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RECENT ADVANCES IN ULTRATRACE ANALYSIS OF DIOXINS AND RELATED HALOGENATED HYDROCARBONS

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Management Perspective

Analytical methodology for the measurement of polychlorinated dioxins and furans at ultratrace levels in environmental samples is extremely challenging. This paper describes methods research and development carrried out at the National Water Research Institute and the National Water Quality Laboratory since 1979. Methodologies for the extraction, clean-up,analysis and confirmation of chlorinated dioxins and furans in biota (fish, clams, gull eggs, clams), sediment, fly ash and water have been developed by NWRI and validated by the NWQL. As a result of this investigation, the NWQL now has the capability for dioxin and furan analysis.

This work has been part of the Water Quality Chemical Methods Development project of NWRI and is a good example of the benefits to be derived from joint NWQL/WQB studies.

Perspective-gestion

Il est très difficile de mettre au point des méthodes de mesure des dioxines polychlorées et des furanes à l'état d'ultratraces dans des échantillons prélevés dans l'environnement. Le présent rapport décrit les travaux de recherche et de développement entrepris en ce sens depuis 1979 par l'Institut national de recherche sur les eaux (INRE) et le Laboratoire national de la qualité des eaux (LNQE). L'INRE a conçu des méthodes d'extraction, de nettoyage, d'analyse et de confirmation de dioxines chlorées et de furanes dans le biote (poissons, palourdes, oeufs de goélands), les sédiments, la suie et l'eau, et ces méthodes ont été validées par le LNQE. Le LNQE possède donc maintenant les compétences lui permettant de se livrer à des analyses de dioxines et du furane.

Ces travaux s'inscrivent dans le cadre du projet d'élaboration des méthodes de détermination de la qualité de l'eau de l'INRE, et constituent un bon exemple des avantages que procurent les études de LNQE et de la DQE lorsqu'elles sont entreprises conjointement.

ABSTRACT

Analytical Methodology for polychlorinated dioxins and furans (PCDDs and PCDFs) in environmental samples is not standardized. Many variations exist for the extraction, cleanup, quantitation and confirmation of these compounds and reported detection limits and recoveries vary over several orders of magnitude. This paper describes research carried out at the Canada Centre for Inland Waters over the past 7-8 years to develop reliable methodology at ultra trace levels (10^{-12} - 10^{-15} g/g) applicable to a wide range of environmental samples.

Extraction techniques are described for biological tissue (fish clams, leaches, eggs), fly ash, sediments and water. Detailed cleanup procedures for the various matrices consist of most or all of the following: gel permeation chromatography; liquid/liquid extraction with Na_3PO_4; liquid/liquid extraction with H_2SO_4; basic alumina chromatography and carbon fibre chromatography. Preliminary screening of the cleaned extracts is achieved by gas chromatography equipped with a mass selective detector and positive identification by gas chromatography/mass spectrometry operated in electron impact and chemical ionization modes and with full scan and multiple ion detection. Custom software permitting the analysis of a wide range of compounds in a single run by GC/MS is reported. The presence of specific isomers of PCDDs and PCDFs is confirmed by high resolution gas chromatography/mass spectrometry.

A radioimmunoassay screening procedure for PCDDs in environmental samples is also described.

INTRODUCTION

The presence of polychlorinated dioxins and furans (PCDDs and PCDFs) in the environment is cause for concern to public, government and municipalities in industrialized societies. This is mainly due to the pronounced toxicity of the 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) isomer to certain biological species (1). Many agencies have established guidelines for these compounds and recently the Ontario Ministry of Environment has recommended a maximum daily human intake for 2,3,7,8-TCDD or its toxic equivalent of PCDDs and PCDFs of less than 10 pg/kilogram of body weight (2).

Methods for determining PCDDs and PCDFs in various types of samples have been well documented (2,3). A recent worldwide survey conducted by the Ontario Ministry of the Environment has shown a proliferation of extraction, cleanup, quantitation and confirmation techniques for analysis of these compounds (4). The reported range of detection limits and recoveries obtained for various types of samples are shown in Table 1 (4). The large range of reported detection limits and recoveries can be attributed to variations in the size of samples, complexity of samples, the amount of determinand in relation to the amount of interfering substances in samples together with variations in the procedures used for cleanup and quantitation of the PCDDs and PCDFs.

Work at the Canada Centre for Inland Waters for the past 7-8 years has been directed at systematic development of analytical

capability to detect and determine hazardous substances including PCDDs and PCDFs down to 10^{-12} g/g with a high degree of reliability and confidence. Developed methodologies had to be applicable to a wide range of environmental samples. This initially involved designing and constructing a special laboratory facility to a) minimize errors due to cross contamination of samples during cleanup and pretreatment and b) provide maximum safety to personnel when handling hazardous chemicals such as 2,3,7,8-TCDD during methods development and/or sample analysis (5). Several methods for extraction, cleanup, quantitation and confirmation of PCDDs and PCDFs at parts-per-trillion (ppt) and parts-per-quadrillion (ppq) levels have been developed to analyze chlorophenol formulations, fish tissues, sediments and fly-ash (6, 7). The methodologies were also applied to a study of the formation and fate of PCDDs and PCDFs during chlorophenol combustion (8).

This paper presents the recent work carried out by the authors in areas of large volume extraction of contaminants at ultra-trace levels (10^{-12} g/g to 10^{-15} g/g) from water, the application of radioimmunoassay as a screening technique for PCDDs, improvements in the cleanup of environmental samples and the use of hydrogen negative ion chemical ionization mass spectrometry to obtain improved precision and increased reliability for PCDDs analysis.

EXPERIMENTAL

APPARATUS AND EQUIPMENT

a) **Glassware:**

Consists of Erlenmayer flasks, separating funnels, Allihn filters, round bottom flasks, micro syringes, centrifuge tubes, disposable glass pipettes and chromatographic columns.

b) **Extraction and Concentration and Filtering Apparatus:**

Consists of rotary evaporators, vortex evaporator, homogenizers, soxhlet apparatus, centrifugation apparatus capable of accommodating 250 mL centrifuge cups and 4500-5000 rpm, gas tight syringes capable of accepting disposable filter units (25 mm diameter x 0.2 µm porocity).

c) **Gel Permeation Liquid Chromatographic System:**

Assembled using suitable injection valve, 5 mL sample loop, 60°A Styragel column, UV detector with 254 nm filter and a solvent delivery system capable of constant flow with medium and low back pressure.

d) **Alumina Column Cleanup Apparatus:**

Consists of 2 cm i.d. x 35 cm glass column with teflon stopcock with a glass wool plug. A 30 g alumina column (Brockman activity 1, 80-200 mesh, activated at 550° for six hours and deactivated with 1%-w:w distilled-deionized water) is used. A 2 cm layer of sodium sulfate was added to cover the alumina in order to maintain its activity. The column must be prepared just prior to use.

e) **Carbon-Fibre System:**

This system consists of a solvent delivery system capable of constant flow at low back pressure, solvent selector value allowing selection of 5-6 solvents, fraction collector and controller, and a 6-port switching valve equipped with 1 mL sample loop. The carbon-fibre column consists of 4 mm i.d. x 7.2 cm annealed glass and fitted with zero dead volume fittings and 2 µm stainless steel frits. The column packing is prepared as described below:

0.6 g of glass fibre filter paper (type GA200, Toyo Roshi Co. Ltd.) is weighed in a suitable size beaker, 0.05 g of activated carbon (PX-21, Amoco Research Corp) is added along with 100 mL methylene chloride. The mixture is shredded and mixed with a Polytron, so that the fibres are of a size capable of retaining the carbon particles. This slurry of carbon fibres is then

packed, using an aspirator, into a 7.2 cm, 6 mm O.D., annealed glass tube, equipped with zero dead volume fittings and 2μ frits.

f) Gas Chromatography-Mass Spectrometry Apparatus:

Preliminary screening and subsequent positive identification, confirmation and quantitative analysis are carried out using a wide variety of instruments and columns. The details of specific instruments and the operating conditions are described in the analytical protocols section of this paper.

g) Continuous-Flow Extractors:

A variety of continuous extractors have been developed to cover the various sampling procedures that may be required. They are designed primarily as portable units for use in the field, however, they can also be setup on board ship or in the laboratory. All the extractors are basically mixer-settlers in which the incoming water is passed through a chamber where it is mixed with solvent.

The extractors were produced in two sizes, "large" to extract up to 1 L per minute and "small" to extract up to 0.5 L per minute. The water can be sampled as 'whole water' i.e. with no separation of particulates or as 'clarified water' by extracting the effluent of a continuous flow centrifuge. The suspended solids can also be

collected and analyzed at the same time. The flow rate through the extractor and total sampling time can be adjusted so that there is sufficient material for analysis of corresponding suspended sediment and clarified water. For long sampling times (one week) the solvent can be stripped from the extractor effluent water and recirculated so that no solvent make up is required.

h) **Apparatus for Radioimmunoassay (RIA):**

Incubator, refrigerator, freezer, gamma counter, and refrigerated centrifuge are essential to carry out the assay.

i) **Reagents and Materials for RIA:**

Antiserum to TCDD, raised in rabbits as described by Albro et al. (9), antiserum to rabbit γ-globulin raised in goats, ^{125}I-labelled 1-N-(5-iodovaleramido)-3,7,8-trichlorodibenzo-p-dioxin, detergent solution, bovine γ-globulin, rabbit γ-globulin, phosphate buffer saline, antibody diluent and second antibody reagent.

j) **Internal (Spiking) Standard**

A mixture of ^{13}C labelled dioxins consisted of 50 pg/μL of 2,3,7,8-^{13}C TCDD, and 100 pg/μL each of 1,2,3,7,8-^{13}C-PCDD, 1,2,3,4,6,7,8-^{13}C-HpCDD and ^{13}C-OCDD in toluene. The ^{13}C hexa isomer was not available at our laboratory.

SAMPLE EXTRACTION TECHNIQUES

a) Tissue Extraction (Fish, Clams, Leeches and eggs)

10 gm of homogenized tissue is subsampled into a 500 mL Erlenmeyer flask and spiked with 25 µL internal standard. After the addition of 100 mL 1N HCl the contents of the flask are mixed by swirling and allowed to stand for 30 min. A 100 mL aliquot of toluene is added and the flask is placed on the wrist shaker overnight. The density of the resulting emulsion will determine the next analytical step. Light to medium emulsions that show some separation are transferred to a separatory funnel and allowed to separate. The lower aqueous layer, solids and emulsion remnants are then placed into stainless steel centrifuge cups and homogenized with an additional 100 mL of toluene, followed by centrifugation to break emulsions. Dense or persistent emulsions may be directly transferred to the centrifuge cups and centrifuged at 4000-4500 rpm for 10-15 minutes prior to decantation into a separatory funnel and subsequent separation. This separation, homogenation centrifugation scheme is repeated twice with 100 mL of toluene and the extracts are combined for further cleanup steps.

b) **Flyash and Sediments**

An appropriate size sample (1-10 g) is spiked with 25 µL internal standard, treated with 100 mLs 1N HCl and allowed to stand 1 hr. The mixture is then filtered and washed with 100 mL of distilled deionized water. The filtrate is extracted three times with 50 mL portions of toluene. The solids are mixed with sodium sulfate to obtain a free flowing mixture and soxhlet extracted for six hours with 300 mL of toluene. The toluene extracts are combined for further cleanup.

c) **Water**

A 50-200 L sample size, depending upon the concentration factor required, is extracted with 300 mL of dichloromethane using a continuous-flow extractor. The extraction is carried out by passing the sample through the extraction chamber where it is mixed with dichloromethane. The water phase is separated and passed to waste through a small packed column, the solvent is recirculated back into the mixing chamber. The effluent water contains dissolved solvent (about 1.5% by volume) which is replenished by adding solvent to the top of the small packed column through which the effluent water is passing, thus giving a counter-current extraction with clean solvent. The packing is 'Teflon' Raschig rings which also serve, by virtue of the solvent wetability of 'Teflon', to break any emulsions and coalesce small solvent drops.

In all extractions a metering pump is used to meter surrogate standard solution into the water sample flow as it enters the extractor. In this way for every sample extracted the recovery of the surrogate standards gives an immediate measure of the material balance obtained in the extraction-analytical process. The surrogate standards are chosen so that they have similar extraction characteristics to the analytes of interest and are compatible with the chromatographic procedure and detection system used. For example, when analyzing for PCDDs, where a mass detector is used, isotopically labelled dioxins such as C^{13} are ideal surrogates.

Extraction of small volumes of water (1-4 L size) is carried out using the separatory funnel technique. A sample is spiked with 25 µL of internal standard solution. The spiked sample is then extracted with dichloromethane. The extract is concentrated and exchanged to hexane or chloroform depending upon the type of cleanup required to eliminate interferences that may be encountered.

CLEANUP PROCEDURES

a) Liquid/Liquid Extraction using Trisodium Phosphate

The sample extracts are treated with 100 mL of 0.05 M trisodium phosphate ($Na_3PO_4.12H_2O$) for two minutes followed by a wash with 100 mL deionized water.

b) Liquid/Liquid Extraction using Sulphuric Acid

The sample extracts are treated with 100 mL of 1N sulphuric acid (H_2SO_4) for two minutes followed by a wash with 100 mL deionized water.

c) Mercury Treatment

Approximately 0.5 mL triple-distilled mercury is added to the concentrated organic extract (5 mL) and vigorously shaken until formation of a black precipitate ceases. The chloroform extract is filtered using a 0.2 µm nylon filter and a 5 mL gas-tight syringe. Additional chloroform is used to wash the mercury until a volume of 12 mL is reached.

d) Gel Permeation Chromatography (GPC)

The sample extract is evaporated to dryness, and the residue, reconstituted in small volumes of chloroform, is filtered through a 0.2 µm nylon filter until a volume of 12 mL is reached. 4.5 mL aliquots are injected into the GPC system operated at 5 mL/minute using chloroform as a mobile phase. The previously calibrated fraction containing the PCDD's and PCDFs is collected. This procedure is repeated for the entire sample volume and the fractions are combined for further cleanup.

e) Alumina Chromatography

The extract from each sample is evaporated to dryness and the residue is dissolved in 1 mL of 1% methylene chloride in hexane. The residue is transferred to the basic alumina column with solvent washings. The column is eluted with a total of 200 mL 1% (V:V) methylene chloride:hexane (Fraction 1) and subsequently with 150 mL 50% (V:V) methylene chloride hexane (Fraction 2). Fraction 2, containing PCDDs and PCDFs, is evaporated and the residue redissolved in 0.9 mL 50% (V:V) methylene chloride:cyclohexane.

f) Carbon Fibre Chromatography

The concentrated extract from the alumina cleanup is injected into the carbon fibre cleanup system and eluted at 4 mL/min in succession as follows:

20 mL 50% (V:V) methylene chloride:cyclohexane, 30 mLs ethyl acetate, 20 mL 4% Benzene in Ethyl Acetate, 20 mL 20% Benene in Ethyl Acetate and 30 mLs 50% Benzene in Ethyl Acetate. The carbon fibre is then back eluted with 30 mL toluene. The toluene fraction containing the PCDDs and PCDFs is concentrated to dryness and reconstituted with 25 µL ^{37}Cl labelled 2,3,7,8-TCDD in toluene for HRGC/LRMS analysis.

ISOLATION OF DIOXINS FROM ENVIRONMENTAL SAMPLES

Isolation procedures vary with type, nature and the concentration of interfering substances in the environmental sample. The detailed sequence of the sample pretreatment, extraction and cleanup procedures is determined by the sample type. Figure 1 illustrates the schematic, depending upon the matrix, employed for isolation of dioxin and related compounds prior to analysis.

ANALYTICAL PROTOCOLS

a) Preliminary Screening using Gas Chromatography with Mass Selective Detector

The system employing an HP5880A gas chromatograph fitted with a 30 m x 0.25 mm i.d. SPB-5 capillary column and HP5970 Mass Selective Detector is used on a routine basis.

The end of the column is inserted directly into the source of an HP 5970 Mass Selective Detector (MSD) used in the electron impact (EI) mode at 70 eV. The electron multiplier is set at 2200 volts and the dwell times at 200 ms.

Screening of the samples for the presence of dioxins is done in the single ion monitoring (SIM) mode by monitoring the three most intense ions in the molecular cluster and the (M-COCl) ion for each congener group over pre-determined time windows (Table 2). These

chomatographic windows are determined by running a mixture of all available dioxin isomers and by monitoring simultaneously the most intense ion for each congener group. The detection of the four specified ions in the correct ratios and at the right retention times provides confirmation of the presence of a particular dioxin isomer in the sample which is then subjected to the quantitation procedure.

The quantitative analysis protocol described here is similar to that reported by Norstrom et al. (10). It involves quantitation by monitoring, for the correct congener group and over its specified time window, the most intense ion in the molecular cluster of the unlabelled and ^{13}C labelled (internal standard) dioxin (Table 3). Use is made of the quantitation and reporting software provided with the HP 59970 data acquisition system. The quantitation is based on the ^{13}C internal standards which automatically correct for losses of material during the clean up and for chromatographic variations between injections. A mixture consisting of equal concentrations of unlabelled and ^{13}C labelled dioxins is first run in order to calibrate the system. The amount of each labelled dioxin is the same as that initially spiked in the sample. After integration of each ion peak, the system automatically updates the calibration table and creates a response ratio (unlabelled/labelled) versus concentration ratio calibration curve for each isomer specified in the table. The ratios found for the sample are then compared with the curves and the concentration of each specified dioxin determined. Recoveries are determined by running an external standard.

b) **Positive Identification**

A Finnigan 4500 gas chromatograph/mass spectrometer is used for routine confirmation. The cleaned sample extracts are injected into the system using a standard H.P. injector in the splitless mode at 260°C. The gas chromatographic separations are performed using a 60 m x 0.32 mm i.d. fused silica column coated with SP2331 to a film thickness of 0.2 μm. To achieve maximum sensitivity, the exit end of the capillary column is inserted directly into the ion source while maintaining the transfer line area at 250°C. Mass spectra are acquired in various modes such as full scan (FS) and multiple ion detection (MID) using both EI and negative ion chemical ionization. The following gas chromatographic and mass spectrometric conditions are used during the preliminary identification of PCDDs.

GC Conditions:

- carrier gas	hydrogen at 14 psi head pressure
- initial temperature	80°C
- initial time	2 minutes
- program at 15°C/minute to	200°C
- program at 4°C/minute to	250°C
- final holding time	40 minutes

MS Conditions:

	EI	CI
- ion source temperature	150°C	50°C
- manifold assembly temperature	100°C	100°C
- reagent gas	-	hydrogen
- ion source pressure	-	0.7 to 1.0 torr
- emission current	0.36 mA	0.36 mA
- electron voltage	70 eV	70 eV
- electron multiplier set at	1100 V, 3 kV on the dynodes for both modes.	
- electron multiplier gain		$x10^{-7}$ amp/volt

The acquired data are processed using a Finnigan SUPER INCOS data system. A supplementary data system, developed under contract for use in our laboratory, is also employed to carry out quantitation using multiple peak scanning mode. The latter system utilizes customized software to enable sufficient sensitivity and selectivity to be obtained for PCDDs through use of an integrated selected ion signal.

c) Custom Software

Custom software permitting the analysis of a wide range of compounds in a single run was developed under contract for use in our laboratory (11). The data system operates on a Zenith Z-100 computer controlling the Finnigan 4500 GC/MS system. The data system is highly automated and yet allows for operator interaction for optimization of sensitivity and selectivity and for verification of results. The sensitivity of the custom software falls between single ion monitoring and full scan mode of operation. It targets in on specific compounds in relationship to an internal standard rather than general time window in the chromatogram. Quantitation is achieved using the response factor of a single characteristic ion (base peak) relative to an internal standard. The identity of the compound is verified by monitoring up to six confirming ions. The system also includes a quality assurance protocol to monitor long-term performance of the GC/MS system and continuously updates the detection limits of individual compounds.

d) Isomer Specific Confirmation

A Varian MAT 311A mass spectrometer directly coupled to a Carlo Erba Model 4160 gas chromatograph is utilized for confirmation. The GC is equipped with a 60 m x 0.25 mm i.d. open tubular column (OTC) coated with Silar 10C. The carrier gas is helium with a linear velocity of 27 cm/s. The MS is equipped with a combination electron impact (EI) and chemical ionization (CI) ion source but operated only in the EI mode. Each four channels of the hardware multiple ion detection (MIS) device can be individually controlled for selection of acceleration voltage, range output, signal bandwidth, background baseline level and integration rate. The intensities of the selected masses are monitored on a four-channel recorder. The adjustable integration rate can accurately reproduce open tubular column peaks of less than 2 sec width at half peak height.

The high voltage unit is monitored using a KEITHLEY Model 191 digital multimeter to calculate the exact acceleration voltage required for the specific masses to be monitored. The magnet is set at perfluorokerosene (PFK) peak at m/z 318.9793 and the resolution is adjusted to between 5,500 and 7,000 mass resolution. The high voltage unit is monitored and used for calculating the exact acceleration voltages for the masses and ions given below.

Ion	Mass (m/z)
$C_{12}H_4O_2{}^{35}Cl_4$	319.8965
$C_{12}H_4O_2{}^{35}Cl_3{}^{37}Cl$	321.8936
$C_{12}H_4O_2{}^{37}Cl_4$	327.8847
$^{13}C_{12}H_4O_2{}^{35}Cl_4$	333.9337

The calculated values are introduced into SIM channels.

Using the Silar 10C column, we are able to resolve 18 baseline separated peaks from a mixture of 22 tetrachlorodebenzo-p-dioxin isomers. The column is also suitable for isomer specific confirmation of 2,3,7,8-TCDD.

e) **Radioimmunoassay Procedure**

A procedure recommended by Albro et al. (9) is employed. The organic extract is passed through appropriate cleanup steps to reduce interferences and concentrated to 10 µL or less per sample. The residue is solubilized by sonication in a detergent-buffer mixture and incubated with a previously optimzed dilution of antiserum from rabbits. A threefold excess of a radiolabelled derivative of trichlorodibenzo-p-dioxin over what can be bound by the amount of antiserum used is added and incubation is continued until equilibrium binding occurs. An optimum amount of antiserum to rabbit gamma-globulin prepared in goats is then added to precipitate the bound radiolabel. After precipitation is complete, the sample is

centrifuged. The supernate is drained off and the pellets radioassayed in a gamma counter. The presence of chlorinated dibenzo-p-dioxins in the sample extract results in decreased radioactivity being precipitated relative to procedural blanks, and the decrease is a measure of the amount of analyte present.

RESULTS AND DISCUSSION

Comparison and Choice of Extraction Techniques

Considerable work has been reported on the concentration and/or extraction of dioxin and related compounds at ultra-trace levels (ng to pg/L, ng/kg) from a wide variety of environmental samples. Adsorption, solvent extraction, homogenization and sonication have been used with numerous combinations of different solvents. In spite of the above, a wide variation in extraction efficiency and inability of certain techniques to quantitatively extract dioxins is universally recognized. Therefore, work was initiated to critically evaluate various techniques and select a narrow range of extraction techniques for different types of matrices. The choice of extraction technique was found to depend upon the type of sample and relative concentration of interferences in the samples. Improved recoveries of PCDDs in fish tissue and sediments have been obtained when samples were pretreated with hydrochloric acid and extracted with toluene using mechanical agitation or soxhlet respectively. Sediments with high sand or gravel

content can be satisfactorily extracted using sonication with organic solvents such as methylene chloride or toluene. Air particulates and flyash must be soxhlet extracted after pretreatment with hydrochloric acid and filtration. Our work confirms the earlier findings of Hutzinger et al. (1) who have critically evaluated various extraction techniques in a wide variety of environmental samples.

Cleanup Procedures

The cleanup scheme utilized for PCDD analysis of environmental samples has evolved from the methodology previously developed for the analysis of 2,3,7,8-TCDD in fish tissue samples (6). Certain fish species and sediment samples require a more comprehensive and exhaustive clean-up scheme to remove interfering compounds. The removal of sulphur compounds prior to gel permeation chromatography is necessary to prevent their deposition within the system and a resulting deterioration of efficiency. Phenolic compounds are one of the most prolific groups of chemical compounds found in nature, and their removal from sediment extracts greatly enhances the stability and resolution of the GPC step. Previously, a micro aluminia column employing neutral alumina had been used to separate aliphatics from aromatics (6,7), however it was found that certain fish extracts were contaminated with polychlorinated biphenyls at a level sufficiently high to result in a break-through of material which in turn led to failure of the carbon fibre isolation procedure. A higher capacity

aluminia column similar to that employed by Norstrom et al. (10), was incorporated into the clean-up process for a more reliable removal of PCBs. It was also found that a more stable elution pattern resulted if a six-solvent elution scheme similar to that established by Stalling et al, (12, 13) was employed with the carbon fibre system.

The detailed sequence of the clean-up procedure is determined by the nature of the sample and the type of its extract (Fig. 1). The choice and the order of cleanup steps, to eliminate interfering substances, is determined by the nature of samples, extraction technique employed and the relative concentration of interfering substances in the extract. Sediment samples, dumpsite samples containing organic matter, sludges and flyash required the most extensive cleanup. However, other types of samples can be analyzed by eliminating some of the cleanup steps described in the experimental part of this paper. Figure 1 illustrates the recommended cleanup steps for various types of samples.

The effectiveness of individual cleanup steps to eliminate interferences is shown in Figure 2. In this example, 25 g of fish tissue extract was carried through the cleanup procedure illustrated in Figure 1. After each major step an aliquot was taken, corresponding to approximately 0.8 g of fish tissue, and injected into a capillary gas chromatograph. It can be seen that each step significantly reduces the background interferences and thus enables more accurate quantitative analysis of PCDD.

Radioimmunoassay Technique for Dioxins

Conventional methods for the analysis of PCDDs, which combine gas chromatography with mass spectrometry following extensive extraction and cleanup procedures, are excessively time consuming and expensive when used for routine environmental monitoring. A gas chromatography-mass spectrometry laboratory with two operators, could be realistically expected to process a maximum of 10 samples a day; with an individual analysis, including isomer confirmation, costing between $500 and $1000. These are serious limitations when considered from the perspective of an environmental surveillance or monitoring program. In the event of an environmental accident involving the release of PCDDs into the environment, a requirement would exist for the short-term analysis of large numbers of environmental and biological samples, to be followed in all probability by an intensive monitoring program. Such demands would severely stress existing PCDD analytical facilities.

The inclusion of an effective screening test in the analytical protocol for PCDDs could resolve some of the foregoing problems by eliminating those samples that are free of PCDD from further time consuming conventional analyses. A screening method for PCDDs need not be isomer specific. Rather, positive samples from the screening step could be subsequently analyzed for specific PCDD isomers, using conventional techniques. Three screening methods have been proposed for the detection of PCDDs: radioimmunoassay (RIA (9), aryl hydro-

carbon hydroxylase (AHH) induction assay (14) and cytosol receptor assay (15). For reasons that have been adequately described by Albro et al. (9), RIA was considered to offer the best potential for eventual incorporation into an analytical protocol for the routine analysis of PCDDs.

Based on the classical antigen-antibody reaction, RIA is a relatively simple, powerful and adaptable technique for the rapid determination of trace levels of organic compounds. Originally developed for the micro-determination of proteinaceous substances, RIA is also extensively used in the detection and determination of steroidal hormones and other low molecular weight organic molecules. Immunoassays have been proposed for the detection of several environmentally important compounds in addition to PCDDs (9): PCBs (16), Dieldrin and Aldrin (17), Atrazin (18), Benomyl and Methyl 2-Benzimidazolecarbamate (19), Diclofop-methyl (20), 2,3,7,8-tetrachlorodibenzofuran (21), and 2,4-D and 2,4,5-T (22). Recently, the International Union of Pure and Applied Chemistry, reporting on improved cost effective approaches to pesticide residues analysis, concluded that immunochemical methods, while being traditionally unfamiliar to the residue chemist, offer exciting possibilities for newer, cost effective approaches (23).

Developed by Albro and co-workers (9), the RIA for PCDDs is a double antibody procedure with a reported detection limit of approximately 25 pg of 2,3,7,8-TCDD, and a reliable sensitivity in human fat of 100 pg ($P < 0.05$) (24). The precision of the RIA for

PCDDs remains to be determined for other sample types. The assay uses detergent micelles to solubilize the highly insoluble PCDD molecules which are then incubated, in a competitive binding reaction, with an iodine-125 labelled dioxin derivative and antibodies raised in rabbits against 2,3,7,8-TCDD. When equilibrium binding has been reached, bound and free labelled hapten are separated, and the bound radioactivity is quantified. A decrease in bound radioactivity, relative to the control tubes, is proportional to the amount of PCDD present. Concentrations of unknowns are interpolated from a standard curve.

A preliminary evaluation of the RIA for PCDDs was undertaken in order to confirm that the procedure has potential for the detection of PCDDs in fish extracts, a matrix routinely analyzed for PCDDs at CCIW. A set of fish samples (Table 4) consisting of a variety of tissue homogenates that had been previously extracted and prepared for TCDD analysis using a deactivated florisil clean-up procedure (R. Thomson, personal communication) was analyzed using RIA and GC-MS. The RIA analyses were undertaken by Dr. C. Mituma, SRI International, Menlo Park, California, USA.

Three of the fish extracts (Lake Trout #2 fraction D, Rainbow Trout fraction D, and Lake Trout #7 fraction E) had high TCDD contents, observations that were confirmed by the RIA analyses. Overloading of the low capacity florisil column probably caused carryover of TCDD from the D fraction, where it would be expected to elute from the column, to the F fraction where it was detected using

RIA in the Lake Trout #2 sample. The discrepancies between the TCDD levels detected in the extracts using GC-MS and RIA are unimportant, since RIA is proposed as a pre-screening method rather than for quantitative determination. Furthermore, RIA is responsive to PCDD isomers other than 2,3,7,8-TCDD (9) and to some PCDF isomers (9).

Several contradictions are apparent between the GC-MS and RIA results. No TCDD was detected in the Lake Trout #1 fraction D, and Ocean Haddock fraction D samples using GC-MS, whereas >20 and 12 ng respectively were detected using RIA. The presence of positive interferences, PCDDs other than 2,3,7,8-TCDD, or cross-reacting PCDFs in the sample extract, or the failure to detect TCDD using the GC-MS protocol in use at that time could cause such an effect. Fraction E of the acid digested Lake Trout #7 sample, was the only sample tested that gave a false negative result using RIA. The remainder of the samples, in which no TCDD was detected using GC-MS, yielded zero or low readings using RIA.

These preliminary data demonstrate that RIA can detect TCDD in fish samples. However, the problem of low level false positive results obtained using RIA requires investigation to clarify whether it is due to positive interferences, assay variability, or the presence of PCDDs other than 2,3,7,8-TCDD. Selection and optimization of an appropriate simplified clean-up procedure would help to eliminate positive interferences from sample extracts. The use of matrix blanks and control samples covering a range of analyte concentrations would further reduce the effect of low level positive

interferences and assay variability and instability in the RIA for PCDDs. The detection of PCDDs other than 2,3,7,8-TCDD, or of PCDFs, is not a problem, since the presence of such compounds in environmental samples is also cause for concern. Samples containing such interferences should be further analyzed using GC/MS.

Efforts to date have focussed on evaluating the potential of RIA for the detection of PCDDs in samples from aquatic environments, interfacing the RIA procedure with appropriate clean-up procedures and evaluating its performance when so interfaced, and investigating various means of improving the responsiveness, and precision of the RIA. Some basic changes to the original RIA procedure have resulted. The modified assay which uses dimethylsulfoxide (DMSO) as the solubilization agent, will be described in a later communication.

Confirmatory Techniques using GC/MS

Standard electron impact (EI) ionization at 70 electron volts is the most widely used form of ionization in conventional mass spectrometry. The majority of GC/MS operators use this mode for the confirmation of PCDDs and PCDFs.

Recently, several other modes of mass spectrometry involving chemical ionization (CI) with reagent gases such as methane, oxygen doped methane, and iso-butane have been used to improve sensitivity and in some cases isomer specificity (25, 26, 27).

All PCDDs available in our laboratory were analysed using full scan (FS) and also multiple ion scanning (MID) modes of detection. Preliminary data acquired using EI and pulsed positive ion negative ion chemical ionization (PPINICI) indicated that TCDDs were most sensitive using the EI-MID mode, while the H_2 NICI/MID mode provided enhanced sensitivity for the other PCDDs ranging from penta to octa chlorinated isomers.

In the past, the use of NICI with hydrogen as reagent gas has not been exploited. Presumably this is because it is not as good a reagent gas as methane or isobutane and in the presence of water molecules the H_3O^+ ions formed would compete for sample molecules and result in poor reproducibility of the CI spectra (28). In addition, hydrogen is a difficult gas to pump from the mass spectrometer system.

In our laboratory, we initially used methane as reagent gas with helium as the GC carrier gas. The use of NICI gave rise to a significant enhancement, compared to the more conventional EI mode, of mass spectrometry for the penta to octa substituted isomers of PCDDs. Because methane contaminates the ion source, the instrument sensitivity declines throughout the day. Substituting hydrogen for methane gives results that are about 70% as sensitive as for methane but additional benefits accrue which more than make up for this decrease in sensitivity. These advantages are:

(a) the use of hydrogen allows the He GC carrier gas to be replaced resulting in improved gas chromatography performance;

(b) the ion source does not become contaminated with the reagent gas and does not show a loss in sensitivity with time. Time consuming source clean ups are eliminated. Changing ion volumes, alternate NICI and EI modes of operation can be used with no apparent loss of sensitivity;

(c) analysis of sample extracts produce significantly lower background noise using H_2NICI mass spectrometry compared to the EI mode. However, similar background noise is observed when standard solutions of PCDDs are run in both modes;

(d) the spectra for PCDDs using EI and H_2NICI are similar in fragmentation of the M and M-Cl clusters. Because of this it is possible to use a common MID descriptor(s) with the data system and later reconstruct a dual display for both modes of operation, thereby enabling a direct comparison of spectra for peaks within ±1% retention time of each other;

(e) the use of multiple ion detection both EI and H_2NICI provides an additional confirmatory step to increase the confidence of PCDD identification.

All PCDDs available in our laboratories are analyzed using FS as well as MID modes. Data acquired for EI, positive ion chemical ionization and negative ion chemical ionization indicates that 2,3,7,8-TCDD is more sensitive using the EI-MID mode, while H_2NICI mode provides enhanced sensitivity for the penta-octa chlorinated PCDDs. Figures 3-5 and Table 5 present data for a selected isomer mix run under different mass spectrometric conditions. These results

indicate that the combination of HP OV-1 and H_2NICI in MID mode provide maximum senstivity for the majority of the higher chlorinated PCDDs. However, the HP OV-1 column does not resolve as many dioxin isomers as the SP2331 column under the conditions described in the experimental section.

With the current protocol, qualitative and confirmatory analysis of PCDDs consist of running the sample extract several times using GC-ECD, GC-MSD, HRGC-LRMS with various modes of ionization followed by a final reconfirmation of results using magnetic sector HRGC/MS.

Analysis of Environmental Matrices

Our earlier work was aimed at developing a reliable analytical method, using mild cleanup steps with least derivatization potential, for TCDDs in environmental samples. The application of this procedure to the analysis of other homologues and complex matrices such as polluted sediments, flyash and samples from dumpsites indicated that certain samples could not be analyzed, as indicated by low recoveries of spiked labelled isomers (6,7).

The modifications reported in this paper enabled us to analyze the above samples with increased accuracy and precision. The modifications which enabled improved recoveries included changes in alumina chromatography, addition of mercury cleanup step and change in the order of cleanup procedures. Table 6 and Figures 6 and 7 illustrate the type of recoveries and mass-chromatograms obtained by

the proposed method using highly contaminated samples. Furthermore, the modified procedure was also found to be satisfactory for analysis of a wide variety of tissue samples (e.g. Herring Gull eggs, clams, leachates and fish with high lipid content). The results of replicate analysis of Herring Gull egg pool samples are shown in Table 7 which illustrates the concentration in ng/kg, of native dioxins found in each replicate. The mean values with standard deviation are also given for each isomer. It should be noted that the native dioxins are reported as the 2,3,7,8-TCDD, 1,2,3,7,8-PCDD and 1,2,3,4,6,7,8-H7CDD isomers. There is only one OCDD. Table 8 lists the recoveries of the method spike and of the samples internal standard. No recoveries are reported for C^{13} hexa since we do not have that labeled isomer.

A few comments can be made about the analysis. The MS chromatograms were very "clean" with no interference present except in the case of samples #1 and #2. An interference was masking the octa M-COCl confirmation ion in those samples. But this was not the case for samples #3 and #4 in which the confirmation ion was clearly detectable thus allowing confirmation of octa in the samples.

Very low levels of native hepta were found in all the samples, typically 1.9 ng/Kg, which correspond to a signal to noise ratio of about 10. Accordingly, the M-COCl confirmation ion could not be detected and confirmation was based on the three most intense ions in the molecular cluster and the retention time of the peak. Traces of the other hepta isomer also appeared to be present in all the samples but its presence was not confirmed.

The lower recoveries for the higher chlorinated dioxins were linked to a loose frit in the carbon fibre column. This resulted in small carbon particles finding their way into the collection flask and sticking to the wall along with minute quantities of dioxins. Since the higher chlorinated dioxins, especially octa, adsorb strongly to carbon, they could not be recovered completely by the toluene washings. To confirm the above, the loose frit in the carbon fibre system was repaired and the performance of the system was tested using the internal (spiking) standard. A 1 mL aliquot of 50% (V:V) methylene chloride:cyclohexane, containing 25 μL of the internal (spiking) solution, was injected into the carbon fibre cleanup system and the toluene fraction containing PCDDs and PCDFs was analyzed using gas chromatography with mass selective detector. The repaired system afforded recoveries of over 95% for all the spiked dioxin isomers.

Overall, because of the very good efficiency of the cleanup and of the use of an internal standard, the results were considered quite reliable. Our laboratory also participated in an Interlaboratory comparison study which demonstrated that the results obtained, using a Herring Gull egg pool, were comparable to the reference values for the sample.

Further work is in progress to validate the above methodology which will include: (a) estimation of practical detection limits for each type of matrix at 95% confidence level, (b) determination of the

mean recoveries of a series of representative samples from each matrix using three levels of labeled dioxin isomers and varying sample size, (c) determining the scope of the methods by analyzing samples across Canada and determining the success rate for the procedure and (d) by "blind" analysis of blanks, reference samples and fortified replicate samples covering the range of matrices described in this paper.

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REFERENCES

1. O. Hutzinger, R.W. Frie, E. Merian and F. Pocchiari (Editors). Chlorinated Dioxins and Related Compounds - Impact on the Environment, Pergamon Press, Oxford, 1982.
2. National Research Council. Polychlorinated Dibenzo-p-Dioxins: Limitations to the Current Analytical Techniques, NRCC Publication No. 18576, 1981.
3. Ontario Ministry of the Environment. Polychlorinated Dibenzo-p-Dioxins and Polychlorinated Dibenzofurans - Scientific Criteria Document for Standard Development No. 4-84, 1985.

4. R.E. Clement and S.A. Lennox. Worldwide Survey on Chlorinated Dibenzo-p-Dioxin and Dibenzofuran Analysis Capabilities. Unpublished Report, Ontario Ministry of Environment, Rexdale, Ontario, Canada, August 1986.

5. B.K. Afghan and J. Lawrence, Canadian Research, (November/ December 1981).

6. B.K. Afghan, Canadian Research, 36 (November 1983).

7. J. Lawrence, F. Onuska, R. Wilkinson and B.K. Afghan, Chemosphere 15(9-12): 1085 (1986).

8. T. Bridle et al., Proceedings of 77th Annual Meeting of the Air Pollution Control Association, 1-15 (1984).

9. P.W. Albro, M.I. Luster, K. Chae, S.K. Chaudhary, G. Clark, L.D. Lawson, J.T. Corbett and J.D. McKinney, Toxicol. and Appl. Pharmacol. 50: 137 (1979).

10. R.J. Norstrom, M. Simon and M.J. Mulvihill, Intern. J. Environ. Anal. Chem., 23: 267 (1986).

11. S. Shrader, Shrader Analytical and Consulting Laboratories (Canada) Ltd., Mississauga, Ontario, Canada, June 1985, unpublished work.

12. L.M. Smith, D.L. Stalling and J.I. Johnson, Anal. Chem., 56(11): 1830 (1984).

13. M.A. Ribick, L.M. Smith, G.R. Dubay and D.L. Stalling, Application and Results of Analytical Methods Used in Monitoring Environmental Contaminants. Submitted at the ASTM Committee E35 on Aquatic Toxicity Symposium, Chicago, Illinois (1979).

14. J.A. Bradlaw and J.A. Caserline, J. Assoc. Off. Anal. Chem., 62: 904 (1979).

15. A. Poland, E. Glover and A.S. Kende, J. Biol. Chem., 251: 4936 (1976).

16. W.H. Newsome and J.B. Shields, Intern. J. Environ. Anal. Chem., 10: 295 (1981).

17. J.J. Langone and H. Van Vunakis, Res. Commun. Chem. Path. and Pharm., 10: 163 (1975).

18. S.J. Huber, Chemosphere, 14: 1795 (1985).

19. W.H. Newsome and J.B. Shields, J. Agric. Food Chem., 29: 220 (1981).

20. M. Schwalbe, E. Dorn and K. Beyermann, J. Agric. Food Chem., 32: 734 (1984).

21. M.I. Luster, P.W. Albro, K. Chae, L.D. Lawson, J.T. Corbett and J.D. McKinney, Anal. Chem., 52: 1497 (1980).

22. D.F. Rinder and J.R. Flecker, Bull. Environ. Contam. Toxicol., 26: 375 (1981).

23. R.J. Hemingway, Pure and Appl. Chem., 56: 1131 (1984).

24. J. McKinney, P. Albro, M. Luster, B. Corbett, J. Schroeder and L. Lawson, In "Chlorinated Dioxins and Related Compounds: Impact on the Environment", O. Hutzinger Ed., Pergamon Press, pp 67-75 (1982).

25. J.R. Hass, M.D. Friesen and M.K. Hoffman, Org. Mass Spectrom., 14: 9 (1979).

26. R.K. Mitchum, G.F. Moler and W.A. Korfmacher, Anal. Chem., 52: 2278 (1980).

27. R.K. Mitchum, W.A. Korfmacher, G.F. Moler and D.L. Stalling, Anal. Chem., 54: 719 (1982).

28. G.W.A. Milne and M.J. Lacey, Crit. Rev. in Anal. Chem., 4: 81 (1974).

Table 1 Reported limits of detection (LOD) and range of percent recovery for PCDDs by laboratories responding to worldwide survey (4) (all values in parts-per-trillion: 10^{-12} g/g).

Sample Type	Compound	Range of LOD	Range of % Recovery
Soil	2,3,7,8-TCDD	0.1-1000	10-179
	OCDD	1.0-500	0-184
Fish	2,3,7,8-TCDD	0.1-1000	6-150
	OCDD	1.0-100	10-110
Water	2,3,7,8-TCDD	0.0005-300	40-110
	OCDD	0-0001-50	40-116
Flyash	2,3,7,8-TCDD	0.1-300	10-118
	OCDD	1.0-100	37-122

Table 2 Nominal masses of the three most intense ions in the molecular cluster of unlabelled dioxins and of the corresponding COCl loss.

Congener	Time Window (min)	Nominal Masses of Monitored Molecular Ions			M-COCl
Tetra	13.0 - 17.0	319.9	321.9*	323.9	258.9*
Penta	17.0 - 20.0	353.9	355.9*	357.8	292.9*
Hexa	20.0 - 23.0	387.8	389.8*	391.8	326.8*
Hepta	23.0 - 27.5	423.8*	425.8	427.8	360.8*
Octa	27.5 - 32.0	457.7	459.7*	461.7	396.8*

*Most intense ion in cluster.

Table 3 Nominal masses of the three most intense ions in the molecular cluster of C^{13} labelled dioxins.

C^{13} Dioxin	Three Most Intense Ions in Molecular Cluster		
Tetra	331.9	333.9*	335.9
Penta	365.9	367.9*	369.9
Hexa	399.9	401.9*	403.8
Hepta	435.8*	437.8	439.8
Octa	469.8	471.8*	473.8

* Most intense ion in cluster.

Table 4 Analysis of fish samples for 2,3,7,8-TCDD using RIA and GC-MS.

Sample	Lipid (%)	Fraction	GC-MS (ng)	RIA (ng)
Lake Trout #1	18.5	D[1]	ND[3]	>20
Lake Trout #1	18.5	F[2]	ND	0
Ocean Haddock	0.4	D	ND	12.0
Ocean Haddock	0.4	F	ND	0.3
Lake Trout #2	24.7	D	3.4	>20
Lake Trout #2	24.7	F	-	>20
Rainbow Trout	19.8	D	3.7	>20
Rainbow Trout	19.8	F	ND	0.6
Lake Trout #7[4]	35.6	E	0.8	0
Lake Trout #7[4]	35.6	F	ND	0.19
Lake Trout #7[4]	35.6	E+F	ND	0
Lake Trout #7	35.6	E	4.0	>20
Lake Trout #7	35.6	F	ND	0.16
Fish Sample #10	ND	E+F	ND	0.5
Fish Sample #13	ND	E+F	ND	0.5
2,3,7,8-TCDD	-	(5)	10.0	>20

[1] 15% ethyl acetate fraction, passed through Al_2O_3 on 40% $MeCl_2$/hexane.
[2] 25% ethyl acetate fraction, passed through Al_2O_3 on 40% $MeCl_2$/hexane.
[3] ND: none detected.
[4] Hexane extraction of HCl digested fish.
(5) Standard run through GPC column, fraction 30-40 min at a flow rate of 5 µl/min, collected.

Table 5 Response ratios for selected PCDDs using various capillary columns and modes of mass spectrometry.

No. of Cl/ Abbreviation	Ions Monitored	GC Columns H_2NICI-FS	FS	MID	H_2NICI
		OV-1/SP2331	H_2NICI/EI	H_2NICI/EI	MID/FS
4/TCDD	257[a],259[a], 320,322*,328[b] 332[c]	ND	0.14	<0.01	ND
5/PnCDD	354,356*,358, 368[c]	2.00	9.70	5.40	30
6/HxCDD	388,390*,392 402[c]	2.70	4.00	5.70	25
7/HpCDD	422,424*,426, 436[c]	3.25	4.80	3.30	22
8/OCDD	456,458,460*, 472[c]	2.25	5.80	1.56	15

ND = Not detected
a = COCl loss in EI mode
b = Ion for C^{37}-labelled isomer
c = Ion for C^{13}-labelled isomer
* = Ion used for quantitation.

Table 6 Percent recovery of labeled dioxins in different matrices

Sample	Amount Spiked/Recovery Observed (ng) (%)			
	T_4CDD	P_5CDD	H_7CDD	O_8CDD
Tap Water (410L)	1.25/105	2.50/106	2.50/119	2.50/124
Suspended Sediment (10g)	1.25/96	2.50/119	2.50/113	2.50/92
Bottom Sediment (10g)	1.25/69	2.50/96	2.50/92	2.50/93
Leeches (13.5g)	1.25/76	2.50/87	2.50/115	2.50/117
Landfill (10g)	1.25/112	2.50/114	2.50/112	2.50/146

Table 7. Levels of dioxins found in Herring Gull egg samples

	Blank	Herring Gull Egg, Replicate No.					
		#1	#2	#3	#4	$\bar{x}$	$\sigma_{\bar{x}}$
Sample Weight	-	25.30g	25.30g	25.30g	28.75g	-	-
TETRA (ng/Kg)	ND	53.5	55.0	47.5	52.7	52.2	3.3
PENTA (ng/Kg)	ND	10.6	9.3	9.5	9.4	9.7	0.6
HEXA (ng/Kg)	ND	13.2	11.6	11.7	12.0	12.1	0.7
HEPTA (ng/Kg)	ND	1.9	2.0	1.4	2.1	1.9	0.3
OCTA (ng/Kg)	ND	23.2	19.5	18.1	19.7	20.1	2.2

ND - Not Detected.

Table 8. Recoveries of method spike and samples internal spike

	Method Spike	% Recoveries Egg Replicate No.			
		#1	#2	#3	#4
C13 TETRA	107	92	100	99	99
C13 PENTA	87	60	78	84	91
C13 HEPTA	85	52	61	65	62
C13 OCTA	70	40	46	47	47

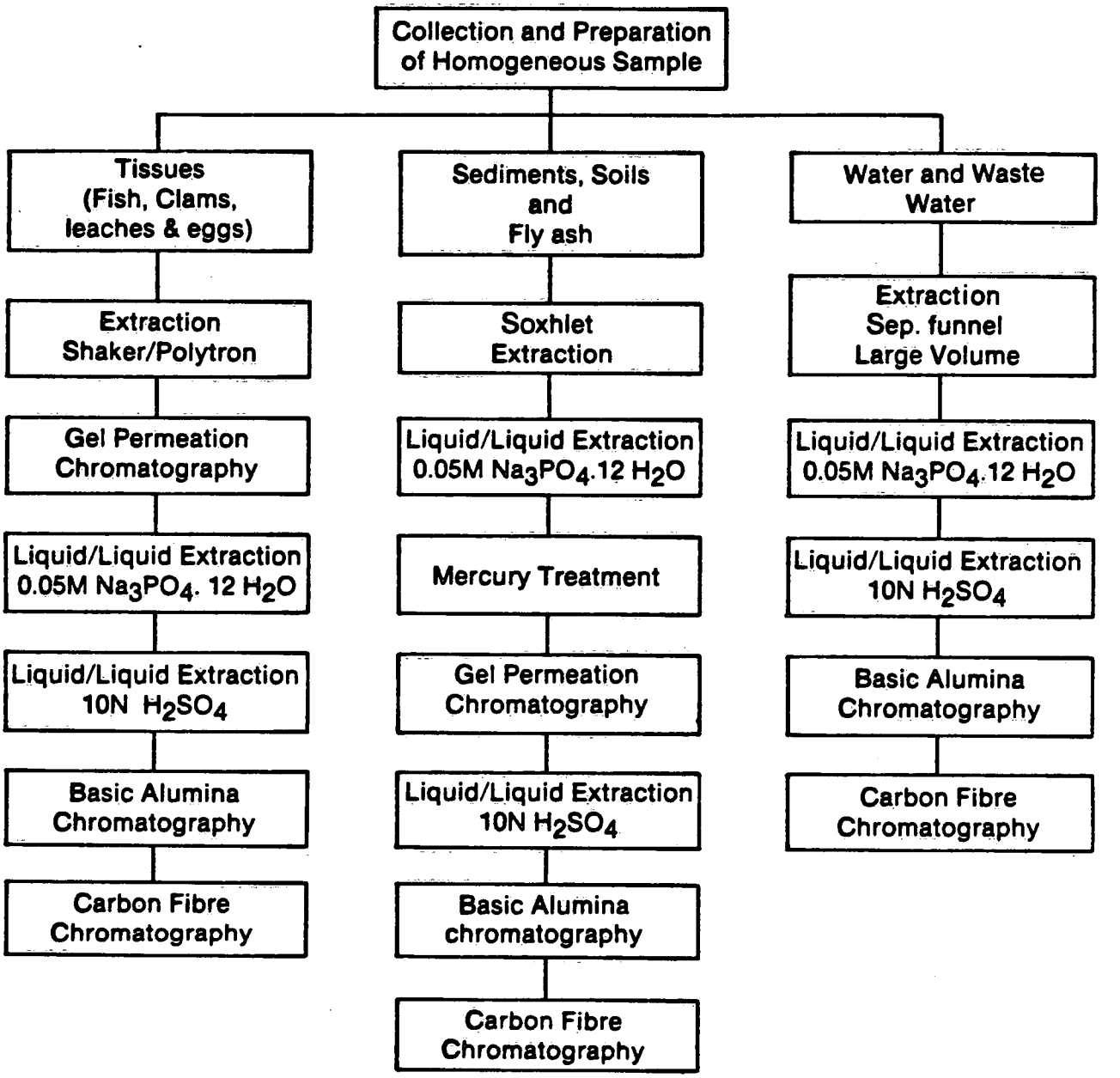
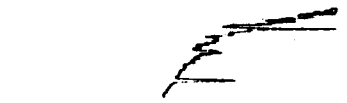
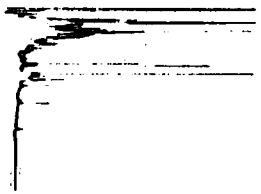


Figure 1 Sample Extraction Schematic

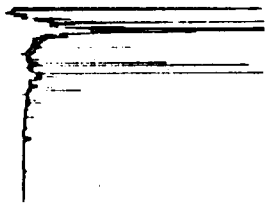


(i) 20 gms of fish tissue, extracted and passed through the GPC column.

(ii) TCDD Fraction from GPC treated with 0.05M Na_2PO_4H 10MH_2SO and AL_2O_3.



(iv) Fraction prior to the TCDD fraction from the carbon-fibre system. Monitoring this fraction provides an indication of the influence of the sample matrix and the effectiveness of the previous cleanup procedures on a routine basis.



(iii) TCDD Fraction from the Al_2O_3 column passed through the carbon-fibre system.

Figure 2 Chromatograms showing effect of sequential clean-up steps.

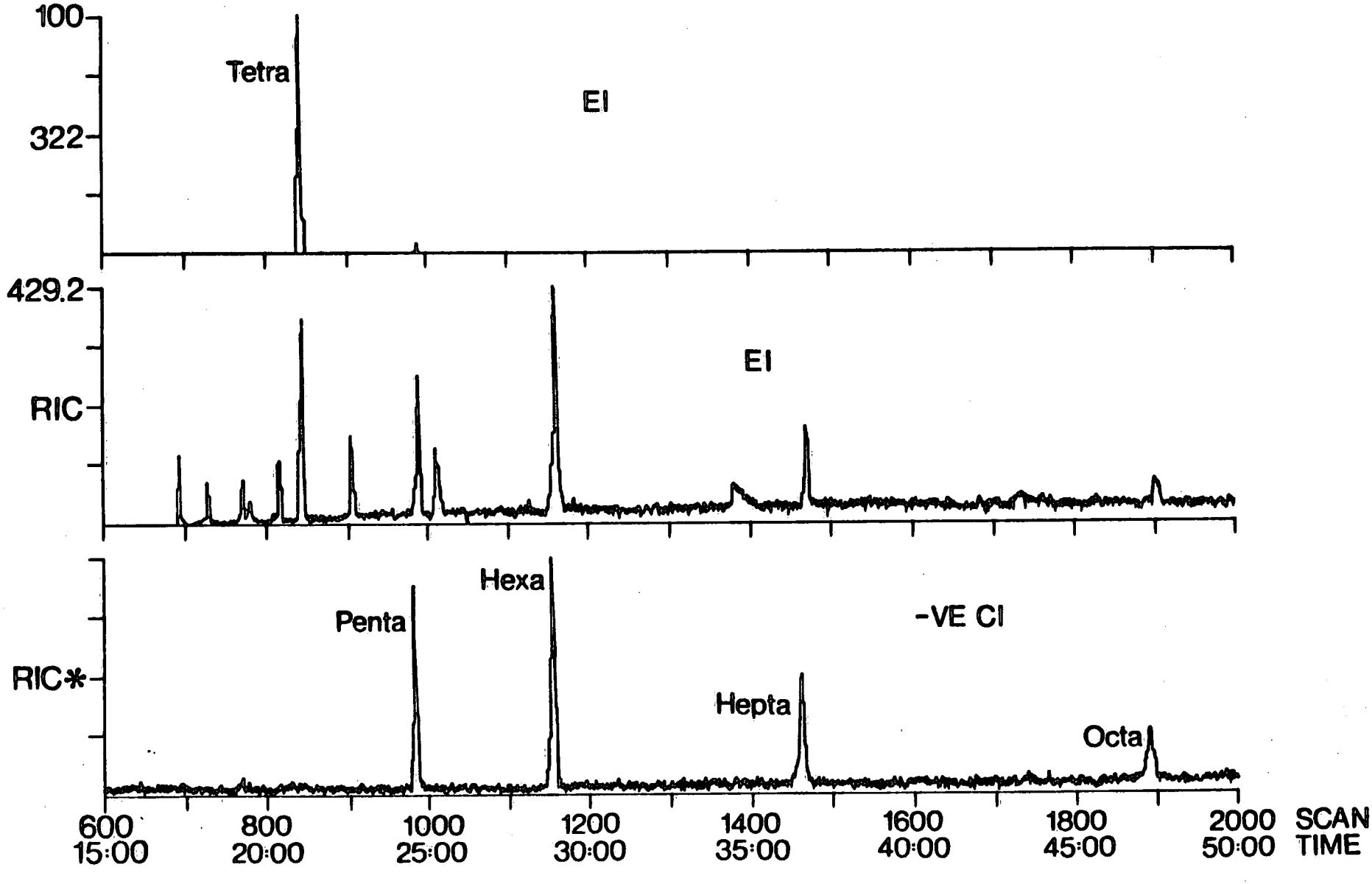


Figure 3 Ion Chromatogram with H_2 NI CI scanning from 240 to 480 amu for Dioxin standard mixture. Tetra shown at mass 322 for top mass chromatogram.

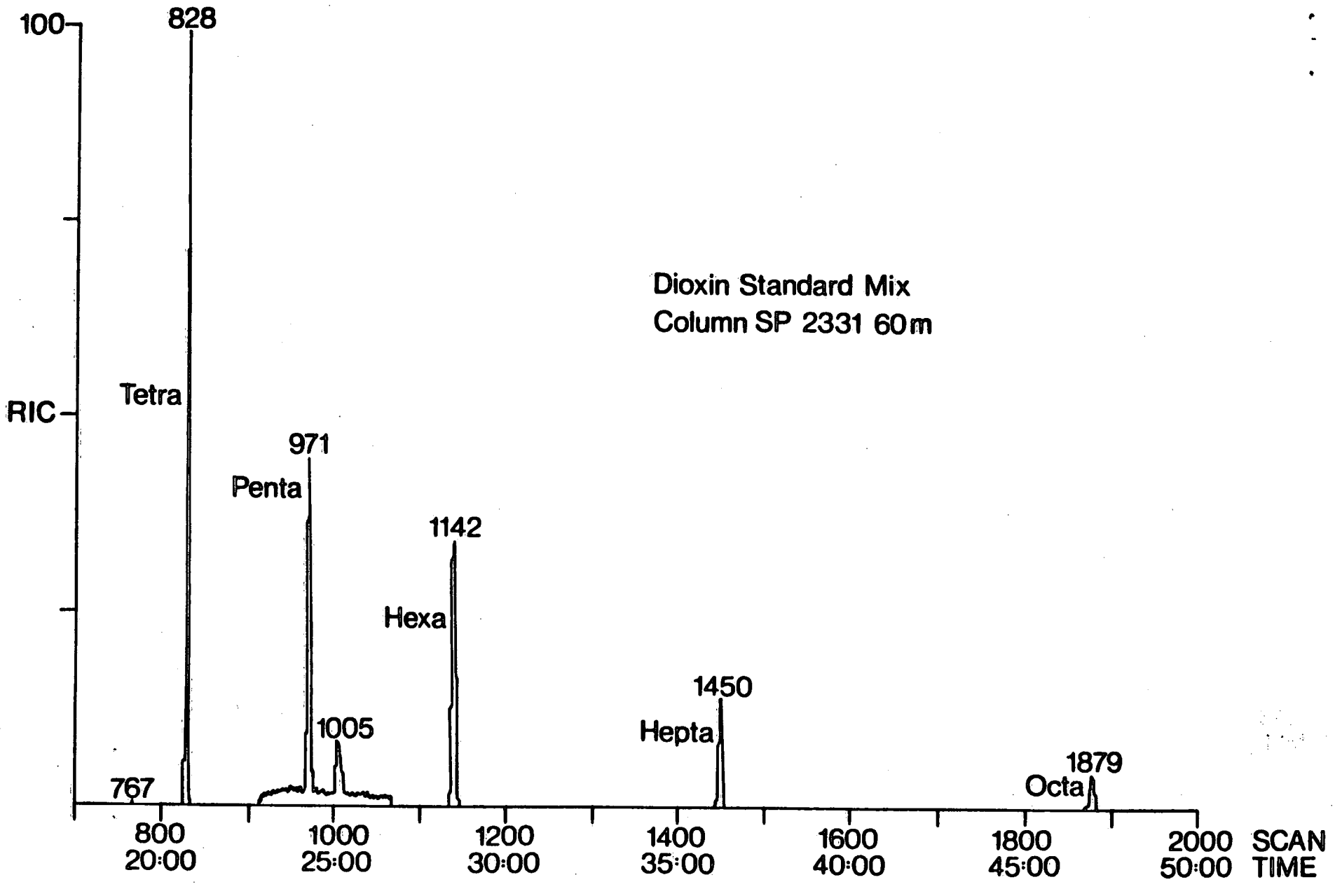


Figure 4 Ion Chromatogram with EI multiple ion scan for Dioxin standard mixture.

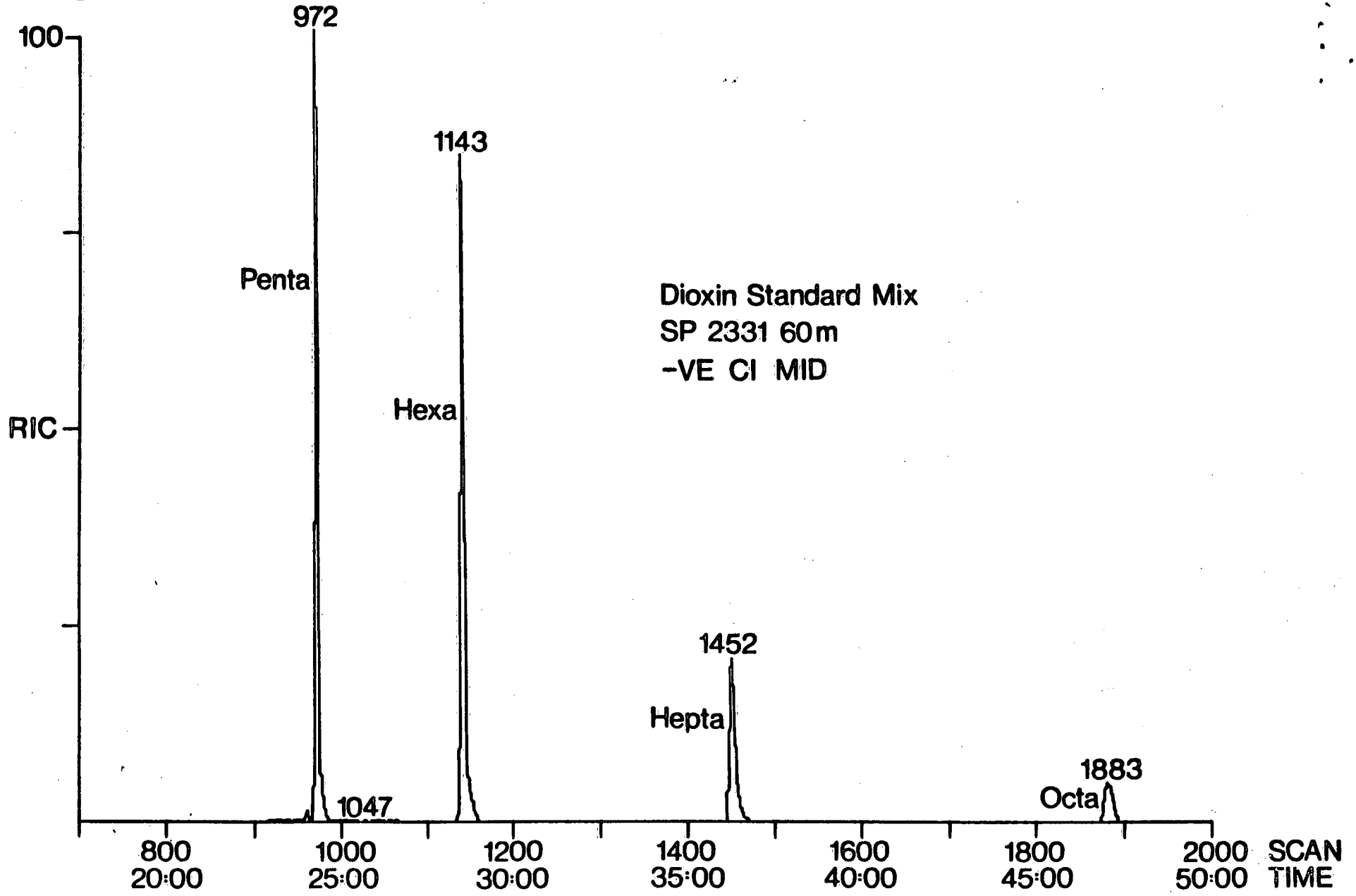
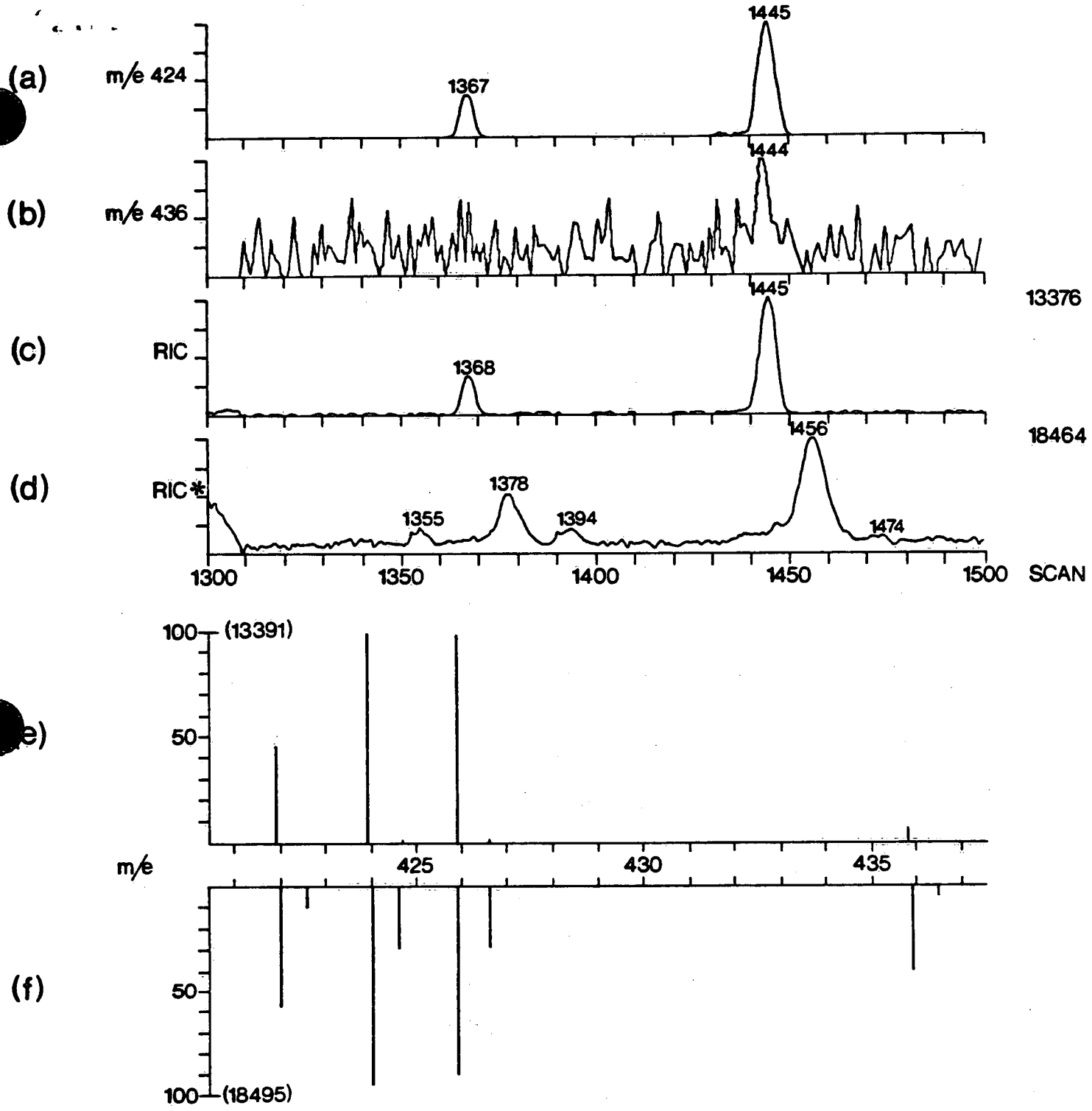


Figure 5 Ion Chromatogram with H_2 NI CI multiple ion scan for Dioxin standard mixture. Note - no response for tetra.



(a) m/e 424, MID EI for Hepta Dioxins

(b) m/e 436, MID EI for C^{13} Internal Standard

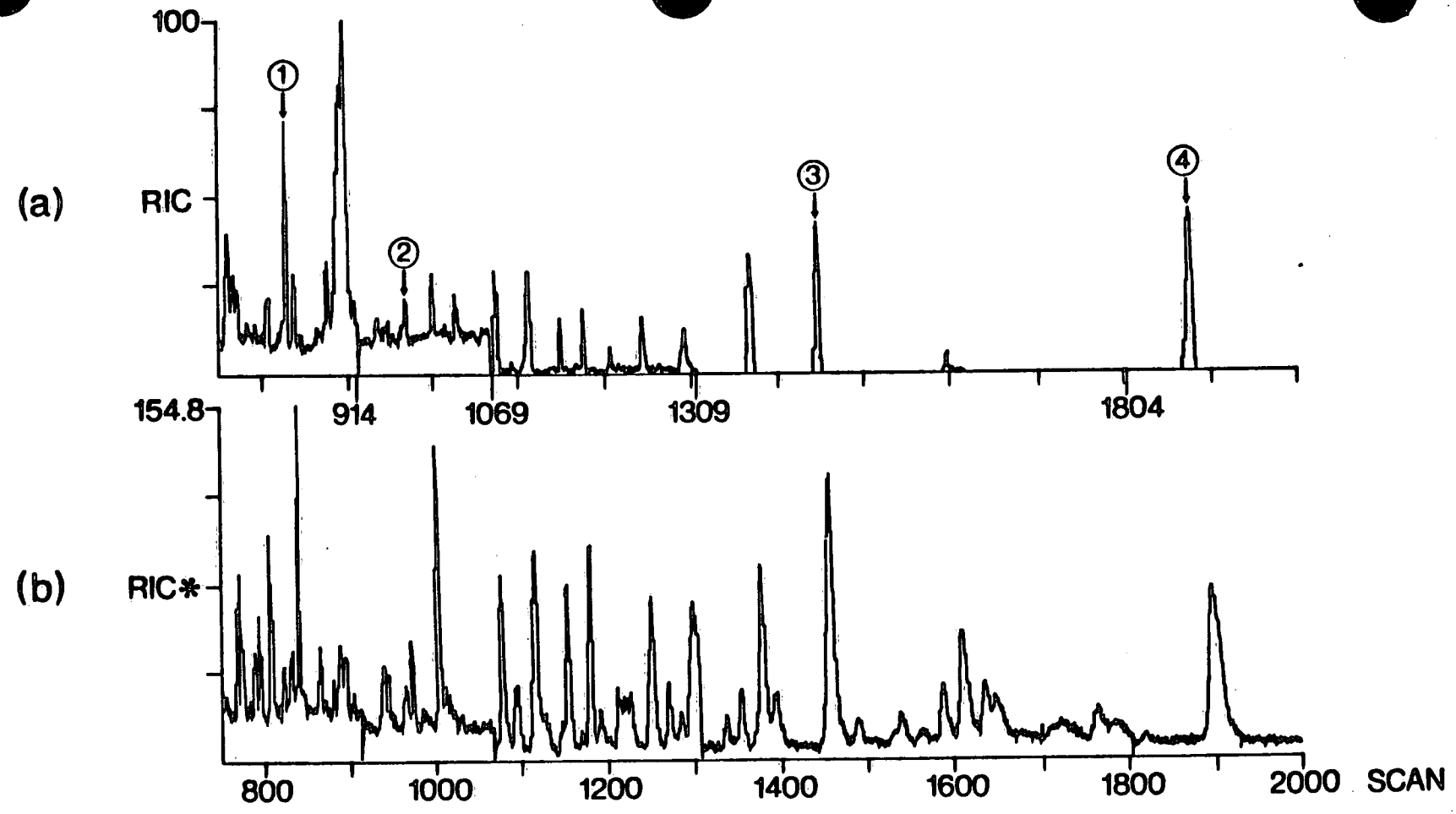
(c) RIC (Reconstructed Ion Chromatogram), MID EI

(d) RIC* (Reconstructed Ion Chromatogram), MID H_2/NI

(e) MID EI Spectra of Scan No. 1445

(f) MID Hz/NI Spectra of Scan No. 1456

Figure 7 Blow up of the Hepta Dioxin region of the mass chromatograph shown in Figure 6.



(a) MID EI run using 5.0 grams of sediment

(b) MID H_2/NI confirmatory run

C^{13} labelled internal stands ① tetra
② penta
③ hepta
④ octa

Figure 6 EI versus CI reconstructed ion chromatogram of contaminated sediment sample.