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Methods Research: Determination of Dioxins
in Fish and Sediment

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PERSPECTIVE-GESTION

Après avoir découvert des quantités infimes de polychloro-dibenzo-p-dioxine (PCDD) dans les poissons, les sédiments, les goëlands argentés et autres maillons de la chaîne alimentaire, on a décidé d'améliorer les méthodes de détection et de quantification courantes de ces composés. Certains isomères étant extrêmement toxiques et cancérogènes, il est important d'établir les limites de détection dans la gamme de parties par trillion.

Ce rapport décrit un exemple de marche à suivre pour isoler et extraire la PCDD dans des échantillons de poissons et de sédiments, pour nettoyer ces échantillons et quantifier la PCDD. Cette méthode a été transmise au Laboratoire national de la qualité de l'eau qui l'utilise maintenant couramment. On continue à chercher des moyens d'améliorer l'extraction de la PCDD dans des sédiments difficiles à analyser et les résultats seront communiqués au laboratoire le plus tôt possible.

Titre : Étude sur des méthodes de détection des dioxines dans les poissons et les sédiments.

METHODS RESEARCH: DETERMINATION OF DIOXINS IN FISH AND SEDIMENT

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ABSTRACT

This communication describes a simple isolation/extraction procedure for fish and sediment samples based on acid digestion, gel permeation chromatography, trisodium phosphate treatment, micro-alumina chromatography and carbon fibre column chromatography. The cleaned extracts are analyzed for tetrachloro dibenzo-p-dioxins using GC-ECD screening and HRGC-MS (multiple ion detection) confirmation.

INTRODUCTION

Concern for dioxin contamination in the environment has necessitated the development of improved methodology for the routine isolation, separation and quantitation of polychlorinated dibenzo-p-dioxins (PCDDs) in fish and sediment. The extreme toxicity and carcinogenicity of some of the isomers (notably those substituted in the 2,3,7,8 positions) requires detection limits in the order of low parts per trillion (pg/g). Analytical difficulties are compounded by the high lipid content of some samples and by the presence of other anthropogenic contaminants at concentrations orders of magnitude higher than the PCDDs.

In our laboratory, various sample treatment, extraction and clean-up procedures have been evaluated for the selective determination and quantitation of PCDDs by high resolution gas chromatography/mass spectrometry (HRGC/MS).

EXPERIMENTAL

a) Extraction and Clean-Up

During the evaluation of various extraction and clean-up processes for fish and sediment samples, it was found that no single set of conditions were optimal for all samples. Matrix variations such as particle size (sediment), the lipid content (fish) and the nature and concentrations of co-extractives resulted in variable extraction efficiencies. The scheme shown in Fig. 1 was found to give consistently good extraction efficiencies for most fish and sediment samples and was found to be suitable for routine application.

Details of the extraction and clean-up steps are provided below. This procedure was developed to be part of a multi-residue analytical scheme for a number of classes of trace organic contaminants.

b) Procedure

All samples must be homogenized utilizing a polytron blender and stirrer. The method of extraction is determined by sample type, i.e. fish or egg samples require an initial acid digestion and subsequent blending by a polytron. Fly ash, the most difficult type of

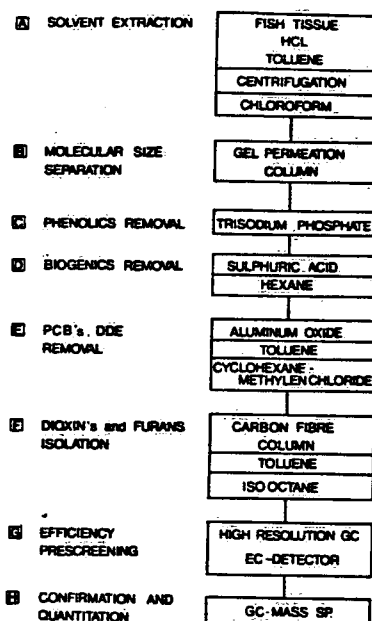


Fig. 1 General Clean-Up Procedure for Dioxins in Fish Tissue

sample to extract should be exhaustively Soxhleted. Sediments, depending on their type, may require either Soxhlet extraction or acid digestion followed by blending the aqueous layer and solids with a polytron by sonification.

The sample is fortified in a 500 mL Erlenmeyer flask with labelled standards (^{13}C -2,3,7,8-TCDD and ^{13}C -octachloro dibenzo-p-dioxin); 100 mL of 1N HCl is added and the mixture allowed to stand for 1 hr. The sample, which is completely dissolved is then extracted with 100 mL toluene and shaken on a wrist action shaker overnight. A gelatinous emulsion sometimes forms with fish tissues which may be broken with concentrated HCl. The separation of the aqueous and organic layers (toluene) is facilitated via centrifugation and blending by the polytron. Separation is carried out in a 500 mL separatory funnel. The organic phase is washed with deionized, organics free water, dried with Na_2SO_4 and then evaporated to dryness. The residue is dissolved in a small amount of CHCl_3 (9 mL) and filtered through a syringe-filter apparatus into a centrifuge tube and the volume is adjusted so that 0.5 to 1.0 g residue per mL of CHCl_3 is not exceeded.

c) Clean-Up and Extraction

Filtered extract is passed through a Styragel gel permeation chromatographic column to remove co-extractives via molecular size exclusion of lipids.

The pre-determined fraction containing the dioxins and furans is then washed with Na_3PO_4 (0.05 M) for the removal of phenolics followed by a H_2SO_4 (10 N) wash for the removal of biogenic constituents.

After reduction in volume of the organic extract and solvent switching to hexane/methylene chloride, the sample is passed through an alumina column to remove PCB's and organochlorine pesticides.

Final polishing and isolation of the dioxin and furan containing fraction prior to GC-screening and subsequent GC/MS quantitation is carried out on a carbon-fibre system (1,2).

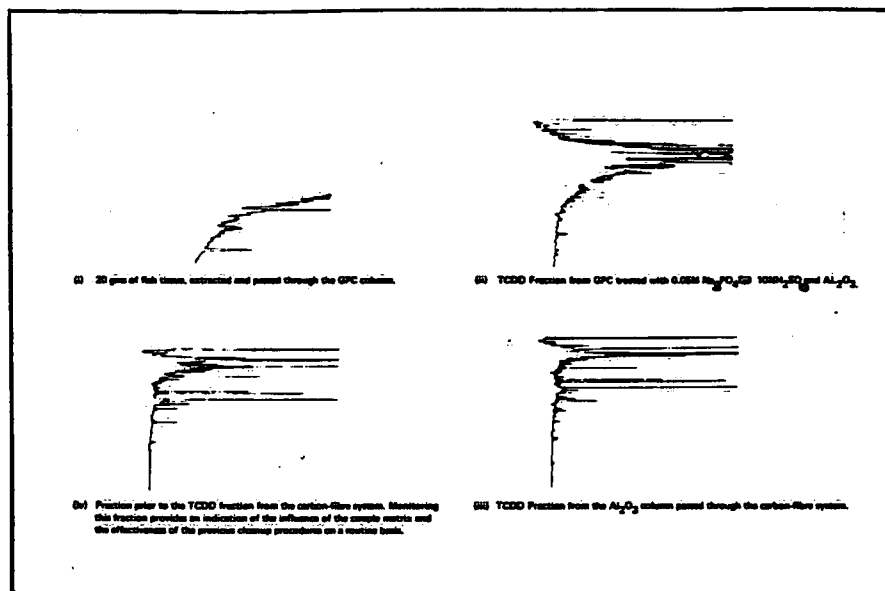


Fig. 2 HRGC-ECD Evaluation of Individual Clean-Up Steps

d) Electron Capture GC-Screening

HRGC/ECD screening of the above extract was carried out using thin film 0.17 μm , 50 m x 0.31 mm i.d. OV-1 FS-WCOT column. A Hewlett-Packard gas chromatograph Model 5880A was used. Samples were injected on-column with a GC-oven temperature of 80°C. After three minutes, the temperature of the oven was rapidly raised to 220°C (30°C/min) and programmed at 4°C/min to 260°C where it was held isothermally for 30 minutes.

The effectiveness of the individual (clean-up) steps is shown in Fig. 2. In this example 25 g of fish tissue extract was carried through the clean-up procedure. After each step an aliquot was taken, corresponding to approximately 0.8 g of fish tissue and injected onto the WCOT column. It can be seen that each step significantly reduces the background interferences and thus enables more accurate quantitation of 2,3,7,8-TCDD.

e) Analysis

Detection of PCDD's was accomplished with a Varian Vista 6000 gas chromatograph equipped with an electron capture detector (ECD) and a cold on-column injection system. A Spectra Physics SP-4200 computing integrator was used to obtain chromatograms and qualitative data for analysis of specific isomers. In these isomer specific analyses, hydrogen was used as the carrier gas. Gas chromatographic conditions were selected to minimize analysis time using a thin film (df=0.15 μm) narrow-bore (106 μm i.d.) WCOT column capable of separating all 22 tetrachloro dibenzo-p-dioxin isomers. The open tubular column, OTC, was 30 m x 0.106 mm i.d. coated with Silar 10c liquid phase. The hydrogen carrier gas was run at 345 kPa and 40 cm/s linear velocity, k' for 2,3,7,8-TCDD was 9.56 and the number of theoretical plates was 455690 at 200°C (3).

Relative retention data for 2:2 type substituted TCDD are given in Fig. 3 and Table 1.

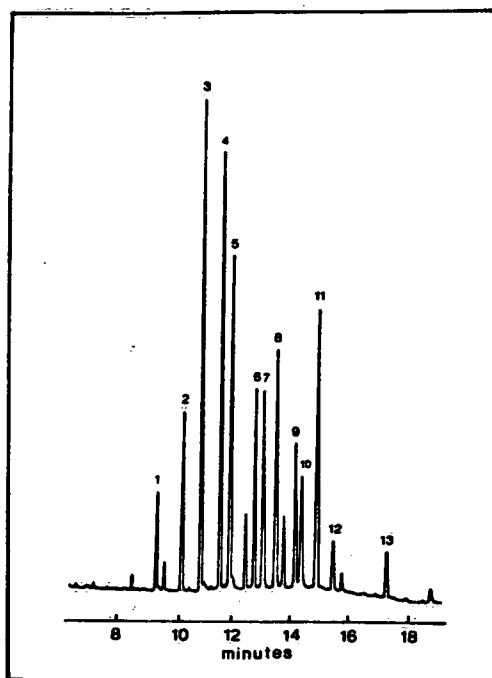


Fig. 3 HRGC-ECD Chromatogram of 2:2 type TCDDs
Column: 20 m x 100 μ m i.d. Silar 10c WCOT column at 200°C.

Table 1. Estimated Elution Order and Relative Retention Times for
2:2 Type Substituted Tetrachlor Dibenzo-p-Dioxin Isomers

Number	Substitution	RRT
1	1368	0.725
2	1379	0.791
3	1368	0.838
4	1369	0.890
5	1268	0.914
6	1478	0.978
7	2378	1.000
8	1279	1.033
9	1278	1.081
10	1469	1.098
11	1269	1.137
12	1267	1.182
13	1289	1.318

f) High Resolution Gas Chromatography/Mass Spectrometry

HRGC/MS analyses were accomplished using a Varian 311A mass spectrometer in tandem with a Carlo Erba 4160 gas chromatograph. The WCOT column, a 50 m x 0.2 mm i.d. fused silica high performance column, was coated with immobilized OV-17 liquid phase from Hewlett-Packard Co. The carrier gas was helium with a linear velocity of 25 cm/s. Temperature programming: the initial temperature was held at 80°C for 2 min, then programmed ballistically to 180°C and then to 250°C at 4°C/min. The rate was later changed to 12°C/min until the oven reached 290°C and the final temperature was held for 10 min. The electron multiplier voltage was held at 2.4 kV; source temperature at 200°C for electron impact ionization; and the manifold temperature at 100°C. The multiple ion detection hardware controlled device permitted the monitoring of a maximum of three ions.

Evaluation of the resolving power of the high performance OV-17 WCOT column by using 2:2 type TCDD isomers and ^{13}C -2,3,7,8-TCDD demonstrated that the procedure employing this column provided a specific analysis for 2,3,7,8-TCDD. The quantitation was checked for internal consistency with a calculation based on both areas and peak heights of m/z 320 and 322. Unlike the confirmation of identity which assumes proper response of all ions, a quantitation can be performed if an ion characteristic of native 2,3,7,8-TCDD and an ion characteristic of the ^{13}C -2,3,7,8-TCDD are observed to elute in the same window.

DISCUSSION

A fused silica OTC was prepared for this study coated with Silar 10c, a most polar cyanopropyl silicone phase that is very difficult to coat over the fused silica surface. The column, about 30 m x 0.1 mm i.d., with a thin film (0.15 μm) could be operated at a temperature 50°C below that for a similar column with a film thickness of 0.25 μm . These narrow-bore capillaries have a low sample capacity for chlorinated dibenzo-p-dioxins. With highest separation efficiencies, they can handle only about 150 picograms of 2,3,7,8-TCDD. Selected ion monitoring (SIM) with very high electron multiplier voltage setting (in our case, over 3.4 kV) can very easily saturate the amplifier and the operator must consider a relatively narrow dynamic range in such instances.

Isothermal conditions were found to be the most suitable for the tetrachloro congeners separation. The column temperature of 200° to 210°C resulted in a retention time window of between 15 and 22 minutes. The most suitable injection was cold on-column injection of the samples utilizing the solvent gap technique (3,4). This technique resulted in excellent peak shapes for all congeners with efficiencies otherwise not possible. Iso-octane was found to be a satisfactory solvent but dodecane or n-tetradecane can also be employed. The WCOT column did not last for more than two to three weeks (approximately 120 injections) without changes in performance becoming apparent. After that, the performance deteriorated rapidly. Better deactivation and surface pre-treatment of the fused silica and glass for the Silar 10c is essential and, perhaps, a stationary phase that would crosslink and bind to the surface silanol groups would be beneficial.

The results in Fig. 2, obtained by GC-ECD, show that good separation of the co-extractives from the compound of interest is obtained by combining the benzene and toluene fractions from the carbon fibre filter column. The recoveries of ^{13}C -labelled 2,3,7,8-TCDD and octachloro dibenzo-p-dioxin under standard conditions carried through the entire clean-up procedure are given in Table 2 for two different operators. These recoveries were better than 75 percent in all cases and better than 89 percent for all cases except sample 1, operator 1. The mean recoveries of real samples (spiked fish tissue homogenates) carried through the procedure was 71.6 percent for ^{13}C -TCDD based on 17 samples analyzed.

Mass spectrometric selected ion monitoring was used in the high resolution MS mode ($R=5000$) to confirm the presence of tetrachloro dibenzo-p-dioxin isomers in a standard solution provided by the U.S. Fish and Wildlife Laboratory (Dr. Stalling). Twenty-one of the 22 peaks could be resolved as shown in Fig. 4. Although GC-ECD showed 22 peaks for this solution, it was concluded that one of these was not a dioxin peak and that one of the tetrachloro isomers, probably 1,2,3,6-TCDD was not present in the standard solution.

Table 2. Recoveries of Labelled 2,3,7,8-TCDD and OCDD Standards from Clean-Up Procedure

Dioxin Recovery*		TCDD (%)	OCDD (%)
Operator 1	Sample 1	75	82
	Sample 2	89	103
	Sample 3	93	99
	Blank	0	5
Operator 2	Sample 1	92	97
	Sample 2	102	100
	Sample 3	102	103
	Sample 4	101	109
	Blank	0	11

*The spiked labelled dioxin standards were carried through all the clean-up procedures to determine the cumulative effect.

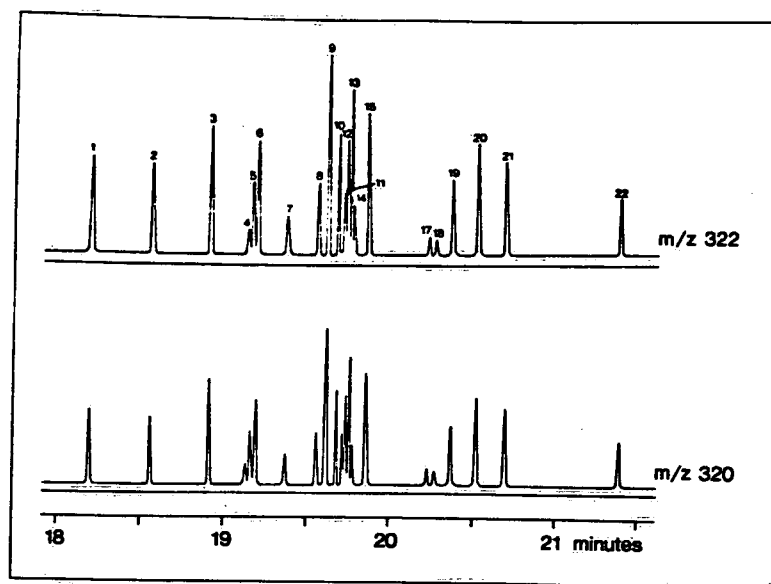


Fig. 4 HRGC/HRMS-SIM traces of m/z 320 and 322 showing the elution of 21 isomers from a 30 m x 0.106 mm i.d. Silar 10c WCOT column.

Elution order:

1=1368; 2=1379; 3=1378; 4=1369; 5=1248; 6=1247; 7=1268; 8=1478; 9=2378;
10=1234; 11=1249; 12=1246; 13=1238; 14=1237; 15=1236; 16=1279; 17=1469; 18=1278;
19=1239; 20=1269; 21=1267; 22=1289.

In conclusion, use of HRGC/LRMS for the determination of TCDD congeners should be used in combination with narrow-bore OTC-gas chromatography. It is preferable to also generate complementary data by HRGC/HRMS ($R > 5000$). This confirmation yields a high degree of confidence in the ability to determine picogram or even femtogram quantities of TCDD, when they are present (5). Exact limits of detection vary with the type of the sample extract and with instrument conditions.

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