ESTIMATION OF THE LEVEL OF PCDD/PCDFs IN SOIL CONTAMINATED WITH A CHLOROPHENOL FORMULATION USING SUPERCRITICAL FLUID EXTRACTION

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Introduction

Chlorophenol formulations, which contain toxic polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) as impurities, were previously widely used as fungicides. Due to the earlier use of wood preservative Ky-5 at Finnish sawmills there are about 300 sawmills that are contaminated with PCDD/PCDFs in Finland. The monitoring of the levels of PCDD/PCDFs at these sites is expensive due to high costs of the analyses, because extensive cleanup steps and use of high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) are needed. Supercritical fluid extraction (SFE) technique can provide an efficient, selective and fast extraction method after which the extract can directly be analyzed without further purification, and it has been applied to some extent in environmental analysis (1). SFE has been reported to be a comparative method to Soxhlet extraction and ultrasonic extraction for the isolation of PCDDs and PCDFs from soil (2). Selectivity in SFE can be achieved e.g. by selecting extraction pressure and temperature. Extracted components can be trapped onto a sorbent, such as activated charcoal, from which PCDD/PCDFs can selectively be eluted (3).

The objective of this study was to develope a SFE-based method by which costs of estimation of the level of PCDD/PCDF in contaminated soil could be reduced compared to those of conventional methods. We tested SFE combined with low resolution MS analyses of the concentration of 1,2,3,4,6,7,8-heptaCDF for the estimation of TCDD equivalent of PCDD/PCDFs ($TEQ_{PCDD/F}$) in soil. HeptaCDFs are among the major PCDD/Fs in Ky-5 and in contaminated soil. The results were compared with HRMS analyses of PCDD/PCDFs in Soxhlet or sonic extracts (purified by column chromatography) of the same samples.

Materials and Methods

Real environmental soil samples were selected to develope a method, since spiked samples may not give reliable recovery data. Soil samples were from Finnish sawmills contaminated by the earlier use of wood preservative Ky-5. One highly contaminated soil (soil A) was used in preliminary tests

to find optimum conditions for SFE. Soil samples with different PCDD/PCDF level were then used to test the suitability of SFE combined with HRGC/LRMS analyses of heptaCDFs for the estimation of the $TEQ_{PCDD/F}$ in soil. These soils had earlier been analyzed for PCDD/PCDFs by conventional methods, which involved Soxhlet or ultrasonic extraction, cleanup by colum chromatography (silica gel, basic alumina and activated carbon) and analyses by HRGC/HRMS (4). In Soxhlet extraction, 1 g of dried soil was extracted with toluene for 24 h and in ultrasonic extraction 1 g of dried soil was sonicated four times with toluene (10 ml) in a glass tube in an ultrasonic bath for 15 min. Soil samples were dried in oven at 40°C before extraction. All tests were performed in duplicates.

SFE was performed with a Suprex AutoPrep 44™ instrument combined with a fraction collector (AccuTrap) and a modifier pump. For SFE, a steel extraction vessel (10 ml) was filled with layers of Na_2SO_4 (5 g; Merck), dried soil (100 mg or 1 g), basic Al_2O_3 (2.5 g; Merck 1097) and Na_2SO_4 (1.5 or 2.5 g). Na_2SO_4 was used to fill the empty space of the vessel, and Al_2O_3 to prevent possible co-extraction of unwanted soil components. SFE was performed at the pressure of 400 atm using SFE grade CO_2 (99.9992 purity, Hamburg, Germany) as the supercritical fluid. The flow rate of CO_2 was 3 ml/min. The extraction chamber was kept at 100°C during the extraction. If methanol was used as a modifier, 0.5 ml was spiked to the vessel via the modifier pump before the static step and 5% methanol was added to CO_2 during dynamic extraction.

The extraction method was tested with soil A using 10 min static extraction and different dynamic extraction times without and with methanol as a modifier. The aim of these tests was to find a long enough time to extract PCDD/PCDFs and to cleanup SFE lines at the same time for the next sample. After each test with soil A, an empty vessel was extracted (dynamic 60 min with methanol) to check whether the lines were clean from PCDD/PCDFs originating from the sample. Each sample vessel was reextracted (dynamic 60 min) to check the efficiency of the first extraction.

For the collection of PCDD/PCDFs during SFE, CO_2 was passed through a solid phase trap which contained a mixture of active carbon (Carbopak C, 60/80 mesh, Supelco) and celite 545 (0.01-0.04 mm; Merck). Two different carbon/celite mixtures were used in SFE: 1:25 or 1:5 (w/w). The latter mixture was used in the comparison of SFE with other methods. The total amount of adsorbent was 0.37 g in the trap, thus the carbon content was 67 mg in carbon/celite 1:5 trap. This material is the same as is used for the carbon column cleanup of PCDD/PCDFs (4), and using this mixture in SFE trap, PCBs can be separated from PCDD/PCDFs, when hexane is used as the first eluent (5). However, if methanol is used as a modifier, the separation of PCDD/PCDFs is not complete, because some PCDD/PCDFs elute with hexane. The temperature of the trap was maintained at 40°C during both collection and desorption and that of the restrictor at 45°C. After the extraction, the PCDD/PCDFs were eluted out from the trap using xylene (10 ml + additional 10 ml for cleanup of the trap and lines) or toluene (10 ml). Toluene was used for carbon/celite 1:5 trap. In this case, a modifier was not used and impurities and PCBs were first collected with hexane (4 ml), and after toluene, the trap and lines were cleaned up with xylene (5 ml) and reconditioned with hexane (5 ml).

For analyses, the PCDD/PCDF extract was concentrated carefully down to dryness and toluene was added (100 or 1000 µl). An aliquot of the extract was mixed with an internal standard solution: PCB 206 for HRGC with electron capture detection (ECD) and decachlorinated diphenyl ether for HRGC/LRMS. HRGC/ECD analyses were performed with a Perkin Elmer AutoSystem gas chromatograph (column DB-5.625: 30 m, 0.25 mm i.d., 0.25 µm phase thickness; temperature program: 100°C (1 min), 20°C/min to 180°C, 4°C/min to 250°C (15 min), 10°C/min to 280°C (5 min); injector: 250°C, detector: 350°C; carrier gas: helium 1 ml/min). HRGC/LRMS analyses were

performed using a HP 5890 Series II gas chromatograph coupled to a HP 5971 series mass selective detector. Because only major PCDD/PCDF impurities in Ky-5 can be determined by HRGC/ECD and HRGC/LRMS, the TEQ in these analyses was calculated from the concentration of 1,2,3,4,6,7,8-heptaCDF by using a correction factor that had been obtained via HRGC/HRMS analyses of contaminated soil samples. The $TEQ_{PCDD/F}$ was calculated using international toxic equivalency factors (I-TEF) (6).

Results and Discussion

An example of the HRGC/ECD chromatogram of soil A is presented in Figure 1. This figure demonstrates that SFE extract of soil without fractionation in the trap or without extra cleanup after SFE is ready for ECD or LRMS analyses of higher chlorinated PCDD/PCDFs. For HRMS analyses of all PCDD/PCDFs after SFE, however, fractionation in the trap and additional cleanup after SFE are needed (5).

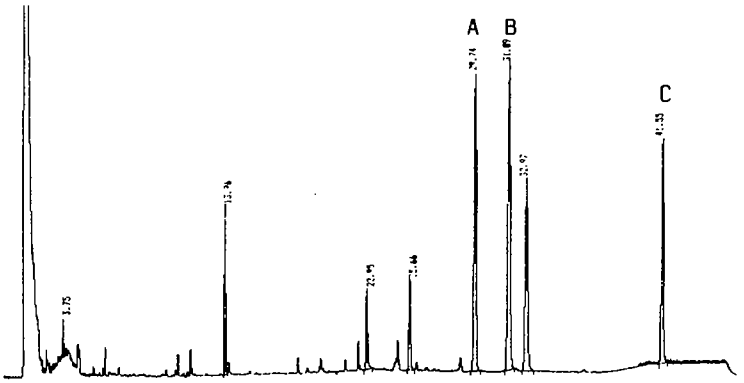

Figure 1. HRGC/ECD chromatogram of soil A extracted using SFE (400 atm, 90 min, no modifier). Peaks A, B and C are PCB 206, 1,2,3,4,6,7,8-heptaCDF, and OCDF, respectively.

Some results of preliminary tests with soil A using different extraction times at 400 atm are presented in Figure 2. Most PCDD/PCDFs were extracted during 60 min: only traces of heptaCDFs and OCDF were measured in the second extract of the sample. 60 min was also sufficient to clean the lines, because only traces of hepta- and octaCDFs were measured in the extract of an empty vessel after the sample. Also the second extraction of soil samples that were examined for the concentration of $TEQ_{PCDD/F}$ showed that 60 min extraction time was sufficient to extract most higher chlorinated PCDD/PCDFs from one gram soil samples. Only traces of heptaCDF and OCDF were detected in the second extraction of these soil.

The concentration of $TEQ_{PCDD/F}$ in some soil obtained both by SFE combined with LRMS analysis and by HRMS analyses of purified Soxhlet or ultrasonic extracts are presented in Table 1. LRMS analyses of SFE extracts can be used to estimate the $TEQ_{PCDD/F}$ in soil contaminated by chlorophenol formulations, when we only need to know whether the soil is clean, slightly or heavily contaminated. Only small sample amounts (100 mg - 1 g) are needed for SFE. In most soil samples analyzed, parallel samples from SFE gave similar results, but in some cases, the error was higher. This could be due to the fact that the quantitation was based on the external standard method and

the results were not corrected for recoveries. The use of an internal standard in SFE will correct this. Furthermore, the inhomogeinity of the sample could have affected the results.

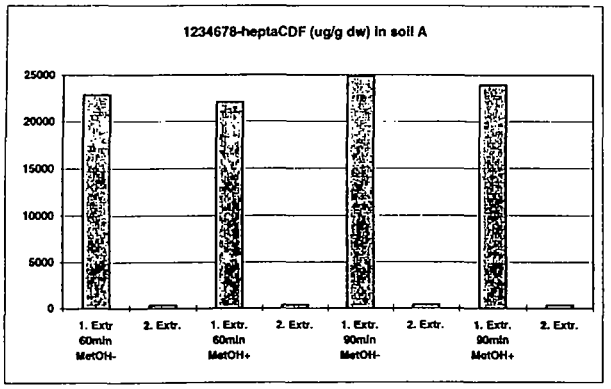

Figure 2. The concentration of 1,2,3,4,6,7,8-heptaCDF (µg/g dry weight) in different SFE tests of soil A (Trap: carbon/celite 1:25 (w/w)).

Table 1. The concentration of $TEQ_{PCDD/F}$ (pg/g dry weight) in three sawmill soil.

Soil	SFE (mean±sd)*	Soxhlet/sonic
1	1020 ± 21	600
2	30300 ±160	18800
3	63700 ± 9500	59900

*n=2; sd=standard deviation; trap: carbon/celite 1:5 (w/w)

SFE is superior, when one quickly needs to know the contamination degree of soil from sawmill sites. SFE can be performed in one hour and direct GC/ECD or LRMS analyses of the extract without concentration will reveal highly contaminated samples. In conventional methods, all toxic PCDD/PCDFs are analyzed by HRMS using expensive ^{13}C-labeled PCDD/PCDFs as internal standards. Analyses costs and analysis time can be reduced remarkedly by the use of SFE. For the estimation of the $TEQ_{PCDD/F}$ in contaminated soil via SFE combined with LRMS analyses, one internal standard for higher chlorinated PCDD/PCDFs is sufficient.

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