DETERMINATION OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN IN SOIL IN THE PRESENCE OF LARGE EXCESS OF CHLORINATED HYDROCARBONS

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The paper describes a method of 2,3,7,8-TCDD determination in soils heavily contaminated with organic compounds (the soil from areas of plants producing pesticides, chlorophenols, etc.). The procedure is based on a sample extraction with a hexane–acetone mixture, cleaning of the extract by washing with potassium hydroxide and sulfuric acid solutions and by liquid chromatography on a multilayer modified silica column and alumina columns, and on GC/MS determination using a labelled internal standard (^{13}C-2,3,7,8-TCDD). The determination limit is 10 ppt and the average recovery 55%.

Production of polychlorinated phenols (PCP) is accompanied by formation of various side products inclusive of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)[1,2]. The most toxic of all 210 PCDD and PCDF congeners – 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) – is mainly contained, up to mg/kg^{-1} levels, in 2,4,5-trichlorophenol (TrCP) and/or in 2,4,5-trichlorophenoxyacetic acid (2,4,5-T)[3] as the final product.

The contamination of soil with 2,3,7,8-TCDD is caused by the substance escaping from the plants producing 2,4,5-TrCP or 2,4,5-T or by application of formulations based on 2,4,5-T to agricultural and other purposes[4–7]. Several methods were developed for the determination of 2,3,7,8-TCDD in soil[4,8–13], but their application to samples of soil heavily contaminated with organic compounds was unsuccessful. The present paper deals with a possibility of 2,3,7,8-TCDD determination in soils containing high concentrations of some chlorinated hydrocarbons (HCH, HCB, PCP, etc.).

EXPERIMENTAL

Chemicals and Adsorbents

All the chemicals used were of the highest purity grade available: NaOH, KOH, Na_2SO_4 an-

hydrous (all p.a., Lachema Brno), conc. H_2SO_4, p.a. (Merck, F.R.G.), $AgNO_3$ (Medika Bratislava). The labelled standard 2,3,7,8-TCDD with all 12 carbon atoms replaced by the ^{13}C isotope (Cambridge Isotope Laboratories, U.S.A.) was dissolved in heptane to give a 0·5 μg ml^{-1} stock solution. Nitrogen (from a pressure cylinder) used for evaporation of solvents was purified by passing through a molecular sieve 5A trap. The solvents (UV-grade): hexane, acetone, methanol (Spolana Neratovice), dichloromethane and benzene (both p.a., Lachema Brno) were rectified in an all-glass apparatus. A silanized glass wool (Supelco, U.S.A.) for sealing liquid chromatography columns was washed with hexane. Silica gel L 100/250 μm (Lachema Brno), placed in a 60 × 2 cm column was washed with 300 ml methanol, 200 ml dichloromethane and 200 ml hexane. The purified adsorbent was activated at 200°C and kept in a desiccator. An adsorbent ICN Alumina B Super I (ICN Biomedicals, U.S.A.) was used in original state. Anhydrous sodium sulfate was purified in the same column as that used for silica gel using 500 ml acetone and 400 ml hexane, whereafter it was baked-out at 700°C for 4 h.

Preparation of Liquid Chromatography Column Packings

Silica gel with sulfuric acid. Approximately 40 g activated silica gel was intensively shaken with 19 ml concentrated H_2SO_4 in a flask until no clumping was observed (loose consistence). The material thus prepared was kept in a capped flask in a desiccator.

Silica gel with sodium hydroxide. The same procedure was applied to 20 g activated silica gel and 10 ml 1M NaOH.

Silica gel with silver nitrate. The same procedure was applied to 20 g activated silica gel and a freshly prepared solution of 0·67 g $AgNO_3$ in 1·5 ml redistilled water.

Preparation of Liquid Chromatography Columns

Modified silica column (*type* I). A glass column (10 mm ID) with a silanized glass wool plug as the bed support was gradually packed with 0·5 g silica gel, 1·5 g silica gel/$AgNO_3$, 1·5 g silica gel/NaOH, 0·5 g silica gel, 4 g silica gel/H_2SO_4, 1 g silica gel, and 2 g anhydrous Na_2SO_4. The column was washed with 30 ml hexane immediately prior to use.

Modified silica column (*type* II). A glass column (20 mm ID) was gradually packed with the same adsorbents as those used in type I, however, their amounts were five times greater. Immediately prior to use the column was washed with 150 ml hexane.

Alumina column (*type* I). A glass column (10 mm ID) was gradually packed with 5 g ICN Alumina B Super I and 5 g anhydrous sodium sulfate.

Alumina column (*type* II). A glass column (4 mm ID) was gradually packed with 1 g ICN Alumina B Super I and 1 g anhydrous Na_2SO_4.

Laboratory glassware was always washed gradually with distilled water, acetone, and hexane and dried in dust-free air. Folded paper filters were washed with acetone and hexane.

Soil Samples and Extraction

The soil samples to be analyzed were taken by means of a probe to 50 cm depth within an area extending to 50 m from a former 2,4,5-TrCP, PCP, and 2,4,5-T production building. Wet samples were dried at 50°C for 8 h. Coarse impurities such as stones, grass, paper, etc. were removed

from the dry sample, and fivefold quartation was used to ensure the sample homogeneity. The sample (50 g) was placed into a 250 ml boiling flask, and 100 μl of $^{13}C_{12}$-2,3,7,8-TCDD standard solution was added. The soil was extracted with three successive portions (70, 30, 30 ml) of a 3 : 1 hexane–acetone mixture using a shaker (30 min for each portion). The extracts were filtered through a folded filter into a separating funnel.

Extract Clean-up

The combined extracts were intensively shaken with successive portions (50, 20, and 20 ml) of 5M KOH. After the last extraction the organic layer was washed with 20 ml portions of redistilled water until neutral. Then the hexane extract was repeatedly washed with 20 ml portions of concentrated sulfuric acid until the organic layer was almost colourless. Finally the hexane layer was again washed with redistilled water until neutral. The extract cleaned in this manner was dried over 40 g anhydrous sodium sulfate on a folded filter.

The solvent was removed using a rotoevaporator until the residue was just dry, whereupon the latter was qualitatively transferred (using several small volumes of hexane) on a modified silica column (type I) and eluated with 40 ml hexane. If the extract — after the purification with conc. H_2SO_4 and after evaporation of the solvent — was considerably coloured and/or there appeared a rather large amount of solid residue, the final clean-up was carried out on the modified silica column (type II) with 200 ml hexane as the eluent.

The eluate from the modified silica column was evaporated just to dryness using a rotoevaporator, and the residue was quantitatively transferred with the minimum amount of hexane on an alumina column (type I). The column was eluated successively with 75 ml 2% dichloromethane in hexane (1st fraction), 50 ml 20% dichloromethane in hexane (2nd fraction), and with 50 ml 50% dichloromethane in hexane (3rd fraction). As the adsorbent activity varies, the elution volumes must be determined from time to time by means of tests with standards. The 3rd fraction containing 2,3,7,8-TCDD was concentrated with rotoevaporation just to dryness.

The residue was quantitatively transferred with a small volume of hexane on an alumina column (type II). The column was successively eluated in the same way as the column type I above using five times smaller elution volumes. The 3rd fraction was taken, concentrated with a rotoevaporator, and the concentrate was transferred with benzene into a conical test tube wherefrom the solvent was removed by evaporation with a stream of nitrogen at room temperature.

GC/MS-SIM Determination

The residue in a conical test tube was dissolved in a known (if the recovery is going to be determined) volume of benzene and analyzed by gas chromatography combined with mass spectrometry in selected ion monitoring mode (GC/MS-SIM). In this study the GC separation was carried out on a OV-17 capillary column. Although any capillary column with nonpolar, intermediate polar or polar polysiloxane phase can be used, for the time being the best PCDD/PCDF separation is achieved on very polar cyanosiloxane columns (SP-2330, CP SIL 88, Silar 10C). The sample extract was injected using splitless or on-column technique. The GC/MS conditions are given in Fig. 1. The detection was accomplished by a Hewlett–Packard HP 5970B Mass Selective Detector operating in SIM mode. The three most intensive molecular ions (i.e. m/z 321·9, 319·9, 323·9) and the characteristic fragment $[M - COCl]^+$ (i.e. m/z 257·0) for TCDD and the three most intensive molecular ions for the $^{13}C_{12}$-TCDD standard (i.e. m/z 333·9, 331·9, 335·9) were monitored.

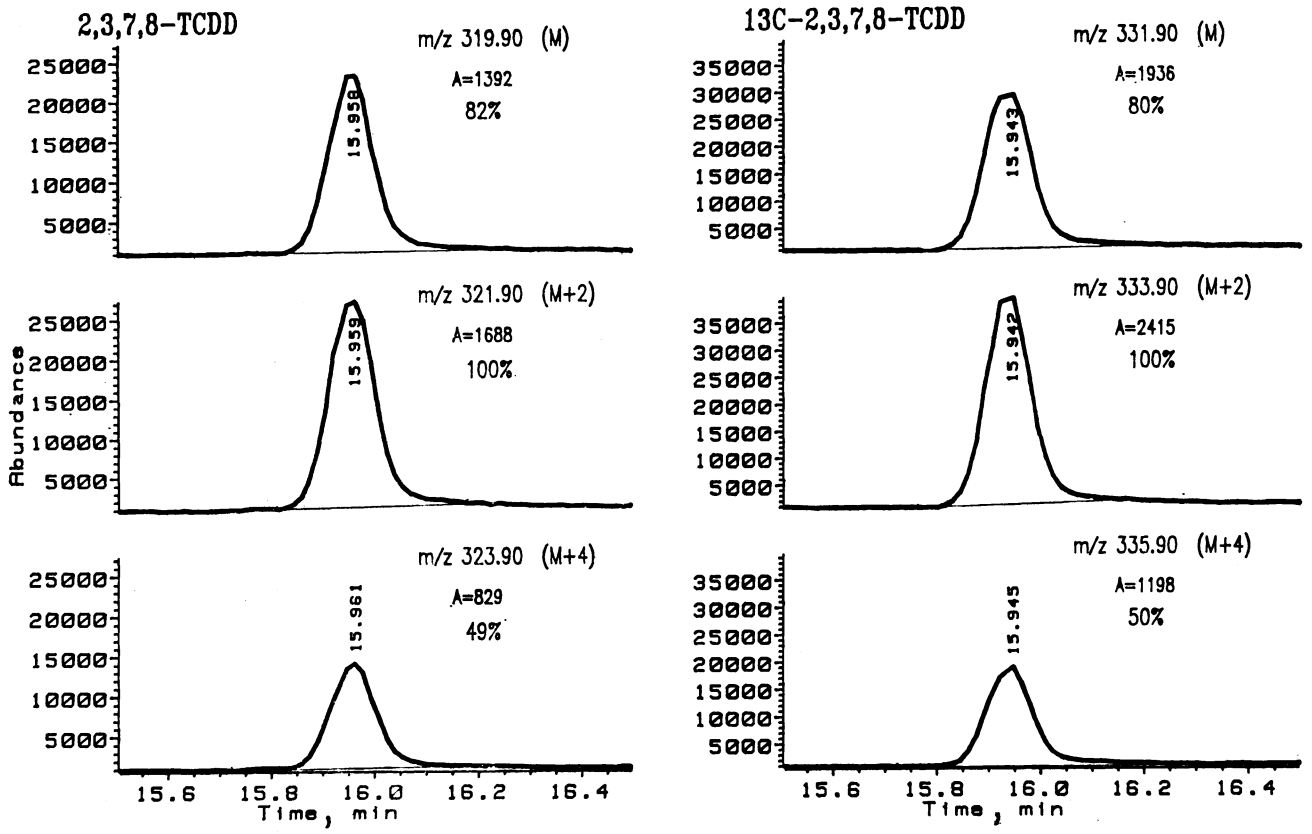

FIG. 1

Reconstructed chromatograms for selected ions (SIM) of 2,3,7,8-TCDD and ^{13}C-2,3,7,8-TCDD in a cleaned-up soil extract. Capillary column 25 m × 0·21 mm, OV-17 immobilized, helium at 130 kPa, oven temperature program: 130°C (0·5 min), 130–240°C at a rate of 30 K min^{-1}; MS detector, electron impact ionization at 70 eV

RESULTS AND DISCUSSION

The soil samples to be analyzed were so complex that no satisfactory results could be obtained without application of a special powerfull clean-up procedure. The main problem consisted in the presence of high content of various coextracts in the soil analyzed. Thus e.g. a random sample of 500 g soil was partially cleaned-up and then extracted to give 6 g white crystals composed mainly of hexachlorocyclohexane (HCH) isomers and hexachlorobenzene (HCB) (according to GC/MS analysis; see Fig. 2), i.e. substances which were produced for a certain period of time in the plant examined. It was just the presence of large amounts of these substances which caused troubles during the sample treatment.

Various solvents or solvent mixtures were tried for the sample extraction, and the 3 : 1 hexane–acetone mixture turned out to be the best. In this combination the acetone enables a better contact of nonpolar hexane with the colloid system of soil and increases the extraction yields.

The sample magnitude had to be gradually decreased from 500 g to 50 g. In consequence of that the amount of interfering components was substantially reduced and they could be removed by liquid chromatography. The currently achieved limit of determination of 2,3,7,8-TCDD with a 50 g sample was 500 pg which corresponds to 10 ppt.

We made use of the unique ability of the special adsorbent ICN Alumina B Super I, viz. that enabling (with application of suitable solvents) the separation of 2,3,7,8--TCDD from all other TCDD isomers, even from all PCDD/PCDF congeners and also from further neutral compounds such as polychlorinated biphenyls, HCH, HCB, etc. Basic and coloured organic components were mainly removed with concentrated sulfuric acid. The expected chlorinated phenols and other components of acidic nature were removed by washing the extract with aqueous potassium hydroxide. The clean-up procedure was completed by application of the multilayer modified silica column.

The use of mass spectrometric detection, besides its advantage of a high selectivity and sensitivity, enabled application of the labelled ^{13}C-2,3,7,8-TCDD as an internal standard. The applied internal standard method compensated the losses during sample treatment, changes in GC/MS reproducibility and sensitivity during one day, inaccuracy in diluting and injecting of the sample extract into a gas chromatograph.

The criteria for identification of 2,3,7,8-TCDD were (see Fig. 1): (i) the coincidence of GC retention time with the labelled internal standard, (ii) correct parent chlorine ion isotope ratio ($\pm 10\%$) for the most intensive masses in the molecular cluster, (iii) a signal:noise ratio higher than 3 : 1.

With reference to this standard we also estimated the recovery of 2,3,7,8-TCDD in each analyzed sample: the mean recovery was 55% ($n = 102$, $s_{rel} = 22{\cdot}7\%$). The high value of relative standard deviation was probably due to the complexity

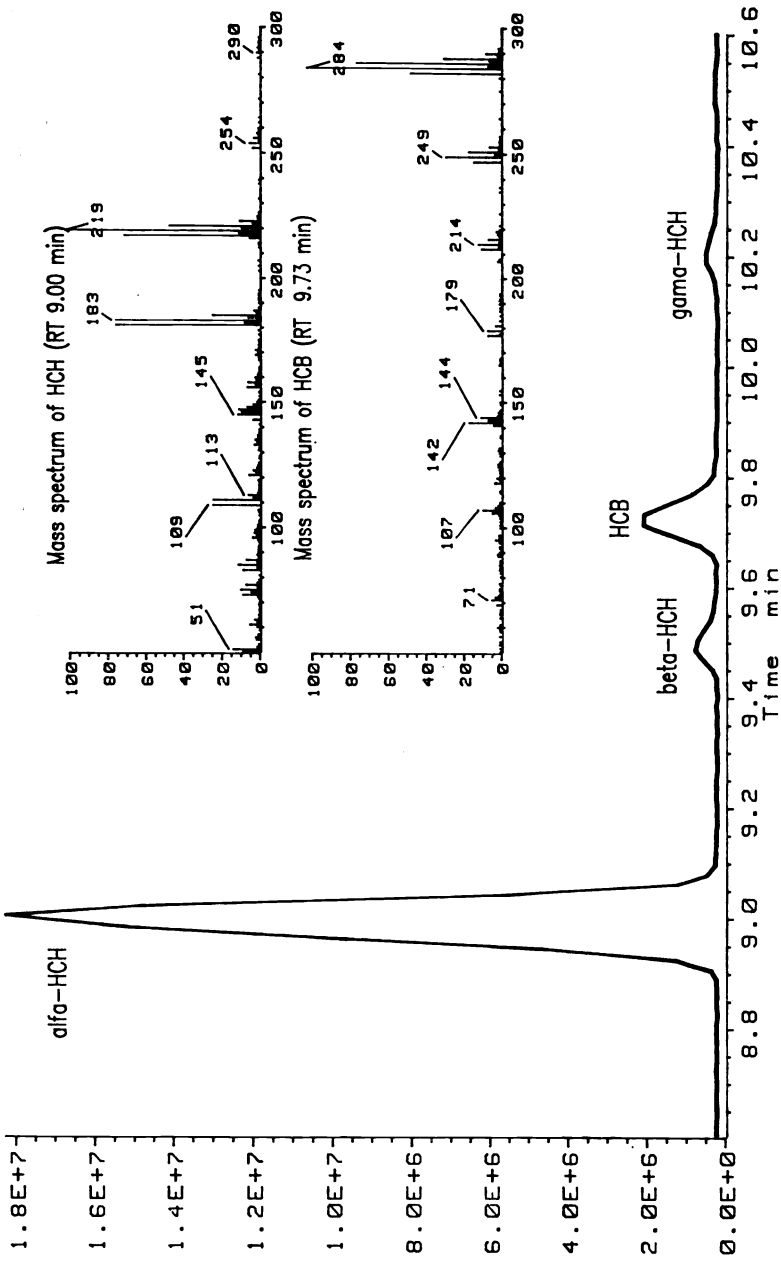

FIG. 2
Reconstructed chromatogram (Total Ion Current) of a partially cleaned-up hexane–acetone extract of a soil sample (for GC/MS conditions see Fig. 1)

of the sample matrix and to the fact that the standard used enabled only the method of direct comparison, i.e. the calibration straight line was supposed to cross the origin of coordinates.

The method described was verified within a concentration range of 0·01 – 30 ng 2,3,7,8-TCDD per 1 g soil.

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