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Assessment of Di-(2-Ethylhexyl) Phthalate (DEHP) in Municipal and Industrial Sludges of Taiwan by Supercritical Fluid Extraction (SFE) and Gas Chromatography with Electron Ionization Detection

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Abstract: In the present study, DEHP in selected municipal sewage (raw and treated) and industrial sludges of Taiwan was quantified by SFE and GC/EID. As a precursor, the optimal operating parameters of SFE were identified by laboratory experiments using DEHP spiked quartz sand. DEHP extraction efficiency was increased by the addition of modifier i.e. methanol (0.5 mL, 7%). DEHP concentration in all sewage sludge (M-1 to M-4) and a textile industry (I-6) samples were above the limit suggested by the European Union (100 mg/kg). The results suggest that excess DEHP concentration observed in the sewage sludge needs considerable attention before disposal.

Keywords: Supercritical fluid extraction, sewage sludge, soil fertilizer, di-(2-ethylhexyl) phthalate

INTRODUCTION

Di-(2-ethylhexyl) phthalate (DEHP) is widely used as a plasticizer in the production of polyvinyl chloride to enhance the flexibility of the plastics. It is commonly found in food plastic wrap, plastic tubing, floor tiles,
DEHP in Municipal Sewage and Industrial Sludges of Taiwan

furniture and automobile upholstery (1). DEHP is highly hydrophobic, persistent, and accumulates in aquatic organisms when it is present in surface water. The potential toxicity of DEHP and its metabolites have increased the focus on likely xeno-estrogenic effects, especially for patients on hemodialysis (2, 3). DEHP is not chemically bonded to the plastic polymer matrix and it can be easily leached into the environment from the plastic products (4). Moreover, residual DEHP may be present in sewage sludge due to the release of DEHP containing wastes into sewers for treatment and disposal. The land application of DEHP containing sewage sludge could lead to bioaccumulation and finally, may produce potential risk for human health (5). Therefore, it is mandatory to evaluate the fate and occurrence of DEHP in the sewage/industrial sludges before its disposal or agricultural utilization.

Many analytical methods for DEHP extraction from sludge samples involve time consuming procedures and require large amounts of toxic solvents. An attractive alternative to overcome these unfavorable characteristics may be the supercritical fluid extraction (SFE), which has increasingly been accepted for extracting organic pollutants from solid samples (6–9). The most common fluid to date has been supercritical CO2 because of its reasonable critical properties (\(T_c = 32^\circ\text{C}, P_c = 72\text{ atm}\)), high purity, low toxicity, and low cost, chemical inertness, and health acceptability. Spike recovery studies can be used for optimizing the analyte collection, but extracting real-world samples (e.g., sewage sludge) under those extraction conditions often give poor recovery (6). Therefore, extraction conditions from the spike recovery studies can only be used as a starting point for further improvement of the extraction efficiencies of real samples.

The main objective of the study was focused on assessing the DEHP in selected municipal sewage and industrial sludges of Taiwan. In addition, considerable attention was paid to optimize the supercritical fluid extraction conditions for the accurate determination of DEHP in the sludge samples.

MATERIALS AND METHODS

Chemicals

High purity DEHP (99%) and methanol (99.8%) were purchased from RDH chemicals (Germany). Hexane (99.35% purity) was obtained from Fisons (UK) and the other chemical solvents i.e. pentane, acetone, acetonitrile, dichloromethane, cyclohexane, and iso-propyl alcohol (HPLC grade) were purchased from local market. Quartz sand and sodium sulfate (\(\text{Na}_2\text{SO}_4\)) (purity 99%) were obtained from SHOWA (Japan). The gases i.e., carbon dioxide (\(\text{CO}_2\)) and nitrogen (\(\text{N}_2\)), used in the experiments were of 99.99% purity.
Sludge Samples

In the present investigation, totally 16 sludge samples were collected from different municipal sewage treatment plants and industries of Taiwan. The source and nature of the sludge samples are reported in Table 1. In addition, a clean sludge (without DEHP) was collected from Livestock Center (anaerobically digested dewatered sludge), Hsinchu, Taiwan and used for the experiments (Table 1). In order to ensure consistent quality of the sludge during the long experimental periods, the aerobic digested sludge of Min-Shen WTP (M-1) located in northern Taiwan was used for the optimization experiments of SFE. As a precursor in the evaluation of the SFE variables, DEHP was spiked in quartz sand and clean sludge (L-1) (each 100 mg/kg). Before SFE, all the samples were air-dried, homogenized, and passed through a 30-mesh sieve (10). During extraction processes, cleaned inert glass wool was placed inside the extraction cell of SFE to support the samples and N₂ gas was used to concentrate the samples as well as the extracts. Furthermore, the samples were immediately extracted (after spiking) to minimize the risk of decomposition and/or loss of analyte.

Table 1. DEHP contents in different types of sludge samples extracted by SFE

<table>
<thead>
<tr>
<th>Type of sludge</th>
<th>Sludge source</th>
<th>Sample name</th>
<th>Nature of sludge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Municipal sewage sludge</td>
<td>Min-Shen WTP</td>
<td>M-1</td>
<td>Aerobic</td>
</tr>
<tr>
<td></td>
<td>De-Hwa WTP</td>
<td>M-2</td>
<td>Anaerobic</td>
</tr>
<tr>
<td></td>
<td>Ba-Li WTP</td>
<td>M-3</td>
<td>Raw sludge</td>
</tr>
<tr>
<td></td>
<td>Nei-Hu WTP</td>
<td>M-4</td>
<td>Raw sludge</td>
</tr>
<tr>
<td>Industrials sludge</td>
<td>Beverage</td>
<td>F-1</td>
<td>Anaerobic</td>
</tr>
<tr>
<td></td>
<td>Fructose</td>
<td>F-2</td>
<td>Anaerobic</td>
</tr>
<tr>
<td></td>
<td>Beverage</td>
<td>F-3</td>
<td>Aerobic/Aerobic</td>
</tr>
<tr>
<td></td>
<td>Chemicals</td>
<td>I-1</td>
<td>Aerobic</td>
</tr>
<tr>
<td></td>
<td>Chemicals</td>
<td>I-2</td>
<td>Aerobic/Aerobic</td>
</tr>
<tr>
<td></td>
<td>Textile</td>
<td>I-3</td>
<td>Aerobic</td>
</tr>
<tr>
<td></td>
<td>Textile</td>
<td>I-4</td>
<td>Chemical (Raw)</td>
</tr>
<tr>
<td></td>
<td>Textile</td>
<td>I-5</td>
<td>Chemical (Raw)</td>
</tr>
<tr>
<td></td>
<td>Textile</td>
<td>I-6</td>
<td>Anaerobic</td>
</tr>
<tr>
<td></td>
<td>Paper</td>
<td>I-7</td>
<td>Aerobic</td>
</tr>
<tr>
<td></td>
<td>Cleaning</td>
<td>I-8</td>
<td>Chemical (Raw)</td>
</tr>
<tr>
<td></td>
<td>products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Livestock</td>
<td>Hsinchu</td>
<td>L-1</td>
<td>Anaerobic</td>
</tr>
</tbody>
</table>
DEHP Measurement and Analyses

Extraction

A Hewlett-Packard 7680T supercritical fluid extractor was used for the extraction of DEHP from various sludge samples. For spiked DEHP studies, 0.5 g of both quartz sands and powdered Na$_2$SO$_4$ were filled into a 7 mL stainless-steel extraction vessel. Exactly, 0.2 or 0.5 g of sludge sample was filled above the quartz sand and Na$_2$SO$_4$. In order to examine the effect of the extraction efficiency, Na$_2$SO$_4$ and methanol (0.5 mL) were added to the vessel at different layers. High purity CO$_2$ was passed through the extraction vessel at various oven temperatures and densities. Finally, the supercritical fluid was depressurized, collected on a Propak-Q solid trap (Hewlett-Packard), and rinsed with 1.5 mL hexane.

The effect of the extraction pressure (77–383 bar), the temperature (40–100°C), the CO$_2$ flow rate (1–4 mL/min), static (0–10 min) and dynamic extraction times (0–30 min), sample weight (0.2 and 0.5 g), DEHP content (50 and 100 mg/kg), modifier, and sample moisture content was investigated and optimal operating conditions were found out. Throughout the study, SFE experiments were carried out in triplicate and the average value was reported in each case. In order to evaluate the efficiency of SFE extraction, DEHP extraction (only with aerobically digested sludge of Min-Shen WTP) was carried out by conventional Soxhlet extraction method (7).

Analytical Technique

DEHP concentration was measured using a gas chromatography fitted with electronic ionization detector (GC/EID). Exactly, 2 μL extracted sample was injected manually into a HP-1800A GCD equipped with HP-1 capillary column (30 m × 0.25 mm LD) (J&W Scientific, Folsom, CA, USA) in splitless mode. The temperatures of the injector and the detector were maintained at 250 and 300°C, respectively. The initial oven temperature was maintained at 70°C for 1 min after sample injection. The oven temperature was then increased to 180°C at a rate of 10°C/min and held for 5 min. Then, the temperature was increased at a rate of 10°C/min to 200°C and held for 5 min. Finally, the temperature was increased at a rate of 20°C/min to 260°C and held for 10 min. Helium was used as a carrier gas at a flow rate of 1.0 mL/min and the injections were made at the splitless mode. The electron ionization detector (EID) was operated at 70 eV and scanned from 30 to 425 u at 1 scan/s. At these conditions, DEHP was identified without any of its isomers/homologues at a retention time of 23.95 min (Fig. 1a). The total ion chromatogram of clean sludge (L-1) (without DEHP) is shown in Fig 1b.

Prior to the analysis of the DEHP samples, a calibration graph was established using known concentrations of standard DEHP solutions (2.5, 7.5, 12.5, 25, 50, 75, and 100 mg/L) ($R^2 = 0.997$). The instrument detection limit (IDL)
and method detection limit (MDL) of DEHP were 0.12 mg/L and 1.74 mg/kg, respectively.

RESULTS AND DISCUSSION

The evaluation of DEHP in the municipal sewage and industrial sludges was carried out in three steps:

1. The parameters of SFE were optimized using spiked quartz sand samples under various operating conditions to obtain the maximum solubility of DEHP in pure supercritical CO$_2$; then,
2. a recovery test with clean sludge (sample L-1) was used to determine the change of extraction efficiency under complex matrix composition (compared with spiked quartz sand); finally,

Figure 1. The total ion chromatograms of DEHP extracted by SFE from (a) DEHP standard solution spiked on quartz sand (b) anaerobically digested sludge sample obtained from livestock center (L-1) (no peak identified excluding solvent peak before 14 min).
3. the sludge samples (Table 1) collected from selected places of Taiwan were analyzed using the optimized SFE parameters. Throughout the study, the experiments were conducted in triplicate and the average value was reported in each case.

**Optimization of Supercritical Fluid Extraction**

**Effect of Pressure**

The fluid pressure (P) is the main parameter governing the fluid strength for extracting analytes from different matrices. The modification in the relationship between pressure and temperature can enhance the solvating power of the supercritical fluid. In order to evaluate the effect of pressure on DEHP extraction, laboratory studies were conducted using DEHP spiked quartz sand at fluid temperature, CO₂ flow rate, static and dynamic extraction times controlled at 40°C, 2.0 mL/min, 2 min and 5 min, respectively. The effect of pressure on the recovery of DEHP is shown in Fig. 2. The increase in fluid density from 0.25 to 0.6 g/mL increased the DEHP recovery drastically from 15.29 to 91.66%. Thereafter, the increase in fluid density (0.7–0.95 g/mL) reduced DEHP recovery considerably. This can be attributed to the increase in solvating power of the fluid at higher density (11). However, the diffusion coefficient decreased at greater density and noticeably decreased the DEHP recovery (0.7 to 0.95 g/mL).

**Effect of Temperature**

At constant pressure the density of fluid decreases with increase in temperature (11). This effect became more pronounced as the compressibility...
increased (Fig. 3). Miller et al. (1997) showed that the density of CO₂ decreased from 847 to 345 kg/m³ when the fluid temperature was increased from 313 to 413 K (12). However, the increase in fluid temperature from 40 to 80°C decreased the density of fluid whereas no significant reduction in DEHP recovery was observed (Fig. 3). The decrease in fluid viscosity increases the contact between fluid and matrix and thereby, increases the diffusion coefficient. On the other hand, diffusion coefficient is the predominant factor that governs the desorption rate of DEHP from matrix and the extraction recovery compared to the solubility of DEHP in fluid.

Although increasing extraction temperature might accelerate the thermal-desorption behavior of organics from different matrix (7), it can lower the fluid strength and adversely affects its ability to accept the analytes escaped from the matrix. However, high extraction temperature can cause sample degradation or boiling during extraction process especially for volatile compounds. Fortunately, DEHP is the plasticizer with excellent stability at high temperatures, hence the effect of thermal degradation of DEHP can be ignored at the extraction temperature of 80°C or higher. From the results, the optimal temperature and extraction pressure for SFE was selected as 80°C and 202 bar, respectively (Figs. 2 and 3).

Effect of Static Extraction Time

The SFE can be performed in static, dynamic, or in combination modes. The static mode allows better penetration of the matrix by the supercritical fluid. Thus, it results in better recoveries and also reduces the dynamic extraction time (13). Hence, SFE was carried out with the combination of static and dynamic modes. The effect of static extraction time on DEHP recovery is shown in Fig. 4. The maximum DEHP recovery was obtained at a static extraction time of 5 minutes.

![Figure 3](image_url)

**Figure 3.** The relationship between extraction temperatures and DEHP recoveries extracted from quartz sand by SFE (Extraction conditions: sample weight 0.5 g, extraction pressure 202 bar, CO₂ flow rate 2.0 mL/min, static/dynamic times 2 and 5 min, respectively).
extraction time of 1 min. The increase in static extraction time beyond 1 min reduced the DEHP recovery (Fig. 4). This might be due to the back-bonding of the extracted analyte in the fluid with the matrix (14, 15).

Effect of Dynamic Extraction Time and CO\textsubscript{2} Flow Rate

For dynamic extractions of SFE (continuous flow), the fluid volume greatly influences the extraction yields and recoveries. The combination of CO\textsubscript{2} flow rate and dynamic extraction time affects both desorption rate and the solubility of analytes. The dynamic extraction stage continuously provides fresh CO\textsubscript{2} to the extraction cell and plays the role of carrier to replace the mixtures remaining in the extraction cell. Hence, CO\textsubscript{2} flow rate becomes an important factor, which affects the fluid volume passing through the extraction cell (CO\textsubscript{2} swept volumes). The effect of dynamic extraction time and CO\textsubscript{2} flow rate on DEHP recovery is shown in Table 2.

For optimizing SFE parameters, the volume of CO\textsubscript{2} consumed by the extraction process is a critical factor. Almost similar DEHP recovery was observed at CO\textsubscript{2} flow rates of 2.0, 3.0, and 4.0 mL/min. Hence, it is necessary to determine the most-fit flow rate with the highest recovery and the smallest quantity of CO\textsubscript{2} sweeping during dynamic extraction stage. The maximum DEHP recovery (>100%) was observed at CO\textsubscript{2} flow rates of 2.0, 3.0, and 4.0 mL/min after 15 minutes of dynamic extraction (Table 2). For the same recovery of DEHP, CO\textsubscript{2} flow rate of 2.0 mL/min consumed approximately 30 mL of CO\textsubscript{2} after 15 min of extraction compared to the flow rates of 3.0 mL/min (45 mL of CO\textsubscript{2} consumption) and 4.0 mL/min (60 mL of CO\textsubscript{2} consumption). Reasonably, CO\textsubscript{2} flow rate of 2.0 mL/min with 30 mL of CO\textsubscript{2} sweeping through the extraction cell with dynamic extraction time of 15 min was considered as the optimal extraction flow rate.
Table 2. Effect of flow rate on DEHP recovery during SFE of spiked quartz sand

<table>
<thead>
<tr>
<th>Dynamic extraction time (min)</th>
<th>Flow rate 1.0 mL/min</th>
<th>Flow rate 2.0 mL/min</th>
<th>Flow rate 3.0 mL/min</th>
<th>Flow rate 4.0 mL/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recovery (%)</td>
<td>RSD (%)</td>
<td>Recovery (%)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>1</td>
<td>8.34 (0.6)</td>
<td>3.36</td>
<td>25.45 (1.2)</td>
<td>10.44</td>
</tr>
<tr>
<td>2</td>
<td>17.71 (1.2)</td>
<td>13.5</td>
<td>69.63 (2.4)</td>
<td>2.03</td>
</tr>
<tr>
<td>5</td>
<td>42.56 (3)</td>
<td>3.14</td>
<td>99.07 (6)</td>
<td>0.71</td>
</tr>
<tr>
<td>10</td>
<td>52.60 (6)</td>
<td>3.90</td>
<td>99.35 (12)</td>
<td>0.38</td>
</tr>
<tr>
<td>15</td>
<td>55.01 (9)</td>
<td>8.52</td>
<td>100.44 (18)</td>
<td>0.71</td>
</tr>
<tr>
<td>20</td>
<td>75.45 (12)</td>
<td>5.46</td>
<td>102.21 (24)</td>
<td>1.45</td>
</tr>
<tr>
<td>25</td>
<td>92.06 (15)</td>
<td>3.01</td>
<td>102.26 (30)</td>
<td>2.03</td>
</tr>
<tr>
<td>30</td>
<td>98.85 (18)</td>
<td>1.37</td>
<td>99.52 (36)</td>
<td>0.53</td>
</tr>
</tbody>
</table>

Note: All results in the Table were obtained at extraction with sample weight 0.5 g, pressure 202 bar, temperature 80°C, and static extraction time 1 min and the data in the brackets are the mass (grams) of CO$_2$ consumed under different flow rates and extraction times where the supercritical CO$_2$ density is 0.6 g/mL at 80°C, 202 bar.
Effect of Sample Weight and DEHP Content

In order to evaluate the optimal sample weight required for SFE and to verify the parameters optimized in the present study, DEHP was spiked (100 mg/kg) into a real sludge sample (L-1). DEHP recovery profile under different sludge sample weight is shown in Fig. 5. At 0.2 g and 0.5 g of sample weight, 0.615 g/min and 0.182 g/min of DEHP recovery rate (100 mg/kg) was observed, respectively. Similar trends in DEHP recovery was observed at an analyte content of 50 mg/kg. Marín et al. (1998) reported an optimal sample weight of 0.2 g when DEHP was extracted from PVC resins by SFE (16). Hence, the optimal sample weight for SFE was selected as 0.2 g for further experiments.

Effect of Modifier and Water Content

Cosolvents and/or modifiers are often used to enhance the solvating power especially pure CO₂ is used as an extraction fluid. Methanol is most commonly used as a modifier in the extraction of environmental samples due to its ability in the enhancement of pollutant desorption rate from the matrix (17–20). The application of the modifier mainly depends on the composition and characteristics of the sample matrix. The solvating power of eight modifiers (n-hexane, iso-propyl alcohol, pentane, acetone, acetonitrile, dichloromethane, cyclohexane, and methanol) with diverse physical-chemical properties on DEHP recovery was investigated. Noticeable improvement in DEHP extraction efficiency was achieved when methanol (0.5 mL, 7%) was used as the modifier (data not shown).

DEHP recovery of 100% was observed after 20 min of dynamic extraction with 0.5 mL of methanol (Fig. 6) whereas, only 60% DEHP recovery

![Figure 5. Effect of sludge weight and analyte content on DEHP recoveries at various dynamic extraction times (Sludge sample used – L-1; Extraction conditions: extraction pressure 202 bar, temperature 80°C, static extraction time 1 min, CO₂ flow rate 2.0 mL/min at various dynamic extraction times).](image-url)
was observed without any modifier even at 30 min of dynamic extraction. In addition to the modifier effect, water in the sludge sample can enhance the polarity of CO₂ during SFE (19, 21). The recovery of DEHP was 73.03% after 30 min of dynamic extraction in the presence water content 5.6%. In contrast, 100% recovery of DEHP was achieved after 30 min of dynamic extraction if no water was present in the sludge sample (water content 0%). The presence of water has proven to have a negative effect and its modifier ability seemed to be limited in this study. On the other hand, Papilloud and Haerdi (1994) extracted herbicides from dried soils (22). The recoveries of herbicides were found to be two-fold when 2% water was added to the samples. The reason for this observation was the increase in solubility of herbicides in the 2% water-contained fluid due to the change in polarity and solvating power of the fluid by water. In contrast, the water content should be maintained less than 5% (22, 23) in order to avoid the formation of water film and to prevent the plugging of SFE restrictor (24).

Assessment of DEHP in Sludge Samples

The sludge samples collected from various sources of Taiwan (Table 1) was extracted using the optimized SFE and quantified by GC/EID. All the sludge samples were extracted at fluid pressure of 202 bar, temperature 80°C, CO₂ flow rate of 2.0 mL/min, static extraction time of 1 min, and dynamic extraction time of 20 min with the addition of 0.5 mL modifier. The total ion chromatogram of sludge sample (M-1) is shown in Fig. 7a and the concentration of DEHP in different sludge samples are shown in Fig. 7b. The maximum DEHP concentration of 333.13 mg/kg was observed in sample M-4 followed by I-6, M-2, M-1, M-3, and F-2. However, the

Figure 6. The effects of water content and the modifier addition on DEHP recoveries at various dynamic extraction times (Extraction conditions: sample weight 0.2 g, pressure 202 bar, temperature 80°C, CO₂ flow rate 2.0 mL/min, static extraction time 1 min at various dynamic extraction times).
DEHP concentration observed in sample F-2 was less than the standard value reported in European Union (EU) guidelines for land disposal of DEHP containing sewage sludge (Fig. 7b). Whereas, the DEHP concentration was below the detectable limit in sludge samples F-1, F-3, I-1 to I-5, and I-7 to I-9.

In order to quantify the relative standard deviation (RSD) of the SFE, the sludge sample M-1 was extracted by conventional Soxhlet extraction process analyzed using GC/EID. The corresponding concentration of DEHP observed in the sludge sample was 124.64 mg/kg (RSD 1.23%). This shows that SFE was effective in the evaluation of DEHP in biological as well as chemical sludge samples.

The concentration of DEHP observed in municipal sewage sludge of other countries is shown in Table 3 and DEHP concentration (3, 25–30) found in municipal sewage and industrial sludges of Taiwan.

![Figure 7](image.png)

**Figure 7.** (a) The total ion chromatograms of DEHP extracted by SFE from sludge sample M-1 (b) DEHP in selected sludge samples of Taiwan.
some of the sludge samples was above the maximum limit suggested by EU for land application (28). The result indicates that DEHP is a common pollutant in the environment and the conventional aerobic and anaerobic sludge treatment processes are unsuccessful in the removal of DEHP from the sewage sludge (samples M-1 and M-2) (Fig. 7b). Hence, post treatment processes such as thermal drying and composting are necessary to ensure the safe disposal/utilization of DEHP containing sludge samples.

CONCLUSIONS

DEHP concentration in municipal sewage and industrial sludges from selected places of Taiwan was appraised by SFE and GC/EID. The extraction efficiency of SFE was promising compared to the conventional extraction method. In all sewage sludge and an industrial sample DEHP levels were above the limit proposed by the EU for land application of sludge. The high DEHP concentration observed in the sewage sludge samples requires post-treatment processes to ensure safe disposal.

REFERENCES

DEHP in Municipal Sewage and Industrial Sludges of Taiwan


