Journal of Chromatography, 153 (1978) 233–238

CHROM. 10,658

CLEAN-UP PROCEDURE FOR THE EXTRACTION OF SOIL SAMPLES IN THE DETERMINATION OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN

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(Received October 20th, 1977)

SUMMARY

This paper describes the method which was developed in relation to analytical work connected with microbial and physico-chemical degradation experiments on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Soil samples are best extracted with methanol plus methylene chloride. Microbial preparations are extracted with light petroleum after boiling with methanolic potassium hydroxide. The clean-up consists of a sulphuric acid treatment and chromatography on a multilayer column (Celite + H_2SO_4/silica gel) followed by alumina column chromatography. The clean-up procedure proved to be suitable for soil samples and microbial preparations even when large quantities of organic matter (hydrocarbons, oils, surfactants) were present.

INTRODUCTION

Following the Seveso accident in 1976[1], a large area south of the trichlorophenol-producing plant was polluted by a chemical mixture consisting primarily of trichlorophenol, but also containing the extremely toxic by-product[2–4] 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The affected area was monitored by means of chemical analyses. In the emergency period, the analyses were carried out by a simple procedure (extraction and mass fragmentography without prior clean-up), but later more refined methods were needed in order to define the borders of the contaminated area, to monitor soil penetration of TCDD and to check laboratory and field decontamination experiments based on suggestions reported in some papers[5–9].

Although several methods have been reported for the determination of trace amounts of TCDD in herbicide formulations[10–16], commercial chlorophenols[17–21], hexachlorobenzene[22], tetrachlorvinphos[23], biological tissue samples[24] and in fats and oils[25,26], no suitable method was available for the determination of trace amounts of TCDD in soil samples, because of the variety and the amount of co-extracted materials. It appears that only Woolson *et al.*[27] have described in detail a procedure for the determination of TCDD in soil samples. However, in their method the clean-up is unsophisticated, so that often the final solution is unsuitable for gas chromatographic–mass spectrometric analysis. Further, the sensitivity of their method

is not satisfactory for field monitoring purposes because of the very high toxicity of TCDD.

Different solvents and solvent mixtures for the extraction of TCDD from soil samples have been tried, and the factors that influence the extraction of organochlorine pesticide residues from soil as reported by Chiba and Morley[28] have been considered. Extraction with methanol followed by methylene chloride proved to be the most efficient procedure.

Several clean-up procedures, which differ according to the type of matrix, have been described previously[10–25]. Typical steps involve treatment of the raw solutions containing TCDD with sulphuric acid, followed by silica gel and/or aluminium oxide column chromatography.

This paper describes a method that has been used for the analyses carried out in connection with microbial and physico-chemical degradation experiments. The method consists of extraction with methanol plus methylene chloride, followed by clean-up with sulphuric acid and passage through two successive chromatographic columns. Quantitation is carried out by gas chromatography–mass spectrometry (mass fragmentography), the three ions at *m/e* 320, 322 and 324 being monitored. This procedure proved to be suitable even for soil samples that naturally contain large amounts of organic matter or to which it is added in microbial degradation experiments. The clean-up step has also been used successfully for the analysis of microbial preparations containing water, oils, surfactants and nutrients.

EXPERIMENTAL

Extraction

Soil samples. Soil samples (400 g) are treated in a beaker with methanol (2 × 300 ml) and then with methylene chloride (4 × 300 ml), with thorough mixing using a glass rod for at least 5 min. After sedimentation, each fraction is filtered through paper into a 2-l separating funnel with a PTFE stopcock. Water (600 ml) is added and the funnel is shaken. After the phases have been separated, the methylene chloride extract is poured into a 1-l round-bottomed flask, and concentrated in several portions in a rotary evaporator (bath temperature 50°, reduced pressure). The aqueous phase in the separating funnel is washed with methylene chloride (3 × 100 ml) and the washings are transferred into the 1-l flask. The methylene chloride extract is evaporated cautiously to dryness and the residue immediately dissolved in 20 ml of light petroleum (b.p. 40–60°).

Aqueous emulsions. To the samples in an erlenmeyer flask are added half their volume of methanol and potassium hydroxide pellets to make a 2 *N* solution with respect to KOH. The mixture is boiled under reflux for 2 h and, after cooling, TCDD is extracted by shaking the mixture with light petroleum (b.p. 40–60°) (6 × 100 ml) and siphoning each fraction into a 1-l round-bottomed flask. The light petroleum extract is concentrated to about 20 ml in a rotary evaporator.

Sulphuric acid treatment

Five millilitres of concentrated sulphuric acid are added to the light petroleum solution in the 1-l flask, which is gently rotated to allow the acid layer to come into contact with the walls. Anhydrous sodium sulphate is added until a free-flowing slurry

is obtained, so that the light petroleum extract can easily be removed with a capillary pipette.

Multi-layer column

Prepare a multi-layer column (glass, 200 × 20 mm I.D., without a stopcock) containing, from bottom to top, anhydrous sodium sulphate (1.0 cm), silica gel (60–200 mesh) (1.5 cm), sodium sulphate–sodium hydrogen carbonate (9:1) mixture (1.5 cm), Celite 545 (6.0 g) impregnated with concentrated sulphuric acid (4.0 ml) pressed with a glass rod to a height of *ca.* 5 cm, and finally anhydrous sodium sulphate (1.5 cm). Prior to the application of the sample, wash the column with 50 ml of light petroleum (b.p. 40–60°). Apply the sample, dissolved in 20 ml of light petroleum (b.p. 40–60°) to the multi-layer column, carefully washing the flask with successive 10-ml volumes of light petroleum. Elute the column with these successive washings until a total volume of 150 ml has been collected. Concentrate the solution to about 10 ml in a rotary evaporator.

Aluminium oxide column chromatography

Partially fill a column (glass, 400 × 20 mm I.D., with a PTFE stopcock) with light petroleum (b.p. 40–60°) and slowly add 16.0 g of aluminium oxide (neutral, Brockmann activity grade I, Merck, Darmstadt, G.F.R., or equivalent). The light petroleum solution from the multi-layer column is transferred quantitatively to the alumina column, which is eluted at 3–5 ml/min with 100 ml of light petroleum (b.p. 40–60°)–methylene chloride (9:1) and then with 100 ml of methylene chloride. The first fraction is discarded, but the second fraction, which contains TCDD, is retained for the TCDD determination.

Mass fragmentography

The second chromatographic fraction (see above) is carefully evaporated to dryness and the residue is immediately dissolved in a suitable volume of isooctane, so that the concentration of TCDD in the final solution is similar to that in the standard solution used for quantitation (0.1 ng/μl). An aliquot of 5–10 μl of the isooctane solution is injected into a gas chromatograph–mass spectrometer equipped with an accelerating voltage alternator. The operating conditions are as follows: glass column, 2 m × 2 mm I.D., packed with 3% OV-101 on Chromosorb G (80–100 mesh); temperatures, oven 230°, injector 270°, separator 250°; carrier gas, helium; flow-rate 25 ml/min; electron energy, 40 eV. TCDD is identified by its chromatographic retention time and the simultaneous presence of the molecular ion at *m/e* 320 and the two isotopic ions at *m/e* 322 and 324, with the correct intensity ratios. Absolute amounts are calculated by comparing the intensity of the *m/e* 322 peak present in the sample with that obtained on injecting a comparable and known amount of TCDD standard.

RESULTS AND DISCUSSION

Several solvents and solvent mixtures were tried for the extraction of TCDD from soil samples. The use of methanol followed by methylene chloride proved to be the simplest and most efficient extraction procedure. Methanol followed by benzene

also gave a satisfactory recovery, but this procedure has some practical drawbacks, especially in the concentration step. The inclusion of methanol in the extraction procedure is very useful in removing water from wet soils and breaking lumps, thus making the extraction easier and more efficient. When soil samples are not too wet, pooled extraction fractions can be concentrated without the need for the liquid–liquid partition with water in the separating funnel. Recovery studies carried out on 20 μg (0.05 ppm) of pure TCDD added to 400 g of uncontaminated soil to which organic matter was added gave the results listed in Table I. Analyses were carried out 10–90 days after the addition of TCDD, in order to reproduce the interactions that might occur in a soil contaminated with TCDD. When some of the samples in Table I were submitted to a second extraction with methylene chloride (3 × 300 ml), a further 5–8% of TCDD could be recovered.

TABLE I

RECOVERY OF 20 μg OF TCDD ADDED TO 400-g SOIL SAMPLES

Sample No.	*TCDD found (μg)*	*Recovery (%)*
1	18.6	93.0
2	17.0	85.0
3	17.7	88.5
4	18.7	93.5
5	18.1	90.5
6	16.9	84.5
7	18.3	91.5
8	18.1	90.5
9	18.5	92.5
10	18.4	92.0
11	18.8	84.0
12	17.2	86.0
13	19.8	99.0
14	19.0	95.0
15	17.2	86.0
Mean		90.1
Standard deviation		±4.4

Recoveries of TCDD from aqueous emulsion containing oils, surfactants, hydrocarbons, microbial nutrients, etc., are given in Table II. Sometimes very troublesome emulsions occur during the extraction, which might account for the recoveries being lower than those obtained in soil sample analyses. Small additions of methanol in the form of a thin jet can help to break these emulsions.

Sulphuric acid treatment and the use of a multi-layer column are very effective in destroying most of the organic materials and removing more polar compounds. The clear, colourless solution thus obtained may contain common soil pollutants, such as PCBs and DDE, which may interfere in the TCDD determination. Alumina column chromatography provides a useful means of separating TCDD from PCBs, DDE and other interfering compounds, which are eluted in the first fraction. The

TABLE II

RECOVERY OF TCDD ADDED TO 300-ml AQUEOUS EMULSIONS

Sample No.	*TCDD added (μg)*	*TCDD found (μg)*	*Recovery (%)*
1	8.19	7.7	94.0
2		8.1	98.9
3		7.5	91.6
4		7.0	85.5
5		4.7	57.4
Mean			85.5
Standard deviation			±16.4
6	11.48	10.4	90.6
7		10.9	95.0
8		8.4	73.2
9		8.9	77.5
10		8.7	75.8
11		7.5	65.3
12		9.5	82.8
13		7.6	66.2
14		8.7	75.8
15		10.0	87.1
16		8.4	73.2
17		9.3	81.0
18		10.9	95.0
19		10.7	93.2
Mean			80.8
Standard deviation			±10.1

second fraction has been found to be sufficiently clean for mass fragmentographic analysis. As we were interested in the determination of the TCDD, the most toxic of the compounds, only this compound was monitored by mass fragmentography. Although the second fraction from the alumina column may contain other polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans, no interference arises in the mass fragmentographic determination of TCDD, as was clearly shown by Buser and Bosshardt[19].

An alumina "macro"-column was preferred to the "micro"-column used by other workers[18,19,26], because several soil samples contained large amounts of low-polarity compounds, which can overload a micro-column and change the chromatographic behaviour of TCDD.

Amounts of sample greater than 400 g can conveniently be processed simply by scaling-up the volumes of solvents used in the extraction step.

ACKNOWLEDGEMENT

The authors thank Mr. E. Verdino for his assistance with the experimental work.

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