Simple and surprisingly effective one-step extraction-cleanup by Soxflo for DDT and its metabolites from environmental samples

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Abstract

A pilot study found that DDT breakdown at the GC inlet was extensive in extracts from some—but not all—samples with high organic carbon contents. However, DDT losses could be prevented with a one-step extraction-cleanup in the Soxflo instrument with dichloromethane and charcoal. This dry-column procedure took 1 h at room temperature. It was tested on spiked soil and peat samples and validated with certified soil and sediment reference materials. Spike recoveries from freshly spiked samples ranged from 79 to 111% at 20–4000 µg/kg concentrations. Recoveries from the real-world CRMs were 99.7–100.2% of DDT, 89.7–90.4% of DDD and 89.6–107.9% of DDE. It was concluded that charcoal cleanups should be used routinely during surveys for environmental DDX pollution in order to mitigate against unpredictable matrix-enhanced breakdown in the GC.
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1. Introduction

Extensive information exists on the negative effects of DDT and its metabolites on the environment and health [1–5]. Recent research revealed that certain edible plants, including some common vegetables, selectively take up persistent organic pollutants (e.g. DDE, chlordane, aldrin, heptachlor, polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans) [6,7]. Only limited research has so far been conducted on one such bioaccumulator, dandelion, which is increasingly being used as a vegetable and in salad within the EU. Since the banning of DDT in the USA and Europe, research into the environmental fate of DDT has decreased and relatively few data now exist on soil DDT levels after long periods of application [8]. It was shown recently that DDT was one of the main pollutants in agricultural soils of central Germany [9] and in Dutch canal sediments [10]. As

DDT binds to soil organic matter and can leach into the soil profile [11,12], soils that had not been cultivated for 13 years had greater concentrations of organochlorine insecticides at the 15–20 cm depth than near the surface [6]. Furthermore, the relatively high volatility of DDT can result in seasonal fluctuations along the soil profile, which may need to be taken into account when devising sampling schemes (our unpublished observations). The long-term persistence of these pesticides, therefore, still poses problems for the environment, for farming in general and for organic farming in particular. Environmental management decisions require accurate data as soil DDT/DDD and DDT/DDE ratios have been used to distinguish between previous and current pesticide inputs [9,13]. Robust methods are, therefore, needed to monitor their environmental fate and potential for bioaccumulation along the whole food chain, i.e. in soils/sediments–plants/animal products [14–16].

The analysis of DDXs still poses surprising challenges and it has been pointed out that high levels may go undetected in many environmental samples [3]. Considerable evi-

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dence exists of analytical problems and poor DDT data: only 71% of participants achieved satisfactory z-scores for DDT analysis in an international proficiency testing scheme for foods and feeds [17]. Amongst the likely reasons for this are slight variations in the water content of the silica used during the cleanup step [18] and DDT breakdown at the GC inlet, which can result from hot injection ports, contamination by co-extracted organic compounds or matrix interferences [3,19–22].

Recent developments for extracting DDXs from soils and sediments include sonication, microwave-assisted extraction [21–23], pressurised liquid extraction (PLE) [24–26] and supercritical fluid extraction (SFE) [27–30]. High soil organic matter (OM) and clay contents tend to pose particular problems for DDX extractions [1,29], but unacceptably low DDX recoveries of 42–70% have also been reported at 100 μg/kg by ultrasonic extraction from a spiked sandy, low OM soil [31]. Hubert et al. [32] suggested that PLE yielded better results than ultrasonic or Soxhlet extractions for DDXs once operating variables were optimised. However, the complexities of method development for PLE and SFE extractions and their high cost pose considerable disadvantages [33]. Although a suite of techniques now exists for fast extractions, cleanup steps still tend to be time-consuming and problematic. One or more cleanup steps are often used and involve activated florisil, silica and sodium sulfate but require careful standardisation [34].

We recently developed a dry-column procedure, the Soxflo (SoF) technique, to extract fat from foods and feeds and terpenoids from plants [35,36]. This proved an efficient and fast technique (ca. 1 h) in which a solvent is passed just once through a sample that is packed into the form of a dry column. As extractions take place at room temperature, it is a mild and environmentally friendly technique requiring neither heating nor cooling water. The SoF technique gave excellent yields with low relative standard deviations compared to Soxhlet extractions and consensus values for CRM samples. Here we report a simple and effective one-step extraction-cleanup, based on the Soxflo technique, for DDT and its metabolites from soil, peat and sediment samples.

2. Experimental

2.1. Reagents and apparatus

Dichloromethane (DCM; HPLC grade), hexane (residue analysis grade) and charcoal (activated grade) were used as received from Fisher Scientific (Loughborough, UK). p,p'-DDT (98%), p,p'-DDD (97%) and p,p'-DDE (99%) and the internal standard, hexachlorobenzene (97%), were from Aldrich Chemicals (Poole, UK). Certified reference materials (CRMs), a sandy loam soil (CRM804-050) and a marine sediment (New York/New Jersey Waterway Sediment, SRM 1944; 4.4% organic carbon), were from LGC Promochem (Teddington, UK). Cellulose disks (Scientific and Technical

Supplies Ltd., Newmarket, UK) were extracted with DCM and air-dried and used in the sample holders of the Soxflo instrument (Scientific & Technical Supplies Ltd., Newmarket, UK). Round bottom flasks and Soxflo stainless steel sample holders were washed in hot water, rinsed with distilled water and oven-dried at 100 °C.

2.2. Soil samples

An unpolluted alluvial soil (Soil Series Black; [37]) was collected from the University Farm at Sonning in October 1999, freeze-dried, sieved (<1 mm) and then ball milled for 5 min. Polluted, weathered coastal soils were provided by the Environment Agency in respect of an investigation of land that had been sprayed with DDT regularly in the 1960s to control mosquitoes. Soils were air dried at r.t. and ground (<2 mm). Soil properties are described in Table 1. Irish moss peat was purchased in a local garden centre (Homebase Ltd., Reading, UK).

2.3. Soxhlet extractions

Samples (10 g) were extracted with DCM (150 ml) for 5 h. Extracts were evaporated just to dryness on a rotary evaporator below 35 °C and taken up in DCM (5 ml), spiked with 5 μl of the internal standard (hexachlorobenzene, 1000 μg/ml) and subjected to gas chromatography–mass spectrometry (GC–MS) analysis.

2.4. Soxflo extractions

First, a layer of charcoal (2 g for soil and peat extractions; 4 g for sediment extractions) was sandwiched between two cellulose disks at the bottom of the stainless steel Soxflo sample holder (25 mm diameter, 65 mm length). Then the samples, soil (10 g), peat (4 g) or CRMs (5 g), were placed above the charcoal layer and capped with a third cellulose disk at the top. This dry column of charcoal plus sample was then firmly compressed by hand with a plunger as described previously [35]. The sample holder was then inserted into the SoF instrument which consists of: (i) a solvent reservoir at the top that is connected to a small pump, (ii) a tightly fitting support for the sample holder, and at the bottom (iii) a connector for a round bottom flask to collect the eluant.

Replicate samples were extracted with DCM (70 ml) at a flow rate of 1 drop/s (extractions lasted for 60 min). The solvent (70 ml) is passed just once through the sample column. Any residual solvent is pushed through the sample by a peristaltic pump at the end of an extraction. Extracts were evaporated just to dryness on a rotary evaporator below 35 °C and taken up in DCM (5 ml), spiked with 5 μl of the internal standard (hexachlorobenzene, 1000 μg/ml) and subjected to GC–MS analysis. External standards ranged from 20 to 1000 (μg/ml) of DDT, DDD and DDE.

Table 1
Soil properties

Soil numbers	Organic C (g/100 g)	Total N (g/100 g)	CEC (meq/100 g)	Sand/silt/clay (%)
Polluted soils				
1	2.19	0.203	14.6	22/56/22
2	3.19	0.281	17.0	38/42/20
3	3.95	0.384	26.5	20/42/38
4	4.80	0.454	22.0	52/38/10
5	6.40	0.681	30.9	44/34/22
Unpolluted soil				
6	6.66	0.705	47.7	33/5/31

2.5. Spiking method

The unpolluted soil no. 6 (50 g, ball milled), the polluted soil no. 1 (50 g, <2 mm) or Irish moss peat (50 g, <2 mm) were placed in a 1 l pearl shaped flask. Standard solutions (50 µl) containing DDT, DDD, DDE (20–4000 µg/ml) were added to 600 ml of hexane to give final spike concentrations of 20–4000 µg/kg soil or peat. The hexane solution was added to the soil or peat and rotated slowly for 1 h on a rotary evaporator at ambient temperature and pressure. Solvent was then evaporated at 40 °C under nitrogen (without vacuum) to remove most of the solvent. When almost dry, nitrogen was switched off and the flask rotated until dry.

2.6. Gas chromatography–mass spectrometry

DCM extracts (2 µl) were injected into the GC–MS system (Carlo Erba GC8000 interfaced with a Fisons MD800 quadrupole mass spectrometer). The GC instrument was fitted with a splitless injector and equipped with a Varian Chrompack WCOT fused silica column (CP-Sil 8CB low bleed/MS, 30 m × 0.25 mm, df = 0.25 µm; cat no. CP5860; Chrompack UK Ltd., London, UK). The injector tempera-

ture was 150 °C; the oven temperature was programmed to rise at 15 °C/min from 150 to 270 °C in the first 8 min and then kept constant for another 5 min. Helium (Premier grade; Air Products, Crewe) was used as the carrier gas set at 140 kPa, flow rate was 1.2 ml/min. Interface temperature was 251 °C. MS conditions were: EI positive mode, ionisation energy was 70 eV, ion source temperature was 200 °C, source current was 967 µA, trap current was 122 µA, filament current was 4.26 A, scan rate was 2 scans/s with 50–210 amu/scan.

Characterisation of compounds was based on GC retention times, computer matching with the Wiley6 library (acceptable matches >900), visual comparison of the fragmentation patterns and by comparison with authentic compounds.

2.7. GC–MS analysis

Positive EI: DDT and DDD were detected by selective ion recording of fragment ions at 235 and 237 m/z and DDE was detected from ions at 246, 248 and 318 m/z.

2.8. Statistical analysis

Data were analysed using INSTAT Plus version 1.5 [38].

Table 2
Comparison of Soxhlet and Soxflo extractions of an unpolluted soil (soil no. 6) spiked with DDT, DDD, DDE (µg/kg)

Spike concentration		DDT Mean	DDT S.D.[a]; R.S.D.	DDD Mean	DDD S.D.; R.S.D.	DDE Mean	DDE S.D.; R.S.D.
Soxhlet							
500	Found	423	76.3; 18.1	473	118.7; 25.1	536	130.1; 24.3
	Recovered (%)	84.5		94.5		107.2	
200	Found	233	58.1; 25.0	244	64.8; 26.6	272	66.2; 24.3
	Recovered (%)	116.4		122.1		136.1	
100	Found	95.8	17.0; 17.7	96.4	17.5; 18.2	104	12.9; 12.4
	Recovered (%)	95.8		96.4		104.2	
Soxflo							
500	Found	443	15.5; 3.5	433	47.2; 10.9	470	30.4; 6.5
	Recovered (%)	88.7		86.6		94.0	
200	Found	179	16.0; 8.9	179	17.1; 9.5	186	14.4; 7.7
	Recovered (%)	89.6		89.5		93.2	
100	Found	89.4	13.2; 14.8	89.4	16.7; 18.6	86.0	12.3; 14.3
	Recovered (%)	89.4		89.4		86.0	

[a] $n = 5$.

3. Results and discussion

3.1. Soil characteristics

Table 1 lists the properties of the soil samples used in this study. These samples included an unpolluted soil and well-weathered, polluted soils. Soil carbon contents ranged from 2.2 to 6.7%, CEC from 15 to 48% and clay contents from 10 to 38%.

3.2. Pilot study: Soxflo versus Soxhlet extractions of a spiked soil sample

The feasibility of the SoF procedure for extracting DDX from soils was tested in a preliminary experiment against Soxhlet extractions. An unpolluted soil from the University farm was spiked with DDT, DDD and DDE concentrations of 100–500 µg/kg. Soxhlet recoveries ranged from 85 to 136% (with R.S.D.-values from 12.4 to 26.6; Table 2) and SoF recoveries from 86 to 94% (with R.S.D.-values from 4 to 19). The SoF technique produced slightly better results as there were no unacceptably high recoveries and R.S.D. values were slightly lower. These results concurred with findings from a previous SoF–Soxhlet comparison [36].

Soxhlet extractions are often used because they give high yields against which new extraction methods are compared. However, Soxhlet extractions can yield large amounts of co-extractants, which may produce too high values for these pesticides despite cleanup and ECD detection [1,27]. For example, Van der Velde et al. found that Soxhlet extractions, compared to solvent extractions at r.t., tended to yield unacceptably high recoveries of DDX (up to 164%) from freshly
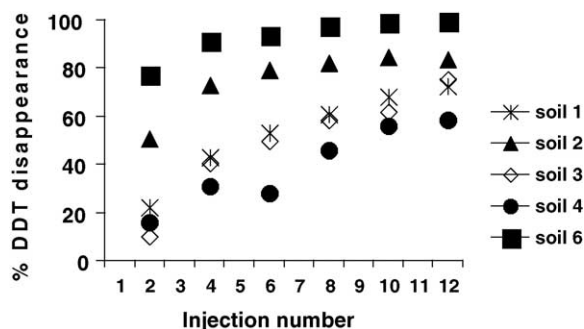

Fig. 1. Effect of soil extracts on measured DDT concentrations in performance evaluation standards (PES). Soils 1–3 had matching PES of 0.20 µg DDT/ml; soil 4 had PES of 2.00 µg DDT/ml; soil 5 had PES of 10.0 µg DDT/ml. Soil dichloromethane extracts were injected in the odd-numbered positions.

spiked sand and peat soils and required additional cleanup steps [27,39,40].

3.3. Matrix-enhanced degradation of DDT in the absence of a cleanup

During a survey for DDX pollution in some coastal soils, which had been sprayed in the 1960s to control mosquitoes, the inclusion of a performance evaluation standard (PES) revealed large losses of DDT in the GC inlet. Matrix-enhanced degradation of DDT occurred in some, but not all, soil extracts and had not been detected during the pilot study that compared SoF and Soxhlet recoveries from a spiked soil (Table 2). These matrix interferences caused PES DDT losses of up to 100% (Fig. 1) when SoF soil extracts without cleanup were injected between PES standards (note: a new GC injection liner was used for each series of soil extracts and PES stan-

Table 3
Effect of soil:charcoal ratios in Soxflo column on measured DDT, DDD and DDE concentrations in soil (µg/kg)

Soil number	Soil:charcoal mass ratio	DDT		DDD		DDE	
		Mean	S.D.[a]; R.S.D.	Mean	S.D.; R.S.D.	Mean	S.D.; R.S.D.
2	10:2	161	16.9; 10.5	19.9	4.65; 23.4	42.3[a]	2.38; 5.6
	5:2	139	20.4; 14.7	18.1	4.68; 25.9	33.7[b]	2.30; 6.8
	5:4	147	15.0; 10.3	16.6	2.33; 14.1	34.0[b]	2.75; 8.1
3	10:2	148	2.0; 1.4	28.9	6.32; 21.9	140	6.1; 4.4
	5:2	140	11.6; 8.3	28.9	3.37; 11.7	128	14.4; 11.3
	5:4	142	23.9; 16.8	25.4	5.83; 23.0	132	27.5; 20.8
4	10:2	1855	99.9; 5.4	244[a]	22.6; 9.3	2245	139.1; 6.2
	5:2	1831	92.0; 5.0	200[b]	15.0; 7.5	2185	84.7; 3.9
	5:4	1848	63.6; 3.4	228[a,b]	19.3; 8.5	2357	99.5; 4.2
5	10:2	5660[a]	1349; 23.8	12262[a]	750; 6.1	836[a,b]	55.2; 6.6
	5:2	8107[b]	278; 3.4	10926[a,b]	810; 7.4	890[a]	28.3; 3.2
	5:4	6241[a]	166; 2.7	9878[b]	234; 2.4	806[b]	21.0; 2.6
Average R.S.D. values							
	10:2		10.3		15.2		5.7
	5:2		7.9		13.1		6.3
	5:4		8.3		12.0		8.9

Values in the same column followed by different letters are significantly different ($p < 0.05$).
[a] $n = 3$.

dards). Interestingly, Foreman and Gates [3] also reported large losses, i.e. up to 65% matrix-enhanced DDT breakdown at the GC inlet, during the analysis of some natural water samples and sediment extracts with concomitant increases in DDD and DDE peaks [3,21].

3.4. Development of a charcoal cleanup procedure

Whilst several techniques exist for fast DDX extractions, cleanups can be time consuming and problematic because silica requires careful standardisation for water content [34,41]. DDT losses were most pronounced in the highly coloured extract from soil 5 (Fig. 1); therefore, charcoal was tested for its suitability in cleaning up these extracts. Charcoal is commonly used for removing organic matter from soil extracts [42]. Soils with organic C contents ranging from 3.2 to 6.4% (Table 1) were subjected to a one-step extraction-cleanup with DCM and charcoal. Table 3 shows the results of three soil to charcoal ratios (10:2, 5:2, 5:4 g/g). Although there were a few significant differences in the amounts of DDT, DDD and DDE extracted from these soils, the trends were not consistent. The 10:2 soil:charcoal ratio yielded slightly higher values in 8 out of 12 data sets, although most of these were not significantly different. Average R.S.D.-values were 8.8 for DDT, 13.4 for DDD and 7.0 for DDE. It was concluded that all soil:charcoal ratios could be used for a one-step extraction-cleanup.

Interestingly, the soil with the highest organic carbon content (soil no. 5) gave the highest DDX values at the 5:2 ratio. More charcoal was also required for the spiked peat (4:2; sample:charcoal ratio) and CRM sediment (5:4) samples (see Sections 3.5 and 3.6). The need for more charcoal in these sample types may indicate that OM could have contributed to DDT breakdown in the GC inlet. The results of another exper-
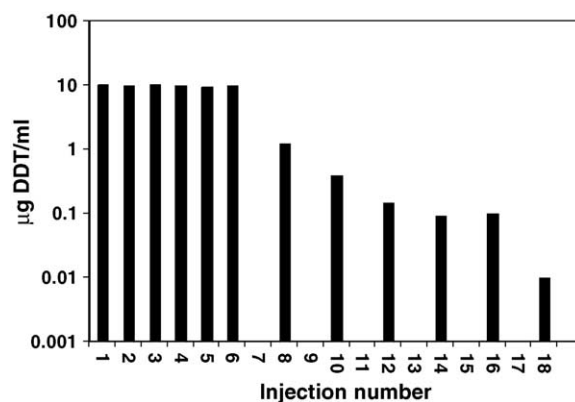

Fig. 2. Effect of peat extract on measured DDT concentrations in a performance evaluation standard (10.0 μg DDT/ml). Dichloromethane peat extracts were injected in positions 7, 9, 11, 13, 15 and 17.

iment are shown in Fig. 2: replicate injections of a 10.0 μg DDT/ml standard yielded reproducible DDT peaks (injections numbered 1–6); but as soon as the Soxflo DCM extract of an unspiked peat sample, which had not been treated with charcoal, was injected between these DDT standards, DDT concentrations decreased logarithmically.

3.5. One-step extraction-cleanup of spiked soil and peat samples

The recoveries achievable with this extraction-cleanup procedure were then investigated in more detail with soil no. 1 (DDX levels were <7 μg/kg) and a peat sample. These samples were spiked with a wider range of DDX concentrations than above (Table 2) to include the Dutch intervention level of 4000 μg DDX/kg. Soil no. 1 was spiked with DDT,

Table 4
Recoveries of spiked DDT, DDD and DDE concentrations from soil (soil no. 1) and peat samples (μg/kg)

Spike concentration		DDT		DDD		DDE	
		Mean	S.D.[a]; R.S.D.	Mean	S.D.; R.S.D.	Mean	S.D.; R.S.D.
Soil							
4000	Found	3610	507.2; 14.1	3653	337.1; 9.2	3733	481.4; 12.9
	Recovered (%).	90.2		91.3		93.3	
1000	Found	888	103.2; 11.6	896	111.4; 12.4	859	145.1; 16.9
	Recovered (%)	88.8		89.6		85.9	
100	Found	84.5	10.22; 12.1	91.9	11.58; 12.6	83.3	8.84; 10.6
	Recovered (%)	84.5		91.9		83.3	
50	Found	47.5	3.07; 6.5	46.1	6.02; 13.1	46.1	6.03; 13.1
	Recovered (%)	94.9		92.1		92.1	
20	Found	20.9	2.13; 10.2	20.2	1.09; 5.4	22.2	2.38; 10.7
	Recovered (%)	104.5		101.0		110.8	
Peat							
1000	Found	921	104.5; 11.3	991	118.3; 11.9	1013	104.9; 10.4
	Recovered (%)	92.1		99.1		101.3	
50	Found	43.8	5.08; 11.6	39.7	4.87; 12.3	40.3	2.55; 6.3
	Recovered (%)	87.6		79.3		80.6	
20	Found	17.3	1.17; 6.7	18.1	1.67; 9.2	17.1	1.98; 11.6
	Recovered (%)	86.5		90.4		85.7	

[a] $n = 6$.

DDD and DDE concentrations of 20–4000 µg/kg. Recoveries ranged from 83 to 111% (average 93%) and R.S.D.-values from 5 to 17 (average 11.4) (Table 4). Garden peat was spiked with 20–1000 µg/kg concentrations; recoveries ranged from 79 to 101 (average 89%) and R.S.D.-values from 6 to 12 (average 10.1). These recoveries from garden peat were excellent compared to values as low as 33% obtained with r.t. solvent extractions of a freshly spiked peat soil (3.3% organic carbon), which the authors attributed to rapid and almost irreversible adsorption to organic matter [27].

The literature contains surprisingly few examples of the use of charcoal in cleaning up pollutant extracts. Charcoal has been recognised as a very efficient adsorbent of DDT and DDD from water, but apparently the presence of water also caused problems with low recoveries [2]. Coconut charcoal has been employed for the separation of DDX from PCBs [2] and as a cleanup for endosulfan [43]. More recently, a method was described which was not too dissimilar to this SoF procedure: sediments containing a wide range of pollutants were extracted by Soxhlet with DCM, and this was followed by a two-step Si gel and charcoal cleanup [44].

It is of interest that our findings of better baselines for DCM versus acetone:hexane (1:1) extractions (Fig. 3) concur with Fitzpatrick and Dean [25] who predicted from the Hildebrand solubility parameter that the ideal extraction solvent for *p,p'*-DDT was DCM and not hexane:acetone (1:1), despite the fact that hexane:acetone is widely used for contaminated soils [1,45]. They experimentally verified this with PLE, whereas we found this to be true also for SoF extractions at r.t. Only a few reports describe the use of DCM for DDX extractions [44,45]. Filek and Lindner [46] substituted DCM with a less toxic solvent, i.e. *tert*-butylmethyl ether, for extracting organochlorine pesticides. It would be interesting to calculate the solubility parameters for this solvent according to the proposed model [25] and to test it experimentally with the SoF extraction procedure.
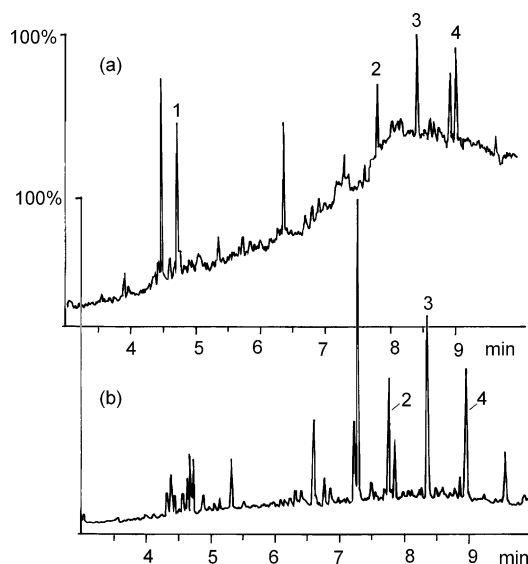

Fig. 3. GC–MS analysis of Soxflo extracts prepared from of soil no. 6 spiked with (a) 100 µg DDX/kg and extracted with hexane/acetone and (b) 200 µg DDX/kg and extracted with dichloromethane. Peak numbers: 1, internal standard; 2, DDE; 3, DDD; 4, DDT.

3.6. Validation of one-step extraction-cleanup with soil and sediment CRMs

Finally, this one-step extraction-cleanup procedure was validated with certified reference materials produced from real-world soil and sediment samples. The observed values covered concentrations from 93 to 1382 µg/kg. The recoveries from the soil CRM were 99.7% for DDT, 90.4% for DDD and 89.6% for DDE with R.S.D.-values of 2.5–4.0 (Table 5). The recoveries from the sediment CRM were 100.2% for DDT, 89.7% for DDD and 107.9% for DDE with R.S.D.-values of 1.2–7.0.

Table 5
Soxflo one-step extraction and cleanup of DDT, DDD and DDE from soil and sediment CRMs prior to GC–MS analysis (µg/kg)

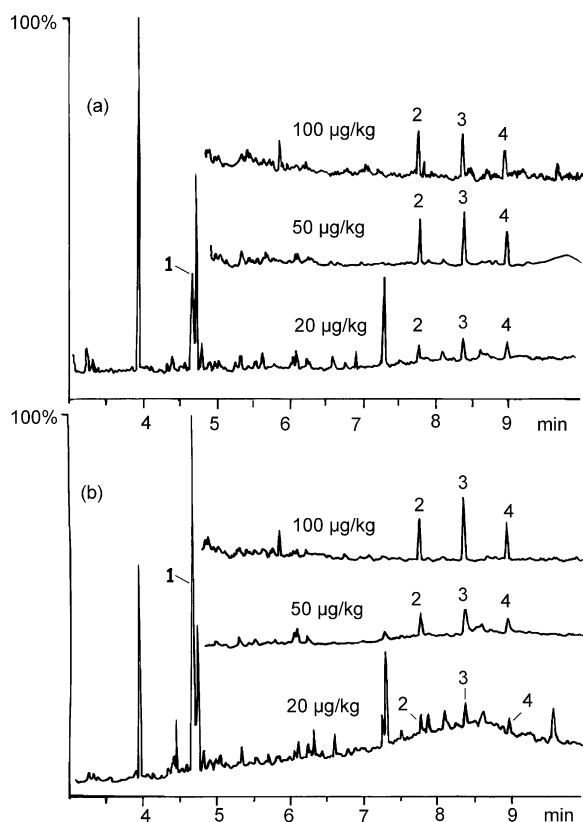| Certified reference materials	DDT	DDD	DDE
Soil CRM			
Observed mean	1057	1382	1362
S.D.[a]	283	558	342
R.S.D.	2.7	4.0	2.5
Recovery (%)	99.7	90.4	89.6
Certified value	1060	1531	1520
95% C.I.	926–1195	1294–1767	1325–1715
New York sediment CRM			
Observed mean	119.2	96.9	92.8
S.D.[b]	3.61	1.20	6.51
R.S.D.	3.0	1.2	7.0
Recovery (%)	100.2	89.7	107.9
Certified value	119	108	86
95% C.I.	108–130	92–124	74–98

[a] *n* = 7.
[b] *n* = 2.

Fig. 4. GC–MS analysis of Soxflo extracts prepared from samples spiked with 20, 50 and 100 μg DDX/kg: (a) spiked soil no. 1 and (b) spiked peat samples. Peak numbers: 1, internal standard; 2, DDE; 3, DDD; 4, DDT.

3.7. Detection limits

The limit of quantification for spiked soil and peat samples was 20 μg DDX/kg (Fig. 4; Table 4). Although DDX peaks were still detectable at 10 μg/kg, there was considerable interference from co-extractants. It would be worth testing if charcoal in combination with other cleanup materials could be used in the SoF sample tube to lower the detection limit further.

The R.S.D.-values for the CRMs ranged from 1.2 to 7.0 (Table 5) and tended to be lower than those obtained with the spiked or weathered samples (range 1.4–25.9; Tables 2–4). It is likely that the lower R.S.D.s from the CRMs can be attributed to their smaller particle size and greater homogeneity [47]. The CRM sediment sample had particle sizes of 61–250 μm whereas our soil samples were either ball milled briefly (Table 2) or sieved to <2 mm (Tables 3 and 4). Figs. 4 and 5 and Tables 2–4 demonstrate what can be achieved with this one-step SoF extraction-cleanup procedure with samples containing naturally incurred DDX that have been ground less finely.

3.8. Soxflo versus other extraction methods

SoF recoveries meet the EU recommendations for accuracy of an analytical method, i.e. 80 to 110% for analyte
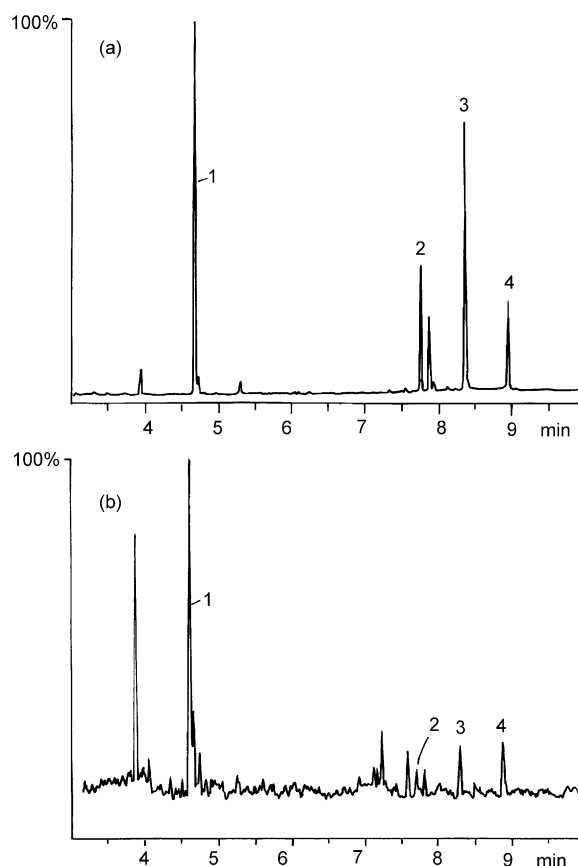

Fig. 5. GC–MS analysis of Soxflo extracts prepared from certified reference materials: (a) sandy loam soil CRM804-050 and (b) New York sediment CRM 1944. Peak numbers: 1, internal standard; 2, DDE; 3, DDD; 4, DDT.

concentrations above 10 μg/kg [39,40]. SoF extractions recovered 83–111% of DDXs from soils and sediments and 79–101% from peat (Tables 2, 4 and 5). Judging from the results presented in Table 5 and Fig. 5, this one-step SoF extraction-cleanup procedure, which was carried out at r.t. with 70 ml DCM and charcoal within 60 min, was just as efficient as several EPA methods that used hexane/acetone (1:1) or DCM/acetone (1:1) and which had been used in the certification of the CRMs: 16–24 h extractions with 300 ml solvent by Soxhlet; 2 h extractions with 50 ml solvent by Soxtec or repeated extractions with 3 × 100 ml solvent by sonication [45]. All values are within the 95% confidence interval given on the CRM certificates. The recoveries were excellent for DDT (99.7% for soil and 100.2% for sediment CRMs); recoveries were 90.4 and 89.7% for DDD and 89.6 and 107.9% for DDE, respectively.

In the SoF technique, the sample is packed into the form of a dry column and the solvent passes through this column just once. This extraction technique, therefore, minimises analyte re-adsorption compared to Soxhlet or conventional solvent extractions whilst at the same time continuously exposing the sample matrix to pure solvent. It was suggested that SoF extractions are based on the same principles as flash column chromatography [35].

Table 6
Interlaboratory comparison of p,p'-DDX in polluted soils (μg/kg)

Soil number	Method	DDT	DDD	DDE	DDT/DDD	DDT/DDE
2	Soxflo[a]	139	18.1	33.7	8.1	3.8
	Contract Laboratory[b]	235	14.0	40.9	16.8	5.7
3	Soxflo	140	28.9	128	5.1	1.1
	Contract Laboratory	221	75.2	111	2.9	2.0
4	Soxflo	1831	200	2185	7.6	0.8
	Contract Laboratory	<0.5	28.7	<0.5	0.02	1.0
5	Soxflo	8107	10926	890	0.5	6.8
	Contract Laboratory	346	4956	893	0.1	0.4

[a] Soxflo data, see Table 3.
[b] DDXs extracted with *iso*-hexane in Soxhlet set-up and extracts cleaned up with activated alumina and silver nitrate.

Van der Velde et al. [27] concluded that SFE gave better recoveries (94%) than solvent or Soxhlet extractions from a peat soil (3.3% organic C) spiked with 1–10 μg/kg concentrations. However, this method was not validated with CRMs, which would have been particularly relevant given the comment that "method development of SFE is laborious because many parameters need optimisation and the strong matrix dependence of the extraction process precludes the transfer of SFE conditions gained in spike experiments to a range of real samples" [33]. Thompson et al. [22] described microwave-assisted extractions (MAE) with DCM followed by several cleanup steps. The procedure was validated with a marine sediment, CRM 1941a, and achieved 102–124% recoveries for DDX. Some reports of new extraction techniques use surprisingly small sample sizes, e.g. 1–2 g for SFE, MAE or PLE extractions [22,24]. The SoF sample tube holds 5–10 g soil and can be modified to include larger quantities.

3.9. An interlaboratory comparison: implications for environmental data

Two of the low and two of the high OM soil samples were sent to a contract laboratory that is well established for organic pollutant analysis. This revealed that soils with <4% C gave comparable results, but data from soils with >4% C were up to 4490-fold higher (Table 6) by the SoF procedure. This suggests that matrix interferences went undetected in the contract laboratory and substantiates concerns that some data relating to DDT degradation in the environment may be of questionable quality because of matrix-enhanced DDT losses in the GC inlet [3]. Further research will be needed to identify the interfering matrix components.

Data obtained with the proposed SoF procedure are more likely to be accurate for the following reasons: (i) DDT levels in soils no. 4 and 5 are considerably higher, which suggests that DDT losses had occurred during the analysis by the contract laboratory (contamination in our laboratory can be ruled out as none was ever detected with blanks); (ii) a very high DDD concentration was found in soil no. 5 with the SoF procedure as expected for a waterlogged site and (iii) a very high DDE concentration was found in soil no. 4 with the SoF procedure as expected for a well-aerated site [8].

The Dutch intervention values are 4000 μg/kg of soil or sediment and refer to the sum of DDT, DDD and DDE concentrations [48]. The contract laboratory would have passed soil no. 4 as satisfactory, whereas the SoF procedure demonstrated that it required treatment. Furthermore, DDT/DDD and DDT/DDE ratios have been used to distinguish between past and present pollutant sources [9]; Table 6 shows large discrepancies amongst these ratios between the two laboratories. Reliable analytical methods will, therefore, be needed to identify pollutant sources and for monitoring the environmental fate of DDT.

4. Conclusions

The Soxflo instrument allowed a one-step extraction-cleanup of 5–10 g environmental samples in 1 h at room temperature. Passing the dichloromethane extracts (70 ml) through charcoal prevented DDT breakdown at the GC inlet. After concentration, these extracts were ready for injection into the GC–MS. Without charcoal cleanup, DDT breakdown at the GC inlet was extensive especially with extracts obtained from peat and some soil samples. It was concluded that charcoal cleanups should be used routinely when monitoring DDX pollution in environmental samples.

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