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Development of supercritical fluid extraction with a solid-phase trapping for fast estimation of toxic load of polychlorinated dibenzo-*p*-dioxins-dibenzofurans in sawmill soil

Mari Mannila[a,1], Jaana Koistinen[a,*], Terttu Vartiainen[a,b]

[a]*Department of Environmental Health, National Public Health Institute, P.O. Box 95, FIN-70701 Kuopio, Finland*
[b]*Department of Environmental Sciences, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland*

Abstract

A method consisting of automated supercritical fluid extraction (SFE) with simultaneous cleanup by a solid-phase trap was developed for fast analysis of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) in soil. SFE was optimised to replace conventional liquid-based methods in routine analyses of PCDD/PCDFs in sawmill soil contaminated by a chlorophenol formulation. PCDD/PCDFs were quantitatively extracted in 60 min using CO_2 at 400 atm and 100 °C without a modifier. A trap containing a small amount of activated carbon mixed with Celite efficiently collected PCDD/PCDFs after SFE. After SFE co-extracted impurities were eluted out from the trap with 4 ml of hexane and PCDD/PCDFs were eluted with 10 ml of toluene. The concentrations and TCDD-equivalent of PCDD/PCDFs corresponded to the results of traditional solvent extraction method (Soxhlet) in six sawmill soils tested. The performance of the trap was maintained over a long period of time (nearly 100 extractions).

Keywords: Sawmill soil; Supercritical fluid extraction; Solid-phase trapping; Polychlorinated dibenzo-*p*-dioxins; Polychlorinated dibenzofurans

1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) are toxic compounds formed as by-products in industrial processes and during combustion. PCDD/PCDFs are found all over the world at background levels and some areas are highly contaminated with these compounds. For example, in Finland, soils of over 250 sawmills are contaminated with PCDD/PCDFs due to the previous use of a wood preservative, KY-5, which is a chlorophenol formulation that contains PCDD/PCDFs as an impurity [1].

For risk evaluation of soil sites, the concentrations of PCDD/PCDFs in the soil are converted to one value, toxic equivalent or TCDD equivalent (TEQ). The TEQ, toxic load, is the sum of the concentrations of each PCDD/PCDF multiplied by its toxicity equivalency factor (TEF) such as I-TEF [2], which describes the toxicity of each congener related to 2,3,7,8-TCDD, the most toxic PCDD/PCDF congener. The Agency for Toxic Substances and Disease Registry (ATSDR) in the US has assigned a threshold value 50 pg TEQ/g for clean residential soil and 1000 pg TEQ/g for residential soil where

*Corresponding author. Fax: +358-17-201-265.
E-mail address: jaana.koistinen@ktl.fi (J. Koistinen).
[1]Present address: Department of Chemistry, University of Idaho, Moscow, ID 83844, USA.

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actions to limit exposure are necessary [3,4]. In Finland, the corresponding threshold values are 20 pg TEQ/g dry weight (dw) for clean soil and 500 pg TEQ/g dw for contaminated soil [5].

When one has to consider the use or remediation of soil sites contaminated with PCDD/PCDFs, large-scale analyses are usually needed. Traditionally liquid-based extraction methods such as Soxhlet and ultrasonic extractions are used in the analyses of PCDD/PCDFs [6]. Because of the unselectivity of these methods, many cleaning steps, which consume large amounts of hazardous organic solvents, are needed to separate the PCDD/PCDFs from co-extracted impurities. Supercritical fluid extraction (SFE) offers a faster and more selective extraction method for environmental analysis [7–10]. CO_2 is the most frequently used fluid in SFE due to its low critical temperature and pressure, as well as low toxicity and suitability for nonpolar pollutants. One important advantage of SFE is a cleaner extract, which may permit the direct detection of extracted compounds without further sample cleanup. Co-extraction of compounds with chemical and physical properties similar to those of target analytes, however, occurs also in SFE. For example, the co-extraction of polychlorinated biphenyls (PCBs) and polychlorinated diphenyl ethers (PCDEs) may cause detection problems in the analyses of PCDD/PCDFs, especially because the levels of PCBs and PCDEs are often two to three orders of magnitudes higher than those of PCDD/PCDFs [6,11]. In traditional sample preparation methods, PCDD/PCDFs are separated from PCBs by open column chromatography using activated carbon [6]. Many PCDD/PCDF methods are based on the enrichment and cleanup procedure developed by Smith et al. [12]. Carbon column chromatography can also be applied for the separation of planar PCBs from other PCBs [13]. When a mixture of Carboback C and Celite 545 is used as a carbon column, PCBs can be eluted out from the column with a mixture of cyclohexane and dichloromethane and PCDD/PCDFs with toluene [14].

Although SFE can offer a fast and selective alternative to conventional methods in sample preparation, its application in analyses of PCDD/PCDFs has not been quite common, so far. For example, there are only some reports on the use of SFE for analyses of PCDD/PCDFs in soil [15–18] and no detailed studies are available concerning the optimisation of SFE for PCDD/PCDFs in soil. The same is true for PCDD/PCDFs in sediment. Onuska and Terry [19] tested SFE for isolation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and Kjeller and Rappe [20] for isolation of all PCDD/PCDFs from sediment. Tetra- and pentabrominated analogues of PCDD/PCDF were reported in sediments when SFE was developed for analyses of polybrominated biphenyls and diphenylethers [21].

The lack of optimisation of SFE for PCDD/PCDFs in soil is not surprising, since there are many factors that have to be considered in their analyses starting from the capacity of SFE instruments. Analyses of PCDD/PCDFs have quite strict requirements and usually relatively large sample amounts are needed to achieve the detection. The detection limit required for most PCDD/PCDF congeners, 0.05 pg/g, is obtained using 10-g samples with 20 ml cells by SFE [22].

PCDD/PCDFs seem to be quite strongly bound to solid material and demand long extraction times and quite drastic conditions (high pressures and high temperatures) or reagents. Pressure and temperature are the most important parameters in SFE. The properties of a supercritical fluid depend on them [7–10]. SFE results of PCDD/PCDFs in soils were comparable to Soxhlet results when SFE was performed in 60 min at 450 atm and 120 °C using CO_2 modified with acetone [18], but not when SFE was performed in 30 min with CO_2 at 300 atm and 40 °C [16]. Better results in the first study could be due to higher temperature and pressure, but also acetone might have affected the results. The addition of a small amount of some solvent, i.e., a modifier, to the extraction fluid or directly on the sample has been reported to increase the extraction efficiency of several analytes [23–28]. The effect of a modifier is based due to its ability to (1) disrupt interactions between analyte and matrix and (2) to enhance the solvent power of the fluid. The extraction of PCDD/PCDFs from fly ash has proven very difficult due to strong interactions between analyte and matrix. A modifier such as benzene or pre-treatment with a strong acid, in addition to high pressure (400 atm), is needed for quantitative extraction of PCDD/PCDFs from fly ash by SFE with CO_2 or NO_2 [29,30].

During SFE, compounds can be collected from the fluid by liquid trapping or solid-phase trapping [7–

10]. With liquid trapping, the sample needs to be cleaned before analyses of PCDD/PCDFs, which makes the total analysis time longer compared to solid-phase trapping. With a solid-phase trap it is possible to automatically clean-up the sample after extraction, because different kind of analytes can be eluted with different solvents. Hengstmann et al. [15] used basic aluminium oxide and von Holst et al. [17] RP18 for solid-phase trapping in SFE of PCDD/PCDFs, but they did not elute PCDD/PCDFs separately from other compounds. Van Bavel et al. [31], instead, successfully separated non-planar and planar pollutants with PX-21 carbon after SFE of human adipose tissue. PX-21 carbon suspended on glass fiber was suggested by Tilio et al. [32] as an efficient trapping material for SFE of PCDDs, but their trap was designed to be disconnected after SFE and reverse elution with toluene was needed to collect PCDD fraction. Solid-phase trapping seems to be ideal for the analyses of PCDD/PCDFs, but the use of a modifier with solid-phase trapping is more critical, since it might disturb the adsorption and fractionation capacity of trap by changing the elution order of extracted compounds [33,34].

The aim of this study was to develop a fast and reliable automated SFE method with an activated carbon trapping to replace a conventional Soxhlet extraction method in the routine analysis of PCDD/PCDFs in soil contaminated by chlorophenols. We aimed to develop a carbon trap from which co-extracted impurities could be flushed out with a small amount of hexane before the collection of PCDD/PCDFs with a small amount of toluene. The first step was an optimisation of the absorption material in the trap, which was performed by SFE of standards. After that the extraction time and the flow-rate of supercritical fluid were optimised with real soil samples. The risk of contamination of samples in sequential extraction and the stability of the trap were also investigated. The developed SFE method was compared to the Soxhlet method.

2. Experimental

2.1. Standards

Standard mixture of [^{12}C]PCDD/PCDFs was from Chemsyn Science Laboratories (Lenexa, Kansas, US) and it contained all toxic PCDD/PCDFs. The solution of [^{12}C]PCBs was obtained from AccuStandard (New Haven, USA) and contained 28 congeners (tri-decachlorinated). The solution of [^{12}C]PCDEs was from the University of Jyväskylä (Finland) and contained 45 congeners (tetra-decachlorinated). Decachlorinated diphenyl ether from the University of Jyväskylä, Finland, was used as a recovery standard for tests with native standards. The mixture of ^{13}C-labelled PCDD/PCDFs that was used as an internal standard for soil was from Campro Scientific (The Netherlands) and contained 16 ^{13}C-labelled PCDD/PCDFs. A mixture of ^{13}C-labelled 1,2,3,4-TCDD and 1,2,3,7,8,9-HxCDD obtained from Campro Scientific (The Netherlands) was used as a recovery standard for samples.

2.2. Solvents and reagents

Solvents (hexane, toluene) were p.a. grade and were obtained from Merck (Darmstadt, Germany). Activated carbon (Carbopack C, 60/80 mesh) was obtained from Supelco (Bellefonte, USA) and Celite 545 (0.01–0.04 mm), Na_2SO_4 (p.a. grade) and Al_2O_3 (Merck 90 standardised) from Merck. Reagents, except carbon, were column-extracted with solvents and activated at 120 °C prior to use. SFE grade carbon dioxide (purity 5.2, AGA, Hamburg, Germany) was used as a fluid in SFE.

2.3. Soil samples

Six soil samples (soils A–F) originating from Finnish sawmill sites were selected for this study. The soils were known to be contaminated with PCDD/PCDFs that originate from a chlorophenol formulation (KY-5). The main compound in KY-5 is a tetrachlorophenol and the dominating PCDD/PCDFs are heptaCDFs and OCDF [1]. The samples were oven dried at 40 °C, homogenised by grinding by hand after removing large particles. Soil A and B were sieved to collect <2-mm particles before taking the subsample for analysis. The organic matter content of each soil was analysed by tempering the sample at 815 °C.

2.4. SFE

The SFE instrument used was a Suprex AutoPrep

44 combined with a fraction collector (AccuTrap) and a modifier pump. SFE conditions were as follows: extraction chamber temperature 100 °C, pressure 400 atm, flow-rate of CO_2 3 ml/min, static extraction time 10 min and dynamic time 60 min. The temperature of the restrictor was 45 °C during SFE. These conditions were based on previous experiments [35,36].

The sample (native standards or soil) was loaded in a stainless steel vessel (10 ml) for extraction. The extraction vessel was first filled with a layer of activated Na_2SO_4 (5 g) on top of which the sample was added (100 μl of solution of standards that contained 2–4 ng of each test compound or 1 g of soil). Internal standards in 100 μl of toluene (115 pg of each ^{13}C-labelled PCDD/PCDFs) were spiked on the top of the soil D before extraction. The extraction cell was finally filled with layers of basic Al_2O_3 (2 g) and Na_2SO_4 (2 g). Duplicate samples of each soil were extracted. Blank samples (extracted between soil samples as quality control for laboratory contamination) consisted of Na_2SO_4, Al_2O_3 and internal standards.

The extracted compounds were collected by a trap filled with a mixture of activated carbon and Celite 545 as a support material (total of 0.37 g of the adsorbent). The ratio of carbon and Celite was 1:5 (w/w). The temperature of the trap was maintained at 40 °C during SFE (collection and desorption steps). After SFE, the trap was flushed with different solvents: 4 ml hexane, 10 ml toluene and 5 ml xylene followed with 5 ml hexane to recondition the system (as illustrated in Table 1). Elution solvent flow-rate was 2 ml/min.

The effect of extraction time on the extraction efficiency of PCDD/PCDFs from contaminated soil was studied by sequential extraction of one soil using four 20-min periods. The effect of fluid flow-rate was studied with three soils.

2.5. Soxhlet

The Soxhlet method used in this study has been accredited by Finnish Accreditation Service and it follows the requirements in standard EN ISO/IEC 170025:2000. The Soxhlet extraction was carried out with 300 ml of toluene for at least 18 h. Extracts were purified before analyses by column chromatography using silica gel, basic aluminium, and activated carbon columns [1].

Table 1
SFE procedure and conditions

Extraction
400 atm, oven 100 °C, restrictor 45 °C
CO_2 3 ml/min, 10 min static, 60 min dynamic
↓
Solid Phase trapping (40 °C)
Activated carbon–Celite (w/w)
(A) 1:25, (B) 1:10, (C) 1:5
↓
Elution
(1) Removal of interferences; 4 ml hexane
(2) Collection of PCDD/PCDFs; 10 ml toluene
(3) Cleaning; 5 ml xylene
(4) Reconditioning; 5 ml hexane

2.6. Concentration

The SFE extracts were concentrated with a centrifugal concentrator (Jouan RC10.22) and Soxhlet extracts by rotary evaporator. The recovery standard solution was added to the toluene fraction of the SFE extracts and to the Soxhlet extracts purified by column chromatography. Decachlorinated diphenylether was used as a recovery standard for SFE of standards and a mixture of ^{13}C-labelled 1,2,3,4-TCDD and 1,2,3,7,8,9-HxCDD (40 pg/congener) for the SFE of soil. The sample was finally concentrated down to about 30 μl using nitrogen flow.

2.7. Analysis

Extracts of native standards were analysed by a high-resolution gas chromatograph (HRGC: HP 5890) coupled to a mass selective detector (HP 5971). The compounds were separated on a HP-5 column (60 m, 0.25 mm, 0.25 μm). Samples were splitlessly injected (2 μl) at 270 °C and helium (purity 5.6, AGA, Finland) was used as a carrier gas. The GC-oven temperature was held at 120 °C for 2 min, increased at 20 °C/min to 180 °C, and increased at 2 °C/min to 270 °C, where it was held for 37 min. Analysis was performed using electron impact (EI) ionisation and selected-ion monitoring (SIM) mode.

Soil samples were analysed with a HRGC (HP 6890, column DB-Dioxin: 60 m, 0.25 mm, 0.15 μm),

which was coupled to a high-resolution mass spectrometer (HRMS: VG 70-250SE, VG Analytical Manchester, UK). The oven temperature was held at 140 °C for 4 min, increased at 20 °C/min to 180 °C, and increased at 2 °C/min to 270 °C, where it was held for 41 min. In all analyses, helium (purity 5.6, AGA, Finland) was used as the carrier gas and the samples were splitlessly injected (2 μl) at 270 °C. Analyses were performed using EI ionisation and SIM mode with 10 000 resolution.

2.8. Quantitation

The concentration of each toxic PCDD/PCDF congener in soil was calculated against ^{13}C-labeled internal standards. The concentration of each congener was then multiplied with its international toxic equivalency factor (I-TEF) [2] and I-TEQ of the PCDD/PCDFs was calculated by summing the converted concentrations. The recoveries of the internal standards were calculated to evaluate the success of SFE.

3. Results and discussion

Since the preliminary studies on SFE of PCDD/PCDFs in soil with CO_2 were promising concerning isolation of PCDD/PCDFs from soil [35,36], 400 atm and 100 °C were selected as a basis for the optimisation of the SFE method. This combination of pressure and temperature results in supercritical CO_2 with a density of 0.75 g/ml, which corresponds to densities of many organic solvents [10]. Furthermore, these conditions were near those which were effective for PCDD/PCDFs in fly ash [29,37] and for PCBs in soil [38] and sediment [39,40].

As the aim was a direct analysis of PCDD/PCDFs after SFE, this study concentrated into optimisation of carbon content in the trap and elution step to obtain an automated cleaning of sample after SFE. Suitability of the optimised trap for real soil analysis was studied by SFE of PCDD/PCDFs in contaminated sawmill soil. Furthermore, the extraction time and fluid flow-rate for quantitative isolation of PCDD/PCDFs from soil was investigated. No modifier was added to CO_2 during SFE, because the earlier studies without methanol had shown comparable results to Soxhlet for PCDD/PCDFs in soil [35,36].

3.1. Trap optimisation

Optimisation of SFE was started with the optimisation of the trap with three different ratios of carbon and Celite in the trap. To find the optimum amount of carbon for the separation of PCDD/PCDFs from disturbing compounds, standards of [^{12}C]PCDD/PCDFs were extracted together with both standards of [^{12}C]PCBs and [^{12}C]PCDEs. The maximum total volume of the solution of native standards (in toluene) that was added into the vessel in each experiment was 200 μl.

Trap A (1:25) and B (1:10; carbon to Celite) proved to have too low carbon content to keep the lower chlorinated PCDD/PCDFs adsorbed during elution with hexane (4 ml), since the recoveries of the PCDD/PCDFs in the toluene (10 ml) fraction were low. Up to 80–90% of tetra- and pentaCDD/CDFs were eluted with hexane from trap A. A lower flow-rate of eluents, 1 ml/min instead of 2 ml/min, did not improve the fractionation properties of trap A. The low amount of carbon in trap A, however, effectively retained the PCDD/PCDFs during 60 min dynamic extraction, because the total recoveries (summed amount in hexane and toluene fractions) were good. The collection efficiency was also studied with this trap using different trap temperatures during collection and desorption step (5/40, 10/40, 20/20, 20/40, 20/80, 40/40, 40/80 °C, respectively), but the tested temperatures seemed not to affect to the collection capacity during dynamic extraction.

The best results concerning the separation of PCDD/PCDFs from PCBs and PCDEs were obtained with trap C (1:5; carbon to Celite) that contained the highest amount of carbon. With this trap none of the PCDD/PCDFs were found in the hexane fraction (4 ml), and the recoveries of the PCDD/PCDFs in the toluene fraction (10 ml) were high. Most of the PCBs and PCDEs eluted with hexane, except some co-PCBs, which partly eluted in the PCDD/PCDF fraction. Because the levels of co-PCBs are generally similar to the PCDD/PCDF levels and the interference they can cause in analyses

of PCDD/PCDFs is minor, this coelution was concluded to be acceptable.

Trap C was used for further optimization of trapping conditions: fractionation capacity and performance with soil samples. Fractionation properties were studied with SFE of standards by dividing the elution into smaller fractions. The elution of trap C with 1 ml fractions of hexane (4×1 ml) and toluene (5×1 ml+1×5 ml) showed that most of the tetra- and hexachlorinated PCDD/PCDFs already eluted with 3 ml toluene. Heptachlorinated PCDD/PCDFs, instead, required 5 ml toluene. This is not surprising, since it is well known that lower chlorinated compounds are more easily eluted from activated carbon than higher chlorinated compounds. OCDD and OCDF were quantitatively eluted from trap C with 10 ml of toluene. The elution of HpCDD, HpCDF, OCDD and OCDF from trap C is illustrated in Fig. 1. Only 2–3% of the amount of OCDD and OCDF found in the toluene fraction was observed in the consecutive 5 ml of xylene. This indicates that toluene is an efficient solvent for elution of PCDD/PCDFs out from trap that contains Carbopak C, whereas van Bavel et al. [31] found xylene much better than toluene for the elution of PCDD/PCDFs from PX-21 carbon.

Trap C worked also well with real soil samples (1-g samples of dried soil): 4 ml hexane was suitable for flushing of co-extracted compounds and 10 ml toluene for the collection of PCDD/PCDFs. In the case of soil samples only the toluene fraction was studied. After extraction, soil D was concentrated as a whole, whereas the extracts of other soils were diluted to 1/10 (soil B, C and F) and 1/20 (soil A and C).

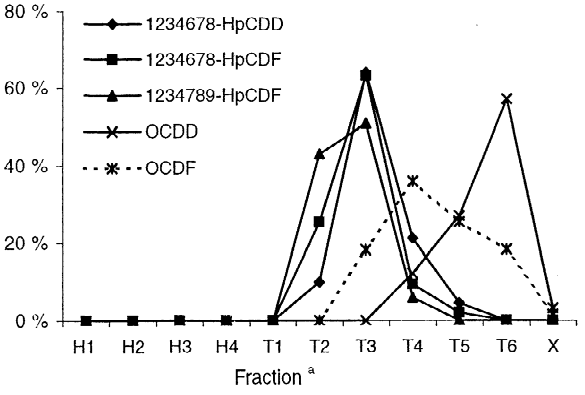

Fig. 1. Elution of higher chlorinated PCDD/PCDFs from trap C (activated carbon content 76 mg) with hexane, toluene and xylene after SFE.

The high-resolution mass chromatograms of SFE samples (directly after concentration) were identical to Soxhlet samples (after purification with three columns) showing no extra peaks. This indicates that SFE samples are clean enough for direct HRGC/HRMS analysis after extraction. Similar HRGC/HRMS chromatograms of soil samples after SFE and Soxhlet with no interfering peaks indicate good selectivity of SFE and the good fractionation capacity of the trap consisting of a mixture of Carbopak C and Celite. Analyses of blanks showed that 5 ml xylene and 5 ml hexane were enough to clean and recondition the trap and lines prior to the next sample.

Parallel to studies of the suitability of trap C for soil, the efficiency of SFE to isolate PCDD/PCDFs from soil using CO_2 without any modifier added to the fluid during dynamic mode was investigated. The effects of extraction time and fluid flow-rate were studied.

3.2. Extraction time

The extraction of PCDD/PCDFs from soil and the optimal extraction time at 400 atm and 100 °C was tested with soil A by dividing the dynamic extraction into four sequential 20-min extractions. Extraction time should be long enough to remove quantitatively extractables from matrix, as well as from the lines that connect the extraction vessel to the restrictor, because these lines are not rinsed with solvents during the elution of the trap. The majority of the extractable PCDD/PCDFs were already extracted during the first 20 min. The result of cumulative sequential SFE of soil A in terms of I-TEQ is compared to Soxhlet extraction in Fig. 2. Second 20-min period (total of 40 min) increased the cumulative results by approximately 10%, but after 40 min, the cumulative extracted amount of PCDD/PCDFs increased only slightly. During the fourth 20-min period, only 1–2% of the total extractable PCDD/PCDFs were observed.

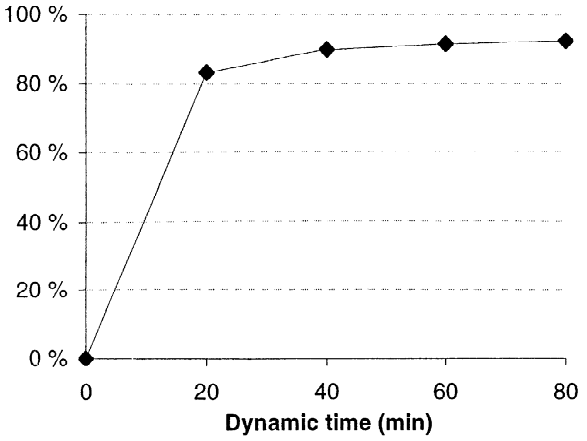

Fig. 2. Effect of dynamic SFE (at 400 atm and 100 °C, after 10 min static time) time on the recovery of PCDD/PCDFs from soil. I-TEQ of PCDD/PCDFs in soil A from SFE compared to Soxhlet result by considering Soxhlet as 100%.

3.3. Fluid flow-rate

The effect of flow-rate on the extraction efficiency of 60 min SFE was studied with three soil samples (soils A–C) by comparing flow-rate 3 ml/min to a lower flow-rate, 1 ml/min. In these tests, each sample vessel was re-extracted using 3 ml/min flow-rate. PCDD/PCDFs were efficiently extracted from soil with both fluid flow-rates tested. Lower flow-rate is usually better concerning the trapping capacity of analytes, but higher flow-rate increases extraction rate and efficiency [9]. A flow-rate of 3 ml/min did not affect the trapping capacity of trap C, since PCDD/PCDFs were not lost during collection step. Both fluid flow-rates gave identical results for I-TEQ of PCDD/PCDFs in soils A–C. The re-extraction of sample C with 3 ml/min flow-rate (after both 1- and 3-ml/min extractions) showed that only 1–5% of the SF-extractable PCDD/PCDFs were retained in the sample after first extraction. All extractable PCDD/PCDFs were efficiently carried out from the vessel even with 1 ml/min, although it does not fulfil the requirements for the amount of fluid needed for quantitative extractions (preferably 10 times the vessel volume) [7,10]. The extractions of blanks after each soil, however, revealed that up to five times more PCDD/PCDFs (mainly HpCDF and OCDF) remained in lines of the instrument after 1 than after 3 ml/min flow-rate. The concentrations of PCDD/PCDFs in the most contaminated blanks (after 1 ml/min flow-rate) on the other hand were only 1% of the PCDD/PCDF concentrations measured in the previous soil sample. Thus when the PCDD/PCDF concentrations of the consecutive samples are at the same level, the contamination is most likely insignificant.

3.4. Reproducibility of SFE and recoveries of [^{13}C]PCDD/PCDFs

The reproducibility of the SFE method was studied by comparing the results of PCDD/PCDFs in five replicates of soil D (Table 2). This sample was selected to study reproducibility, because it contained the lowest levels of PCDD/PCDFs. Furthermore, with this sample unlike other soils the internal standard addition was possible before SFE because the low levels of PCDD/PCDFs allowed a direct MS analysis without the dilution of the sample. The developed SFE method showed good reproducibility: the relative standard deviations (RSD) of the individual PCDD/PCDFs varied between 3 and 20% and the RSD of I-TEQs of PCDD/PCDFs was 14%. The RSDs of the recoveries of the internal standards of soil D and blanks were acceptable being below 10% for most PCDD/PCDFs.

The recoveries of [^{13}C]PCDD/PCDFs in toluene

Table 2
Recoveries of [^{13}C]PCDD/PCDFs for soil D and its blanks after SFE with trap C (for SFE conditions see text)

	Soil D (n=5) Mean (SD)	Blank (n=5) Mean (SD)
[^{13}C]2,3,7,8-TCDD	83 (3)	85 (7)
[^{13}C]1,2,3,7,8-PeCDD	97 (3)	88 (8)
^{13}C-1,2,3,4,7,8-HxCDD	69 (5)	88 (1)
[^{13}C]1,2,3,6,7,8-HxCDD	75 (4)	87 (2)
[^{13}C]1,2,3,4,6,7,8-HpCDD	76 (10)	85 (3)
[^{13}C]OCDD	62 (6)	78 (3)
[^{13}C]2,3,7,8-TCDF	50 (7)	76 (9)
[^{13}C]1,2,3,7,8-PeCDF	62 (3)	86 (10)
[^{13}C]2,3,4,7,8-PeCDF	91 (2)	82 (9)
[^{13}C]1,2,3,4,7,8-HxCDF	79 (3)	92 (6)
[^{13}C]1,2,3,6,7,8-HxCDF	78 (3)	89 (1)
[^{13}C]2,3,4,6,7,8-HxCDF	72 (2)	86 (3)
[^{13}C]1,2,3,7,8,9-HxCDF	73 (4)	81 (5)
[^{13}C]1,2,3,4,6,7,8-HpCDF	65 (6)	86 (4)
[^{13}C]1,2,3,4,7,8,9-HpCDF	71 (3)	81 (4)

fraction were comparatively good for all congeners (62–97%), except for 2,3,7,8-TCDF (50%) (Table 2). The lower recoveries of ^{13}C-labeled 2,3,7,8-TCDF in soil D could be due to the matrix effect, because this congener was not found in the hexane fraction and the recoveries in the blank were good (67–85%). We have noticed with some samples that certain PCDD/PCDFs are not ionised well in mass spectrometer if there are matrix impurities that coelute at the same time. In some cases better ionisation is obtained in a re-run of the sample, but usually the problem is solved by an extra clean-up. However, also the Soxhlet recovery of ^{13}C-labeled 2,3,7,8-TCDF from soil D was low (50%), even though the sample was purified with three columns before the MS analysis. This indicates the challenge of PCDD/PCDF analysis due to the low level of analyte and thus increased effect of co-elutive compounds from matrix.

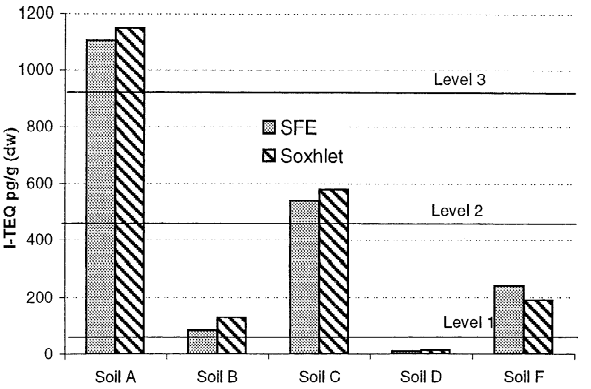

Fig. 3. Comparison of SFE and Soxhlet in the determination of I-TEQ of PCDD/PCDFs in sawmill soil. Level 1, 50 pg/g; ATSDR limit value for clean soil [3,4]. Level 2, 500 pg/g, Finnish limit value for contaminated soil [5]. Level 3, 1000 pg/g; ATSDR limit value for contaminated soil [3,4].

3.5. Performance of trap during use

The trapping and fractionation ability of carbon trap over a long period of time was studied by repeating the extraction of soil F with the same trap. Five extractions of soil F were performed during 11 months. The repeated extractions of soil F with the same trap showed that the same adsorption material can be used for several samples without loosing the trapping and fractionation properties of the trap. There were no significant differences between the results of extractions performed with a comparatively new trap (after extraction of 20 samples) or with a trap that had been used for over 70 samples. This indicates that the adsorption capacity of the trap can maintain good over a long period of time. This finding is similar to the results of van Bavel et al. [32]. The trap they used worked well over 40 human adipose samples.

3.6. SFE versus traditional methods

The measured concentrations of PCDD/PCDFs in soils and the reproducibility of SFE corresponded to those of Soxhlet extraction (Fig. 3). SFE values were between 65 and 126% of the value obtained by Soxhlet. In soil D the concentrations of PCDD/PCDFs expressed as I-TEQs were below the US (ATSDR) and Finnish threshold values for clean soil (below 50 or 20 pg I-TEQ/g dw). Soil C was close to the Finnish limit defined for contaminated soil (500 pg I-TEQ/g dw), whereas the level in soil A corresponded to the ATSDR limit (1000 pg I-TEQ/g dw). Also soils B, E and F contained elevated levels of PCDD/PCDFs. The TEQ was highest in soil E (7000 pg I-TEQ/g dw), being over five times greater than the value assigned by ATSDR for contaminated soil (1000 pg I-TEQ/g dw). SFE reproducibility in the estimation of the toxicity of the studied samples was comparable to the Soxhlet; RSDs of I-TEQ with both methods were between 5 and 25%. These results show that developed SFE method is reliable for the estimation of PCDD/PCDF level in both high and low contaminated soil samples. SFE has earlier proven competitive to traditional methods in terms of both accuracy and precision of PCDD/PCDFs in municipal fly ash [37,41], as well as for PCBs in soil [38] and sediment [39,40,42].

Organic matter content did not have any correlation to the SFE efficiency with the studied soil samples. The amount of organic material was 43% in soil A, 13% in soil C, 26% in soil F, and below 5% in soils B, D and E. The nature of organic matter (such as its rigidity and polarity) is believed to be a

more important factor causing sorption of organic compounds to the matrix than the quantity of organic matter [43]. The extraction of PCDD/PCDFs from soil is generally considered challenging due to strong adsorption between the matrix and analytes. The binding strength of the organic contaminant in a sample matrix, however, is dependent on multiple sample characteristics, and simple projections about the extractability of certain compounds from the matrix cannot be made [43,44].

Similar results of SFE and Soxhlet indicate that addition of a modifier, such as methanol, which is the most widely used modifier in SFE [7], is not necessary to CO_2 during SFE for the quantitative extraction of PCDD/PCDFs from soil contaminated by a chlorophenol formulation like KY-5. Furthermore, in the case of soils contaminated with chlorophenols, the extraction efficiency of PCDD/PCDFs might be affected by other substances that can act as a modifier.

Our results showed that addition of toluene to the vessel as a modifier is not necessary for the isolation of PCDD/PCDFs from Finnish sawmill soil contaminated by chlorophenols. Also PCBs have been successfully extracted from soil without a modifier by Lee and Peart [39], whereas Windal et al. [45] reported that SFE of PCDD/PCDFs from fly ash was not successful without addition of toluene as a solvent for internal standards.

Comparable results of the developed SFE method to conventional methods both in terms of the TEQ of PCDD/PCDFs and reproducibility indicate that SFE method is suitable for the fast determination of the dioxin load in sawmill soil contaminated by chlorophenols. Even with 1-g sample, it is possible to estimate the 'purity' of soil (i.e., the I-TEQ is <20 pg/g dw). If the levels of PCDD/PCDFs in the consecutive samples are similar, the risk of contamination of the instrument is insignificant, but if there are orders of magnitudes differences in the levels of PCDD/PCDFs, the cleaner sample should be extracted first. This is, however, not possible in the case of unknown samples. If a high-level sample has been extracted before a low-level sample, the extraction of cleaner sample should be repeated. This, of course, lowers to some extent the capacity of SFE performance, but the reduction in solvent consumption and time are so high with SFE, that it still is beneficial method over liquid-based techniques.

4. Conclusions

The developed SFE method with an automated extraction, trapping and on-line cleaning by fractionation worked well with soil samples and can offer a fast and economic way to analyse PCDD/PCDFs in sawmill soil. SFE at pressure 400 atm and temperature 100 °C using CO_2 without a modifier is sufficient for the quantitative isolation of PCDD/PCDFs from soil contaminated by chlorophenols and for the estimation of the TEQ of PCDD/PCDFs. No additional clean-up procedure is needed with this SFE method and the use of reagents and the exposure of laboratory personnel to hazardous chemicals are minimal. An adsorption material containing only 76 mg of Carbopak C carbon (mixed with Celite 545 in the ratio of 1:5) is sufficient to collect PCDD/PCDFs during SFE and allows their elution with a minimal amount of solvents (total consumption 25 ml). Most critical interfering compounds, PCBs and PCDEs, are effectively isolated from PCDD/PCDFs using the developed procedure. Tests with standards showed that the addition of internal standards to the sample can be performed in 200 μl toluene and still maintain fractionation capacity of the trap. The capacity of the trap is good permitting over 70 extractions with the same trap.

The major critical point of the developed method is that each sample goes through the same lines and adsorption material and the contamination of the following sample can occur. The risk can be minimised by (1) using sufficiently high flow-rate (3 ml/min) and (2) extracting blank samples between soil samples. To keep lines pure and the amount of possible re-extractions reasonable, we suggest a new blank after a sequence of three soil samples. With the developed SFE method soil having PCDD/PCDFs below 20 pg I-TEQ/g, the lowest limit value for contaminated soil, can be reliably analysed using only 1 g of soil. If lower detection limits for PCDD/PCDFs are, however, desired, higher sample amounts are needed and the method including trap-

ping and cleaning steps should be re-optimised at new conditions.

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