United States
Environmental Protection
Agency

Environmental Research
Laboratory
Duluth MN 55840

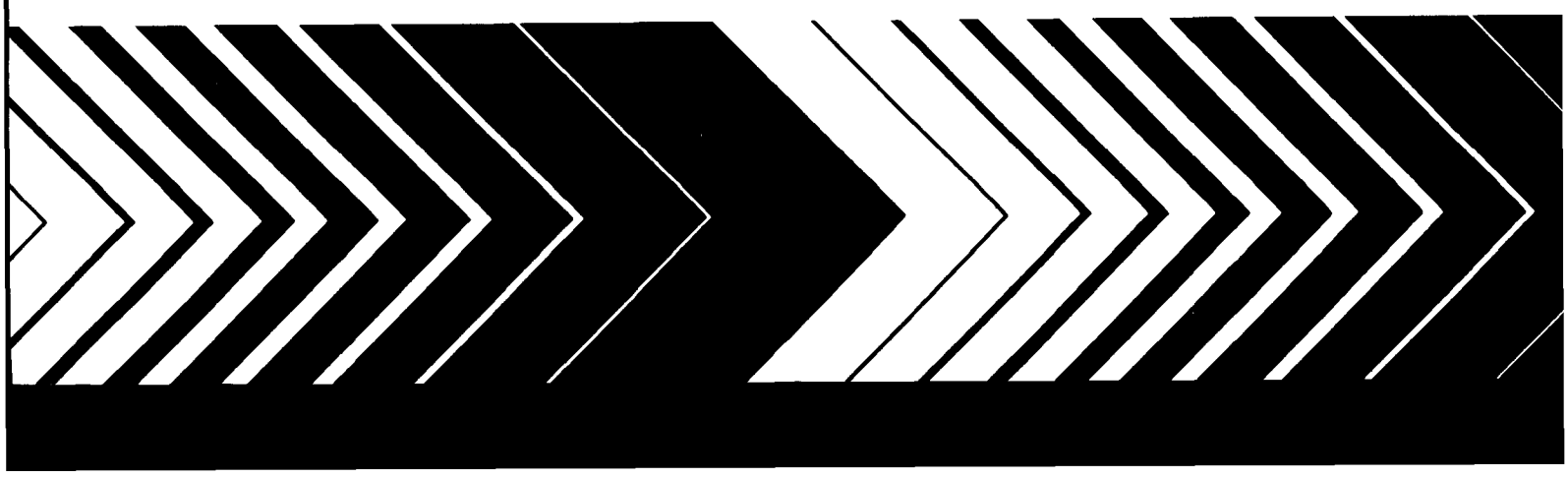
EPA/600/3-85/019
May 1986

Research and Development

EPA

National Dioxin Study

Analytical Procedures and Quality Assurance Plan for the Analysis of 2,3,7,8-TCDD in Tier 3-7 Samples of the U.S. Environmental Protection Agency National Dioxin Study



EPA/600/3-85/019
May 1986

National Dioxin Study

Analytical Procedures and Quality Assurance Plan for the Analysis of 2,3,7,8-TCDD in Tier 3-7 Samples of the U.S. Environmental Protection Agency National Dioxin Strategy

Environmental Monitoring Systems Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Environmental Chemistry Laboratory
U.S. Environmental Protection Agency
NASA/NSTL, Building 1105
Bay St. Louis, MS 39529

Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Duluth, MN 55804

Notice

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Analytical methodology used by EMSL-RTP, ECL-BSL, and ERL-D in the analysis of samples for the study of Tiers 3-7 in the U.S. EPA National Dioxin Strategy is provided. The methods are based on low and high resolution mass spectrometry using stable isotope labeled internal standards. The methods were peer reviewed by the Quality Assurance Committee for the National Dioxin Strategy, as revised in November 1984. The methods are to serve only as a guideline for these analyses and may be modified as required to successfully meet target detection limits.

Contents

Section I

Analytical Procedures and Quality Assurance Plan for Tiers 3 through 7 of the National Dioxin Study. Environmental Monitoring Systems Laboratory, Environmental Chemistry Laboratory

Contents (Continued)

Section II

Analytical Procedures and Quality Assurance Plan for the National Dioxin Study. Environmental Research Laboratory-Duluth

Contents (Continued)

Section I
Analytical Procedures and Quality Assurance Plan for Tiers 3 Through 7 of the National Dioxin Study

Environmental Monitoring Systems Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina 27711

Environmental Chemistry Laboratory
U.S. Environmental Protection Agency
NASA/NSTL, Building 1105
Bay St. Louis, Mississippi 39529

I. Introduction

The identification and quantitative measurement of part-per-trillion (ppt) to part-per-quadrillion (ppq) levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2378-TCDD) TCDD isomers, other CDDs and CDFs in various sample matrices is currently performed by only a limited number of laboratories in the U.S. and in foreign countries. Efficient sample preparation procedures and sensitive and specific detection techniques are required for the analytical determinations. Credibility and validity of results at this level have been the subject of intense scrutiny by the scientific community. Therefore, stringent measures have been used by EPA since 1975 to validate the analytical methodology/results derived for quantitative measurement of 2378-TCDD by participating laboratories. Four basic principles used for this purpose were: comprehensive studies were performed to demonstrate and validate the methods; analysis of blind quality assurance QA samples during the analysis of test samples; multiple laboratory participation; and defined analytical criteria used for confirmation of 2378-TCDD. Participants have included two EPA laboratories, Environmental Monitoring Systems Laboratory (EMSL-RTP) and the Environmental Chemistry Laboratory-Bay St. Louis, Mississippi (ECL); Wright State University; University of Nebraska; Harvard University; University of Utah and Dow Chemical Company. Sample preparation procedures and detection techniques utilized by the various laboratories are quite different, yet the analytical results have been in remarkably close agreement as a result of the application of these principles. Therefore, the credibility and validity of each laboratory's method and the results are enhanced. These four principles will also be used in the recently created National Dioxin Study.

In order to satisfy the urgent need for special analytical services, existing facilities, equipment and personnel of ORD and OPP, which are currently involved in 2378-TCDD analysis are being detailed and expanded into a consolidated two year effort by establishment of a National Trace Analysis TROIKA. The TROIKA consists of components from the Environmental Research Laboratory-Duluth (ERL-D) and the Environmental Monitoring Systems Laboratory-Research Triangle Park (EMSL-RTP) of the Office of Research and Development (ORD) and the Environmental Chemistry Laboratory-Bay St. Louis (ECL) of the Office of Pesticide Programs (OPP). These three laboratories have two major functions: (1) provide immediate and valid ppt and ppq level analytical results for the National Study and (2) serve as the central nucleus for the incorporation of laboratories from the private sector into the national program after they have demonstrated and validated their analytical capabilities in the EMSL-LV certification program. It is estimated that direct TROIKA involvement will be minimal by October 1, 1985 and the analysis thereafter will be handled directly by the State or Regional Offices and contract laboratories.

The primary efforts of the TROIKA will be devoted to analytical determinations for 2378-TCDD. It is estimated that 100 to 150 analyses for 2378-TCDD per month can be generated. Analyses will also be performed for determination of penta- through octa-chlorinated dibenzo-*p*-dioxins (CDDs) and tetra- through octa-chlorinated dibenzofurans (CDFs) in samples from special studies. These type of analyses require additional time and effort and pose some problems due to unavailability of sufficient reference standards.

The portion of this plan concerned with TCDD isomers, other CDDs and CDFs represents a unique endeavor for the Agency. The complexity of the analytical task to extract, separate and identify the many CDDs and CDFs combined with detection and quantification at parts per trillion levels yields a study of such a scope as to defy comparison. No reference point exists for so many analyses at such low levels. Compounding the complexity and adding to the uncertainty is the lack of certified reference standards for many of the CDDs

and CDFs and the unknown extraction properties of the matrices to be tested. For these reasons, portions of this document pertaining to CDDs and CDFs can only be considered an "expectation plan." It defines the quality of work expected. Only the assessment of the actual analytical data will determine if the expectations exceeded the current analytical capabilities.

Responsibility for performing the Tier 4 analytical work was assigned to the ECL-BSL and EMSL-RTP laboratories during the last year of the study. Sample preparations are performed by the ECL laboratory and the HRGC-HRMS analyses for tetra through octa CDDs and CDFs are performed by the EMSL-RTP laboratory. The sample preparation procedures and HRGC-HRMS methods of analyses are fully described in the Tier 4 attachment to this document.

II. Objective

The objective of this specific portion of the National Study is to determine the absence and/or degree of environmental contamination by ppt levels of 2378-TCDD in soil, sediment, fish and ppq levels in water. Analytical data generated in this study must be scientifically sound and valid in order for it to be used for meaningful assessment purposes. Although a brief description of the organization and responsibilities of various laboratories and offices is presented, the primary purpose of this document is to present the analytical methodology and the quality assurance procedures that will be used to insure that the quality of data is sufficient to satisfy the study objective.

This QA plan for the analytical portion of the work is designed for use by experienced EPA laboratories for a period of two years. The analytical methods that will be used by the EPA laboratories are described fully and those used by specific universities are described in peer reviewed journals. The described methods will be used for the major part of the work. However, based on experience, it is emphasized that many "troublesome" samples will be encountered during this two-year study. Therefore, the laboratories will make any necessary modifications and/or develop the methods required to provide valid analytical results for the Agency. At the conclusion of this effort in October 1985, a specific QA plan for use by private contract laboratories will be prepared by appropriate EPA personnel.

Collection, storage, shipment and prioritization of samples are described in a separate document prepared by OW.

III. Description of Organizational Structure and Route of Samples, Analysis and Data Flow

The organizational structure that describes this portion of the National Study is shown on page 1-3.

Briefly, requests for analysis are submitted to the OW sample management office for prioritization. The requestor is then notified about the status of the request, the date and the analytical laboratory that the samples should be sent to. Simultaneously, the information is also transmitted to the analytical laboratory, i.e., the Environmental Chemistry Laboratory (ECL), located at Bay St. Louis, Mississippi. After receipt of samples, appropriate quality assurance (QA) samples and test samples are fortified with labeled compounds of interest and subjected to specific extraction and clean-up procedures. Extracts from the sample preparation procedures (60 μl extracts) are then shipped by Federal Express to the EMSL-RTP for HRGC-HRMS analysis. Analytical results for the set of samples are verbally transmitted to the ECL. Any problems that may be encountered must be resolved before proceeding to the next sample set. If QA results are within acceptable limits the EMSL-RTP submits a memorandum describing analytical results to the ECL. The ECL inserts theoretical QA values and a signature and submits the memorandum to ERL-D. This can now be considered valid data upon which tentative actions may be formulated, i.e., confirmation analysis by a referee laboratory or collection of more samples to determine extent of contamination. Results generated in each quarter will be subjected to a peer review prior to releasing them to EPA-HQ.

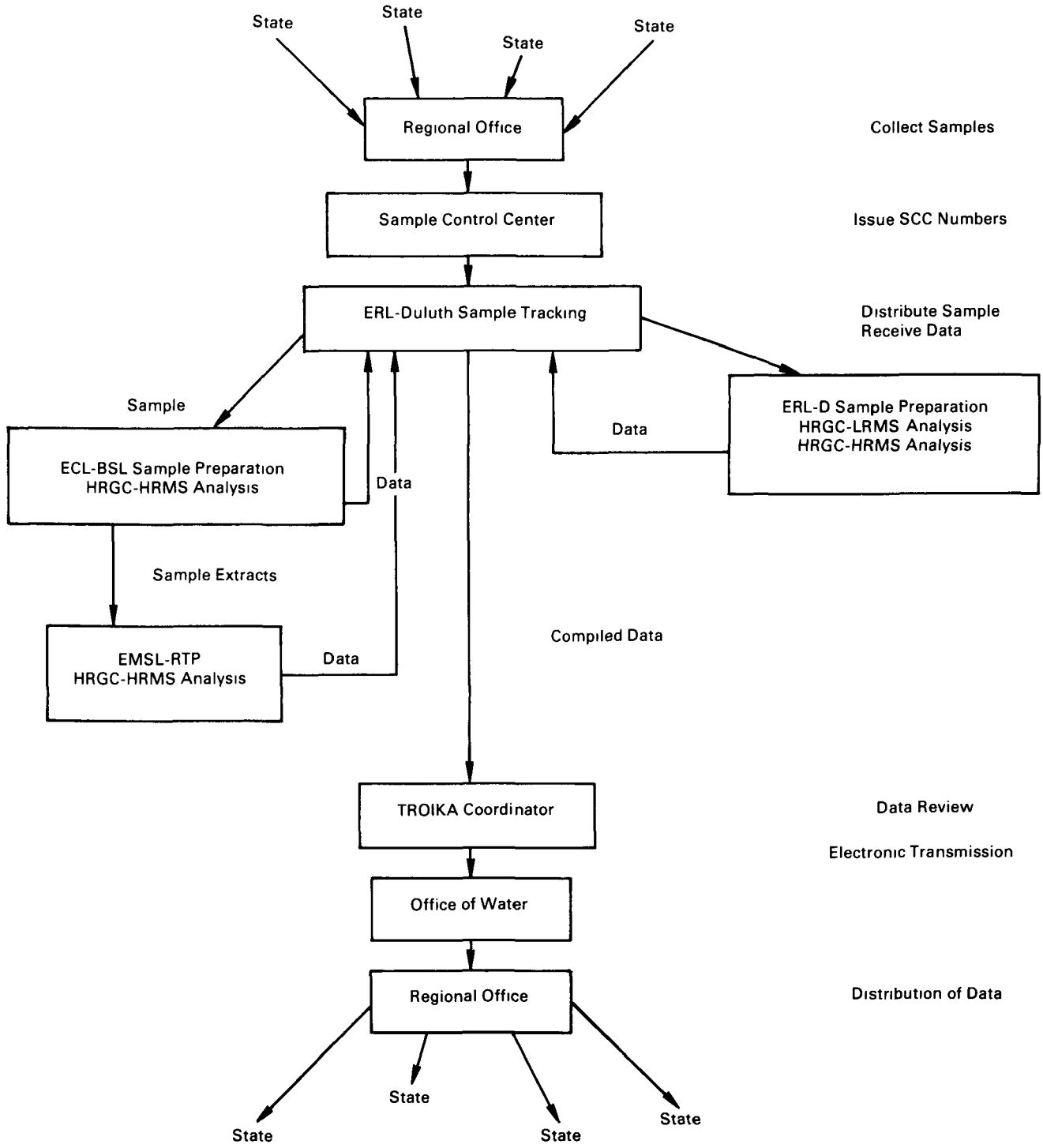
IV. Sample and Data Flow

(see diagram on page 1-3)

V. Safety

2378-TCDD is toxic and can pose health hazards if handled improperly. Techniques used for handling radioactive and infectious materials are applicable to 2378-TCDD. Only highly trained individuals who are thoroughly versed in appropriate laboratory procedures and familiar with the hazards of 2378-TCDD should handle this substance. A good laboratory practice involves routine physical examinations and blood checks of employees working with 2378-TCDD. Also, facial photographs using oblique photoflood lighting should be periodically taken to detect chloracne, which is an early indication of 2378-TCDD exposure.

Safety and health protocols and a well ventilated, controlled access laboratory are required for work involving 2378-TCDD. Persons working with 2378-TCDD should be enrolled in a health monitoring program and subjected to specific medical examinations on a semi-annual or annual basis. Other requirements are: appropriate safety clothing and eye



protection equipment should be provided; exhausts from vacuum pumps should be vented through appropriate filters to the atmosphere; analytical standards and sample extracts should be stored in locked refrigerators; work with 2378-TCDD should be performed in the hood; bench tops and floor of hood should be covered with teflon sheets and/or plastic backed adsorbent paper; the surfaces of bench tops,

hood floor, laboratory floor, and apparatus should be periodically subjected to wipe tests utilizing filter paper wetted with appropriate solvent, such as hexane; filter papers should be subjected to sample preparation prior to HRGC-HRMS analysis for 2378-TCDD (filter paper extracts may be analyzed without clean-up to determine the extent of contamination if an accident should occur); procedures to deal with accidents should be clearly defined in the safety and health protocols; inventories of 2378-TCDD and other toxic compounds in the laboratory should be performed on a semi-annual basis to insure that large amounts are not allowed to accumulate (analytical standards that cannot be used and the remainder of sample extracts should be removed from the laboratory and stored in appropriate room or building to await disposal); liquids should be concentrated to dryness and all disposable items such as absorbent paper and towels that may be contaminated with low levels of 2378-TCDD should be stored in steel drums fitted with heavy gauge polyethylene liners to await proper disposal. In summary, careful attention to details, log books, inventories, analytical reports, safe laboratory practices and good judgment are required in order to document and perform the necessary work in a safe and acceptable manner.

VI. Background Information Regarding EMSL-RTP and ECL-Bay St. Louis, Mississippi Methods of Analyses for CDDs and CDFs

The EMSL-RTP and ECL-Bay St. Louis, Mississippi laboratories have performed analyses for 2378-TCDD for EPA since 1973. Basic procedures and methods of analysis used today were developed in early 1977 and are described fully in Analytical Chemistry, pp. 1239-1245, 1980. They have been applied for the determination of 2378-TCDD in a wide variety of sample matrices. For example, human milk, human adipose tissue, beef, deer and elk adipose tissue and muscle, fish, water, soil, sediment, fly ash, stack gas emissions, chemical products, etc. The credibility and validity of these methods have been demonstrated and established by participation in numerous national and international method validation studies such as the Canadian/American Study regarding 2378-TCDD in fish from the Great Lakes.

These basic sample preparation procedures and HRGC-HRMS methods of analysis have been modified over the years to perform analyses for tetra- through octa-chlorinated CDDs and CDFs and to incorporate knowledge, wisdom and advances such as fused silica SP-2330 capillary columns as they become available. The major modifications are: $^{13}C_{12}$-2378-TCDD and $^{13}C_{12}$-OCDD are used as internal standards to determine method efficiency achieved for each sample. These two internal standards are also used in quantification of CDDs and CDFs and for determining the minimum limits of detection for each series of tetra through octa CDD and CDF isomers; mild extraction procedures are used because hot KOH digestion will destroy hexa through octa CDDs; fused silica polar HRGC capillary columns are used for resolution of components; analytical criteria for confirmation of 2378-TCDD have been expanded and/or modified to use for other CDDs and CDFs as described in specific portions of the analytical methods. The credibility and validity of these procedures for tetra through octa CDDs and CDFs have been demonstrated and validated by participation in method validation studies with Wright State University—water samples, Dow Chemical—soil samples, and an international study—human adipose tissue samples. These analyses for tetra through octa CDDs and CDFs are complex, time consuming and have specific limitations due to unavailability of sufficient labeled and native reference standards. Analytical standards of available CDDs and CDFs are prepared by ECL-Bay St. Louis, Mississippi and submitted to EMSL-RTP for HRGC-HRMS analyses and certification for use in the National Dioxin Study. The analytical capabilities and the limitations of these analyses are described in specific portions of the following analytical methods.

The QA program is instituted simultaneously with sample preparation. Aliquots (specific grams) of QA and test sample matrices are fortified with 2.5, 5.0 or 10 ng $^{13}C_{12}$-2378-TCDD in order to determine and document the efficiency achieved in sample preparation (NOTE: 20 ng $^{13}C_{12}$-OCDD are also added at this point if tetra through octa CDD and CDF analyses are required). QA samples are also fortified with various levels of native 2378-TCDD and various levels of 2378-TCDF and penta through octa CDDs and CDFs if these analyses are also required. Extracts of QA and test samples are submitted in a "blind" fashion to the analytical laboratory for HRGC-HRMS analysis, i.e., the identity of the QA samples in the set of extracts is unknown to the mass spectroscopist.

VII. Sample Preparation Procedures for 2378-TCDD, Other CDDs and CDFs

A. Grinding, Blending and Storage of Samples

Samples received at ECL-Bay St. Louis will be pre-ground and/or blended at the region or state laboratories. If not, homogeneous samples will be prepared at ECL. Fillet, whole fish or specific organs of animal analyses will be determined by the region or state submitting the sample. The major portion of all samples will be stored for reference. Fish and animal tissue will be stored at -15°C. Water, sediment and soil samples will be stored in the dark at 4°C.

B. Extraction Procedures

1. Soil

Weigh 10.0 g of soil into a 33 mm x 80 mm cellulose thimble. Add 5.0 ng of $^{13}C_{12}$-2378-TCDD directly onto the soil (NOTE: add 20 ng $^{13}C_{12}$-OCDD if other CDD and CDF analysis required), cover with a plug of glass wool and place the thimble into a 40 mm soxhlet extraction apparatus fitted with a 250 mL Erlenmeyer flask. Add 175 mL of benzene and place the apparatus on a hot plate. Adjust the heat until the benzene drips at a rate of two drops per second. Extract for 16 hours. Allow the apparatus to cool. Remove the extractor and place a three-bulb Snyder column onto the flask containing the benzene extract. Place on a hot plate and concentrate the benzene to 10 mL (do not let go to dryness). Add 100 mL of hexane and again concentrate to 10 mL. Add a second 100 mL portion hexane and concentrate to 10 mL. Let cool and add 25 mL hexane. The extract is ready for the acid/base cleanup at this point.

2. Sediment

Place a 11.0 cm round medium porosity filter paper on a 15 cm x 15 cm piece of screen wire, weigh and record the weight. Weigh 10.0 g of wet sediment onto the filter paper and spread evenly into a 1-2 mm layer and record the weight. Add 5.0 ng of $^{13}C_{12}$-TCDD directly onto the sediment layer (NOTE: add 20 ng $^{13}C_{12}$-OCDD if other CDD and CDF analysis required). Let the sediment dry in a chemical fume hood at room temperature for 24 hours. Re-weigh the filter paper, dry sediment and the screen wire. Transfer the filter paper and dry sediment into a 33 mm x 80 mm cellulose thimble and cover with a plug of glass wool. Proceed with the soil procedure at the point where the thimble is placed in the 40 mm soxhlet extraction apparatus.

3. Water

Measure exactly 1000 mL of water and pour into a 2 L separatory funnel. Add 2.5 ng of $^{13}C_{12}$-2378-TCDD directly to the water (NOTE: add 20 ng $^{13}C_{12}$-OCDD if other CDD and CDF analysis required). Add 100 mL of methylene chloride (CH_2CL_2) to the separatory funnel and shake vigorously for about one minute. Allow the layers to separate and drain the lower layer (CH_2CL_2) into a 500 mL Erlenmeyer flask. Reextract the water with 2 more 100 mL portions of CH_2CL_2 and combine all the CH_2CL_2 extracts in the 500 mL Erlenmeyer flask. Place a three-bulb Snyder column on the 500 mL Erlenmeyer flask, add a few large granules of sodium sulfate and concentrate to ~10 mL on a steam bath. Add 100 mL of hexane and again concentrate to 10 mL. Allow the flask to cool and add 25 mL hexane. The extract is ready for the acid/base cleanup at this point.

4. Fish and Tissue

Weigh 10.0 g of a homogeneous fish or tissue sample into a 100 mL round bottom boiling flask. Add 5.0 ng of $^{13}C_{12}$-2378-TCDD directly to the sample (NOTE: add 20 ng $^{13}C_{12}$-OCDD if other CDD and CDF analysis required). Add 20 mL of ethyl alcohol, 40 mL of a 45% potassium hydroxide solution, a teflon-coated stir bar and stir at room temperature for 16 hours. Transfer the alcoholic-base solution to a 250 mL separatory funnel. Rinse the boiling flask with 10 mL of ethanol and add the rinse to the separatory funnel. Rinse the boiling flask with 25 mL of hexane and transfer the hexane wash to the separatory funnel. Shake the separatory funnel vigorously for one minute. Allow the hexane layer to separate and drain the aqueous layer (lower layer) into a second 250 mL separatory funnel. Drain the hexane extract from the first separatory funnel into a 125 mL Erlenmeyer flask and save. Add 25 mL of hexane to the second separatory funnel and shake vigorously for one minute. Allow the layers to separate and drain the lower layer back into the first separatory funnel. Drain the hexane into the 125 mL Erlenmeyer flask. Repeat the extraction step with two more 25 mL portions of hexane. Combine the four 25 mL hexane extracts into one of the separatory funnels. Proceed with the acid/base cleanup at the point where the combined hexane is washed with 30 mL of 2 N potassium hydroxide.

C. Cleanup Procedures

1. Acid/Base Cleanup

Transfer the hexane extract to a 250 mL separatory funnel with 2-25 mL portions of hexane. Wash the combined hexane with 30 mL of 2 N potassium hydroxide. Allow layers to separate and discard the aqueous layer. Carefully add 50 mL of concentrated sulfuric acid. Shake vigorously for 1 minute, allow layers to separate and discard the acid layer. Repeat the acid wash with two more 50 mL portions of concentrated sulfuric acid. Carefully add 25 mL of distilled water, shake, allow layers to separate and discard the water. Transfer the hexane through a 42 mm x 160 mm filter funnel containing a plug of glass wool and 3 cm of sodium carbonate into a 250 mL Kuderna-Danish (KD) concentrator fitted with a 15 mL catch tube. Rinse the filter funnel with 2-25 mL portions of hexane. Place a three-bulb Snyder column on the KD concentrator and

concentrate on a steam bath to 1-2 mL. The extract is ready for the alumina column cleanup at this point, but it can be stoppered and stored in the dark if necessary.

2. **Alumina Column Preparation**

Gently tamp a plug of glass wool into the bottom of a 5-3/4 inch disposable Pasteur pipet. Pour Woelm neutral alumina into the pipet while tapping the column with a pencil until a height of *4.5* cm of alumina is packed into the column. Top the alumina with 0.5 cm of anhydrous granular Na_2SO_4. Prewash the column with 3 ml CH_2CL_2. Allow the CH_2CL_2 to drain from the column, then force the remaining CH_2CL_2 from the column with a stream of dry nitrogen. Place prepared columns in an oven set at 225°C. Store columns in the oven until ready for use, at least overnight. Remove only the columns needed and place them in a dessicator over Drierite until they have equilibrated to room temperature. Use immediately.

3. **Alumina Column Cleanup**

Prewet the alumina column with 1 mL of hexane. Transfer the 2 mL hexane extract from acid/base cleanup onto the column. Wash the column with 6.0 mL of carbon tetrachloride and discard. Elute the column with 4.0 mL of methylene chloride and catch the eluate in a 12 mL distillation receiver. Place a micro-Snyder column on the receiver and evaporate the methylene chloride just to dryness by means of a hot water bath. Add 2 mL of hexane to the receiver and evaporate just to dryness. Add another 2 mL portion of hexane and evaporate to 0.5 mL. The extract is ready for the carbon column cleanup at this point.

4. **Carbon Column Preparation**

Weigh 9.5 g of Bio-Sil-A (100-200 mesh) silica gel, which has been previously heated at 225°C for 24 hours, into a 50 mL screw cap container. Weigh 0.50 g of Amoco PX-21 carbon onto the silica gel, cap and shake vigorously for one hour. Just before use, rotate the container by hand for at least 1 minute. Break a glass graduated 2.0 mL disposable pipet at the 1.8 mL mark and fire polish the end. Place a small plug of glass wool in the pipet and pack it at the 0.0 mL mark using two small solid glass rods. Add 0.1 mL of Bio-Sil-A 100-200 mesh silica gel. If more than one column is made at one time it is best to add the silica gel to all the columns and then add the carbon-silica gel mixture to all the columns. Add 0.45 mL of the carbon-silica gel mixture to the column. The top of this mixture will be at the 0.55 mL mark on the pipet. Top the column with a small plug of glass wool.

5. **Carbon Column Cleanup**

Place the carbon column in a suitable clamp with the silica gel plug up. Add approximately 0.5 mL of 50% benzene-methylene chloride (v/v) to the column. Fit a 10 mL disposable pipet on the top of the carbon column with a short piece of extruded teflon tubing. Add an additional 9.5 mL of the 50% benzene-methylene chloride. When approximately 0.5 mL of this solvent remains, add 10 mL of toluene. After all the toluene has gone onto the column, remove the 10 mL reservoir and add a total of no less than 2.0 mL of hexane to the column. When approximately 0.1 mL of the hexane is left on the top of the column, transfer the sample extract (0.2 mL to 0.4 mL in hexane) onto the column with a Pasteur pipet. Rinse the distillation receiver that contained the extract with two separate 0.2 mL portions of hexane and transfer each rinse onto the column. Allow the top of each transfer layer to enter the glass wool before adding the next one. When the last of the transfer solvent enters the glass wool, add 0.5 mL of methylene chloride, replace the 10 mL reservoir and add 4.5 mL of methylene chloride to it. When approximately 0.5 mL of this solvent remains, add 10 mL of 50% benzene-methylene chloride. When all this solvent has gone onto the column, remove the reservoir, take the column out of the holder, rinse each end with toluene, turn it over and put it back in the holder. All previous elution solvents are discarded. Place a suitable receiver tube under the column and add 0.5 mL of toluene to the top of the column. Fit the 10 mL reservoir on the column and add 9.5 mL of toluene to it.

When all the toluene has eluted through the column and has been collected in the receiving tube, add 3 microliters of tetradecane and concentrate just to dryness using a stream of nitrogen and a heating block of 60°C. Just before the sample reaches dryness, it should be put into a solvent such as benzene or iso-octane which is suitable for GC/MS analysis.

D. Glassware Cleanup Procedures

In this procedure each piece of glassware is taken through the cleaning separately except in the oven baking process. The 100 mL round bottom flasks, the 250 mL separatory funnels, the KD concentrators, etc., that are used in the digestion and extraction procedures are washed three times with hot water, two times with acetone and two times with hexane. This glassware is then baked in a forced air oven which is vented to the outside for 16 hours at 450°C. The teflon stopcocks are cleaned as above except for the oven baking step. All glassware is rinsed with acetone and hexane immediately before use.

E. Soil Dry Weight Determination

Weigh a 15 cm diameter aluminum pie pan and record the weight. Weigh 20.0 g of soil into the pan and spread evenly in the bottom and record the weight. Place the pan containing the soil in a chemical fume hood and dry at room temperature for 48 hours. Re-weigh the dry soil and pan and record the weight.

% Dry Weight Calculation

$$\frac{\text{wt. dry soil}}{\text{wt. wet soil}} \times 100 = \text{Dry Weight}$$

VIII. HRGC-HRMS Methods of Analysis for 2378-TCDD, Other CDDs and CDFs

A. Instrumentation

1. *EMSL-RTP*—A Varian/MAT 311A mass spectrometer (MS) directly coupled (open split interface) to a Varian Model 2700 gas chromatograph (GC) is utilized for these analyses. The GC is equipped with polar or non-polar WCOT glass or fused silica capillary columns. The MS is equipped with an eight-channel hardwired multiple ion selection (MIS) device. The MIS output signals are recorded on an eight-channel Soltec strip chart recorder. This HRGC-HRMS manual control MIS device is fully described in the described Analytical Chemistry publication. NOTE: Computer controlled MIS acquisition and processing programs will be installed in October 1984. This will hopefully provide for much more efficient analysis for tetra through octa CDDs and CDFs. For example, the analysis for tetra through octa CDDs and CDFs can be performed on one sample injection.
2. *ECL-BSL*—A Varian/MAT 312 mass spectrometer (MS) directly coupled (modified open split coupler) to a Varian Model 3700 gas chromatograph (GC) is utilized for these analyses. The GC is equipped with polar or non-polar WCOT fused silica capillary columns. The MS is equipped with an eight-channel hardwired multiple ion selection (MIS) device. The MIS output signals are recorded on an eight-channel Rikadenki strip chart recorder.

B. Calibration of HRGC-HRMS Instrumentation

Perfluorokerosene (PFK), m/z 318.9793, is used in daily operations to establish mass resolution and initial sensitivity. 250 pg $^{13}C_{12}$-2378-TCDD and 10 pg native 2378-TCDD are injected at the beginning of each day of operation. Parameters are optimized to provide approximately 60% full scale deflection on the strip chart recorder for the 10 pg 2378-TCDD. The m/z 320/322 chlorine isotope ratio must be between 0.67 and 0.87:1.0 before initiation of analysis of test samples. Standards are analyzed at intervals during daily operation to provide the data required for quantification of 2378-TCDD in test samples.

The instrumentation is calibrated for analysis of other CDDs and CDFs in a similar manner. For example, PFK m/z 330.9793 is used as the reference mass to establish mass resolution and initial sensitivity. 250 pg $^{13}C_{12}$-2378-TCDD and 50 pg each of penta and hexa CDF are injected at the beginning of each day of operation. Parameters are optimized to provide approximately 60% full scale deflection on the strip chart recorder for the 50 pg each of penta and hexa CDF. The chlorine isotope ratio achieved for the respective compounds must be ±20% of their theoretical values. Standards are analyzed at intervals during daily operation to provide the data required for quantification of penta and hexa CDFs in test samples.

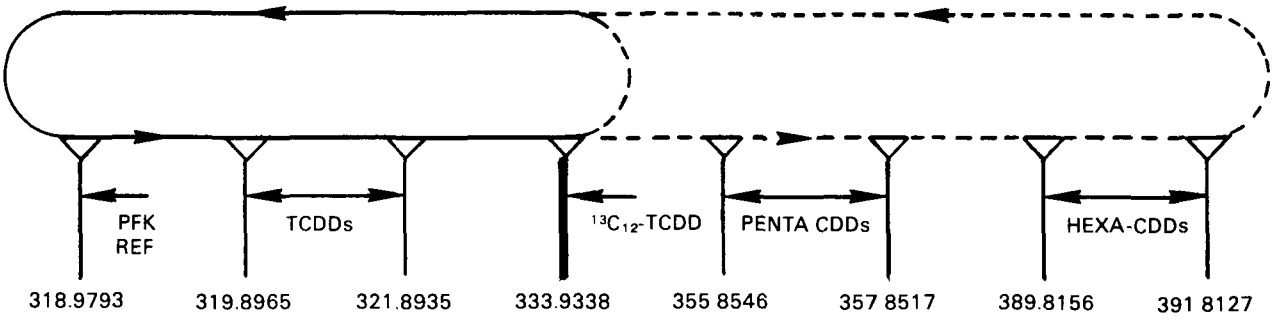
A diagram of the HRGC-HRMS MIS analysis for CDDs is shown on the next page.

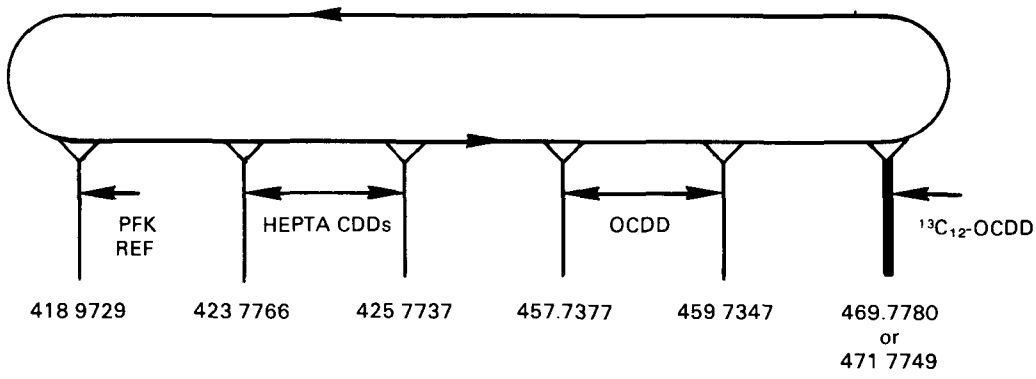
C. Multiple Ion Monitoring Analysis

(see diagram on page 1-8)

D. HRGC-HRMS MIS Operation Parameters

EMSL-RTP	ECL-BSL
1. HRGC Parameters	
60 m SP-2330 fused silica capillary column	Same
Helium carrier gas—1.2 ml/min	Same
Injection port temperature—275°C	Same
Injection technique—splitless	Same
Initial column temperature—100°C	140°C
Hold for seven minutes at 100°C after injection	Hold for 2 min/140°C
Program to 240°C at 25°C/min	Program to 240°C at 20°C/min
Hold at 240°C for 20 to 40 minutes	Same





INTEGRATION TIME 30 msec/mass

GC transfer lines to ion source—270°C	Same

2. HRMS Parameters

Electron energy, 70 eV	~40 eV
Filament emission, 1mA	2mA
Acceleration voltage, 3 kV maximum	Same
Mass resolution, 8000 to 10,000	Same
Multiplier gain greater than 10^6	Same
Ion source temperature, 270°C	Same

3. Hardwired MIS Parameters

Repetitive integration rate, 30 milliseconds per mass	Same
Jump time between masses, 12 milliseconds	Same

E. CDD and CDF Analytical Standards

Analytical standards of CDD and CDF reference isomers described below were prepared by ECL-Bay St. Louis, Mississippi. An aliquot of each standard was submitted to EMSL-RTP for HRGC-HRMS analysis and certification for use in the National Dioxin Study. These analytical standards were subjected to comprehensive HRGC-HRMS analysis to determine: the presence or absence of pg amounts of native isomers in ng amounts of the labeled standards; the presence of more than one isomer and lower and higher chlorinated isomers; the concentrations of specific analytical standards were compared with those prepared by FDA, Dow Chemical, Wright State University and the University of Nebraska. The analytical standards and several qualitative reference standards that are used in the National Dioxin Study are listed as follows:

$^{13}C_{12}$-2378-TCDD	13478-penta-CDF
$^{13}C_{12}$-2378-TCDF	124679-hexa-CDF
$^{13}C_{12}$-OCDD	123478-hexa-CDD
$^{37}CL_4$-2378-TCDD	1234689-hepta-CDF
2378-TCDD	$37Cl_4$hepta-CDD
1234-TCDD	1234678-hepta-CDD
1368-TCDD	OCDD
2378-TCDF	OCDF

Qualitative standards used for establishing retention time windows and for identification purposes:

- Mixture of 22 TCDD isomers
- 2:2 type TCDD isomers
- 3:1 type TCDD isomers and 1234-TCDD
- 1368/1379-TCDDs
- 1469/1269/2348/1289/2468-TCDFs
- 12378-penta CDD
- Mixture of tetra through hexa-CDF isomers
- Extracts of municipal incinerator fly ash that contains all tetra through octa CDDs and CDFs

F. Analyses of Sample Extracts for CDDs and CDFs

Each sample extract may be subjected to one or all of the following six analyses. The reference mass, exact masses of respective CDD and CDF compounds and the type of capillary column that is typically used are listed as follows:

1. PFK, 318.9793; TCDDs, 319.8965/321.8936; $^{13}C_{12}$-TCDD, 333.9338.
   60 m fused silica SP-2330 capillary column.
2. PFK, 292.9825; TCDFs, 303.9016/305.8987; TCDDs, 319.8965/321.8936; $^{13}C_{12}$-TCDD, 333.9338.
   60 m fused silica SP-2330 capillary column.
3. PFK, 330.9793; $^{13}C_{12}$-TCDD, 333.9338; PCDDs, 355.8546/357.8517; HxCDDs, 389.8156/391.8127.
   30 m fused silica SP-2330 capillary column.
4. PFK, 330.9793; $^{13}C_{12}$-TCDD, 333.9338; PCDFs, 339.8597/341.8567; HxCDFs, 373.8207/375.8178.
   30 m fused silica SP-2330 capillary column.
5. PFK, 418.9729; Hepta CDDs, 423.7766/425.7737; OCDD, 457.7377/459.7348; $^{13}C_{12}$-OCDD, 471.7750.
   20 m fused silica SP-2330 or 15 m SE-54 WCOT glass capillary column.
6. PFK, 404.9761; Hepta CDFs, 407.7817/409.7788; OCDF, 441.7428/443.7398; $^{13}C_{12}$-OCDD, 471.7750.
   20 m fused silica SP-2330 or 15 m SE-54 WCOT glass capillary column.

HRGC-HRMS Methods of Analysis Utilizing a Digital PDP-11/34 Computer and Associated MIS Acquisition and Processing Programs.

The computer based MIS programs were installed and put into service during the last year of the study. This concept is similar but more efficient than the previously described hardwired MIS technique. For example, the analysis for total tetra through octa CDDs and CDFs are performed on one sample injection utilizing a 30m or 60m OV-101 or DB-5 fused silica capillary column. A standard or standards containing all of the previously described labeled and native tetra through octa CDDs and CDFs are used for determination of response factors and for quantification purposes. The peak area of each tetra, penta, and hexa CDD and CDF is used to determine the response factor of each compound relative to the labeled $^{37}CL_4$ or $^{13}C_{12}$-2378-TCDD. Response factors for the hepta and octa CDDs and CDFs are developed in a similar manner relative to the peak area of $^{13}C_{12}$-OCDD. This method of analysis is described fully in the attachment regarding Tier 4.

Aliquots, 1 to 3 μl, of 60 μl extracts are injected for analysis. Typical amounts of analytical standards injected for quantification purposes are:

- 100 pg $^{13}C_{12}$-TCDD, 2.5 pg 2378-TCDD
- 250 pg $^{13}C_{12}$-TCDD, 10 pg 2378-TCDD
- 250 pg $^{13}C_{12}$-TCDD, 10 pg 2378-TCDD, 16 pg 2378-TCDF
- 250 pg $^{13}C_{12}$-TCDD, 50 pg each of penta and hexa CDF
- 250 pg $^{13}C_{12}$-TCDD, 50 pg of hexa CDD
- 1000 pg $^{13}C_{12}$-OCDD, 100 pg each of hepta CDD and OCDD
- 1000 pg $^{13}C_{12}$-OCDD, 100 pg each of hepta CDD and OCDF

A standard containing all of these compounds in similar concentrations/amounts is used in computer MIS analysis.

- NOTE: nanogram amounts used for determination of high concentrations

G. Analytical Criteria Used for Confirmation of 2378-TCDD

Since many compounds can interfere with the determination of CDDs and CDFs, it is of the utmost importance that positive identifications be made. Compounds such as PCBs (hepta and nonachlorobiphenyl), chlorinated methoxybiphenyls, DDT, DDE, chlorinated phenyl-benzoquinones, chlorinated xanthenes and hydroxychlorinated dibenzofurans interfere at the m/z's of interest and necessitate a resolution of at least 8,000. Chromatographic separation of the 22 TCDD isomers must be achieved and verified by injection of isomer mixes to confirm resolution of 2378-TCDD. It is crucial that the 2378-TCDD isomer be separated from all other isomers. The criteria for positive 2378-TCDD confirmation are listed below. Criteria 1-4 must be met before the presence of 2378-TCDD can be considered valid. These criteria will be met for all positive samples

reported by the EPA laboratories performing the analyses. Supplemental criteria, a or b are applied to a representative number of positive samples as needed for conclusive confirmation purposes. The mass spectroscopist will use the appropriate and necessary technique and criteria to provide the required valid data for the Agency.

1. Correct HRGC-HRMS retention time of 2378-TCDD (±3 sec) on a 2378-TCDD isomer specific column relative to the labeled internal standard.

   GC peak resolution should not exceed 35% valley for TCDD isomers of equal concentrations eluting before and after 2378-TCDD.

2. Correct chlorine isotope ratio of molecular ion, m/z 320/322 (0.67-0.87).

3. Correct HRGC-HRMS multiple ion monitoring response for 2378-TCDD masses and $^{13}C_{12}$ or $^{37}Cl_4$-TCDD mass (simultaneous response, ±3 sec, for elemental compositions m/z 320, 322, and 334 or 328).

4. Response of m/z 320/322 must be greater than 2.5 times the noise level.

Supplemental Criteria:

a. COCL loss indicative of TCDD structure.

b. HRGC-HRMS peak matching analysis of m/z 320/322 in real time to confirm exact masses that correspond to TCDD elemental compositions.

H. Analytical Criteria Used for Confirmation of Other CDDs and CDFs

1. HRGC-HRMS retention time of specific CDD or CDF isomers available relative to the labeled internal standards.
2. HRGC-HRMS retention time window of respective CDD or CDF series of isomers based on reference fly ash extract.
3. Chlorine isotope ratio of previously described molecular ions of respective CDD or CDF isomers within ±20% of theoretical values:

   penta—1.54 octa—0.88
   hexa—1.23 hepta—1.03

   Supplemental criteria: a representative number of samples may be subjected to the following analyses if needed for confirmation purposes:

a. HRGC-HRMS peak matching analysis of molecular ions in real time to confirm masses that correspond to the elemental compositions of respective CDDs or CDFs.

b. Comparison of sample analysis to analysis of reference fly ash sample that contains all of the tetra through octa CDDs and CDFs.

c. HRGC-HRMS analysis to confirm the absence of specific chlorinated diphenylethers at appropriate retention times.

I. Quantification Procedures

The manual control MIS has specific limitations in regard to quantification over a wide range. Therefore, standards in a very narrow range of interest are used for quantification purposes.

1. Method Efficiency, percent recovery of $^{13}C_{12}$-2378-TCDD and $^{13}C_{12}$-OCDD.

   Aliquots of external standards containing specific amounts of $^{13}C_{12}$-2378-TCDD and native 2378-TCDD are analyzed periodically during the analysis of sample extracts in daily operations. The peak height response produced by the specific amount of $^{13}C_{12}$-2378-TCDD in the standard is used to calculate the amount of $^{13}C_{12}$-2378-TCDD in the aliquot of sample extract analyzed. The amount present in the aliquot is used to determine the amount of $^{13}C_{12}$-2378-TCDD in the total volume of extract. Typical responses produced by sample and standard are shown along with other pertinent data:

Sample	Peak Height Response	Standard	Peak Height Response
m/z 334	195 mm	m/z 334	200 mm for 250 pg $^{13}C_{12}$-2378-TCDD
m/z 322	170 mm	m/z 322	150 mm for 10 pg native 2378-TCDD
m/z 320	133 mm	m/z 320	117 mm for 10 pg native 2378-TCDD

Aliquot analyzed, 2 µl from 60 µl
10 ng $^{13}C_{12}$-2378-TCDD added to 5 gram sample prior to extraction

Example of calculation to determine % recovery—

$$\text{Standard / Sample}\quad \frac{200\text{ mm}}{195\text{ mm}} = \frac{250\text{ pg}}{x} \qquad x = 244\text{ pg}$$

$$\frac{244\text{ pg}}{x} = \frac{2\ \mu\text{l}}{60\ \mu\text{l}} \quad \frac{7.32\text{ ng}}{10\text{ ng}} = 73\%$$

$$x = 7.32\text{ ng } {}^{13}C_{12}\text{-2378-TCCD}$$

This type of calculation is also used to determine the % recovery of $^{13}C_{12}$-OCDD.

2. **Quantification of 2378-TCDD and TCDD Isomers**

   EMSL-RTP

   The concentrations of 2378-TCDD and TCDD isomers in a sample extract are determined by

comparing the labeled and native 2378-TCDD peak height responses to those produced by the external standard (previously shown in calculation of % recovery).

Example of calculation to determine ppt levels of 2378-TCDD in sample extract—

Standard Sample $\frac{117\text{ mm}}{133\text{ mm}} = \frac{10\text{ pg 2378-TCDD}}{x}$

$x = 11.4$ pg

$$\frac{11.4\text{ pg}}{x} = \frac{244\text{ pg } ^{13}C_{12}\text{-TCDD}}{10\text{ ng } ^{13}C_{12}\text{-TCDD}}$$

$x = 467$ pg native 2378-TCDD

$$\frac{467\text{ pg}}{5\text{ grams}} = 93\text{ ppt}$$

This calculation and respective peak heights are also used to determine the concentration of TCDD isomers.

ECL-BSL

Equation 1: Response Factor (RRF) for native 2378-TCDD using $^{13}C_{12}$-2378-TCDD as an internal standard.

$RRF_d = (A_sC_{is}/A_{is}C_s)$

where: A_s = SIM response for 2378-TCDD ion at m/z +322

A_{is} = SIM response for $^{13}C_{12}$-2378-TCDD internal ion at m/z 334

C_{is} = Concentration of the internal standard (pg/μl)

C_s = Concentration of the 2378-TCDD (pg/μl)

Equation 2: Calculation of concentration of native 2378-TCDD using $^{13}C_{12}$-2378-TCDD as internal standard .

Concentration, $pg/g = (A_s)(I_s)/(A_{is})(RRF_d)(W)$

where: A_s = SIM response for 2378-TCDD ion at m/z + 322

A_{is} = SIM response for the $^{13}C_{12}$-2378-TCDD internal standard ion at m/z 334

I_s = Amount of internal standard added to each sample (pg)

W = Weight of sample in grams

RRF_d = Relative response factor from Equation 1

3. Calculation of Minimum Limit of Detection (MLD) for 2378-TCDD

The minimum limit of detection is defined as the amount of 2378-TCDD that will produce clearly defined peak shapes for the masses m/z 320/322 in the proper isotope ratio and with a signal to noise ratio greater than 2.5:1.0. The sample weight, aliquot of extract used in analysis, sample preparation efficiency, sample matrix effects and noise, and the HRGC-HRMS sensitivity are variables that influence and determine the MLD generated for each sample extract.

Example of calculation to determine the minimum limit of detection utilizing the peak height responses and pertinent data shown in calculation of % recovery.

2.5 x 2 mm baseline noise = 5.0 mm

instrument sensitivity for standard

$$\frac{10\text{ pg}}{117\text{ mm}} = 0.09\text{ pg/mm}$$

5.0 x 0.09 pg/mm = 0.45 pg 2378-TCDD

$$\frac{0.45\text{ pg}}{x} = \frac{244\text{ pg } ^{13}C_{12}\text{-2378-TCDD}}{10\text{ ng } ^{13}C_{12}\text{-2378-TCDD}}$$

$x = 18$ pg 2378-TCDD

$$\frac{18\text{ pg}}{5\text{ gram sample}} = 4\text{ ppt MLD}$$

J. Quantification of TCDFs, Penta Through Octa CDDs and CDFs and Determination of Minimum Limits of Detection

The calculations previously described are also used in quantification of the other CDDs and CDFs. An example is shown below:

Sample	Peak Height Response	Standard	Peak Height Response
m/z 334	200 mm	m/z 334	185 mm for 250 pg $^{13}C_{12}$-2378-TCDD
m/z 340	190 mm	m/z 340	175 mm for 100 pg Penta-CDF
m/z 342	125 mm	m/z 342	115 mm for 100 pg Penta-CDF
m/z 374	4 mm	m/z 374	125 mm for 90 pg Hexa-CDF
m/z 376	2 mm	m/z 376	113 mm for 90 pg Hexa-CDF

aliquot analyzed, 2 μl from 60 μl
10 ng $^{13}C_{12}$-2378-TCDD added to 5 gram sample prior to extraction.
amount of $^{13}C_{12}$-TCDD in 2 μl aliquot analyzed

$$\frac{185\text{ mm}}{200\text{ mm}} = \frac{250\text{ pg }^{13}C_{12}\text{-TCDD}}{x}$$

$x = 270\text{ pg }^{13}C_{12}\text{-TCDD}$

Penta - CDF

$$\frac{175\text{ mm}}{190\text{ mm}} = \frac{100\text{ pg penta-CDF}}{x} \quad x = 109\text{ pg}$$

$$\frac{109\text{ pg}}{x} = \frac{270\text{ pg }^{13}C_{12}\text{-TCDD}}{10\text{ ng}} \quad x = 4.04\text{ ng}$$

$$\frac{4.04\text{ ng}}{5\text{ gm}} = 808\text{ ppt penta CDF}$$

Hexa - CDF

2.5 x 2 mm baseline noise = 5 mm

$$\frac{90\text{ pg}}{125\text{ mm}} = 0.72\text{ pg/mm}$$ instrument sensitivity

5 x 0.72 pg/mm = 3.6 pg hexa-CDF

$$\frac{3.6\text{ pg}}{x} = \frac{270\text{ pg }^{13}C_{12}\text{-TCDD}}{10\text{ ng }^{13}C_{12}\text{-TCDD}}$$

$x = 133$ pg hexa-CDF

$$\frac{133\text{ pg}}{5\text{ grams}} = 27\text{ ppt MLD}$$

K. HRGC-HRMS Analytical Data

Some examples of typical analytical data are shown in Figures 1, 2 and 3.

1. Analysis of analytical standard.
2. Analysis of TCDDs in soil extract.
3. Analysis for hepta-CDDs and OCDD in soil extract.

IX. Internal Quality Control/Quality Assurance

Extraction, clean-up and analysis of samples will be done in sets of twelve.

1. Labeled $^{13}C_{12}$ or $^{37}CL_4$-2378-TCDD in known amounts, 2.5 to 10 ng, is added to each test sample, QC sample and method blank prior to extraction and clean-up in order to determine the analytical methodology efficiency and for quantification purposes. 20 ng $^{13}C_{12}$-OCDD will be added to those samples that require analysis for other CDDs and CDFs.
   Frequency: every sample.
2.

a. Method Blank: A blank extraction apparatus is prepared in the laboratory and subjected to same

Figure 1. Analysis of analytical standard (simultaneous responses).

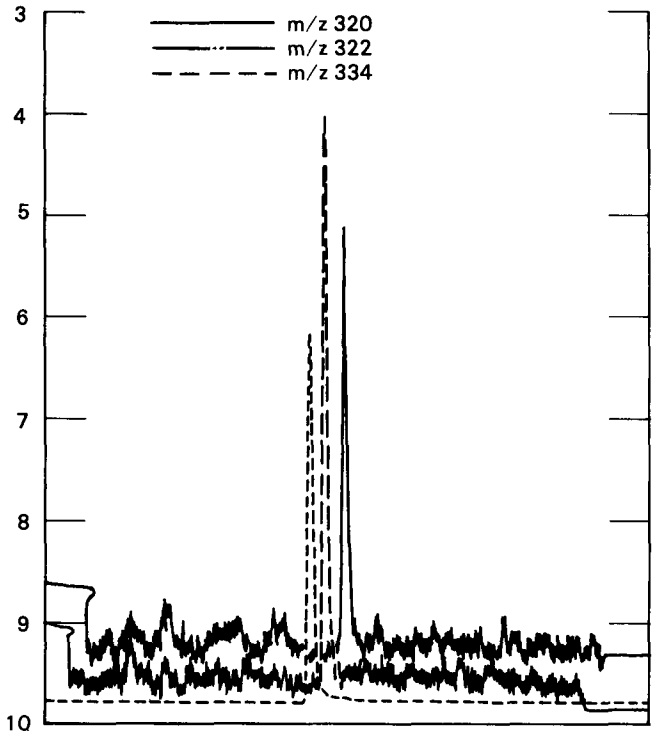


Figure 2. Analysis for TCDDs in Soil.

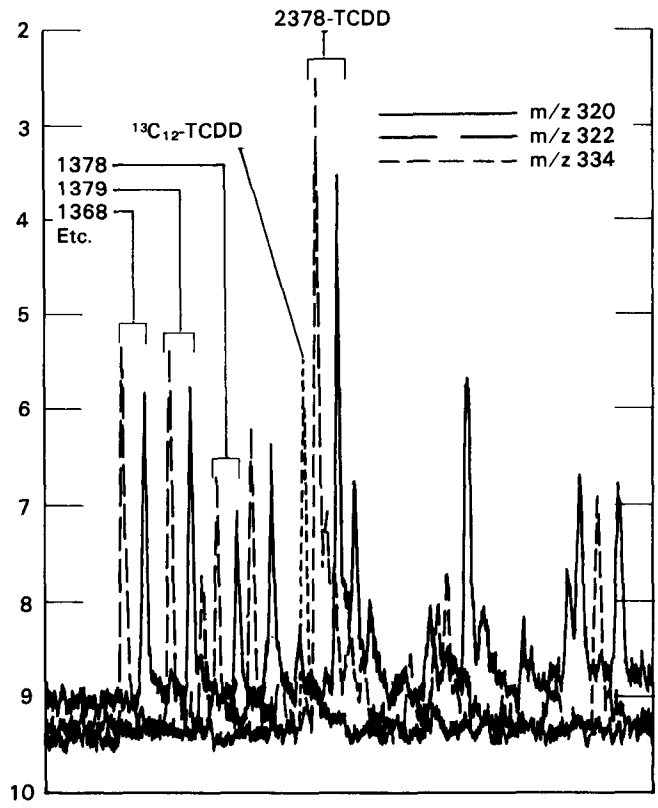
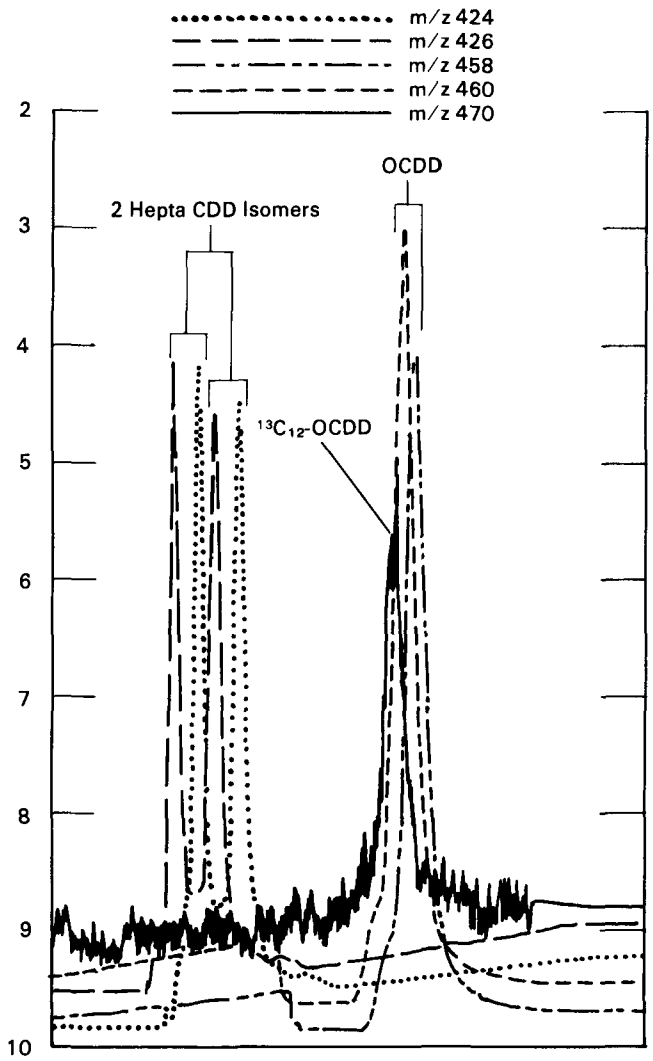


Figure 3. Analysis for hepta CDDs and OCDD in soil.



sample preparation procedures as test samples.
Frequency: one every other extraction set, alternate with matrix blank.

b. Matrix Blank: Portions of respective sample matrix previously analyzed and known to be free of TCDD contamination will be subjected to sample preparation and HRGC-HRMS analysis.
Frequency: one every other extraction set, alternate with method blank.

3.

a. Fortified Matrix Blank: Exact amounts of native 2378-TCDD and/or other compounds are added to a known amount of the respective matrix described above or to a specific environmental sample previously analyzed.
Frequency: one every other extraction set, alternate with control sample.

b. Control Sample: Portions of a respective sample matrix previously analyzed and known to contain 2378-TCDD and/or TCDD isomers will be subjected to sample preparation and HRGC-HRMS analysis.
Frequency: one every other extraction set, alternate with fortified matrix blank.

4.

a. Duplicate sample (lab): Two separate portions of the same sample will be subjected to respective sample preparation procedures and HRGC-HRMS analysis.
Frequency: one every other extraction set, alternate with composite duplicate (field).

b. Composite duplicate (field): Two separate portions of the same field sample will be subjected to respective sample preparation procedures and HRGC-HRMS analysis.
Frequency: one every other extraction set, alternate with duplicate sample (lab).

5. Solvent Blank: Aliquots of solvent will be injected into HRGC-HRMS and analyzed.
Frequency: after every high level positive sample analysis and/or as needed.

6. Analytical Standards: Concentration of working standards will be compared with a primary standard and fresh standards will be prepared as needed.

7. Aliquots of analytical standards ($^{13}C_{12}$-TCDD, 2378-TCDD, etc.) used in fortification of test samples and QA samples will be submitted along with the extracts to the respective analytical laboratories by the ECL. This concept provides a common quantification base for all participating laboratories.

8. Analytical standards used by ECL in the National Dioxin Program will be validated and certified by EMSL-RTP.

9. All sample extracts will be submitted to the mass spectrometry laboratory in a "blind" fashion.

X. Quality Assurance/Quality Control Requirements for Analytical Data

1. Each set of twelve samples must satisfy the following criteria in order for it to be considered valid data:

Criteria	Requirements
● Method efficiency achieved for $^{13}C_{12}$-2378-TCDD	50 to 120%
● Method efficiency achieved for $^{13}C_{12}$-OCDD	40 to 120%
● Analytical criteria used for confirmation of 2378-TCDD and other CDDs and CDFs	Satisfies the criteria previously stated
● Accuracy and precision for 2378-TCDD, and other specific CDDs and CDFs in laboratory fortified sample or control sample	50 to 150% @ $\geq$ 6 x MDL. Accuracy below 6 x MDL may have greater variability
● Method blank and matrix blank free of CDD and CDF contamination at target minimum limits of detection	Described below

Compounds		Target Minimum Limits of Detection[a] Soil, Sediments, Fish, etc. (ppt)	Water (ppq)
2378-TCDD	2378-TCDF	1-5	10-40
21 TCDD Isomers	37 TCDF Isomers	1-5	10-40
14 Penta CDDs	28 Penta CDFs	20-40	20-40
10 Hexa CDDs	16 Hexa CDFs	30-60	30-60
2 Hepta CDDs	4 Hepta CDFs	40-80	40-80
OCDD	OCDF	50-100	50-100

[a]per isomer, which is based on previously described isomers available for coinjection purposes.

2. Accuracy is defined as the degree to which the analytical measurement reflects the true level of 2378-TCDD present in the sample. Laboratory fortified matrices are used to determine accuracy.

3. Precision is defined as the measure of mutual agreement among individual measurements for a specific compound in a sample. Control samples, field duplicates, duplicate and triplicate of test samples split in the laboratory will be used to determine precision.

4. Method efficiency, percent recovery, is determined by dividing the amount of labeled TCDD and/or OCDD present in the extract by the amount that was added prior to extraction.

5. **Corrective Actions**

Certain types of problems can be encountered in the evaluation of analytical data achieved for a set of twelve sample extracts. Potential problems and the corrective action that will be applied are shown:

Problems	Action
● If method blank or matrix blank is positive	Reextract and reanalyze blank and all positives
● If detection limit for blank is > the level of 2,3,7,8-TCDD in a sample	Reextract and reanalyze blank and all positives which are at levels below the mld for the blank
● If spiked matrix or reference sample is outside QA for 2,3,7,8-TCDD accuracy	Reextract and reanalyze all positives
● If method efficiency is outside QA for sample	Reextract and reanalyze sample
● If method efficiency is outside QA for blank	Reextract and reanalyze blank and all positives
● If method efficiency is outside QA for fortified matrix or reference sample	Reextract and reanalyze spiked matrix
● If 2,3,7,8-TCDD level is outside QA for duplicates	Reanalyze duplicates
● If 2,3,7,8-TCDD level exceeds calibration standard range	Reextract and reanalyze with smaller portion of sample, or extend calibration range with additional standard
● If GC resolution is outside QA for isomer specific analysis	Reanalyze complete set on alternate column
● If GC relative retention time between $^{13}C_{12}$ 2,3,78-TCDD and/or $^{37}Cl_4$ 2,3,7,8 and 2,3,7,8-TCDD are outside QA for fortified matrix or reference sample	Reanalyze complete set on alternative column

6. Completeness is defined as the percentage of valid (meets all quality assurance requirements) data compared to the total number of samples analyzed. For the National Dioxin Study the "TROIKA" will not release to the Office of Water any data that has not been reviewed by the "TROIKA" to assure validity of data. Therefore, completeness is defined as 100% for this study and will not otherwise be calculated.

7. Representativeness is dependent on the sampling plan, and, therefore, is not covered in this plan. Assessment of representativeness by eval-

uation of blind coded analyses of field duplicates is the charge of the regional laboratory dioxin coordinators and not the "TROIKA."

8. Comparability is defined as the extent to which the sample results can be verified or duplicated by another independent laboratory or compared against results previously found. No interlaboratory studies are currently planned by the Office of Water for the TROIKA labs to participate in. However, the TROIKA labs will routinely exchange blind coded samples between themselves and Dr. Mike Gross, University of Nebraska, Lincoln, Nebraska, who is currently working with the TROIKA under an EPA cooperative agreement.

XI. Capabilities, Qualifications, and Limitations of Methods of Analysis for Tetra Through Octa CDDs and CDFs

The previously described limited supply of labeled and native CDD and CDF analytical standards and the qualitative standards provide the base required for:

- Determination of method efficiency for tetra through octa CDDs and CDFs.
- Identification and quantification of 2378-TCDD in the presence of the other 21 TCDD isomers.
- Identification and quantification of each of the other 21 TCDD isomers.
- Identification and quantification of 2378-TCDF in the presence of the other 37 TCDF isomers.
- Identification and quantification of the 2 hepta CDD isomers, 4 hepta CDF isomers, OCDD and OCDF.
- Establishing the specific retention time windows and identification and quantification of TCDFs, penta and hexa CDDs and CDFs.

Qualifications—

- The 2378-TCDD analytical standard is used for quantification of the other 21 TCDD isomers.
- 2378-TCDF and the specific penta through octa CDD and CDF isomers previously described are used for quantification of the respective CDD and CDF isomer series.
- The response factor, sensitivity of other isomers and the extraction and clean-up efficiency for other isomers may not be identical to those reference isomers on hand.
- Therefore, the concentration of TCDD, TCDF, penta through hexa CDD and CDF isomers should be considered to be a "semi-quantitative" estimate. Additional labeled and native CDD and CDF isomers are needed in order to provide more accurate and valid quantification of these compounds.

Limitations—

- Conclusive and valid isomer assignment of all TCDFs, penta through hexa CDDs and CDFs cannot be made with the limited supply of reference compounds available today.
- Concentrations are reported as total TCDFs, penta CDDs and CDFs, hexa-CDDs and CDFs.

Most of these qualifications and limitations will be removed after additional reference standards are made available.

XII. External Quality Assurance/Multiple Laboratory Precipitation

1. Specific test samples and QA samples 2A, 2B, 3A, 3B, 4A and 4B described under the Internal QC/QA will be submitted to other validated laboratories such as the ERL-D, University of Nebraska, Wright State University and University of Umea for sample preparation and analysis.
   Frequency: multiple laboratory participation, numbers, etc. will be worked out and determined at a later date.
2. Extracts and/or "split extracts" from the laboratories above will be submitted to EMSL-RTP for HRGC-HRMS analysis.
   Frequency: as needed for confirmation/validation purposes.
3. Private sector laboratories from the EPA certification program will initially receive a very large percentage (30 to 50%) of QA samples in their shipment of test samples in order to demonstrate and validate their analytical capabilities and results.
4. Extracts and/or "split extracts" from 3 above will be submitted to EMSL-RTP for HRGC-HRMS analysis.
   Frequency: as needed for confirmation/validation purposes.
5. Analytical standards will be shipped along with extracts to insure that all laboratories will have a common base for quantification purposes.

XIII. Analytical Report Format, Reporting of Data and Review Panel

Method efficiency will be reported as the percent recovery of $^{13}C_{12}$-2378-TCDD and also $^{13}C_{12}$-OCDD if it was used. CDDs and CDFs will be reported in ppt on weight basis. Compounds not detected will be reported as ND with minimum limit of detection in parenthesis, for example ND (2). A text that describes and summarizes the analysis and analytical results will accompany each report that is submitted to the Director,

ERL-D. ERL-D will submit the complete report, text and results, to the region or state at a later date. Examples of typical reports and a table of results for 2378-TCDD and for tetra through octa CDDs and CDFs are shown in Figures 4, 5 and 6.

The data and other pertinent information will be subjected to a review by the participants and experienced scientists at the end of each quarter. Dr. Jaworski will submit the review and validated data to HQ by 20 April, 20 July, 20 October and 20 January following each quarter.

The raw data, mass spectrum, chromatograms and calculations remain in the analytical laboratory that performed the analyses and are filed for reference.

Review Panel: An independent review panel consisting of recognized experts in HRGC-HRMS analysis of dioxins will be formed to evaluate the effectiveness of the analytical method and QA program detailed in this plan. Members of this panel will include representatives from EPA ERL-Duluth, an independent commercial laboratory, and a third party, yet to be determined.

XIV. Sampling Procedures

Sampling protocol and field QC procedures are detailed in a separate document prepared by OW. All field sample containers will be pre-cleaned with alconox, denatured alcohol and trichloroethylene. Samples will be kept iced at 4°C during storage and transport to the ECL. No holding time has been established for soils, but samples in this study will be sent to the laboratory as soon as possible after homogenization and extracted within 30 days. Samples will be kept refrigerated during storage and extracts will be stored in capillary tubes and kept frozen. Once soil samples have been homogenized and split, CDD/CDF aliquots will be kept at ambient temperature in the dark. Others will be refrigerated.

XV. Sample Custody

Samples and accompanying information under chain of custody are submitted to the ECL or ERL-D laboratory by the regions for sample preparation and analysis. Extracts of test samples and QA samples are then coded and shipped by Federal Express to respective analytical laboratories under a chain of custody that has been used since 1975. An example of the chain of custody form is shown below. The form is completed, copied and the original is returned to the submitting laboratory. Pertinent information required by the analytical laboratory (sample weights, type, labeled TCDD fortification level, etc.) are also provided in an accompanying memorandum.

NOTE: All shipments of extracts must conform to DOT specifications and are shipped by commercial cargo planes, such as Federal Express.

CHAIN OF CUSTODY RECORD

Date Samples Shipped _____

Samples Shipped From _____

_____

(Signature, Affiliation)

Samples Received by _____

(Signature, Affiliation)

Date Samples Received _____

Condition of Samples _____

How Samples Transferred _____

Samples Transferred: _____

_____

_____

Figure 4. Analytical Report Form.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
Research Triangle Park, NC 27711

Date: *October 5, 1983*

Subject: Analyses for 2,3,7,8-TCDD Residues

From:

To: Director, ERL-D

Text: Description and summary of results, TCDD isomers, qualifications, etc

Sample ID	Sample TYPE	Sample Weight (g)	$^{13}C_{12}$-TCDD Fortification Level (ng)	$^{13}C_{12}$-TCDD % recovery	ppt Levels of 2,3,7,8-TCDD Detected/Minimum Limit of Detection (MLD)	ECL Entry QA PPT
			Example			
RTP-1[a]	soil[a]	10[a]	2.5[a]	88[b]	ND(1)[b]	

[a]information supplied with extract [b]experimental results

Column Description _____

Retention Time of 2,3,7,8-TCDD _____

Figure 5.

Table 1 (Set 2 thru Set 6)

Analytical Results for 2378-TCDD in Soil Samples

Sample ID	Sample Weight (g)	Set 2[a] Method Efficiency. % Recovery of 5 ng $^{37}Cl_4$-TCDD	2378-TCDD Detected (ppt)	2378-TCDD Minimum Limit of Detection (ppt)	ECL Entry QA PPT
13312	10	96	26	3	
13313	10	92	13	1	
13314	10	108	12	1	
13315	10	72	17	2	
13316	10	100	18	1	
13317	10	86	26	1	
13318	10	94	ND	1	
13319	10	92	28	1	
13320	10	50	12	3	
13321	10	80	2	1	
13322	10	100	ND	1	
13323	10	78	29	2	

ND = not detected at specified minimum limit of detection.
[a]received at EMSL-RTP, June 22, 1984.
NOTE: $^{13}C_{12}$-TCDD has replaced $^{37}C_4$-TCDD.

Figure 6.

Table 2. Analytical Results for CDDs and CDFs in Soil Samples (Continuation)

Compounds	Sample I.D., Method Efficiency, Concentrations Detected and Minimum Limits of Detection (ppt)[a]					
I.D.s	13398	13399	13400	13401	13406	13412
% Recovery of 5 ng $^{13}C_{12}$-TCDD	75	80	98	90	100	100
% Recovery of 20 ng $^{13}C_{12}$-OCDD	89	90	95	72	--	70
1368-TCDD	ND	95	ND	ND	--	105
1379-TCDD	ND	62	ND	ND	--	76
1378-TCDD	ND	25	ND	ND	--	40
1369-TCDD 1247-TCDD 1248-TCDD	ND	19	ND	ND	--	20
1268-TCDD	ND	9	ND	ND	--	13
1478-TCDD	ND	7	ND	ND	--	ND
2378-TCDD	ND(4)	103(18)	4(2)	ND(4)	3500(39)	271(7)
1234-TCDD 1237-TCDD 1238-TCDD 1246-TCDD 1249-TCDD	ND	19	ND	ND	--	26
1236-TCDD 1279-TCDD	ND	14	ND	ND	--	39
1278-TCDD 1279-TCDD	ND	ND	ND	ND	--	ND
1289-TCDD	ND	ND	ND	ND	--	ND
2378-TCDF	ND(6)	34(7)	ND(4)	ND(4)	450(60)	27(7)
Penta-CDDs	ND(27)	268(91)	ND(44)	ND(24)	--	236(67)
Penta-CDFs	ND(8)	321(42)	ND(16)	ND(8)	--	895(137)
Hexa-CDDs	31(27)	2400(91)	72(44)	ND(24)	--	4040(67)
Hexa-CDFs	35(20)	939(40)	ND(36)	ND(22)		3092(130)
Hepta-CDDs	68(21)	38000(1600)	200(167)	150(24)	--	75000(900)
Hepta-CDFs	ND(27)	5600(240)	ND(23)	ND(31)	--	15400(383)
OCDD	126(22)	120000(1900)	10600(233)	340(26)	--	375000(1300)
OCDF	ND(44)	5600(780)	ND(56)	ND(51)	--	8580(475)

[a]Qualifications stated in text.

Section II
Analytical Procedures and Quality Assurance Plan for the National Dioxin Study

Environmental Research Laboratory
U.S. Environmental Protection Agency
6201 Congdon Boulevard
Duluth, Minnesota 55804

Introduction

This section contains the analytical methods and quality assurance procedures to be used at ERL-D for the analysis of 2,3,7,8-TCDD in environmental tissue and water samples. The samples will be a part of the National Dioxin Study, Tiers 3-7 of the National Dioxin Strategy. ERL-D will join EMSL-RTP and ECL-BSL Labs of the U.S. EPA to form a National Dioxin Study Trace Analysis Group, referred to as the TROIKA.

Although specific analytical procedures will vary somewhat between the three labs, the quality assurance protocol will be consistent and controlled by internal checks. A separate Analytical Procedures and Quality Assurance Plan has been developed for EMSL-RTP and ECL-BSL. Our entire QA/QC plan (December 1983) has been peer reviewed by scientists within and without the Agency. Their comments, along with information developed by the TROIKA through further methods development work, have been reviewed and incorporated into this draft when appropriate. A list of TROIKA responses to reviewer comments have been forwarded to both the reviewers and the Office of Water.

The methodology described in this section is to serve only as a guideline for the methods of analysis. Changes in specific methods may be required due to changes in reagents, instrumental parameters, sample matrix, etc., however, all guidelines for quality assurance will be met before data is released.

A diagram showing the involvement of the TROIKA in the development of sampling/analytical protocols, sample traffic flow, analyses of samples, data review, and data reporting is presented in the section described by ECL/BSL and EMSL/RTP.

I. Sample Preparation for 2,3,7,8-TCDD Analyses

A. Grinding, Blending, and Storage of Samples

1. Biological Tissue

In some instances, samples received at ERL-D will be pre-ground at the Region or state labs. Samples to be ground at ERL-D will be ground in a stainless steel meat grinder by putting the sample through three times. Specific organ or whole animal analysis will be determined by the Region or state submitting the sample. After grinding, all samples will be stored in a solvent-rinsed glass jar fitted with an aluminum seal at -15°C.

2. Water

Water will be stored, in the container in which it is received, in the dark at 4°C.

B. Extraction Procedures

1. Biological Tissue

Subsamples (20g) of previously ground, homogenized, and frozen biological tissue are blended in a 400 mL beaker with enough anhydrous sodium sulfate (~ 60g) to dry the sample. Two-thirds of the dried sample is transferred to a course-frit Soxhlet extraction thimble. The sample is spiked with 100 μL each of 5 pg/μL $^{13}C_6$ 1,2,3,4-TCDD and 1 pg/μL 1,2,3,4-TCDD in iso-octane and the remaining sample is added to the thimble. The sample is extracted for 24 hours in a Soxhlet extraction apparatus fitted with a 500 mL round bottom flask using 250 mL of a 1:1 mixture of hexane and methylene chloride. The sample is then quantitatively transferred to a 500 mL Kuderna-Danish (KD) apparatus and 5 mL of iso-octane is added. The apparatus is then fitted with a 3-ball Snyder column and the volume is reduced to 5 mL on a steam bath. The extract is sealed and stored in a freezer.

2. Water

Water (900 mL) is added to a solvent rinsed separatory funnel (1000 mL), spiked with 100 μL of 5 pg/μL $^{13}C_6$ 1,2,3,4-TCDD in dimethylformamide and shaken for 5 min. Methylene chloride (120 mL) is then added to the separatory and the water is vigorously extracted. The phases are allowed to separate and the methylene chloride

(at least 100 mL) is drained through a funnel of anhydrous sodium sulfate into a Kuderna-Danish apparatus. The water is extracted twice more with methylene chloride (2 x 100 mL), and the extracts along with iso-octane (5 mL) are added to the Kuderna-Danish apparatus. The extract is reduced in volume to 5 mL, sealed, and stored in a freezer.

C. Isolation Procedures

1. Reagents

a. Solvents

Only pesticide grade distilled-in-glass solvents are to be used. They are: hexane, iso-octane, methylene chloride, benzene, toluene, acetone, and methanol (Burdick and Jackson).

b. Sodium Sulfate

Sodium sulfate (Baker reagent grade anhydrous) is Soxhlet extracted eight hours with 1:1 hexane/acetone, air dried for 12 hours, and vacuum oven dried (100°C) for 24 hours. The sodium sulfate is then baked at 600°C in a furnace for 24 hours, cooled, and stored in an empty hexane solvent bottle.

c. Alumina

Basic alumina (Merck-Darmstadt) is Soxhlet extracted eight hours with methanol, air dried for 12 hours, and vacuum oven dried (100°C) for 24 hours. It is then stored in an empty hexane solvent bottle. It is then activated at 225°C for 24 hours and stored at 105°C until used.

d. Silica-Gel

Silica-Gel-60 (Merck-Darmstadt), is Soxhlet extracted eight hours with methanol, air dried for 12 hours, and vacuum oven dried (105°C) for 24 hours. It is then stored in an empty hexane solvent bottle. Just before use, it is activated at 105°C for 24 hours.

e. Sulfuric Acid/Celite

Sulfuric acid (Baker Chemical Company, Ultrex) (5 mL) is blended in a 250 mL beaker with Celite 545 (Baker) 10 g.

f. Potassium Silicate

High purity potassium hydroxide (Aldridge Chemical Co.) (56 g) is dissolved in 500 mL methanol. Silica-Gel (100 g) is added to the mixture and refluxed with stirring for 1 hour. Cool the mixture, drain the solvent, and transfer the solids to a Soxhlet thimble. Extract with methanol for 4 hours, cool, air dry, and place in a hexane solvent bottle until use.

g. Silica Gel/Carbon

Silica gel (100 g) (Merck-Darmstadt) is Soxhlet extracted with methanol (200 mL) for 24 hours, air dried, and activated at 150°C for 24 hours. To a portion of silica gel (20 g), add Amaco PX-21 carbon (500 mg) (carbon courtesy of Dr. David Stalling, U.S. FWS, Columbia, MO) and blend until uniform color.

2. Xenobiotic-Biological Molecule Separation

The sample extract in 5 mL iso-octane is quantitatively transferred to a 30 cm x 2.5 cm glass chromatography column fitted with a 300 mL reservoir on top and a course glass frit on the bottom, which has been packed with (bottom to top) 2 g silica gel, 2 g potassium silicate, 2 g sodium sulfate, 10 g celite/sulfuric acid and 2 g sodium sulfate, and washed with 100 mL hexane. The column is eluted with 200 mL hexane into a KD apparatus. Iso-octane (1 mL) is added to the KD and the sample is concentrated to 0.5 mL for PCDD separation.

3. Isolation of 2,3,7,8-TCDD from other Xenobiotics

a. Alumina Chromatography

Alumina (1.5 g) is packed into a disposable pipette, washed with methylene chloride (4 mL), allowed to drip dry, and activated at 225°C for 24 hours prior to use. The sample extract in 0.5 mL iso-octane is transferred to the cooled alumina column previously washed with hexane (1 mL). The column is eluted with 6 mL carbon tetrachloride and a fraction containing 2,3,7,8-TCDD is eluted with methylene chloride (4 mL).

b. Silica Gel/Carbon Chromatography

The methylene chloride fraction from the alumina column procedure is transferred to a 4 mm x 200 mm column containing 200 mg silica gel/carbon. The column is eluted with methylene chloride (15 mL) and benzene/methylene chloride (1:3, 15 mL) in the forward direction of flow, and toluene (7 mL) in a reverse direction flow. The toluene fraction is collected and reduced in volume to 0.5 mL using a micro-Snyder apparatus on a heating block. The final extract is stored in a freezer until mass spec analysis.

II. GC/MS Parameters

A. Quantification Standards

Standards of 2,3,7,8-TCDD are to be provided by ECL-BSL. Standards of $^{13}C_6$ 1,2,3,4-TCDD and $^{13}C_{12}$ 1,2,3,4-TCDD are purchased from Cambridge Isotope Inc., Cambridge, MA, in 50 μg/mL

solutions. All standards are verified for use by EMSL-RTP. $^{13}C_6$ 1,2,3,4-TCDD will be used as the internal standard spiked into the sample at the beginning of extraction. $^{13}C_{12}$ 1,2,3,4 will be used as the internal standard spiked into all samples at the end of the clean-up procedure. All analytical standards contain an equal amount of $^{13}C_{12}$ 1,2,3,4-TCDD with varying amounts of $^{13}C_6$ 1,2,3,4-TCDD and 2,3,7,8-TCDD. By using response factor ratios to calculate concentrations (see section of quantification procedures), the percent recovery of the overall clean-up procedure, and the level of 2,3,7,8-TCDD can be determined.

Standard	$^{13}C_{12}$ 1,2,3,4	$^{13}C_6$ 1,2,3,4	2,3,7,8
A	25 pg/μL	2.5 pg/μL	2.5 pg/μL
B	25 pg/μL	12.5 pg/μL	12.5 pg/μL
C	25 pg/μL	25 pg/μL	25 pg/μL
D	25 pg/μL	25 pg/μL	50 pg/μL

B. GC/MS Column Performance Standard

A column performance dioxin mixture has been prepared by ERL-Duluth with all 22 TCDD isomers. This standard contains equal amounts of all TCDD isomers *except* 2,3,7,8-TCDD but includes $^{37}Cl_4$ 2,3,7,8-TCDD. The column performance standard is evaluated by MIS monitoring of ions m/z 320 and 328.

C. GC/MS Instrumentation Parameters

Two GC/MS systems will be used for dioxin analysis at ERL-D, a Finnigan-MAT 4500 MS/INCOS DS high resolution GC/low resolution MS system, and a Finnigan-MAT 8200 MS/SS300 DS high resolution GC-high resolution MS system. Both instruments will be used in the multiple-ion-selection mode (MIS). Tuning of these instruments will, therefore, optimize the signals for 2,3,7,8-TCDD (m/z 320 and 322). Ions to be monitored are shown in the table below.

Mode	$^{13}C_{12}$ 1,2,3,4	$^{13}C_6$ 1,2,3,4	2,3,7,8
Low Resolution	334	328	320/322
High Resolution	333.9338	327 9166	319.8965/321.8936

Confirmation of 2,3,7,8-TCDD by high resolution MS using five ions (shown in the table below) will be done for 10% of all positive analyses, whether originally analyzed by low or high resolution mass spectrometry.

Ion	M	M+2	M+4	M-ClCO	M+2-ClCO
Mass	319.8965	321.8936	323.89	256.9327	258.9298
Ratio	.67 - .87	1.00	.39 - .59	At least 25% M	At least 25% M

The 4500 MS should be tuned and mass calibrated each day, and the 8230 MS should be tuned each day. A complete set of analytical standards will be run when necessary to establish sensitivity and linearity. Subsequently, a limited subset of standards will be run each day to establish instrument performance. Immediately before analysis, each sample is spiked with 10 μL of 50 pg/μL $^{13}C_{12}$ 1,2,3,4-TCDD, and the volume adjusted to 20 μL. Typical instrument operating parameters are given in Appendices A and B.

D. Quantification Procedure

1. Percent Recovery $^{13}C_6$ 1,2,3,4-TCDD

The percent recovery of $^{13}C_6$ 1,2,3,4-TCDD is determined by calculating the amount of $^{13}C_6$ 1,2,3,4 present in the final sample extract and dividing by the amount spiked into the sample at the start of the clean-up procedure. This is done by using the mean (3 values) relative response factor for the ion response ratios between $^{13}C_{12}$ 1,2,3,4 and $^{13}C_6$ 1,2,3,4 (Eq 1) and using this response factor to calculate the concentration in the final solution (Eq 2). Concentration in the final solution times the final volume equals the total amount present.

Standard

$$RF\ 328/334 = \frac{A328 \times C334}{A334 \times C328} \qquad \text{Eq 1}$$

Sample

$$C328 = \frac{A328 \times C334}{A334 \times RF\ 328/334} \qquad \text{Eq 2}$$

Where: RF 328/334 = response factor for ions 328 and 334.

A328 = Area for peak of m/z 328
A334 = Area for peak of m/z 334
C328 = Concentration of $^{13}C_6$ 1,2,3,4-TCDD
C334 = Concentration of $^{13}C_{12}$ 1,2,3,4-TCDD

2. Quantification of 2,3,7,8-TCDD

The quantification of 2,3,7,8-TCDD is determined by calculating the mean response factor between 2,3,7,8-TCDD and $^{13}C_6$ 1,2,3,4-TCDD from each standard (Eq 3), and using the response factor to calculate the level of 2,3,7,8-TCDD (Eq 4).

Standards

$$RF\ 322/328 = \frac{A322 \times C328}{A328 \times C322} \qquad \text{Eq 3}$$

Sample

$$V322 = \frac{A322 \times S328}{A328 \times RF\ 322/328} \qquad \text{Eq 4}$$

Where: RF 322/328 = response factor for ions 322 and 328.

A322 = Area for peak of m/z 322
A328 = Area for peak of m/z 328
C322 = Concentration of 2,3,7,8-TCDD
C328 = Concentration of $^{13}C_6$ 1,2,3,4-TCDD
S328 = Spike level of $^{13}C_6$ 1,2,3,4-TCDD (pg/g)
V322 = Final level of 2,3,7,8-TCDD in original sample (pg/g)

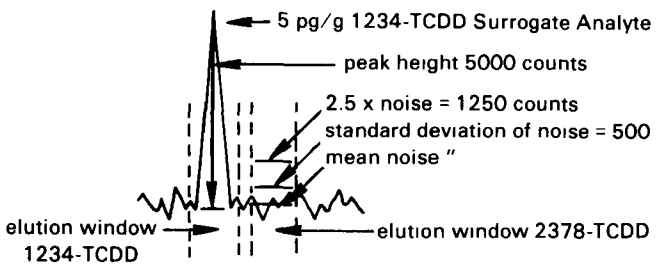
3. Determination of Minimum Detection Limit (MDL)

The MDL for each sample will be statistically calculated according to procedures outlined in Anal. Chem. *55*, 842-847 (1983) and "Confidence Limits for Isotope Dilution-Gas Chromatography/Mass Spectrometric Determination of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in Environmental Samples" in Chlorinated Dioxins and Dibenzofurans in the Total Environment, Eds. G. Choudhary, L. Keith, and C. Rappe, Butterworth Press, 1983. Those techniques are not currently available to the TROIKA, however, OW has contracted with Finnigan Corporation to implement the appropriate software into the SS-300 and Incos data systems used by TROIKA labs. In the interim, all data will be generated in a format appropriate for reanalysis when the statistical package is implemented. In addition, the MDL for biological tissue will be determined by two methods. First, a MDL will be established for each sample using a "surrogate analyte present" technique; and second a "verified" MDL will be established for a representative subset of each matrix type (muscle, walleye, duck, etc.).

4. Surrogate Analyte Present Technique

The target detection limit for biological tissue is 1 pg/g. Since it has been established that biological tissue contains only 2,3,7,8-TCDD and not the other 21 TCDD isomers, a "surrogate analyte" can be added to each sample near the target detection limit to aid in establishing the 2,3,7,8-TCDD MDL. The method used is presented below

If 2,3,7,8-TCDD is Not Detected Then:

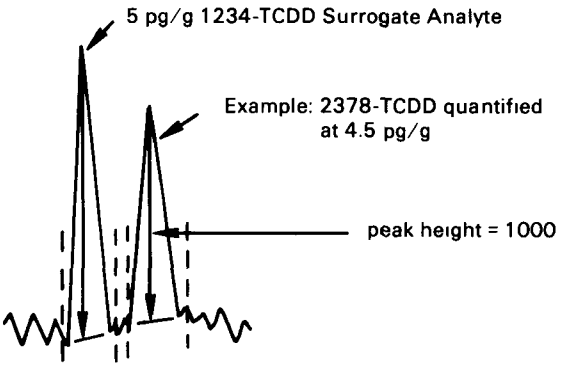


Measure the peak height of the 5 pg/g surrogate analyte, 1,2,3,4-TCDD. Determine 1 standard deviation of noise through the retention time window of 2,3,7,8-TCDD, and calculate a signal level 2.5X noise. (Both of these measurements are computer controlled commands.) Using a response factor between 1,2,3,4-TCDD and 2,3,7,8-TCDD of 1.0 (previously determined) calculate MDL by:

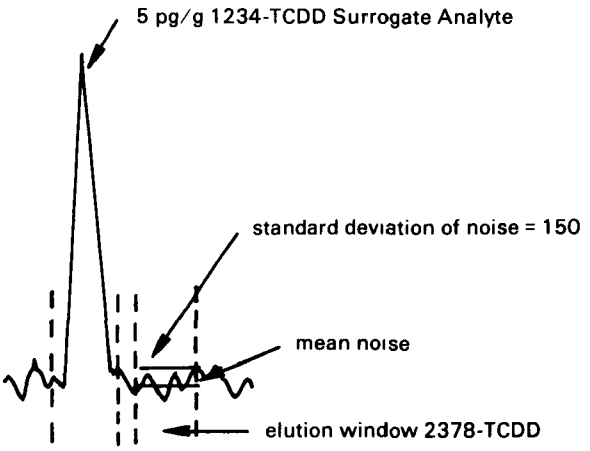
$$\text{MDL} = \frac{1250 \text{ counts peak height}}{(5000 \text{ counts peak height})} \quad (5 \text{ pg/g}) = 1.3 \text{ pg/g}$$

If 2,3,7,8-TCDD is Present in the Sample Then:

Measure the peak height of the quantified 2,3,7,8-TCDD in the sample.



Next, measure the standard deviation of noise through the retention time window of 2,3,7,8-TCDD in the "instrument blank" solvent injection.



The MDL is then calculated by the following equation:

$$\text{MDL} = 2.5 \times \frac{\text{Standard Deviation of Noise Peak Height}}{\text{peak height of quantified 2,3,7,8-TCDD}} \times \text{level of 2,3,7,8-TCDD}$$

Example:

$$2.5\,[\,150/1000\,]\,4.5 = 1.7 \text{ pg/g}$$

5. **Representative Matrix Technique:**

As one of the QA samples analyzed with each sample set, one sample previously analyzed as "not detected" will be spiked at 5X, the target detection limit, with 2,3,7,8-TCDD before being routinely processed for 2,3,7,8-TCDD quantification. This sample will not be used to evaluate QA/QC for each sample set, however, a record of quantification of 2,3,7,8-TCDD in the spiked sample, and MDL in each of the spiked and non-spiked samples will be kept at ERL-D for evaluation during the course of the study.

III. Quality Assurance/Quality Control

A. General Procedures of Operation

Analysis of samples will be done in sets of twelve.

1. **Method Blank:** A blank extraction apparatus is prepared in the laboratory and subjected to the same sample preparation procedures as test samples; or a *Matrix Blank*: A sample previously analyzed and known to be free of TCDD contamination (at a level below the accepted detection limit for that sample matrix). The Method Blank and Matrix Blank will be used in alternate sample sets.

2. **Fortified Matrix:** Exact amounts of native 2, 3,7,8-TCDD and/or other compounds to be analyzed for are added to a sample previously shown not to be contaminated. This sample may be substituted for a reference sample that has been analyzed by at least three labs and a mean value of contamination has been established. The level of contamination of 2,3,7,8-TCDD in the matrix spike will be at least 10X the target detection limit.

3. **Detection Limit Verification Sample:** One sample from a previously analyzed sample set will be spiked with 2,3,7,8-TCDD at 2.5X the target detection limit and analyzed with the next sample set. The addition of this QA/QC sample will be done for only the first three sample sets of any matrix type to establish that the calculated MDL is achievable. If analytical results show difficulty in obtaining the MDL, then this QA/QC sample must be in each set. If no problem is experienced, then this QA/QC sample may be dropped.

4. **Duplicate Sample:** Two separate portions of the same sample will be processed and analyzed.

5. **Environmental Samples:** The total number of environmental samples analyzed will be eight if the detection limit verification sample is used, otherwise nine samples will be analyzed.

6. **Sequence of Logging and Labeling Samples:**

a. Logging Incoming Samples:

The Sample Control Center (SCC) notifies ERL-D when samples have been shipped. Upon arrival, the samples are checked to make sure they are in good condition and the Dioxin Shipment Records are complete. ERL-D informs SCC that samples arrived safely or if there were any problems with the samples (example: mislabeled, no species identification). The samples are then put in a freezer until they are homogenized. After a sample is homogenized, it is put into the double-door freezer. Samples that have been extracted are put into the single-door freezer. After acceptable data is generated for a sample, it is brought to a locker plant for permanent storage. A Locker Plant Log is kept with episode # and SCC # of all National Dioxin Study samples stored there.

A computer program has been developed for sample tracking and data storage (see Appendix D). The episode #, SCC #, date sample received, and matrix type are entered into the Dioxin Survey Sample Log and the computer. The following additional information is also entered into the computer: analysis lab, latitude, longitude, and site location.

b. Logging and Labeling Samples During Preparation:

Since only one set of 9 samples and 3 QA samples is started each day, a lab ID number is assigned so that the samples go to the MS lab blind coded. A letter, A through L, is used for the individual samples followed by the date the set was started and the initials of the person doing the prep work, e.g., A053084CS.

As each sample is weighed, the assigned lab ID #, episode #, SCC #, matrix description, weight of sample, and amounts of the surrogate spike and internal standard are entered into the log book and onto the Dioxin Study data sheet (see Appendix E). The lab ID # is written on label tape and placed on the beaker containing the appropriate sample before weighing another. Soxhlet thimbles with respective beakers are placed in a row. Transfer from the beaker into the thimble occurs in alphabetical order and the empty beaker is placed behind the thimble. After the thimble is placed in the Soxhlet, the label tape is immediately put on the round bottom flask. During each transfer throughout the procedure, the label is transferred to the receiving vessel. Before the final transfer, the lab ID # is written on the microvial with a permanent marker. The lab ID # is recorded in the GC/MS sample log book along with the GC/MS file number at the time of analysis.

7. **Format:** All samples will be blind coded by the sample preparation chemist and analyzed as such by the mass spectrometry lab. Once during each working day, acquire data on an injection of solvent to obtain an "instrument" blank. ECL-BSL and ERL-D will also routinely exchange blind coded samples to verify quality of data. The schedule for this will be approximately every six months.

B. Instrumentation

Typical instrumental parameters are shown in Appendices A and B.

1. Maintenance

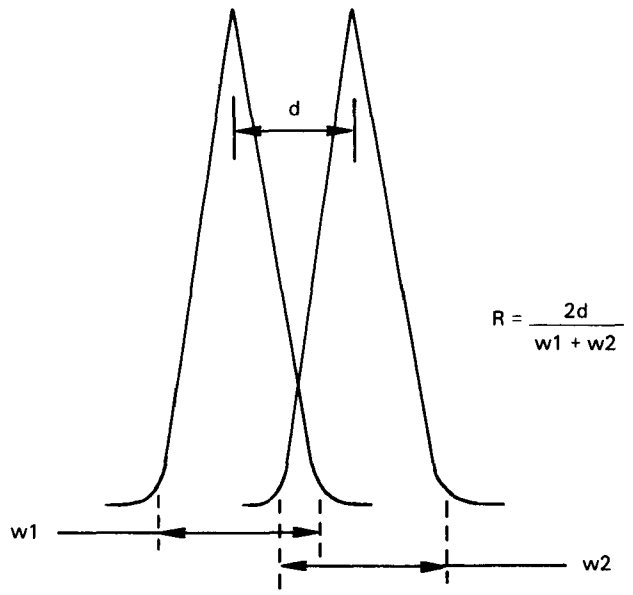
A rigorous maintenance schedule of the gas chromatograph, mass spectrometer, and computer maintenance has been established for all GC/ MS/COM systems at ERL-D. This schedule is closely followed and all maintenance is logged into proper log books. Corrective measures are taken immediately upon identification of an instrument malfunction.

2. Quality Control Parameters

a. GC Column Performance

The ability of the GC column to resolve 2,3,7,8-TCDD from the other TCDD isomers will be evaluated on an SP2230 column using the GC Column Performance Standard when 2,3,7,8 needs to be confirmed. The ion current profile of m/z 328 must be resolved from non-2,3,7,8-TCDD isomers (m/z 322) eluting both before and after the 328 peak by a resolution of 0.75 (87.5% resolved) or greater.

Method:



Quality Assurance:

$$R \geq 0.75$$

Quality Control:

If resolution fails, the column must be either cleaned or replaced, and samples reanalyzed with a column that meets resolution requirements.

In addition, the absolute retention time difference between $^{13}C_6$ 1,2,3,4-TCDD and 2,3,7,8-TCDD must be checked throughout the analysis set. For 2,3,7,8-TCDD retention time confirmation, $^{37}Cl_4$ 2,3,7,8-TCDD will be added to the sample at 25 ppt.

Method:

Calculate the absolute time (sec.) difference between $^{13}C_6$ 1,2,3,4 and 2,3,7,8, or $^{37}C_4$ 2,3,7,8 and 2,3,7,8 for standards and samples.

Quailty Assurance:

The retention time change between standard and sample should not exceed 3 sec.

Quality Control:

Examine GC for leaks, perform routine maintenance on GC, etc., to correct the problem. Verify proper performance before starting sample analysis.

b. Mass Spectral Qualitative Identification of 2,3,7,8-TCDD

Initial MIS analysis will be done by monitoring m/z 320 and 322 (M and M + 2 respectively) and confirmation will be done by also monitoring m/z 324, 257, and 259 (M + 4, M - COCl, and M + 2 -COCl respectively) at or above 10,000 resolution.

Method:

Multiple Ion Selection Gas Chromatography-Mass Spectrometry.

Quality Assurance:

Ion intensity ratios for the molecular ion is M = 0.67 - 0.87, M + 2 = 1.0, M + 4 = 0.39 - 59. M -COCl and M - COCl must be 25% $<$ M $<$ 100%.

Quality Control:

If all ions do not meet intensity ratio requirements, identification is rejected.

c. Mass Spectral Quantitative Analysis

After 2,3,7,8-TCDD has been qualitatively identified in a sample, it must be quantified. The method of quantification is the method of using peak areas and response factors. The analytical

standards used allow the calculation of a response factor for 2,3,7,8-TCDD: $^{13}C_6$ 1,2,3,4-TCDD at a high, medium, and low signal level. The response factor used for the quantification of 2,3,7,8-TCDD in environmental samples will be the mean value of all measurements at these three levels. Peak areas will be computer software determined.

Method: See Section II.D.

Quality Assurance: See Section III.B.

Quality Control: See Section III.B. In addition, samples in which the level of 2,3,7,8-TCDD is greater than the highest calibration standard will be reanalyzed with a smaller sample size.

d. Evaluation of Detection Limits for 2,3,7,8-TCDD

The method to be used by the TROIKA for the evaluation of the limits of detection is a statistical evaluation of signal levels described by Dr. Ronald Mitchum, U.S. FDA, Jefferson, AK. The software to do this will be provided to the TROIKA by Finnigan Corp. through a contract with U.S. EPA, Office of Water. The QA/QC for the M.D.L. for biological tissue used at ERL-D is described.

Method: See II.D.3

Quality Assurance Requirements: The signal level for 2,3,7,8-TCDD in all positive samples must be at least 2.5X the background noise level. The matrix blank and lab blank shall not contain detectable levels of 2,3,7,8-TCDD.

Quality Control: If the matrix blank or laboratory blank contain detectable levels of 2,3,7,8-TCDD, all samples in that set that contain detectable levels of 2,3,7,8-TCDD will be reanalyzed in the next sample set. In addition, appropriate measures must be taken to identify the source of contamination and eliminate the contamination.

C. Evaluation of Data

All data generated by the TROIKA will meet all quality assurance requirements, and be reviewed by the TROIKA before being released to the Office of Water. In addition to all QA requirements listed in sections above, data must also meet guidelines of accuracy and precision discussed below. A summary is shown in the table below.

Parameter	Target Detection Limit	Accuracy*	Precision*
Biological Tissue	1 pg/g	50-150%	50-150%
Water	10 fg/g	50-150%	50-150%

*at ≥6 target detection limit.

1. Accuracy of 2,3,7,8-TCDD Quantification

Accuracy, the degree to which the analytical measurement reflects the true level present, will be evaluated in two ways for each sample set (8 samples and 4 QA). These are: (1) the variance of 2,3,7,8-TCDD spiked into a blank sample matrix, and (2) the recovery of the internal standard $^{13}C_6$ 1,2,3,4-TCDD for each sample.

Method:

$$\% \text{ Accuracy } 2,3,7,8 = \frac{\text{Measured } 2,3,7,8}{\textit{Actual } 2,3,7,8} \times 100$$

$$\% \text{ Recovery } {}^{13}C_6\,1,2,3,4 = \frac{\text{Measured concentration of } {}^{13}C_6\,1,2,3,4}{\textit{level of } {}^{13}C_6\,1,2,3,4 \text{ spike}} \times 100$$

Quality Assurance Requirements:

50% < % accuracy 2,3,7,8 < 150%

50% < % recovery $^{13}C_6$ 1,2,3,4 < 120%

Quality Control Action:

If % accuracy is out of range, whole sample set will be reanalyzed. If $^{13}C_6$ 1,2,3,4-TCDD is out of range for any individual sample, then that sample must be reanalyzed in the next set. Note: The spike level of 2,3,7,8-TCDD will be 10X the expected detected limit, and the spike level of $^{13}C_6$ 1,2,3,7-TCDD will be 25X the expected detection limit.

2. Precision of 2,3,7,8-TCDD Quantification

Precision, a measure of mutual agreement among individual measurements of the same pollutant in a sample, will be evaluated for each sample set as the relative percent difference between lab duplicate measurements of 2,3,7,8-TCDD.

Method:

I. If 2378-TCDD is measured at ≥6X the detection limit, then:

relative percent difference =

$$\frac{|\text{QA dup 1} - \text{QA dup 2}|}{\text{QA dup}} \times 100$$

QA dup 1 and 2 = levels of 2378-TCDD in duplicates.

$\overline{QA\ dup}$ = mean value of 2378-TCDC measurements.

II. If 2378-TCDD is measured at ≤6X the detection limit, then:

relative percent difference =

$$\frac{\text{detection limit}}{\overline{QA\ dup}} \times 100$$

III. If 2,3,7,8-TCDD is reported as "ND" then precision is recorded as 0%.

IV. If 2,3,7,8-TCDD is ND for one sample, and positive for duplicate, then calculate precision using detection limit for ND sample.

Quality Assurance Requirements:

I. ≤50% if measured ≥6X detection limit
II. ≤100% if measured ≥6X detection limit

Quality Control Action:

If relative percent difference is out of range, reextract and reanalyze duplicates to meet QA.

3. Completeness

Completeness is defined as the percentage of valid (meets all quality assurance requirements) data compared to the total number of samples analyzed. For the National Dioxin Study the "TROIKA" will not release to the Office of Water any data that has not been reviewed by the "TROIKA" to assure validity of data. Therefore, Completeness is defined as 100% for this study and will not otherwise be calculated.

4. Representativeness

Representativeness is dependent on the sampling plan, and, therefore, is not covered in this plan. Assessment of representativeness by evaluation of blind coded analyses of field duplicates is the charge of the regional laboratory dioxin coordinators and not the "TROIKA."

5. Comparability

Comparability is defined as the extent to which the sample results can be verified or duplicated by another independent laboratory or compared against results previously found. No interlaboratory studies are currently planned by the Office of Water for the TROIKA labs to participate in. However, the TROIKA labs will routinely exchange blind coded samples between themselves and Dr. Mike Gross, University of Nebraska, Lincoln, Nebraska, who is currently working with the TROIKA under an EPA cooperative agreement.

6. Additional Potential Problems and Corrective Action:

Additional problems with a sample set not specifically identified in the above discussions are described below along with the corrective action. No data will be reported until corrective action satisified QA.

Problem	Corrective Action
If method blank or matrix blank is positive	Reextract and reanalyze blank and all positives
If detection limit for blank is > the level of 2,3,7,8 in a sample	Reextract and reanalyze blank and all positives which are at levels below the mld for the blank
If spiked matrix or reference sample is outside QA for 2,3,7,8-TCDD accuracy	Reextract and reanalyze all positives and matrix spike sample
If method efficiency is outside QA for sample	Reextract and reanalyze sample
If method efficiency is outside QA for blank	Reextract and reanalyze blank and all positives
If method efficiency is outside QA for fortified matrix or reference sample	Reextract and reanalyze spiked matrix
If 2,3,7,8-TCDD level is outside QA for duplicates	Reanalyze duplicates
If 2,3,7,8-TCDD level exceeds calibration standard range	Reextract and reanalyze with smaller portion of sample, or extend calibration range with additional standard.
If GC resolution is outside QA for isomer specific analysis	Reanalyze complete set on alternate column
If GC relative retention time between $^{37}Cl_4$ 2,3,7,8 and 2,3,7,8-TCDD are outside QA for fortified matrix or reference sample	Reanalyze complete set on alternative column

IV. Percentage Lipid of Fish Tissue

The percent lipid will be determined for all fish that show a detectable level of 2378-TCDD. To determine percent lipid, a separate aliquot of fish (2 g) will be blended with 6 g anhydrous sodium sulfate and extracted with methylene chloride (25 mL). The extract will be reduced to volume (2 mL) with an air line, and transferred to a previously weighed, dried aluminum boat. The remainder of the methylene chloride is allowed to evaporate in a hood. The aluminum

boat is placed in an oven at 50°C for 2 hours, cooled and weighed. The percent lipid is calculated from the difference in weights.

V. Age of Fish

Aging of fish will be done only for samples with detectable levels of 2378-TCDD. Scales will be sampled before fish are ground and preserved. Determination of age will be done by qualified aquatic biologists at the University of Wisconsin-Superior through a cooperative agreement with ERL-D.

VI. Sampling Procedures

Fish and water sampling procedures will be developed by the Office of Water, EPA Headquarters and reviewed by each of the TROIKA labs.

VII. Sample Custody

Chain-of-custody procedures will be established by the Office of Water, implemented by Sample Control Center, Headquarters, and used by the TROIKA when required.

VIII. Data Reporting Format

A standardized reporting form will be used at ERL-D to report quantitative results for fish, water and sediments. See Appendix C.

IX. Dioxin Isostereomer Analysis

A. PCDD/PCDF Congeners

The TROIKA will perform isomer specific analysis for all tetra-octa PCDDs and tetra-octa PCDFs when agreed upon with the Office of Water. These analyses are limited, however, to only congeners available to the TROIKA. Moreover, these analyses are limited by the assumption that all congeners reported behave similar during sample preparation to the stable isotope labeled congeners available for internal standards. Analytical methodology will basically follow that described for 2378-TCDD, but has been modified as described in the ECL/EMSL section.

B. Other Polychlorinated Planar Molecules

The TROIKA will perform qualitative analyses for other TCDD isostereomers when agreed upon with the Office of Water. These molecules will be defined as those co-eluting with TCDD in the sample preparation scheme. These molecules may include naphthalenes, biphenylenes, acenaphthalenes, anthracenes, phenanthrenes, fluorenes, carbazoles, pyrenes and chrycenes.

Analytical methodology will basically follow that described for 2378-TCDD, but may need to be modified. Basically this will involve the elimination of the alumina column (PCBs/PNCs separation from PCDDs/PCDFs) and the use of an alternate 2nd internal standard to replace $^{13}C_{12}$ 2378-TCDD.

X. Validation of Methodology and Evaluation of Data Comparability

The validation of the methodology used at ERL-Duluth for biological tissue and water was extensive and followed the outline given below:

A. Evaluation of concentration of quantification standards: A solution of 2,3,7,8-TCDD was received from ECL-Bay St. Louis and quantified.
Result: Expected 26pg, found 25pg.

B. Evaluation of sample preparation bias between internal standard and analyte: The ratio of analyte (2,3,7,8-TCDD) and internal standard (13C6 1,2,3,4-TCDD) was compared before and after sample preparation when spiked into fish at 5, 10 and 25pg/g.
Result: Before (m/z 322/328) 1.0 ± .08
After (m/z 322/328) 1.0 ± .08

C. Demonstrate that calculated minimum level of detection was actually achievable: Samples of fish were spiked at 2.0, 0.5 and 0.1pg/g and analyzed:
Result: All samples were quantified within the QA/QC criteria for the National Dioxin Study.

D. Demonstrate accuracy and precision with 2,3,7,8-TCDD spiked fish: Spike six (6) fish at 5.0pg/g and analyze.
Result: mean 7.0pg/g, range 3.7 to 11pg/g, s.d. 2.40

E. Demonstrate accuracy and precision with environmental samples contaminated with 2,3,7,8-TCDD: Analyze one fish 15 times.
Result: mean 14.5pg/g, range 11-18pg/g, s.d. 1.85

F. Demonstrate credibility of results for 2,3,7,8-TCDD reported as "not detected": Spike seven different environmental samples previously analyzed as N.D. with 2,3,7,8-TCDD at 5pg/g.
Result: The 5pg/g spike was quantified with sufficient S/N to show that the previously reported level of detection for the N.D. determination was achievable.

G. Evaluate data comparability: Exchange a blind coded environmental sample used as a 2,3,7,8-

TCDD contaminated reference fish. (The fish exchanged was ERL-Duluth reference fish #3).

Result:

ERL-Dululth	- 15pg/g
ECL-Bay St. Louis/ EMSL-RTP	- 12pg/g
University of Nebraska (through EPA Region VII)	- 16pg/g
Dow Chemical Company	- 18pg/g
California Analytical (through EPA Region VII)	-11pg/g

The mean of the labs without ERL-Duluth is 14.2pg/g.

The mean of the labs with ERL-Duluth is 14.5pg/g.

Exchange blind coded reference fish and fish from the National Dioxin Study with a highly respected lab outside the USEPA. The lab selected was Dow Chemical Company, Midland, MI.

Result:

Sample Description	2,3,7,8-TCDD found (pg/g) Dow Chemical	ERL-Duluth
Reference fish	13	14
Reference fish 2	32	35
Reference fish 3	18	15
Blank fish	ND (1.4)	ND (1.0)
EPA 13239	2.2 (1.2)	ND (1.8)
EPA 13273*	170	190
EPA 13272	16	17
EPA 13271	76	70
EPA 13243	4.1 (1.4)	1 2 (.5)

*mean of duplicate

A review of the analytical methodology used by the TROIKA labs for the National Dioxin Study was held May 18 and 19, 1985 in Bay St. Louis, MS. Review comments specific to the procedures used at ERL-Duluth primarily concerned the identification of 2,3,7,8-TCDD by relative GC retention time (12 ±2 seconds past $^{13}C_6$-1,2,3,4-TCDD), and quantification of 2,3,7,8-TCDD relative to $^{13}C_6$ 1234-TCDD instead of using $^{13}C_{12}$ 2378-TCDD. For several reasons, primarily Health and Safety related, restrictions placed upon ERL-Duluth did not allow for routine use of 2,3,7,8-TCDD in sample preparation areas. This in turn prompted the use of $^{13}C_{12}$ and $^{13}C_6$ labeled 1,2,3,4-TCDD. As a result the very extensive validation of the methodology and a thorough evaluation of data comparability was necessary. As can be seen from the above discussion, 2,3,7,8-TCDD data generated by ERL-Duluth will meet all QA/QC requirements established for the Dioxin Study.

During the course of the past year, it has been shown that the hazards of exposure to 2,3,7,8-TCDD in the lab are extremely small. Because of this, and also partly because of improved instrumental sensitivity, ERL-Duluth will now be allowed to spike each sample with 25pg/g $^{37}C_{14}$ 2,3,7,8-TCDD. Procedures for quantification of 2,3,7,8-TCDD will remain the same for consistency of data from start to finish of this study. However a comparison of the ratio of $^{13}C_6$ 1,2,3,4,-TCDD to $^{37}C_{14}$ 2,3,7,8-TCDD (m/z 328) will be made for each analyses to determine if a bias has developed between 1,2,3,4-TCDD and 2,3,7,8-TCDD. As a QA requirement the peak area of $^{37}C_{14}$ 2,3,7,8-TCDD (m/z 328) must be within ± 25% of the peak area of $^{13}C_6$ 1,2,3,4-TCDD. The QC requirement is that if the peak area is outside of the QA requirement, the analyses must be done over. All other QA/QC requirements will also be maintained.

Appendix A
Capillary GC Conditions

Column	A(30m DB5)*	B(30m SP2340)**
Helium Carrier linear velocity	40 cm/sec at 100°C	40 cm/sec at 100°C
Capillary head pressure	30 psi	30 psi
Injection***	Grob type splitless	Grob type splitless
Septum purge flow	5 mL/min	5 mL/min
Split flow	25 mL/min	25 mL/min
Injector temp.	300°C	300°C
Splitless time	1.0 min	1.0 min
Initial temp.	80°C (hold 2 min)	80°C
Program rate	4°C/min	4°C/min
Final temp.	250°C	250°C
Hold time	30 min	30 min

*Column used for identification and quantification.
**Column used for isomer specific confirmation.
***GC will have both splitless and on-column capability.

Appendix B

Mass Spectrometric Conditions

LRMS

Instrumentation: Finnigan Mat 4500 w/INCOS data system.

Inlet System: Capillary direct to ionizer.

Ionization Mode: Electron impact at 70 eV, 150°C, 2×10^{-6} torr.

Scan Mode: Computer controlled selected ion monitoring.

Scan Time: 1.0 sec (0.25 sec/each of 4 mass intervals).

Mass Intervals:

319.771-320.021	m^+	TCDD
321.769-322.019	$[m+2]^+$	TCDD
327.790-328.040	$[m+2]^+$ $^{13}C_6$	TCDD
333.809-334.059	$[m+2]^+$ $^{13}C_{12}$	TCDD

HRMS

Instrumentation: Finnigan Mat 8230/SS300 data system.

Inlet: Capillary column directly inserted into ionizer.

Ionization: Electron impact, 70eV, 1mA emission current.

Source Pressure: 1×10^{-5} torr.

Ionizer Temperature: 250°C.

Resolution: 5000, 10% valley.

Data Acquisition: Multiple Ion Selection (MIS) of the following ions:

319.8964	$[m]^+$	TCDD
321.8934	$[m+2]^+$	TCDD
323.8904	$[m+4]^+$	TCDD (optional)
327.9138	$[m+2]^+$ $^{13}C_6$	TCDD
333.9338	$[m+2]^+$ $^{13}C_{12}$	TCDD
330.9793	PFK Lock Mass	

Scan Rate: 15 MIS cycles per 10 sec.

Supplemental criteria for HRMS confirmation
Resolution: 8000-10000, 10% valley
Additionally, monitor COCl loss ions, 256.9327 $[COCl]^+$ and 258.9298 $[(m+2)\text{-}COCl]^+$

Appendix C

Date: _____

National Dioxin Study

United States Environmental Protection Agency
Environmental Research Laboratory
6201 Congdon Blvd.
Duluth, MN 55804

2378-TCDD Analysis

Episode Number _____

SCC #	Matrix	2378-TCDD (d 1.) pg/g	% Rec. Int. Std. pg/g	% Accuracy pg/g	% Precision pg/g	% Moisture Lipid

KEY

SCC # = Sample Control Center sample identification number.
d.L. = detection limit.
% Rec. Int. Std. = Percent recovery of internal standard . pg/g = level of spike.
Accuracy = measured/actual]100 : pg/g = level at which accuracy determined.
Precision = [(|sample A-duplicate A|)/mean of A values]100 : pg/g = level at which precision determined.
P2-NA = Priority two sample not analyzed because priority one below detection limit.

Appendix D

NATIONAL DIOXIN STUDY: Sample Tracking System ERLD Loc = 158

1-EPISODE #: _____ 2-SCC #: _____ 3-Tier #: 0

4-Date Received Troika: 0/ 0/ 0 5-Ana. Lab: _____ 6-Extraction Date: 0/ 0/ 0

7-2378-TCDD: _____ 8-d.l.: 0.0 9-% Recovery (INT. STD.): 0

10-Lab ID: _____ 11-GC/MS ID: _____

12-Matrix Type: _____ 13-WT: 0.00

Spike Levels 14-NATURAL 1234: 100 15-$^{13}C_6$ 1234: 500

16-320/322 Ratio: .00 17-S/N Ratio: 0.0

18-% Lipid or Moisture: 0 0 19-% Anal. Done: 1 20-Other TCDD: 0

21-Latitude: __ 0 0' 0" 22-Longitude: ___ 0 0' 0"

23-Location: _____

24-Coment: _____

_____

ENVIRONMENTAL RESEARCH LABORATORY
NATIONAL DIOXIN STUDY
DULUTH, MINNESOTA
1984

DATE STARTED: _____ DATE COMPLETED: _____ ANALYST: _____

MASS SPECTRAL RESPONSE FACTORS $^{13}C_6$ 1,2,3,4-TCDD/$^{13}C_{12}$ 1,2,3,4-TCDD = _____

2,3,7,8-TCDD/$^{13}C_6$ 1,2,3,4-TCDD = _____

PRECISION: % Difference of Duplicate = _____ at _____ pg/g

ACCURACY: % Difference from Spike = _____ at _____ pg/g

Lab ID #	GC/MS#	Episode # SCC # Matrix	Wt of Sample (g)	Spike Level 1,2,3,4-TCDD (pg)	Spike Level $^{13}C_6$ 1,2,3,4-TCDD (pg)	% Recovery $^{13}C_6$ 1,2,3,4-TCDD	pg/g of 2,3,7,8-TCDD (d.l.)	320/322	S/N 322	% Lipid/ Moisture	Comments

☆ U.S. GOVERNMENT PRINTING OFFICE 1986 - 646-116/20818