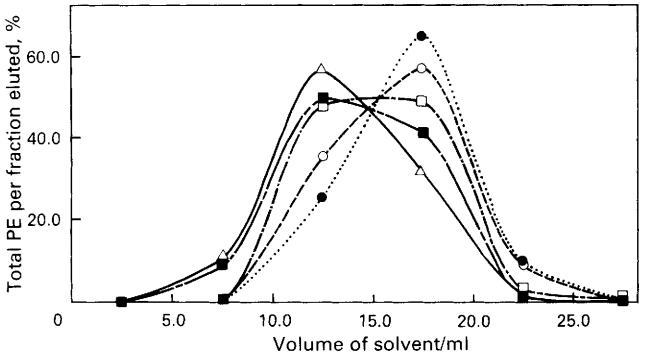

Fig. 4. Elution of PEs from Florisil with 5% acetone in CH_2Cl_2. ●, DMP; ○, DEP; □, DBP; ■, BBP; and △, DEHP

components.[15] Thus the response of the less volatile IS decreases due to discrimination and the R_F value of the more volatile PEs will decrease, as can be calculated using equation (1) and can be seen in Fig. 1. This influence of losses inside the syringe needle decreases as the size of the sample injected increases. It can also be seen that the R_F values of BBP and DEHP almost follow the ideal line. This leads to the assumption that the extent of the discrimination is very similar for BBP, DEHP and the IS BrP. For this reason BrP can be used for the quantification of BBP and DEHP but cannot be used as an IS for DMP, DEP and DBP.

An alternative IS was therefore required. As for the first IS the R_F values for the second IS, DMIP, were plotted against the injection volumes (see Fig. 2). In this instance, consequently the opposite effect is observed. The R_F values of DMP, DEP and DBP follow the ideal line fairly well, whereas those of BBP and DEHP show considerable deviation. Now the R_F values of the less volatile PEs increase with decreasing injection volumes, as the analyte responses decrease [see equation (1) and Fig. 2]. Because of the discrimination effects mentioned above DMIP was finally chosen for the quantification of DMP, DEP and DBP, whereas BBP and DEHP were determined with BrP as the IS.

Development of an LC Method for PE Clean-up

Generally sewage sludge contains large amounts of dissolved and suspended organic material. Strachan *et al.*[14] found that an average of 10.6% of sludge dry mass and 36% of the total organic carbon in sludge can be extracted by use of non-aqueous solvents. These high levels of co-extracted organic compounds, which lead to intensely coloured sample extracts, present a considerable challenge to precise and accurate determination of PEs in sludges. Also compounds interfering with the GC-ECD analysis, such as PCBs or pesticides, have to be separated from the PEs. Therefore a very selective and efficient extract clean-up procedure is necessary to produce final extracts of sufficient quality for reliable GC-ECD determination.

Table 2. Recovery of PEs from the Florisil column (n = 5 in all instances)

PE	Recovery, %		
	x	s	s/x
DMP	101.6	1.4	1.4
DEP	100.4	1.8	1.8
DBP	100.7	3.2	3.2
BBP	99.6	4.5	4.6
DEHP	103.1	5.8	5.6

Most of the methods described for the clean-up prior to GC analysis, however, were elaborated for sample matrices other than sewage sludge[16,17] or for techniques other than GC-ECD[12] and therefore are not suitable for sludge extract clean-up under the present conditions. Others require large volumes of eluents,[18] show only poor recovery[19] or use benzene,[20] which should be avoided if possible because of its carcinogenic effect. Several methods with only one LC column with different solid phases were tested in this study but none of them resulted in sufficiently clean extracts without undesirable interferences with the GC-ECD analysis. Therefore, following Russel and McDuffie[20] a modified dual LC clean-up procedure was developed. First 15% de-activated (water, m/m) alumina was used and various solvents or solvent mixtures were evaluated in order to establish a procedure for the elution of the PEs. Initially these experiments were carried out with standard solutions. After a suitable elution pattern had been found, it was then tested for clean-up efficiency and separation of PAHs, PCBs and pesticides.

To remove extremely non-polar compounds the column was flushed with 30 ml of hexane and 30 ml of 10% methylene chloride in hexane. It was established that all pesticides, PCBs and PAHs used in this study eluted in the first fraction with 30 ml of hexane. This first fraction can be further separated using a silica gel column chromatography procedure as described by McIntyre *et al.*[10] or Russel and McDuffie[20] to obtain one fraction containing the PCBs and another containing pesticides and PAHs. Another 30 ml of 50% methylene chloride in hexane eluted all of the PEs from the column. The elution patterns for the five PEs tested are shown in Fig. 3. Recovery studies were performed for the alumina column and the results are given in Table 1. Examination of the table reveals that the recoveries of the five PEs are quantitative with a small standard deviation. It was found, however, that the third fraction containing the PEs was still coloured and that interference with the GC analysis occurred. Hence the use of Florisil as a second LC column packing material was evaluated for further clean-up of the last alumina fraction. The experiments with the Florisil columns were carried out in the same manner as those with the alumina columns.

The Florisil column was eluted first with 30 ml of 50% methylene chloride in hexane and then with 30 ml of pure methylene chloride. No PEs were present in either fraction as GC-ECD revealed. For the third fraction several mixtures of acetone in methylene chloride were tested with regard to elution, recovery of the PEs and clean-up efficiency. The optimum result was found at 30 ml of 5% acetone in methylene chloride. The fractional elution of the different PEs from the Florisil column with 5% acetone in methylene chloride and the recoveries are shown in Fig. 4 and Table 2, respectively. In the final procedure the third fraction from the alumina column was introduced directly on to the Florisil column so that time could be saved by not evaporating this fraction prior to passing it through the Florisil column. This LC clean-up procedure has the following advantages compared with other methods: the solvent consumption can be

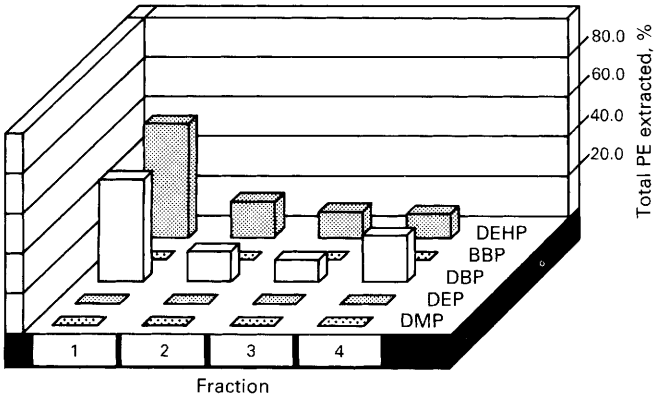

Fig. 5. Percentage recoveries of PEs from unspiked sewage sludge with successive extractions using ultrasonication

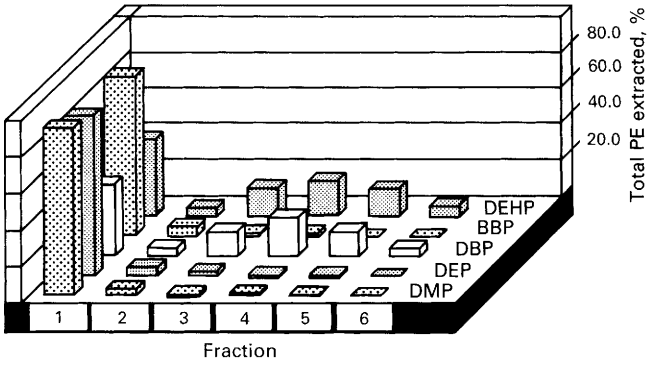

Fig. 6. Percentage recoveries of PEs from spiked sewage sludge with successive extractions using ultrasonication

kept fairly low if the complexity of the matrix is taken into account; the method allows five PEs to be determined in only one fraction for the GC analysis; it allows the separation of PEs from PCBs, pesticides and PAHs and, using the methods described above, separates PCBs from pesticides and PAHs; the recovery of the PEs is complete. Hence, a quantitative GC-ECD analysis of sewage sludge is possible without the interference of accompanying components owing to this rigorous clean-up procedure.

Optimisation of the Ultrasonic Extraction Method

Several methods are described for the extraction of sewage sludge or soil samples with organic solvents: (*i*) homogenisation and centrifugation of the liquid sludge[11,20]; (*ii*) Soxhlet extraction of liquid or dried sludge[10,21]; and (*iii*) ultrasonic extraction of dried sludge.[12,22] The first of these extraction methods was examined, but as it was hindered by the formation of resistant emulsions it was no longer taken into consideration. The ultrasonic extraction technique was then examined and optimised. Methylene chloride was chosen as the solvent because as has been previously described[11,15,22,23] it exhibits the best extraction efficiency for non-liquid matrices. To test the extraction efficiency of ultrasonication a sub-sample was extracted four times and the solvent extracts were collected and analysed separately. The percentage recoveries of the four extractions are presented in Fig. 5. Examination of the results indicates that a great proportion is recovered in the first extraction. However, a considerable amount is still recovered in the fourth extraction, which, with respect to DBP, is even greater than in the third extraction. Another sub-sample which was spiked before analysis was therefore treated in the same manner as the unspiked sub-sample (see Fig. 6).

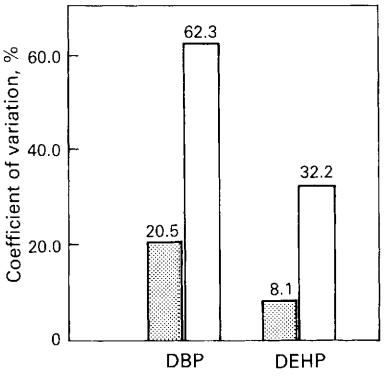

Fig. 7. Comparison of the coefficients of variation for the extraction of ground and unground sewage sludge. ▨, Ground sewage sludge (n = 5); and □, unground sewage sludge (n = 4)

Examination of these results indicates that those PEs which could not be detected in the unspiked samples, namely DMP, DEP and BBP, were detected mainly in the first two extractions with negligible amounts being recovered in the other fractions. The behaviour of DBP and DEHP is analogous to that of the unspiked sub-sample. Considerable amounts are detected in the fourth and even fifth extractions which are higher than the amounts in the second and third extractions.

The results lead to the conclusion that the spiked compounds are incorporated on to the sample matrix in a different way than the compounds already present in the sample and that both the spiked and the unspiked compounds cannot be extracted equally. Both DBP and DEHP are bound to the sludge matrix during the treatment of wastewater and, therefore, are more strongly adsorbed on to the different non-extractable organic compounds in the sludge than are the spiked PEs. These sorptive forces have different intensities. A large proportion (40–50%) can be extracted in the first extraction as can be seen in Fig. 6 and therefore behaves similarly to the spiked PEs. A more intensive or longer ultrasonication is however needed to extract the other 50–60% of the PEs. Even though only negligible and non-detectable amounts of DBP and DEHP could be extracted in the sixth and seventh extractions, respectively, the possibility that a portion of the PEs may still remain in the sludge after such an exhaustive extraction cannot be excluded. Ogner and Schnitzer[24] have found that fulvic acids can complex phthalates and that these PEs could not be re-extracted with organic solvents. In general it is therefore not possible to perform a complete extraction or recovery of the PEs originally existing in the sewage sludge. On the other hand it can be expected that the relative percentage of the PEs extracted is fairly high because of the small amounts of fulvic acids present in anaerobically digested sludges.[25] Because the total recovery cannot be determined, as mentioned above, it is important that the reproducibility of the extraction and analysis procedure is ensured. To examine the reproducibility and the influence of sludge grinding, sub-samples of ground and unground sludge were analysed and the coefficients of variation of the results for DBP and DEHP were determined (Fig. 7).

It is evident that grinding the sludge strongly reduces the coefficient of variation. This is because the heterogeneity of the untreated dried sludge is offset by grinding. Comparison with other investigations shows that in this study the reproducibility of the analysis of ground sludge is equal to or better than that reported previously for DBP and DEHP.[10,11]

Comparison of Ultrasonic and Soxhlet Extraction Techniques

In Table 3 the two extraction techniques are compared in terms of extraction efficiency, blank levels and extraction

Table 3. Comparison of Soxhlet and ultrasonic extraction of sludge samples

PE	Blank level/μg g^{-1} Soxhlet extraction	Ultrasonic extraction
DBP	50.3	1.6
DEHP	3.3	2.9
	Concentration/μg g^{-1} (standard deviation)	
	Soxhlet extraction	Ultrasonic extraction
DEHP	92.5 (6.4)	109.4 (8.1)
Extraction time/h	72	5

Table 4. Concentrations of PEs in sewage sludge samples

Location	Concentration/μg g^{-1} (dry mass) DMP	DEP	DBP	BBP	DEHP
Sonnefeld	ND*	ND	2.8	0.6	224.1
Coburg	ND	ND	2.6	0.3	73.5
Naila	ND	ND	3.1	ND	229.8
Weidhausen	ND	ND	2.3	0.5	65.8
Mitwitz	ND	ND	17.4	0.7	149.5
Rehau	ND	ND	24.1	ND	74.7
Rodach	ND	ND	236.0	0.3	133.9
Hof	ND	ND	4.1	0.2	480.6
Bayreuth	ND	ND	25.7	ND	180.7

* ND: Not detected.

time. The blank levels especially for DBP are very high for Soxhlet extraction. This is probably due to gross contamination of the extraction thimbles although they were pre-extracted for 24 h. Similar blank level problems with Soxhlet extraction have been reported by Peterson and Freeman.[22] The comparison of the extraction efficiency is therefore limited to DEHP only. It can be seen that ultrasonication was slightly better than Soxhlet extraction with almost the same standard deviations. Considering the laborious preparation time and larger solvent volumes required for Soxhlet extraction it is obvious that the Soxhlet extraction technique is not useful for the determination of PEs in sewage sludge samples.

Results for Several Sewage Sludge Samples

The analytical method described has been used to determine the amounts of PEs in several sewage sludge samples of wastewater treatment plants near the town of Bayreuth in the north-east of Bavaria, FRG. The results are given in Table 4. It can be seen that only DBP and DEHP and trace amounts of BBP are present in these sludges. These results are in agreement with those of other workers. Strachan *et al.*[14] reported concentrations of DBP and DEHP from 120 to 600 μg g^{-1}. In addition Petrasek *et al.*[27] demonstrated that DMP and DEP are almost completely degraded during the wastewater treatment process whereas only 44% of DBP and none of the DEHP are degraded. The high levels of DBP and particularly DEHP are explicable if it is taken into account that these two compounds are the most commonly used and therefore are the most widely distributed PEs in the environment.

Conclusion

The method of analysis discussed here combines a rapid and reproducible ultrasonic extraction with a rigorous clean-up procedure for the determination of PEs in complex matrices such as sewage sludge. The precision and accuracy of the method were verified and the problems with discrimination in the injector of the GC-ECD system were solved. This method is economical and has been used successfully to determine the amounts of PEs in several sewage sludge samples.

This investigation was funded by the Umweltbundesamt (UBA) Berlin. This report does not necessarily reflect the views and policies of the UBA. The author also acknowledges the assistance of S. Bär in performing some of the analytical procedures.

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Paper 0/01327G
Received March 27th, 1990
Accepted May 4th, 1990