Loureiro Engineering Associates, Inc.

January 29, 2008

United States Environmental Protection Agency
Region 1 - New England Regional Office
One Congress Street, Suite 1100 (HBO)
Boston, Massachusetts 02114-2023

Attn: Anna Krasko, Project Manager

RE: Addendum 1 - Response to Additional Comments
November 13, 2007 Monitoring Well Installation Work Plan
Centredale Manor Restoration Project Superfund Site
North Providence, Rhode Island

Dear Ms. Krasko:

On January 28, 2008, Loureiro Engineering Associates, Inc. (LEA) received written comments provided by the United States Environmental Protection Agency (EPA) on the above-referenced Work Plan. The written comments are in addition to those provided by EPA on November 21, 2007 which were addressed by LEA in the revised Work Plan submitted to EPA on January 7, 2008. On behalf of Emhart Industries, Inc., LEA is providing this correspondence to address EPA's additional comments provided on January 28, 2008. This correspondence constitutes Addendum 1 to the revised Work Plan.

To facilitate your review of the information presented in this addendum, EPA's comments are provided below in italicized text, followed by the corresponding response.

Comments to Field SOPs

1. *Inconsistency: SOP 10007 (MW Develop), Section 5.3, indicates that parameters will be measured at least every 3 well volumes, while Attachment 1, comment 7, states "at least every well-volume." Please clarify which is correct.*

   In developing the monitoring wells to be installed at the site, parameters will be measured and recorded at least every well-volume.

2. *On the field data record groundwater form for purge/collection, include a start time for purge, a stop time for purge/collection and a total volume purged/collected. This allows for an accurate pumping rate to be calculated.*

For each monitoring well that is sampled, the time at which groundwater purging is initiated (start time) and completed (stop time), the total volume of groundwater purged, the total volume of groundwater collected, and the time at which the groundwater samples are collected will be recorded on the low-flow field sampling record.

Comments to Analytical SOPs

1. *DAT methods based on EPA Method 8290 and Battelle methods based on EPA Method 1613. DAT detection limits not as sensitive as Battelle - but shouldn't be a problem for this site.*

   The detection limit stated for EPA Method 8290 and EPA Method 1613 may indeed be different. The detection limit achievable will be technically the same as long as similar instrumentation is used. The detection limit is calculated on a per sample basis for both EPA Method 8290 and EPA Method 1613. EPA Method 1613, Table 2, has a minimum level for solids without interferences of 1 part per trillion (ppt), while EPA Method 8290, Table 1, has the same minimum level of 1 ppt. Thus, technically there is little difference in the methodology detection limits.

2. *Dioxin Extraction SOP-155: it is unclear in Section 11.2 what happens to the other 5-mL of extract (5 mL of the 10 mL sample extract removed for acid/base cleanup).*

   The other 5 milliliters of extract are reserved and archived.

3. *Inconsistency between the extraction SOP-155 and analysis SOP-92: The extraction SOP says to add 10 uL of RS and 50uL, of nonane (Sections 11.4.4 and 11.4.5) and the analysis SOP says to "Remove the sample extract (blown down to dryness) or blank from storage. Add 20 uL of recovery standard and mix thoroughly." (Section 12.1). Please clarify.*

   There is an inconsistency between the extraction SOP 155 and the analysis SOP 92. The SOPs have been revised to be consistent with 10 microliters (µl) of RS and 20 µl of nonane. The revised SOPs are attached to this addendum.

Additional Comments

1. *With respect to the field and analytical data collected for this investigation, please submit data in a format to merge into the existing project database. Plan to coordinate with Battelle so that the data submission meets database requirements. Also, submit copies of the complete, raw data packages and third party validation reports to USEPA for inclusion in the Site file.*

The field and analytical data will be submitted in a form that is consistent with that needed to merge the data into the existing site database. Copies of the complete, raw data packages and third party validation reports will be submitted to EPA as part of the report that is prepared to document the investigation and findings.

2. *Please notify Centredale Manor about the temporary storage of IDW on their property.*

The Centerdale Manor property Manager, Ms. Ann Viccaro, has been notified that we will temporarily store several drums of investigation-derived waste (IDW) on Cap No. 1, enclosed within the fence and located at the southern extent of the Centerdale Manor property.

3. *Please incorporate these and my January 23rd comments below into the WorkPlan and notify EPA when the field work is scheduled.*

As you can see from the MW-05S log, that well was screened from about 3 feet - 8 feet, not 7 - 12 feet as you originally assumed. We hope to review the SOPs you submitted in the next few days. With respect to LEA's responses provided to our original comments, determination of the vertical position of the screened interval should be based on the portion of the saturated zone that has the highest likelihood of contamination. This was determined during previous site investigations based on field screening data in conjunction with soil characterization and visual observation of staining or other evidence of potential contamination -- See page 3-5 of January 2002 TTNUS report (Technical Memorandum Source Area Investigation). Simply screening the well on a pre-determined depth would be inconsistent with previous work/data and may result in 'missing' the contamination. As you need to revise the portion of proposal to acknowledge the correct depth of the existing screen in well MW-05S, please also include a revised procedure to determine well screen depths in the field, consistent with previous field investigations and MW-05S installation.

In reviewing the boring log and monitoring well construction log for monitoring well MW-05S, it appears that the approach that was taken was to screen the saturated horizon that exhibited the highest level of contamination. Monitoring well MW-05S is screened from three to eight feet below the ground surface (bgs). We will use the same approach, constructing each well with a 5-ft section of screen: We will set the screen for each monitoring well to span the horizon within the saturated zone that exhibits the highest level of contamination, based on field photoionization detector (PID) readings and visual and olfactory evidence gathered during an examination of the soil split-spoon samples. To be consistent with this approach, each boring will be advanced to a depth of approximately eight feet bgs. If the highest level of contamination is observed above eight feet or if no contamination is observed to this depth, then we will set the screen from three to eight feet bgs. Please note that this is the shallowest interval that the wells

could be screened while ensuring an adequate seal from the surface/well-head area. If the level of contamination is observed to increase with depth, then the boring will be advanced beyond eight feet, up to a depth of twelve feet bgs, and the screen will be set across the interval that exhibits the highest level of contamination.

We trust that the information provided in this addendum adequately addresses your comments. We look forward to receiving your final approval of the revised Work Plan and Addendum 1. We are tentatively scheduled to install the three, proposed monitoring wells on Thursday, January 31, 2008. Should you have any questions or concerns, please feel free to contact me directly at (860) 410-2976.

Sincerely,

LOUREIRO ENGINEERING ASSOCIATES, INC.

David N. Scotti, P.G.
Project Manager

Copy to: Eve Vaudo (EPA)
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D A T Laboratory , Inc.
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Approved by Tamara Cavender
Date 01/29/2008

SOP - 155

STANDARD OPERATING PROCEDURE (SOP) FOR THE EXTRACTION OF SAMPLES FOR DETERMINATION OF PCDDs AND PCDFs BY HRGC/HRMS USING METHOD 8290

Reviewed by: _____ Date: 1/29/08
Quality Assurance Officer

Approved by: _____ Date: 1/29/08
President, DAT Inc.

DATA ANALYSIS TECHNOLOGIES, INC (DAT)
7715 CORPORATE BLVD.
PLAIN CITY, OH 43064

Date:	Reviewed by:	Date	Reviewed by:

PROCEDURE

1.0 SCOPE AND APPLICATION

1.1. This SOP provides procedures for the sample preparation and extraction of water, soil, sediment, sludge, tissue and other sample matrices to be analyzed by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS).

1.2. Following extraction, the analysis is performed with high resolution mass spectrometry as described in DAT SOP #092.

2.0 SUMMARY OF METHOD:

2.1. This procedure uses matrix specific extraction and analyte specific cleanup for HRGC/HRMS analysis techniques.

2.2. A specified amount of a sample of soil, sediment, fly ash, water, sludge (including paper pulp), still bottom, fish tissue, or human adipose tissue is spiked with a solution containing specified amounts of nine isotopically ($^{13}C_{12}$) labeled PCDDs/PCDFs listed in Column 1 of Table 1. The sample is then extracted according to a matrix specific extraction procedure. Aqueous samples that are judged to contain 1 percent or more solids, and solid samples that show an aqueous phase are filtered, the solid phase (including the filter) and the aqueous phase extracted separately, and the extracts combined before extract cleanup. The extraction procedures are:

2.2.1. Toluene: Soxhlet extraction for soil, sediment, fly ash, and paper pulp samples.

2.2.2. Methylene chloride: liquid-liquid extraction for water samples.

2.2.3. Toluene: Dean-Stark extraction for fuel oil and aqueous sludge samples.

2.2.4. Toluene extraction for still bottom samples.

2.2.5. Hexane/methylene chloride: Soxhlet extraction or methylene chloride, soxhlet extraction for fish tissue samples.

2.2.6. Methylene chloride extraction for human adipose tissue samples.

2.2.7. As an option, all solid samples (wet or dry) may be extracted with toluene using a Soxhlet/Dean Stark extraction system.

2.3. The extracts are submitted to an acid-base washing treatment and dried. Following a solvent exchange step, the extracts are cleaned up by column chromatography on alumina, silica gel, and activated carbon.

2.3.1. The extracts from adipose tissue samples are treated with silica gel impregnated with sulfuric

acid before chromatography on acidic gel, neutral alumina, and activated carbon.

2.3.2. Fish tissue and paper pulp extracts are subjected to an acid wash treatment only, prior to chromatography on alumina and activated carbon.

2.4. The preparation of the final extract for HRGC/HRMS analysis is accomplished by adding 10 to 50 uL (depending on the matrix) of a nonane solution containing 50 pg/uL of the recovery standards $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDD (Table 1). The former is used to determine the percent recoveries of tetra- and penthachlorinated PCDD/PCDF congeners, while the later is used to determine the percent recoveries of the hexa-, hepta-, and octachlorinated PCDD/PCDF congeners.

3.0 DEFINITIONS

3.1. See Section 13.0.

4.0 CONTAMINATION AND INTERFERENCES

4.1. Solvents, reagents, glassware and other sample processing hardware may yield discrete artifacts or elevated baselines that may cause misinterpretation of the chromatographic data. A method blank must be analyzed to prove that this hardware is free from contamination. Analysts should avoid using PVC gloves.

4.2. The use of high purity reagents and solvents helps minimize interference problems. Purification of solvents by distillation in all glass systems may be necessary.

4.3. Interferants co-extracted from the sample will vary considerably from matrix to matrix. PCDDs and PCDFs are often associated with other interfering chlorinated substances such as polychlorinated biphenyls (PCBs), polychlorinated diphenyl ethers (PCDPESs), polychlorinated napthalenes, and polychlorinated alkyldibenzofurans, that may be found at concentrations several orders of magnitude higher than the analytes of interest. The clean-up technique described in this SOP must be used to reduce interference.

4.4. Proper cleaning of glassware is extremely important, because glassware may not only contaminate the samples but may also remove the analytes of interest by adsorption on the glass surface.

4.4.1. All glassware used in extractions for Dioxins/Furans should be rinsed with the last solvent used, and then methanol.

4.4.2. Wash all glassware with warm tap water and a detergent solution as soon after use as practical. Rinse with tap water. Sonication of glassware containing a detergent solution for approximately 30 seconds may aid cleaning. Glassware with removable parts, particularly separatory funnels with fluoropolymer stopcocks, must be disassembled prior to washing.

4.4.3. After detergent washing, glassware must be rinsed immediately with the following: organic-

free reagent water, methanol, methylene chloride, toluene, and then methanol.

4.4.4. Allow the glassware to drain/dry in a fume hood.

4.4.5. Rinse again with organic-free reagent water.

4.4.6. Do not bake reusable glassware in an oven as a routine part of cleaning. Baking may be warranted after particularly dirty samples are encountered but should be minimized, as repeated baking of glassware may cause active sites on the glass surface that will irreversibly adsorb CDD/ CDFs.

4.4.7. If baking is needed, bake at a temperature of 180^0C for 15 minutes to remove residue.

4.4.8. After glassware has dried, cap ends with clean aluminum foil and store in designated locations in the wet laboratory.

4.4.9. The glassware shall only be used for Dioxins/Furans and Halowax sample preparation.

4.4.10. Immediately prior to use, the Soxhlet apparatus and all separatory funnels are rinsed with methanol, methylene chloride, and toluene.

5.0 SAFETY

5.1. All personnel are required to comply with DAT's Health and Safety Documents as described in the Quality Assurance Manual.

5.2. Because of the extreme toxicity of many of these compounds, the analyst must take the necessary precautions to prevent exposure to materials known or believed to contain PCDDs or PCDFs.

6.0 APPARATUS AND MATERIAL

6.1. Laboratory fume hood of sufficient size to contain the sample preparation equipment listed below.

6.2. Glove box.

6.3. Tissue homogenizer-with stainless steel Macro-shaft and Turbo-shear blade.

6.4. Meat grinder –Hobart, or equivalent, with 3 to 5 mm holes in inner plate.

6.5. Equipment for determining percent moisture.

6.6. Oven-Capable of maintaining a temperature of 110 +/- 5^0 C.

6.7. Dessicator.

6.8. Balances.

6.8.1. Analytical-Capable of weighing 0.1 mg.

6.8.2. Top loading-Capable of weighing 10 mg.

6.9. Extraction Apparatus for Water Samples

6.9.1. pH meter, with combination glass electrode

6.9.2. pH paper, wide range (Hydrion Papers, or equivalent)

6.9.3. Graduated cylinders, one-L capacity and 100 mL capacity

6.9.4. Liquid/liquid extraction-Separatory funnels, 2-L capacity, with fluoropolymer stopcocks.

6.9.5. 500 mL round bottom flasks with funnels

6.10. Soxhlet/Dean-Stark (SDS) extractor for filters and solid/sludge samples.

6.10.1. Soxhlet-50 mm ID, 200 mL capacity with 500 mL round bottom flask

6.10.2. Thimble-43 x 123 to fit Soxhlet.

6.10.3. Moisture trap-Dean Stark or Barret with fluoropolymer stopcock, to fit Soxhlet.

6.10.4. Heating mantle-Hemispherical, to fit 500 mL round bottom flask.

6.10.5. Variable transformer-Powerstat (or equivalent), 100 volt, 10 amp.

6.10.6. Beakers-400 to 500 mL.

6.11. Apparatus for extraction of tissue

6.11.1. Bottle for extraction (if digestion/extraction is using HCl is used)-500-600 mL wide-mouth clear glass with fluoropolymer-lined cap.

6.11.2. Bottle for back extraction-100-200 mL narrow mouth clear glass with fluoropolymer-lined cap.

6.11.3. Ann Arbor Shaker Table- platform-type rotary shaker that produces vigorous agitation.

6.11.4. Rack attached to shaker table to permit agitation of four to nine samples simultaneously.

6.11.5. Beakers-400 to 500 mL.

6.11.6. Spatulas-Stainless steel.

6.12. Filtration Apparatus

6.12.1. Pyrex glass wool-Solvent extracted by SDS for three hours minimum.

6.12.2. Glass funnel- 125 to 250 mL.

6.12.3. Glass-fiber filter paper- Whatman GF/D (or equivalent), to fit glass funnel in Section 8.8.2.

6.12.4. Drying column- 15 to 20 mm ID Pyrex chromatographic column equipped with coarse-glass frit or glass-wool plug.

6.12.5. Buchner funnel-15 cm.

6.12.6. Glass-fiber filter paper-to fit the Buchner funnel in Section 8.8.5.

6.12.7. Filtration flasks- 1.5 to 2.0L, with side arm.

6.12.8. Pressure filtration apparatus-Millipore YT30 142 HW, or equivalent.

6.13. Centrifuge Apparatus

6.13.1. Centrifuge- Capable of rotating 500 mL centrifuge bottles or 15 mL centrifuge tubes at 5,000 rpm minimum.

6.13.2. Centrifuge bottles-500 mL, with screw-caps, to fit centrifuge.

6.13.3. Centrifuge tubes-12 to 15 mL, with screw-caps, to fit centrifuge.

6.14. Cleanup Apparatus

6.14.1. Automated gel permeation chromatograph

6.14.1.1. Column-600 to 700 mm long x 25 mm ID, packed with 70 g of SX-3 Bio-beads.

6.14.1.2. Syringe-10mL, with Luer fitting

6.14.1.3. Syringe filter holder-stainless steel, and glass-fiber or fluoropolymer filters (Gelman 4310, or equivalent).

6.14.1.4. UV detectors-254 nm, preparative or semi-preparative flow cell

6.15. Pipets

6.15.1. Disposable, Pasteur-150 mm long x 5-mm ID

6.15.2. Disposable, serological-10mL (6 mm ID), for the preparation of the carbon columns.

6.16. Glass chromatographic columns

6.16.1. 300 mm 150 mm long x 10.5 mm ID (Kontes K-420155, or equivalent) with coarse-glass frit or glass-wool plug and 250 mL reservoir.

6.16.2. 200 mm long x 1.5 mm ID, with coarse-glass frit or glass-wool plug and 250 mL reservoir.

6.16.3. 300 mm long x 25 mm ID, with 300 mL reservoir and glass or fluoropolymer stopcock.

6.17. Stirring apparatus for batch silica cleanup of tissue extracts.

6.17.1. Mechanical stirrer.

6.17.2. Bottle-500 to 600 mL wide-mouth clear glass.

6.18. Oven-For baking and storage of adsorbents, capable of maintaining a constant temperature (+/_ 5^0C) in the range of 105-250^0C.

6.19. Concentration Apparatus

6.19.1. Buchi Rotavapor R-114 evaporator equipped with a variable temperature water bath.

6.19.2. Vacuum source for rotary evaporator equipped with shutoff valve at the evaporator and vacuum gauge.

6.19.3. Carbon dioxide, CO_2, is used as the coolant, and tap water is used to maintain the vacuum.

6.19.4. Round bottom flask- 500 mL or larger, with ground-glass fitting compatible with the rotary evaporator.

6.19.4.1. Amber glass-2 to 5 mL with fluoropolymer-lined screw cap.

6.19.4.2. Glass - 0.3 mL, conical, with fluoropolymer-lined screw or crimp cap.

6.20. Nitrogen blowdown apparatus – Equipped with water bath controlled in the range of 30-60° C (N-Vap, organomation)

7.0 REAGENTS AND STANDARDS

7.1. pH Adjustment and Back-Extraction

7.1.1. Potassium hydroxide, KOH, ACS grade, 20 percent (w/v) -Dissolve 20 g reagent grade KPH in 100 mL organic-free reagent water.

7.1.2. Sulfuric acid, H_2SO4, concentrated, ACS grade (specific gravity 1.84).

7.1.3. Sodium chloride, NaCl, analytical grade, prepare at 5% (w/v) solution in organic free reagent water.

7.1.4. Potassium carbonate, K_2CO_3, anhydrous, analytical reagent.

7.2. Dessicating agent

7.2.1. Sodium sulfate (powder, anhydrous), Na_2SO4. Purify by heating at 400^0C for 4 hours in a shallow tray, cooled in a dessicator, and stored in a pre-cleaned glass bottle with screw-cap that prevents moisture from entering. If, after heating, the sodium sulfate develops a grayish cast (due to the presence of carbon in the crystal matrix), that batch of reagent is not suitable for use and should be discarded. Extraction with methylene chloride (as opposed to simple rinsing) and baking at a lower temperature may produce sodium sulfate that is suitable for use.

7.2.2. Tissue drying-Sodium sulfate, reagent grade, powdered, treated and stored as above.

7.2.3. Pre-purified nitrogen.

7.3. Solvents for Extraction

7.3.1. Methylene chloride, CH_2Cl_2, high purity.

7.3.2. Hexane, C_6H_{14}, high purity.

7.3.3. Methanol, CH_3OH, high purity.

7.3.4. Nonane, C_9H_{20}, high purity.

7.3.5. Toluene, $C_6H_5CH_3$, high purity.

7.3.6. Cyclohexane, C_6H_{12}, high purity.

7.3.7. White quartz sand, For Soxhlet/Dean-Stark extraction. Bake at 450^0C for four hours minimum.

7.4. Stock Standard Solutions-Purchased pre-mixed from Cambridge Isotope Laboratories with certification to their purity, concentration, and authenticity.

7.4.1. Internal Standard Stock Solution: catalog #EDF-4053 @ 1000 ng/mL in nonane, stored at room temperature.

7.4.2. Surrogate Standard Stock Solution: catalog #EDF-4054 @ 1000 ng/mL in nonane, stored at room temperature.

7.4.3. Recovery Standard Stock Solution: catalog #EDF-4055 @ 500 ng/mL in nonane, stored at room temperature.

7.4.3.1. Sample extracts are spiked with 10 uL or 5 ng/total of 7.4.3.

7.4.4. Alternative Recovery Stock Solution: catalog #PS-46-1,2,3,7,8,9-HxDF$^{13}C_{12}$ @ 50 ng/mL, stored at room temperature.

7.4.5. Method 8290 Matrix Spiking Solution: catalog # EDF-5008, @ 100-500 ng/mL.

7.4.5.1. The Matrix Spike sample extracts are spiked with 40 uL or 4-20 ng/total of 7.4.5.

7.5. Working Standards.

7.5.1. Internal Standard Working Solution- 100 ng/mL standard is prepared by adding 100 uL of 7.4.1 to 900 uL of nonane.

7.5.2. Surrogate Standard Working Solution- 100 ng/mL standard is prepared by adding 1.0 mL of 7.4.2 to 9.0 mL of nonane.

7.5.3. Alternate Recovery Standard- Prepare a 10 ug/mL by pipetting 100uL of the Alternate Recovery Stock Solution (7.4.4) into a screw top dram vial containing 400 uL of nonane.

7.5.3.1. Alternative Recovery Spiking Solution- Prepare a 100 ng/mL standard by adding 10uL of 7.5.3 to 990 uL of nonane.

7.6. Adsorbents for Sample Cleanup

7.6.1. Silica Gel

7.6.1.1. Activated silica gel-80/200 mesh rinsed with methylene chloride, baked at 180^{0}C for a minimum of one hour, cooled in a dessicator, and stored in a pre-cleaned glass bottle with screw-cap that prevents moisture from entering.

7.6.1.2. Alumina-Acidic Silica gel (30% w/ w)-Thoroughly mix 44.0 g of concentrated sulfuric acid with 100.0 g of activated silica gel in a clean container. Break up aggregates with a stirring rod until a uniform mixture is obtained. Store in a bottle with a fluoropolymer-lined screw-cap.

7.6.1.3. Basic silica gel-Thoroughly mix 30 g of 1N sodium hydroxide with 100 g of activated silica gel in a clean container. Break up aggregates with a stirring rod until a uniform mixture is obtained. Store in a bottle with a fluoropolymer-lined screw-cap.

7.6.1.4. Potassium silicate

7.6.1.4.1. Dissolve 56 g of high purity potassium hydroxide in 300 mL of methanol in a 750-1000 mL flat-bottom flask.

7.6.1.4.2. Add 100 g of silica gel and a stirring bar, and stir on a hot plate at 60-70^{0}C for one to two hours.

7.6.1.4.3. Decant the liquid and rinse the potassium silicate with 100 mL portions of methanol, followed by a single rinse with 100 mL of methylene chloride.

7.6.1.4.4. Spread the potassium silicate on solvent-rinsed aluminum foil and dry for two to fours hours in a hood.

7.6.1.4.5. Activate overnight at 200-250^{0}C.

7.6.2. Alumina-Either one of two types of alumina, acid or basic, is used in the cleanup of sample extracts. The same type of alumina must be used for all samples, including those used to demonstrate initial precision and recovery and ongoing precision and recovery.

7.6.2.1. Acid alumina-Activate by heating to 130^{0}C for a minimum of 12 hours.

7.6.2.2. Basic alumina- Activate by heating to 600^{0}C for a minimum of 24 hours. Store at 130^{0}C in a covered flask. Use within five days of baking.

7.6.3. Carbon

7.6.3.1. PX-21 Active Carbon

7.6.3.2. 5.5% w/w PX-21 Silica

7.6.3.3. ID ¼" x 2"L HPLC Column.

7.6.4. Anthropogenic isolation column-Pack the column in Section 6.16.3 from bottom to top with the following:

7.6.4.1. 2 g silica gel

7.6.4.2. 2 g potassium silicate

7.6.4.3. 2 g granular anhydrous sodium sulfate

7.6.4.4. 10 g acid silica gel

7.6.4.5. 2 g granular anhydrous sodium sulfate

7.6.5. Florisil column

7.6.5.1. Florisil-60 to 100 mesh. Soxhlet extract in 500 g portions for 24 hours.

7.6.5.2. Insert a glass wool plug into the tapered end of a graduated serological pipet. Pack

with 1.5 g of Florisil topped with approximately 1 mL of sodium sulfate and a glass wool plug.

7.6.5.3. Activate in an oven at 130-150^{0}C for a minimum of 24 hours and cool for 30 minutes. *Use within 90 minutes of cool.*

7.7. Reference Matrices-Matrices in which the CDDs/CDFs and interfering compounds are not detected by the analytical method.

7.7.1. Organic-free water –water demonstrated to be free from the analytes of interest and potentially interfering substances at the method detection limit for the analyte(s) of interest.

7.7.2. High-solids reference matrix-Playground sand or similar material. Prepared by extraction with toluene.

7.7.3. Paper reference matrix-Glass-fiber filter, Gelman Type A or equivalent. Cut paper to simulate the surface area of the paper being tested.

7.7.4. Tissue reference material-Corn or other vegetable oil. May be prepared by extraction with toluene.

7.7.5. Other matrices-This method may be verified on any reference material by performing the tests given in Section 9.2.

8.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

8.1. Collect samples in amber glass containers following conventional sampling practices. Aqueous samples that flow freely are collected in refrigerated bottles using automatic sampling equipment. Solid samples are collected as grab samples using wide-mouth jars.

8.2. Storage and holding times-All samples, except fish and adipose tissue samples, must be stored at 4°C in the dark, and must be extracted within 30 days of collection. Fish and adipose tissue samples must be stored at -20^{0}C in the dark, and must be extracted within 30 days of collection

8.3. *Fish and tissue samples must* be frozen upon receipt at the laboratory. Recommended holding times are demonstrated below:

8.4. Grinding or blending of fish samples-If not otherwise specified in a project plan, the whole fish (frozen) should be blended or ground to provide a homogenous sample. The use of a stainless steel meat grinder with a 3 to 5 mm hole size inner plate is recommended. In some circumstances, analysis of fillet or specific organs of fish may be requested. If so requested, the above fish requirement is superseded.

8.5. Aqueous, soil and air samples must be shipped and stored at less than 6°C. Fish and tissue samples must be shipped at less than 4°C and stored in deep freeze at less than -20°C.

8.6. Phase separation-This is a guideline for phase separation for very wet (> 25 percent water) soil, sediment, and paper pulp samples. Place a 50-g portion of sample in a suitable centrifuge bottle and centrifuge for 30 minutes at 2,000 rpm. Remove the bottle and mark the interface level on the bottle. Estimate the relative volume of each phase. With a disposable pipet, transfer the liquid layer into a clean bottle. Mix the solid with a stainless steel spatula and remove a portion to be weighed and analyzed for percent dry weight determination, extraction). Return the remaining solid portion to the original sample bottle (empty) or to a clean sample bottle that is properly labeled, and store it as appropriate. Analyze the solid phase by using only the soil, sediment, and paper pulp method. Take note of, and report, the estimated volume of liquid before disposing of the liquid as a liquid waste.

9.0 QUALITY ASSURANCE/QUALITY CONTROL

9.1. A Method Blank (MB) must be extracted with each analytical batch of twenty samples or less. Extraction and analyses of method blanks are required to demonstrate freedom from contamination.

9.2. A Laboratory Spike (LS) must be extracted with each analytical batch of twenty samples or less.

9.3. A Matrix Spike (MS) shall be extracted with each analytical batch of twenty samples or less.

9.4. The laboratory shall spike all samples with labeled compounds to monitor method performance.

9.5. The laboratory shall spike all samples with 40 µL of the internal working standard (7.5.1) and 40 µL of the surrogate standard working solution (7.5.2) to assess method performance on the sample matrix.

9.6. In each batch of samples, locate the sample specified for duplicate analysis, and analyze a second 10g soil or sediment sample portion or 1L water sample or an appropriate amount of the type of matrix under consideration

10.0 SAMPLE PREPARATION

10.1. Sample preparation involves modifying the physical form of the sample so that the CDDs/ CDFs can be extracted efficiently. In general, the samples must be in a liquid form or in the form of finely divided solids in order for efficient extraction to take place.

10.1.1. For samples that contain particles, percent solids and particle size are determined using the procedures in Sections 10.6, 10.7, and 10.8 respectively.

10.1.2. Aqueous samples-Because CDD/CDFs may be bound to suspended particles, the preparation of aqueous samples is dependent on the solids content of the sample.

10.1.2.1. Aqueous samples visibly absent of particles are prepared per Section 10.9 and extracted directly using the separatory funnel technique in Section 11.0.

10.1.2.2. Aqueous samples containing visible particles and containing one percent suspended solids or less are prepared using the procedure in Section 10.7. After preparation, the sample is filtered per Section 10.9.3. After filtration, the particles and filter are extracted using the SDS procedure in Section 11.5 and the filtrate is extracted using the separatory funnel procedure in Section 11.0.

10.1.2.3. For aqueous samples containing greater than one percent solids, a sample aliquot sufficient to provide 10 g of dry solids is used, as described in Section 10.10.

10.2. Solid samples are prepared using the procedure described in Section 10.10 followed by extraction via the SDS procedure in Section 11.5.

10.3. Multi-phase samples-The phase(s) containing the CDDs/CDFs is separated from the non-CDD/CDF phase using pressure filtration and centrifugation, as described in Section 10.11. The CDDs/CDFs will be in the organic phase in a multi-phase sample in which an organic phase exists.

10.4. Procedures for grinding, homogenization, and blending of various sample phases are given in Section 10.12.

10.5. Tissue samples- Preparation procedures for fish and other tissues are given in Section 10.13.

10.6. Determination of Percent Suspended Solids

10.6.1. Aqueous liquids and multi-phase samples consisting of mainly an aqueous phase.

10.6.1.1. Dessicate and weigh a GF/D filter (Section 6.12.3) to three significant figures.

10.6.1.2. Filter 10.0 +/- 0.02 mL of well mixed sample through the filter.

10.6.1.3. Dry the filter a minimum of 12 hours at 110 +/- 5^0C and cool in a dessicator.

10.6.1.4. Calculate percent solids as follows:

$$\% \text{ solids} = \frac{\text{weight of sample aliquot after drying (g) – weight of filter (g)}}{10 \text{ g}} \times 100$$

10.7. Non-aqueous liquids, solids, semi-solid samples, and multi-phase samples in which the main phase is not aqueous, but not tissues.

10.7.1. Weigh 5-10 g of sample to three significant figures in a tared beaker.

10.7.2. Dry a minimum of 12 hours at 110 +/- 5^0C, and cool in a dessicator.

10.7.3. Calculate percent solids as follows:

$$\% \text{ solids} = \frac{\text{weight of sample aliquot after drying}}{\text{weight of sample aliquot before drying}} \times 100$$

10.8. Determination of Particle Size.

10.8.1. Spread the dried sample from Section 10.7.2 on a piece of filter paper or aluminum foil in a fume hood or glove box.

10.8.2. Estimate the size of the particles in the sample. If the size of the largest particles is greater than 1 mm, the particle size must be reduced to 1mm or less prior to extraction using the procedures in Section 10.12.

10.9. Preparation of Aqueous Samples Containing 1% Suspended Solids or Less

10.9.1. Aqueous samples visibly absent of particles are prepared per the procedure below and are extracted directly using the separatory funnel technique in Section 11.1.

10.9.2. Preparation of sample and quality control aliquots

10.9.2.1. Mark the original level of the sample on the sample bottle for reference. Weigh the sample plus the bottle to +/- 1 g.

10.9.2.2. Add 40 uL of the Surrogate Standard Working Solution (Section 7.5.2) into the sample bottle. Cap the bottle and mix the sample by careful shaking. Allow the sample to equilibrate for one to two hours, with occasional shaking.

10.9.2.3. For each sample or sample batch (to a maximum of 20 samples) to be extracted during the same 12-hour shift, place two 1.0 L aliquots of organic-free reagent water in clean sample bottles or flasks.

10.9.2.3.1. Add 40 uL of the Surrogate Standard Working Solution (Section 7.5.2) into both organic free reagents water aliquots. One of these aliquots will serve as the method blank.

10.9.2.4. Add 40 uL of the method 8290 Matrix Spiking Solution (Section 7.4.5) into the remaining reagent water aliquot. This aliquot will serve as the Ongoing Precision and Recovery (OPR) standard.

10.9.2.5. If the sample(s) contain visible particles, proceed to the following section for filtration of particles.

10.9.3. Filtration of Particles

10.9.3.1. Assemble a Buchner funnel on top of a clean filtration flask. Apply vacuum to the flask, and pour the entire contents of the sample bottle through a glass-fiber filter in the Buchner funnel, swirling the sample remaining in the bottle to suspend any particles.

10.9.3.2. Rinse the sample bottle twice with approximately 5 mL portions of organic-free

reagent water to transfer any remaining particles onto the filter.

10.9.3.3. Rinse any particles off the sides of the Buchner funnel with small quantities of organic-free reagent water.

10.9.3.4. Weigh the empty sample bottle to +/- g. Determine the weight of the sample by difference. Save the bottle for future use.

10.9.3.5. Extract the filtrate using the separatory funnel procedure in Section 11.1.

10.9.3.6. Extract the filter containing the particles using the Soxhlet-Dean Stark (SDS) extraction procedure in Section 11.5

10.10. Preparation of Samples Containing Greater Than 1% Solids

10.10.1. Weigh a well-mixed aliquot of each sample (of the same matrix type) sufficient to provide 10 g of dry solids (based on the solids determination in Section 10.7) into a clean beaker or glass jar.

10.10.2. Add 40 uL of the Surrogate Standard Working Solution (Section 7.4.5) into the sample in Section 10.10.1

10.10.3. For each sample or sample batch (to a maximum of 20 samples) to be extracted during the same 12-hour shift, weigh two 10 g aliquots of the appropriate reference material (Section 7.7.2) into clean beakers or glass jars.

10.10.4. To one of the 10 g aliquots from Section 10.10.3, add 40 uL of the Surrogate Standard Working Solution (Section 7.4.5). This will serve as the Method Blank.

10.10.5. To the remaining 10 g aliquot, add 40 uL of the method 8290 Matrix Spiking Solution (Section 7.4.5). This will serve as the Ongoing Precision and Recovery Standard.

10.10.6. Stir or tumble and equilibrate the aliquots for one to two hours.

10.10.7. Decant excess water. If necessary to remove water, filter the sample through a glass- fiber filter and discard the aqueous liquid.

10.10.8. If particles > 1 mm are present in the sample (as determined by Section 10.8), spread the sample on clean aluminum foil in a hood. After the sample is dry, grind to reduce the particle size (Section 10.12).

10.10.9. Extract the sample and QC aliquots using the SDS procedure in Section 11.5.

10.11. Multi-phase Samples

10.11.1. Using the percent solids determined in Section 10.7, determine the volume of sample that will provide 10 g of solids, up to 1 L of sample.

10.11.2. Pressure filter the amount of sample determined in Section 10.11.1 through Whatman GF/ D glass-fiber filter paper (Section 6.12.3). Pressure filter the blank and OPR aliquots through GF/ D papers also. If necessary to separate the phases and / or settle the solids, centrifuge these aliquots prior to filtration.

10.11.3. Discard any aqueous phase (if present). Remove any non-aqueous liquid present and reserve the maximum amount filtered from the sample (Section 10.11.1) or 10 g, whichever is less, for combination with the solid phase (Section 10.10).

10.11.4. If particles > 1 mm are present in the sample (as determined in Section 10.8) and the sample is capable of being dried, spread the sample and QC aliquots on clean aluminum foil in a hood. After the aliquots are dry or if the sample cannot be dried, reduce the particle size using the procedures in Section 10.12 and extract the reduced particles using the SDS procedure in Section 11.5. If particles > 1mm are not present, extract the particles and filter in the sample and QC aliquots directly using the SDS procedure in Section 11.5.

10.12. Sample Grinding, Homogenization, or Blending-Samples with particle sizes greater than 1 mm (as determined in Section 10.8.2) are subjected to grinding, homogenization, or blending. The method of reducing particle size to less than 1 mm is matrix dependent. In general, hard particles can be reduced by grinding in a Wiley mill or meat grinder, by homogenization, or in a blender.

10.12.1. Each size-reducing preparation procedure on each matrix shall be verified by running the Initial Precision and Recovery (IPR) tests required by the method before the procedure is employed routinely.

10.12.2. The grinding, homogenization, or blending procedure shall be carried out in a glove box or fume hood to prevent particles from contaminating the work environment.

10.12.3. Grinding-Certain papers and pulps, slurries, and amorphous solids can be ground in a Wiley mill or heavy duty meat grinder. In some cases, reducing the temperature of the sample to freezing or to dry ice or liquid nitrogen temperatures can aid in the grinding process. Grind the sample aliquots from Section 10.10.7 or 10.11.4 in a clean grinder. Do not allow the sample temperature to exceed 50^0C. Grind the blank and reference matrix aliquots using a clean grinder.

10.12.4. Homogenization or blending-Particles that are not ground effectively , or particles greater than 1 mm in size after grinding, can often be reduced in size by high speed homogenization or blending. Homogenize and/or blend the particles or filter from Section 10.10.7 or 10.11.4 for the sample, blank, and OPR aliquots.

10.12.5. Extract the aliquots using the Soxhlet extraction procedure in Section 11.5.

10.13. Fish and Other Tissues-Prior to processing tissue samples, the laboratory must determine the exact tissue to be analyzed. Common requests for analysis of fish tissue include whole fish- skin removed, edible fish fillets (filleted in the field or in the laboratory), specific organs, and other portions. Once the appropriate tissue has been determined, the sample must be homogenized.

10.13.1. Homogenization

10.13.1.1. Samples are homogenized while still frozen, where practical. If the laboratory must dissect the whole fish to obtain the appropriate tissue for analysis, the unused tissues may be rapidly refrozen and store in a clean glass jar for subsequent use.

10.13.1.2. Each analysis requires 10 g of tissue (wet weight). Therefore, the laboratory should homogenize at least 20 g of tissue to allow for re-extraction of a second aliquot of the same homogenized sample, if re-analysis is required. When whole fish analysis is necessary, the entire fish is homogenized.

10.13.1.3. Homogenize the sample in a tissue homogenizer or grind in meat grinder. Cut tissue too large to feed into the grinder into smaller pieces. To assure homogeneity, grind three times.

10.13.1.4. Transfer approximately 10 g (wet weight) of homogenized tissue to a clean, tared, 400 to 500 mL beaker. For the alternate HCL digestion/extraction, transfer the tissue to a clean, tared 500 to 600 mL wide-mouth bottle. Record the weight to the nearest 10 mg.

10.13.1.5. Transfer the remaining homogenized tissue to a clean jar with a fluoropolymer-lined lid. Seal the jar and store the tissue at $< -10^0$C. Return any tissue that was not homogenized to its original container and store at $< -10^0$C.

10.13.2. QC aliquots

10.13.2.1. Prepare a method blank by adding approximately 10 g of the oily liquid reference (Section 7.7.4) to a 400-500 mL beaker. For the alternate HCL digestion/extraction, add the reference matrix to a 500-600 mL wide-mouth bottle. Record the weight to the nearest 10 mg.

10.13.2.2. Prepare a precision and recovery aliquot by adding approximately 10 g of the oily liquid reference matrix (Section 7.6.4) to a separate 400-500 mL beaker or wide-mouth bottle, depending on the extraction procedure to be use. Record the weight to the nearest 10 mg. If the initial precision and recovery tests is to be performed, use four aliquots; if the ongoing precision and recovery tests is to be performed, use a single aliquot.

10.13.3. Spiking

10.13.3.1. Add 40 uL of the Method 8290 Matrix Spiking Solution (Section 7.4.5) into the sample, blank and OPR aliquots.

10.13.4. Extract the aliquots using the procedures in Section 11.7.

11.0 EXTRACTION AND CONCENTRATION OF SAMPLES

11.1. Separatory funnel extraction of filtrates and of aqueous samples visibly absent particles. Allow the sample to come to an ambient temperature before beginning the extraction process. Mark the level of sample on the exterior of the sample bottle/container for later determination of the sample volume.

11.1.1. Rinse separatory funnels with methylene chloride, toluene, and another rinse with methylene chloride.

11.1.2. A one liter graduated cylinder, a 100 mL graduated cylinder, and 500-mL round bottom flask are rinsed with the same solvents.

11.1.3. Transfer a well-shaken one liter sample into the rinsed 2-liter separatory funnel. Rinse the sample container twice with 15 mL of methylene chloride, and add the rinsates to the separatory funnel.

11.1.4. Add 40 uL of the Internal Standard Solution (7.5.1).

11.1.5. Pour one liter of reagent free organic water to a separatory funnel and designate as the Method Blank (13.11).

11.1.6. Pour one liter of organic free water into a separatory funnel and designate as the Laboratory Spike (Sections 13.9 and 13.14).

11.1.6.1. Add the LS to the sample for dioxins analysis.

11.1.7. Add 60 mL of methylene chloride to each separatory funnel and shake for approximately two minutes with periodic venting to relieve pressure.

11.1.8. Allow the separatory funnel to set for at least ten minutes.

11.1.9. Filter the sample into a 500 mL round bottom flask through a funnel with a glass filter filled two-thirds with sodium sulfate.

11.1.10. Repeat the extraction procedures in Sections 11.1.7, 11.1.8, and 11.1.9 two more times for each sample, the method blank, and the LS.

11.1.11. Drain each separatory funnel, and rinse the inside of each funnel with two 20-mL aliquots of methylene chloride. Add the rinsates to each corresponding 500-ml round bottom flask containing the extracts.

11.2. Roto-Vap Procedure

11.2.1. Each extract from Section 11.1 is carried through a roto-vap solvent exchange procedure into hexane.

11.2.2. Add 30 ml of hexane to the 500-mL round bottom flask containing the sample and attach the flask to the Buchi Rotavapor R-114 (Section 6.19.1).

11.2.3. Assemble the rotary evaporator according to the manufacturer's instructions, and warm the water bath to 45^{0}C. Before use, pre-clean the rotary evaporator by concentrating 100 mL of clean extraction solvent through the system. Archive both the concentrated solvent and the solvent in the catch flask for a contamination check if necessary.

11.2.4. Between samples, three 2-3 mL aliquots of solvent should be rinsed down the feed tube into a waste beaker.

11.2.5. Reduce the sample extract volume to approximately 2 to 3 mL and transfer to a 40 mL VOA vial. Bring to a final volume of 10 mL with hexane.

11.2.6. Repeat the procedures in Section 11.2.1 through 11.2.6 for the Method Blank and the LS.

11.2.7. Add 40 uL of the cleanup standard/alternate standard (7.5.3) to each sample aliquot. After this addition, aliquot 5 mL to a test tube for acid/base clean-up. Archive the remaining extract (5 mL) as a back up extract.

11.3. Acid/Base Clean-up.

11.3.1. Add approximately 5 mL of concentrated sulfuric acid (Section 7.1.2) to the sample contained in the labeled test tube in Section 11.2.7 and agitate.

11.3.2. Bubble the sample through the acid layer by means of a nine-inch glass pipet with bulb.

11.3.3. Allow the layers to separate. The use of a centrifuge is allowed.

11.3.4. Remove the acid (bottom) layer.

11.3.5. Repeat the procedures in Section 11.3.1 through 11.3.3 until the acid layer remains clear.

11.3.6. Add 5 mL of 5% Sodium Chloride, NaCl, (Section 7.1.4) and bubble the sample through, and then remove the sodium chloride layer.

11.3.7. Add 5 mL of potassium hydroxide, KOH, (Section 7.1.1). Bubble the sample through, and then remove the KOH layer. Make sure not to leave the sample on the KOH layer for too long, in order to prevent degradation of the sample.

11.3.8. Repeat the Sodium chloride wash in Section 11.3.6.

11.3.9. Add sodium sulfate (Section 7.2.1) to dry the sample. Add until the sodium sulfate is free flowing.

11.3.10. Repeat the above procedure for the Method Blank and the LCS.

11.3.11. The dried sample and quality control aliquots (Method Blank and LCS) are then ready for carbon column clean-up.

11.4. Carbon Column Clean-up of Extracted Samples

11.4.1. Submit the dried extract (Section 11.3.11) for further column clean-up. See Table 2 attached for the column conditions and collection of cleaned extract as well as the addition of the recovery standards.

11.4.2. As the sample is injected into the column the "waste loop" is placed into the test tube and the "Sample Collect" is placed into a previously cleaned 250 mL Erlenmeyer flask.

11.4.3. Roto-vap solvent exchange the Dioxins/Furans using the Buchi Rotavapor R-114 (Section 6.19.1) into hexane, then reduced to approximately 2- 3 mL, and transferred to a concentrator tube.

11.4.4. Add 10 uL of the Recovery Standard Solution (Section 7.5.3) and 20 uL of nonane.

11.4.5. The samples are further reduced with nonane to a final volume of 20 uL and transferred to a 1.5 mL labeled autosampler vial with a 50 µL liner.

11.5. Soil/Sediment Extraction

11.5.1. After the percent dry weight is determined in Section 10.7, aliquot 10 g of the sample based on its dry weight into a cleaned and dry 250-mL beaker.

11.5.2. Add 10 g of sodium sulfate (Section 7.2.1) to the beaker and mix with a clean and dry stainless steel spatula. This is done to absorb any moisture in the sample. Additional sodium sulfate may be used until the sample has no clumps and appears free-flowing.

11.5.3. Add 100 g of pre-extracted play sand (Section 7.7.2) to the sample, stirring until the sample has no clumps and appears free-flowing.

11.5.4. In a pre-extracted thimble, place 5 g of pre-extracted silica gel. Place the play sand (Section 7.5.2) containing the sample and sodium sulfate on top of the silica gel. Be careful not to disturb the silica gel layer.

11.5.5. The thimble is then placed into a Soxhlet Dean/Stark apparatus (Section 6.10).

11.5.6. The soxhlet is attached to a 500 mL flat bottom flask with approximately 300-250 mL toluene.

11.5.7. Spike the sample with 40 uL of the 100 ng/ml internal standard working solution (Section 7.5.1) and 40 uL of the 100 ng/mL surrogate standard working solution (Section 7.5.2).

11.5.7.1. This results in a 4.0 ng/total spike addition.

11.5.8. Add 2- 25 mL of toluene to the soxhlet, set the reflux rate to five times per hour, and extract for eighteen hours.

11.5.9. After the eighteen hour extraction, the thimble is allowed to drain into the soxhlet.

11.5.10. The soxhlet is then drained into the flat bottom flask, with a rinse of toluene.

11.5.11. The extraction flask is then filled with 300-350 mL methylene chloride. Twenty to 25 mL of methylene chloride is added to the thimble in the soxhlet.

11.5.12. The sample is then extracted with methylene chloride for approximately eighteen hours.

11.5.13. The toluene and methylene chloride fractions are then combined by rotary evaporation and reduced.

11.5.14. The sample in the flat bottom flask is then transferred to a 500 mL round bottom flask through a funnel fitted with a filter two-thirds filled with sodium sulfate (Section 7.2.1).

11.5.15. The sample in the 500 mL round bottom flask is solvent exchanged into hexane using the Buchi Rotavapor R-114 (Section 6.19.1) and bring the sample to a final volume of 5 mL with hexane.

11.5.16. Place the extract into a 16 x 25 mm test tube (Section 6.22).

11.5.17. Add 40 ul of the 100ng/mL Alternate Standard Solution (Section 7.5.3).

11.5.17.1. This results in a 4.0 ng/total spike addition.

11.5.18. The sample is now ready for the clean-up procedure.

11.6. Soil/Sediment clean-up procedure.

11.6.1. The clean-up procedure for soil/sediment samples is the same as the clean procedure for aqueous samples discussed in Section 11.3 (Acid/Base Clean-up) and Section 11.4 (Column Clean-Up).

11.6.2. See Table 4 for the Fractionization Cleanup Procedure.

11.7. Extraction of Tissue Samples by Soxhlet Extraction.

11.7.1. Add 30-40 g of powdered anhydrous sulfate (Section 7.2.1) to each of the beakers (Section 10.13.1.2) and mix thoroughly. Cover the beakers with aluminum foil and allow to equalibrate for 12-24 hours. Remix prior to extraction to prevent clumping.

11.7.2. Assemble and pre-extract the Soxhlet apparatus per Sections 6.10.1 through 6.10.6. except use the methylene chloride; hexane (1:1) mixture for the pre-extraction and rinsing and omit the quartz sand. The Dean-Stark moisture trap may also be omitted, if desired.

11.7.3. Reassemble the pre-extracted Soxhlet apparatus and add a fresh charge of methylene chloride; hexane to the reflux flask.

11.7.4. Transfer the sample/sodium sulfate mixture (Section 11.7.1) to the Soxhlet thimble, and install the thimble in the Soxhlet apparatus.

11.7.5. Rinse the beaker with several portions of solvent mixture and add to the thimble. Fill the thimble/receiver with solvent. Extract for 18-24 hours.

11.7.6. After extraction, cool and disassemble the apparatus.

11.7.7. Quantitatively transfer the extract to the Buchi Rotavapor-114 macro-concentration device (Section 6.19.1) and concentrate to dryness. Set aside the concentration apparatus for re-use.

11.7.8. Complete the removal of the solvent using the nitrogen blowdown apparatus (Section 6.20), and a water bath temperature of 60^0C. Weigh the receiver, record the weight, and return the receiver to the blowdown apparatus, concentrating the residue until a constant weight is obtained.

11.7.9. Percent lipid determination- The lipid content is determined by extraction of tissue with the same solvent system (methylene chloride: hexane) that was used in EPA's National Dioxin Study so that lipid contents are consistent with that study.

11.7.9.1. Re-dissolve the residue in the receiver in hexane and spike with 1.0 mL of the cleanup standard (Section 7.5.3) into the solution.

11.7.9.2. Transfer the residue/hexane to the anthropogenic isolation column (Section 11.8.1) or bottle for the acidified silica gel batch cleanup (Section 11.8.2.), retaining the boiling chips in the concentration apparatus. Use several rinses to assure that all material is transferred. If necessary, sonicate or heat the receiver slightly to assure that all material is re-dissolved. Allow the receiver to dry. Weigh the receiver and boiling chips.

11.7.9.3. Calculate the lipid content to the nearest three significant figures as follows:

$$\text{Percent lipid} = \frac{\text{weight of residue (g)}}{\text{weight of tissue (g)}} \times 100$$

11.7.9.4. It is not necessary to determine the lipid content of the blank, IPR, or OPR aliquots.

11.7.10. HCl digestion/extraction and concentration

11.7.10.1. Add 200 mL of 6 N HCl and 200 mL of methylene chloride: hexane (1:1) to the sample, method blank, and Lab Spike.

11.7.10.2. Cap and shake each bottle one to three times. Loosen the cap in a hood to vent excess pressure. Shake each bottle for 10-30 seconds and vent.

11.7.10.3. Tightly cap and place on shaker. Adjust the shaker action and speed so that the acid, solvent, and tissue are in constant motion. However, take care to avoid such violent action that the bottle may be dislodged from the shaker. Shake for 12-24 hours.

11.7.10.4. After digestion, remove the bottles from the shaker. Allow the bottles to stand so that the solvent and acid layers separate.

11.7.10.5. Decant the solvent through a glass funnel with glass-fiber filter (Sections 6.12.2. through 6.12.3.) containing approximately 10 g of granular anhydrous sodium sulfate (Section 7.2.1) into a macro-concentration apparatus (Section 6.19.1). Rinse the contents of the bottle with two 25 mL portions of hexane and pour through the sodium sulfate into the apparatus.

11.7.10.6. Concentrate the solvent to near dryness by macro-concentration using the Roto-Vap procedure (Section 11.2).

11.7.10.7. Complete the removal of the solvent using the nitrogen blowdown apparatus (Section 6.20) an d a water bath temperature of 60 degrees C. Weigh the receiver, record the weight, and return the receiver to the blowdown apparatus, concentrating the residue to until a constant weight is obtained.

11.7.10.8. Percent lipid determination-The lipid content is determined in the same solvent system {(methylene chloride: hexane (1:1)} that was used in the EPA's National Dioxin Study so that the lipid contents are consistent with that study.

11.7.10.8.1. Re-dissolve the residue in the receiver in hexane and spike 1.0 mL of the alternate recovery standard (Section 7.5.3) into the solution.

11.7.10.8.2. Transfer the residue/hexane to the narrow-mouth 100-200 mL bottle retaining the boiling chips in the receiver. Use several rinses to assure that all material is transferred, to a maximum hexane volume of approximately 70 mL. Allow the receiver to dry, weigh the receiver and boiling chips.

11.7.10.8.3. Calculate the percent lipid per Section 11.7.9.3. It is not necessary to determine the lipid content of the blank, IPR, and OPR aliquots.

11.8. Clean-up of Tissue Lipids Extracts-Lipids are removed from the Soxhlet extract using either the anthropogenic isolation column (Section 11.8.1) or acidified silica gel (Section 11.8.2), or removed from the HCl digested extract using sulfuric acid and base back-extraction (Section 11.8.3).

11.8.1. Anthropogenic isolation column-Used for removal of lipids from the Soxhlet/SDS extraction (Section 11.7).

11.8.1.1. Prepare the column as given in Section 7.6.4.

11.8.1.2. Pre-elute the column with 100 mL of hexane. Drain the hexane layer to the top of the column, but do not expose the sodium sulfate.

11.8.1.3. Load the sample and rinses onto the column by draining each portion to the top of the bed. Elute the CDDs/CDFs from the column into the apparatus used for concentration (Section 11.2) using 200 mL of hexane.

11.8.1.4. Concentrate the cleanup extract (Section 11.7) to constant weight. If more than 500 mg of material remains, repeat the cleanup using a fresh anthropogenic isolation column.

11.8.1.5. Re-dissolve the extract in a solvent suitable for the additional cleanups to be used.

11.8.1.6. Add 40 uL of the Alternate Recovery Standard Spiking Solution (Section 7.5.3.1) into the residue/solvent.

11.8.1.7. Additional cleanups of either Florisil (Section 11.9) or Carbon (Section 11.4) are recommended as minimum additional cleanup steps.

11.8.1.8. Following cleanup, concentrate the extract to 10 uL.

11.8.2. Acidified silica gel-Procedure alternate to the anthropogenic isolation column (Section 7.6.4) that is used for removal of lipids from the Soxhlet extraction (Section 11.7).

11.8.2.1. Adjust the volume of hexane in the bottle to approximately 200 mL.

11.8.2.2. Add 40 uL of the Alternate Recovery Standard Spiking Solution (Section 7.5.3.1) into the residue/solvent.

11.8.2.3. Drop the stirring bar into the bottle, place the bottle on the stirring plate, and begin stirring.

11.8.2.4. Add 30-100 g of acid silica gel (Section 7.6.1.2) to the bottle while stirring, keeping the silica gel in motion. Stir for two to three hours.

11.8.2.5. After stirring, pour the extract through approximately 10 g of granular anhydrous sodium sulfate (Section 7.2.1) contained in a funnel with glass-fiber filter into a macro concentration device (Section 11.2). Rinse the bottle and sodium sulfate with hexane to complete the transfer.

11.8.2.6. Concentrate the extract per Sections 11.2.1 through 11.2.7 and clean up the extract using the procedures in Sections 11.3.1 through 11.3.11.

11.8.3. Sulfuric acid and base back-extraction-Used with HCl digested extracts.

11.8.3.1. Add 10 mL of concentrated sulfuric acid to the bottle. Immediately cap and shake one to three times. Loosen cap in a hood to vent excess pressure. Cap and shake the bottle so that the residue/solvent is exposed to the acid for a total time of approximately 45

seconds.

11.8.3.2. Decant the hexane into a 250 mL separatory funnel making sure that no acid is transferred. Complete the quantitative transfer with several hexane rinses.

11.8.3.3. Back extract the solvent/residue with 50 mL of potassium hydroxide solution followed by two organic free reagent water rinses.

11.8.3.4. Drain the extract through a filter funnel containing approximately 10 g of anhydrous sodium sulfate in a glass fiber filter into the Roto-Vap macro concentration device (Section 6.19.1).

11.8.3.5. Concentrate the cleaned up extract to a volume suitable for the additional cleanups given in Sections 7.4.

11.8.3.6. Following cleanup, concentrate the extract to 10 uL.

11.9. *Florisil Cleanup*

11.9.1. Pre-elute the activated Florisil column (Section 7.6.5) with 10 mL of methylene chloride followed by 10 mL of hexane: ethylene chloride (98:2 v/ v) and discard the solvents.

11.9.2. When the solvent is within 1 mm of the packing, apply the sample extract) in hexane to the column, Rinse the sample container twice with 1 mL portions of hexane and apply to the column.

11.9.3. Elute the interfering compounds with 20 mL of hexane: ethylene chloride (98:2) and discard the eluate.

11.9.4. Elute the CDDs/CDFs with 36 mL of methylene chloride and collect the eluate. Concentrate the eluate. Concentrate the eluate via macro concentration for further cleanup.

12.0 EXTRACTION AND CONCENTRATION PROCEDURES FOR ASH SAMPLES

12.1. Weigh out 10 g of sample to the second decimal point and place sample into the extraction bottle.

12.2. Add 150 mL of 1M HCl to the sample. Cap the extraction bottle tightly, and shake with the Ann Arbor Shaker Table (6.11.3) for three hours @ room temperature.

12.3. Rinse a glass fiber filter with toluene, and filter the sample through the filter, placed on a Buchner funnel, into a 1 L flask.

12.4. Rinse the filter cake with 500 mL of organic free reagent water, dry the filter cake at room temperature overnight in a dessicator.

12.5. Scrap the filter cake into a clean beaker and set aside for later use.

12.6. To the sample in the beaker, add 10 g of anhydrous sodium sulfate (Section 7.2.1), mix thoroughly, cover the beaker and let sit for approximately one hour.

12.7. Mix again, and let sit for approximately one hour.

12.8. Place the filter paper from Section 12.5 and the sample (mixed with sodium sulfate) into an extraction thimble.

12.9. Place the extraction thimble into the Soxhlet attached to a 500 mL flat bottom flask filled with 300-350 mL of toluene.

12.10. Add 40 uL of the Internal Standard Working Solution (Section 7.5.1).

12.11. Extract the sample for 16-18 hours.

12.12. After extraction, suspend the thimble to drain the toluene.

12.13. Remove the Soxhlet, dispose of the thimble, and filter toluene through a glass funnel filled two-thirds full with anhydrous sodium sulfate (Section 7.2.1) into a 500 mL round bottom flask.

12.14. Roto-vap the solvent exchange into hexane.

12.15. Reduce to approximately 2-3 mL, transfer to a 40 mL VOA vial, and bring to a final volume of 10 mL with hexane.

12.16. Add 40 uL of the Alternate Recovery Standard Spiking Solution (Section 7.5.3.1).

12.17. The extract is now ready for the Acid/Base Cleanup procedure in Section 11.3.

12.18. After the Acid/Base cleanup procedure is performed, the extract is ready for the Carbon Column Cleanup in Section 11.4.

12.19. Roto-vap solvent exchange into hexane. Reduce to approximately 2-3 mL and transfer to a concentrator tube.

12.19.1. Add 10 uL of the Alternate Recovery Standard Solution (Section 7.5.3) and 50 uL of nonane (7.3.4).

12.19.2. Reduce to a final volume of 50 uL nonane, and place sample in a 2-mL autosampler vial with liner.

13.0 DEFINITIONS AND ACRONYMS

13.1. Analyte-A CDD or CDF tested for this method.

13.2. CDD-Chlorinated Dibenzo-*p*-ioxin-The isomers and congeners of tetra-through octa chlordibenzo-*p*-dioxin.

13.3. CDF-Chlorinated Dibenzofuran-The isomers and congeners of tetra-through chlorodibenzofuran.

13.4. Field Blank-An aliquot of reagent water or other reference matriz that is placed in a sample container in the laboratory or the field, and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation, and all analytical procedures. The purpose of this field blank is to determine if the field or sample transporting procedures and environments.

13.5. GC-Gas chromatograph or gas chromatography.

13.6. HRGC- High Resolution GC.

13.7. HRMS-High Resolution MS.

13.8. Laboratory Control Sample (LCS) - see ongoing precision and recovery standard (OPR).

13.9. Laboratory Reagent Blank-See method blank.

13.10. May- This action, activity, or procedural step is neither required nor prohibited.

13.11. Method Blank- An aliquot of reagent water that is treated exactly like as a sample including exposure to glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with samples. The method blank is used to determine if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

13.12. OPR-Ongoing precision and recovery standard (OPR); a laboratory blank spiked with known quantities of analytes. The OPR is analyzed exactly like a sample. Its purpose is to assure that the results produced by the laboratory remain within the limits specified in this method for precision and recovery.

13.13. PAR –Precision and recovery standard; secondary standard that is diluted and spiked to form the IPR and OPR.

13.14. Preparation Blank-See method blank.

13.15. Organic Free Reagent Water- Water demonstrated to be free from the analytes of interest and potentially interfering substances at the method detection limit for the analyte(s) of interest.

13.16. SDS-Soxhlet/Dean Stark extractor; an extractor device applied to the extraction of solid and semi-solid materials.

13.17. Should-This action, activity, or procedural step is suggested but not required.

13.18. Stock Solution-A solution containing an analyte that is prepared using a reference material traceable to EPA, the National Institute of Science and Technology (NIST), or a source that will

attest to the purity and authenticity of the reference material.

13.19. TCDD-Tetrachlorodibenzo-*p*-dioxin.

13.20. TCDF-Tetrachlorodibenzofuran.

14.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

14.1. All appropriate pollution prevention procedures and waste management procedures as described in the DAT Waste Management Plan must be utilized by all personnel involved in the preparation of the samples.

15.0 REFERENCES

15.1. Polychlorinated Dibenzodioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by High-Resolution Gas Chromatography/High Resolution Mass Spectrometry (HRGC/HRMS), EPA Method 8290, Revision 1, January 1998.

15.2. DAT Quality Assurance Manual, Revision 4.3, May 2007.

15.3. DAT Waste Management Plan, September 2001.

TABLE 1:
COMPOSITION OF THE SAMPLE FORTIFICATION AND RECOVERY STANDARD SOLUTIONS[a]

Analyte	Sample Fortification Solution Concentration (pg/μL)	Recovery Standard Solution Concentration (pg/μL)
$^{13}C_{12}$-2,3,7,8-TCDD	10	--
$^{13}C_{12}$-2,3,7,8-TCDF	10	--
$^{13}C_{12}$-1,2,3,4-TCDD	--	500
$^{13}C_{12}$-1,2,3,7,8-PeCDD	10	--
$^{13}C_{12}$-1,2,3,7,8-PeCDF	10	--
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	10	--
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	10	--
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	--	500
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	10	--
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	10	--
$^{13}C_{12}$-OCDD	20	--

[a] These solutions should be made freshly every day in nonane or other appropriate solvent because of the possibility of adsorptive losses to glassware. If these solutions are to be kept for more than one day, then the sample fortification solution concentrations should be increased ten fold, and the recovery standard solution concentrations should be doubled. Corresponding adjustments of the spiking volumes must then be made.

TABLE 4
FRACTIONIZATION CLEANUP

Equivalents:

L - load- load position
I - inject position
L-I - switch from load to inject
F - forward flow
R - reverse flow

Solvent system:
1-Hexane
2- 1:1 Cyclohexane: Methylene Chloride
3- 75:20:5 Methylene Chloride : Methanol: Toluene
4- Toluene

Column packing: PX-21/Silica 5.5% w/w

Conditioning the column

Step	Injection valve	Flow direction	Valve Solvent system	Valve Run time minutes
1.	L	F	2	4:00
2.	I	F	1	4:00

Sample cleanup

Step	Injection valve	Flow direction	Valve Solvent system	Valve Run time minutes
3.	L-I	F	I	7:00 collect loop waste
4.	L-I	F	I	5:00
5.	I	F	2	5:00
6.	L	F	3	3:00
7.	L	R	4	10:00 collect Sample

Repeat steps 1 and 2 and inject next sample.

8. Solvent exchange cleaned extract to hexane using an 80 mL pear flask bring to a nominal 2.0 -3.0 mL. Place into a blown concentrator tube and add recovery standard and 50 uL Nonane.

9. Bring to a final volume of 50 uL nonane.

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Date 01 / 29 / 2008

SOP - 092

STANDARD OPERATING PROCEDURE (SOP) FOR THE DETERMINATION OF PCDDs AND PCDFs BY HRGC/HRMS METHOD 8290

Reviewed by: _____ Date: 1/29/08
Quality Assurance Officer

Approved by: _____ Date: 1/29/08
President, DAT Inc.

DATA ANALYSIS TECHNOLOGIES, INC (DAT)
7715 CORPORATE BLVD.
PLAIN CITY, OH 43064

Date:	Reviewed by:	Date	Reviewed by:

PROCEDURE

1.0 SCOPE:

1.1. This method provides procedures for the analysis of polychlorinated dibenzo-p-dioxins (tetra- through octachlorinated homologues; PCDDs), and polychlorinated dibenzofurans (tetra- through octachlorinated homologues; PCDFs) from extracts of samples prepared according to SW 846 Method 8290. Air samples should be prepared as described in Method 23, DAT SOP 115.

1.2. Prior to analysis the laboratory must demonstrate proficiency using the preparative method DAT SOP 155 and the analysis using DAT SOP 92.

1.3. Sample specific Estimated Detection Limits (EDLs) are calculated for each 2,3,7,8 and each non 2,3,7,8- substituted congener according to Method 8290.

2.0 APPLICATION:

2.1. This method uses high resolution gas chromatography and high resolution mass spectrometry (HRGC/HRMS) on purified sample extracts. Samples with concentrations of specific congeneric analytes that are greater than ten times the upper MCLs (method calibration limits) must be analyzed by Method 8280 or other method.

3.0 DETECTION LIMITS:

3.1. Detection limits differ depending on the individual matrix and its complexity. However a 1L water sample will have a lower DL of 0.01 ppt for 2,3,7,8-TCDD. See Table 9 for a list of method calibration limits.

4.0 SUMMARY OF METHOD:

4.1. This procedure uses matrix specific extraction, analyte specific cleanup and HRGC/HRMS analysis techniques.

4.2. A specified amount (See Table 9) of a sample of a specific matrix is spiked with a solution containing specified amounts of nine isotopically ($^{13}C_{12}$) labeled PCDDs/PCDFs. The sample is then extracted according to a matrix specific extraction procedure.

4.3. The extracts are submitted to an acid-base washing treatment and dried. Following a solvent exchange step, the extracts are cleaned up by column chromatography on alumina, silica gel, and activated carbon.

4.4. The preparation of the final extract for HRGC/HRMS analysis is accomplished by adding 10 to 50 µl of a nonane solution containing 50 pg/µl of the recovery standards.

4.5. Two µl of the concentrated extract are injected into an HRGC/HRMS system capable of performing selected ion monitoring at resolving powers of at least 10,000 (10 percent valley definition).

4.6. The identification of OCDD and nine of the fifteen substituted congeners for which a 13C-labeled standard is available in the sample fortification and recovery standard solutions is based on their elution at their exact retention time and the simultaneous detection of the two most abundant ions in the molecular ion region. Identification is also based on a comparison of the ratios of the integrated ion abundance of the molecular ion species to their theoretical abundance ratios.

4.7. Quantitation of the individual congeners, total PCDDs and total PCDFs is achieved in conjunction with the establishment of a five point calibration curve for each homologue, during which each calibration solution is analyzed once.

5.0 SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE:

5.1. Shipping and storage temperatures and holding times must be documented for review.

5.2. Grab and composite samples should be collected in glass containers. Whole fish should be ground or blended to provide a homogeneous sample.

5.3. Holding times are matrix specific and are as noted:

5.4. Aqueous, soil and air samples must be shipped and stored at less than 6°C. Fish and tissue samples must be shipped at less than 4°C and stored in deep freeze at less than -10°C.

5.5. Sodium thiosulfate must be added to aqueous samples before extraction if it is determined that residual chlorine is present. This can be done in the field or in the laboratory.

6.0 INTERFERENCES:

6.1. Solvents, reagents, glassware and other sample processing hardware may yield discrete artifacts or elevated baselines that may cause misinterpretation of the chromatographic data. A method blank must be analyzed to prove that this hardware is free from contamination. Analysts should avoid using PVC gloves.

6.2. Interferants coextracted from the sample may be found at concentrations several orders of magnitude higher than the analytes of interest. The clean-up technique described in this SOP must be used to reduce interference.

7.0 SAFETY:

7.1. All personnel are required to comply with DAT's Health and Safety Documents as described in the

Quality Assurance Manual.

7.2. Because of the extreme toxicity of many of these compounds, the analyst must take the necessary precautions to prevent exposure to materials known or believed to contain PCDDs or PCDFs.

8.0 EQUIPMENT AND SUPPLIES:

8.1. Reference EPA Method 8290 for a complete list of all equipment and supplies necessary for the extraction and clean-up of samples for the analysis of PCDDs and PCDFs.

8.2. **HRGC/HRMS/DS:** (High-Resolution Gas Chromatograph/High-Resolution Mass Spectrometer/Data System). The GC is equipped for temperature programming and capillary columns.

8.2.1. GC Injection Port - The GC injection port is designed for capillary columns. 2 μL injection volumes are used unless otherwise noted.

8.2.2. Gas Chromatograph/Mass Spectrometer (GC/MS) Interface - The GC/MS interface components can withstand 350°C. The interface has been designed so that the separation of 2,3,7,8-TCDD from the other TCDD isomers achieved in the gas chromatographic column is not appreciably degraded. The GC column is fitted directly into the mass spectrometer ion source without being exposed to the ionizing electron beam. Graphite ferrules should be avoided in the injection port because they may adsorb the PCDDs and PCDFs. Vespel®, or equivalent, ferrules are recommended.

8.2.3. Mass Spectrometer - The static resolving power of the instrument must be maintained at a minimum of 10,000 (10 percent valley).

8.2.3.1. The mass spectrometer must be operated in a selected ion monitoring (SIM) mode with a total cycle time (including the voltage reset time) of one second or less. At a minimum, the ions listed in Table 6 for each of the five SIM descriptors must be monitored. Note that with the exception of the last descriptor (OCDD/OCDF), all descriptors contain 10 ions. The selection (Table 6) of the molecular ions M and M+2 for 13C-HxCDF and 13C-HpCDF rather than M+2 and M+4 (for consistency) was made to eliminate, even under high-resolution mass spectrometric conditions, interferences occurring in these two ion channels for samples containing high levels of native HxCDDs and HpCDDs. It is important to maintain the same set of ions for both calibration and sample extract analyses. The selection of the lock-mass ion is left to the performing laboratory.

NOTE: **At the option of the analyst, the tetra- and pentachlorinated dioxins and furans can be combined into a single descriptor.**

8.2.3.2. The recommended mass spectrometer running conditions are based on the groups of monitored ions shown in Table 6. By using a PFK molecular leak, tune the instrument to meet the minimum required resolving power of 10,000 (10 percent

valley) at m/z 304.9824 (PFK) or any other reference signal close to m/z 303.9016 (from TCDF). By using peak matching conditions and the aforementioned PFK reference peak, verify that the exact mass of m/z 380.9760 (PFK) is within 5 ppm of the required value. Note that the selection of the low- and high-mass ions must be such that they provide the largest voltage jump performed in any of the five mass descriptions (Table 6).

8.2.4. Data System - A dedicated data system is employed to control the rapid multiple-ion monitoring process and to acquire the data. Quantitation data (peak areas or peak heights) and SIM traces (displays of intensities of each ion signal being monitored including the lock-mass ion as a function of time) must be acquired during the analyses and stored. Quantitation may be reported based upon computer generated peak areas or upon measured peak heights (chart recording). The data system is set to acquire data as low as 10 ions in a single scan. The data system is set to switch to different sets of ions (descriptors) at specified times during an HRGC/HRMS acquisition. The data system provides hard copies of individual ion chromatograms for selected gas chromatographic time intervals. It also acquires mass spectral peak profiles and provides hard copies of peak profiles to demonstrate the required resolving power. Measurements of noise on the base line are performed using the hard copies of individual ion chromatograms provided by the data system.

NOTE: *The detector ADC zero setting must be set to allow peak-to-peak measurement of the noise on the base line of every monitored channel and allow for good estimation of the instrument resolving power.*

8.2.5. GC Columns

a. In order to have an isomer specific determination for 2,3,7,8-TCDD and to allow the detection of OCDD/OCDF within a reasonable time interval in one HRGC/HRMS analysis, the 60 m DB-5 fused silica capillary column is used. Minimum acceptance criteria must be demonstrated and documented. At the beginning of each 12 hour period (after mass resolution and GC resolution is demonstrated) during which sample extracts or continuing calibration solutions will be analyzed, column operating conditions must be attained for the required separation on the column to be used for samples. Operating conditions known to produce acceptable results with the recommended column are shown in Table 2.

b. 2,3,7,8-TCDF isomer must be confirmed on a DB-Dioxin 60m x 0.25mm x 0.15mm bonded phase fused silica capillary column (which resolves the 2378-TCDF isomer), when 2,3,7,8-TCDF is detected on the DB-5 column at a level greater than or equal to the target detection limit. An equivalent column may be substituted.

9.0 REAGENTS AND STANDARDS:

9.1. Reagents and standards used for sample extraction and cleanup are addressed in DAT SOP

155.

9.2. Calibration Solutions (Table 1) - Five nonane solutions containing 17 unlabeled PCDDs and PCDFs and 18 $^{13}C_{12}$ -labeled PCDDs and PCDFs at known concentrations are used to calibrate the instrument. The concentration ranges are homologue dependent, with the lowest values for the tetrachlorinated dioxin and furan (0.5 pg/μL) and the highest values for the octachlorinated congeners (1000 pg/μL).

9.3. Recovery Standard Solution - This nonane solution contains two recovery standards, $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDD, at a nominal concentration of 100 pg/μL per compound. 20 μL of this solution is spiked into each sample extract before the HRGC/HRMS analysis.

9.4. GC Column Performance/Retention Window Check Solution (RTCHK) - This solution contains the first and last eluting isomers for each homologous series from tetra- through heptachlorinated congeners. The solution also contains a series of other TCDD isomers for the purpose of documenting the chromatographic resolution.

9.5. All the components and concentrations of each calibration standard, recovery standard, internal standard, and matrix spike solution is verified prior to use for sample analyses. Testing consists of back to back analysis of the "test" solution and a "control" solution. Each component of the test solution must be within 80-120% of the true concentration when calculated versus the control standard.

9.6. Standards are stored in 1/2 dram amber glass vials at room temperature.

10.0 QUALITY CONTROL, ACCEPTANCE CRITERIA AND CORRECTIVE ACTIONS:

10.1. System Performance Criteria:

At the beginning of each 12-hour period during which samples are to be analyzed, an aliquot of the 1) GC column performance check solution and 2) high resolution concentration calibration solution No.3 (See Table 1) shall be analyzed. A mass resolution check is also performed using an appropriate reference compound such as PFK. A continuing calibration and mass resolution check must be performed at the end of a 12 hour period or at the end of the analysis batch, however, the performance check solution must be analyzed only once, at the beginning of the 12 hour period.

10.1.1. GC Column Performance

Inject 2 μl of the column performance check solution and acquire selected ion monitoring data. The chromatographic separation between 2,3,7,8-TCDD and the peaks representing any other unlabeled TCDD isomers must be resolved with a valley of ≤ 25%, where:

Valley percent = (x/y) * (100)

x = measured as in Figure 4 from the 2,3,7,8-closest TCDD eluting isomer
y = the peak height of 2,3,7,8-TCDD.

10.1.2. Mass spectrometer Performance

The mass spectrometer must be operated in the electron ionization mode. A static resolving power of at least 10000 must be demonstrated at appropriate masses before any analysis is performed. Static resolving power checks must be performed at the beginning and at the end of each 12-hour period of operation. Corrective action must be implemented whenever the resolving power does not meet the requirement.

A mass drift correction must be performed. Select a lock-mass ion from the reference compound (PFK). The selection of the lock-mass ion is dependent on the masses of the ions monitored within each descriptor. The level of the PFK metered into the ion chamber during analysis should be adjusted so that the amplitude of the most intense selected lock-mass ion signal does not exceed 10 percent of the full scale deflection for a given set of detector parameters.

The instrument resolving power must be documented.

Results of at least one analysis of the GC column performance check solution and of two mass resolution and continuing calibration checks must be reported with the sample data collected during a 12-hour period. Deviations from criteria specified for the GC performance check or for the mass resolution check invalidate all positive sample data collected between analyses of the performance check solution and the extracts from those positive samples shall be reanalyzed.

10.2. Quality Control Samples

10.2.1. Method Blank:

An HRGC/HRMS method blank run is required between a calibration run and the first sample run. The sample method blank extract may by analyzed more than once if the number of samples within a batch requires more than 12 hours of analyses.

- Unlabelled analyte concentrations in the method blank must be 1) ≤1 ppt for TCDD and TCDF, 2) ≤ 5 ppt for penta-heptaCDD and CDF and 3) ≤ 10 ppt for OCDD and OCDF.
- Internal standard recoveries in the blank must be within 1) 40-135% for tetra-octa, and have a S/N ≥ 10:1.
- Detection limits obtained for each blank cannot exceed 1) ≤1 ppt for TCDD and TCDF, 2) ≤ 5 ppt for penta-heptaCDD and CDF and 3) ≤ 10 ppt for OCDD and OCDF.

If method blank criteria cannot be met, the sample set must be reextracted and reanalyzed.

10.2.2. Matrix Spike and Matrix Spike Duplicate

A matrix spike must be analyzed with the sample set. The results obtained from the MS and MSD samples should agree within 20% RPD. Results reported outside the criteria

must be flagged and explained.

10.2.3. Internal Standard Recoveries

For each sample and QC sample, calculate the percent recovery of the internal standard. Internal standard recoveries must be within 1) 40-135% for tetra-octa and have a S/N ≥ 10:1. If internal standard recovery criteria cannot be met, the sample must be reanalyzed.

10.2.4. In each batch of samples, locate the sample specified for duplicate analysis, and analyze a second 10g soil or sediment sample, or an appropriate amount of the type of matrix under consideration.

10.2.4.1. The results of the laboratory duplicates (percent recovery and concentrations of 2, 3, 7, 8-substituted PCDD/ PCDF compounds) should agree within 25 percent relative difference (difference expressed as percentage of the mean). Report all results.

11.0 CALIBRATION AND STANDARDIZATION:

11.1. Initial Calibration (ICAL) - Initial calibration of the instrument is required before any samples are analyzed for PCDDs and PCDFs. Initial calibration is also required if any continuing calibration does not meet the required criteria.

11.1.1. All five calibration solutions listed in Table 1 must be used for the initial calibration.

11.1.2. Tune the instrument with Perfluorokerosene (PFK) to achieve a static resolving power of at least 10,000 (10% valley).

11.1.2.1. Documentation of the mass spectrometer resolving power is accomplished by recording the peak profile of the high-mass reference signal (typically m/z 330.9792) obtained during a peak examination experiment by using the low-mass PFK ion at m/z 292.9825 (or lower in mass) as a reference.

11.1.2.2. The format of the peak profile representation allows manual determination of the peak resolution. The peak width (at 5% peak height) of the high-mass reference ion must not exceed 100 ppm (resolving power: 10,000) for samples analyzed under the 8290 protocol. Peak width is determined by triangulation with no more than 10% allowance for sampling error. Instrumental ion transmission and resolution will be checked, adjusted and documented in case the resolution is below the minimum required 10,000 resolving power.

11.1.3. Inject 2 µL of the GC column performance check solution and acquire SIM mass spectral data as described earlier in Section 8.2.3.1. The total cycle time must not exceed 1 second.

11.1.4. Using the same GC and MS conditions that produced acceptable results for the column performance check solution (i.e. acquisition windows and TCDD resolution), analyze a 2

µL portion of each of the five calibration solutions with the following spectrometer operating parameters.

11.1.4.1. The ratio of integrated ion current for the ions appearing in Table 5 (homologous series quantitation ions) must be within the indicated control limits (set for each homologous series).

11.1.4.2. The ratio of integrated ion current for the ions belonging to the carbon-labeled internal, surrogate, alternate, and recovery standards must be within the control limits stipulated in Table 5.

NOTE: *Ion ratios for all 17 native analytes and all 18 carbon-labeled internal and recovery standards must be within the specified control limits simultaneously in one run for each of the five (5) calibration standard solutions. If the ion abundance ratios are outside the limits, corrective action must be taken and acceptable abundance ratios achieved before any samples may be analyzed.*

11.1.4.3. For each SICP and for each GC signal representing the elution of a target analyte the signal-to-noise ratio (S/N) must be better than or equal to 2.5.

11.1.4.4. Referring to Tables 6, 7, and 8, calculate the 17 relative response factors (RRF) for unlabeled target analytes relative to their appropriate internal standards, the nine RRFs for the labeled $^{13}C_{12}$ internal standards and the seven labeled surrogate and alternate standards relative to the two recovery standards according to the following formulae:

$$RRF\ (n) = \frac{A_x \times Q_{is}}{Q_x \times A_{is}}$$

$$RRF\ (m) = \frac{A_{is} \times Q_{rs}}{Q_{is} \times A_{rs}}$$

Where:

RRF (n)	=	Analyte RRF
RRF (m)	=	Standard RRF
A_x	=	sum of the integrated ion abundances of the quantitation ions (Table 3) for unlabeled PCDDs/PCDFs,
A_{is}	=	sum of the integrated ion abundances of the quantitation ions (Table 3) for the labeled internal standards,
A_{rs}	=	sum of the integrated ion abundances of the quantitation ions (Table 3) for the labeled recovery standards,
Q_{is}	=	quantity of the internal standard injected (pg),
Q_{rs}	=	quantity of the recovery standard injected (pg), and
Q_x	=	quantity of the unlabeled PCDD/PCDF analyte injected (pg).

The RRF (n) and RRF (m) are dimensionless quantities; the units used to express Q_{is}, Q_{rs} and Q_x must be the same.

11.1.4.5. Calculate the average RRF and their respective percent relative standard deviations (%RSD) for the five calibration solutions.

11.1.4.6. The relative response factors to be used for the determination of the concentration of total isomers in a homologous series are calculated as follows:

- For congeners that belong to a homologous series containing only one isomer (e.g., OCDD and OCDF) or only one 2,3,7,8-substituted isomer TCDD, PeCDD, HpCDD, and TCDF), the mean RRF used will be the same as the mean RRF determined in Section 11.1.4.7.

NOTE: *The calibration solutions do not contain $^{13}C_{12}$-OCDF as an internal standard. This is because a minimum resolving power of 12,000 is required to resolve the $[M+6]^+$ ion of $^{13}C_{12}$-OCDF from the $[M+2]^+$ ion of OCDD (and $[M+4]^+$ from $^{13}C_{12}$-OCDF with $[M]^+$ of OCDD). Therefore, the RRF for OCDF is calculated relative to $^{13}C_{12}$-OCDD.*

- For congeners that belong to a homologous series containing more than one 2,3,7,8-substituted isomer the mean RRF used for those homologous series will be the average of the mean RRFs calculated for all individual 2,3,7,8-substituted congeners.

NOTE: *HRGC/HRMS responses of all isomers in a homologous series that do not have the 2,3,7,8-substitution pattern are assumed to be the same as the responses of one or more of the 2,3,7,8-substituted isomer(s) in that homologous series.*

11.1.4.7. Relative response factors [RRF (m)] to be used for the determination of the percent recoveries for the internal surrogate and alternate standards are calculated as follows:

$$\text{RRF (m)} = \frac{A_{is} \times Q_{rs}}{Q_{is} \times A_{rs}}$$

$$\text{RRF (m)} = \frac{1}{5} \sum_{j=1}^{5} \text{RRF}$$

Where:

A_{is} = sum of the integrated ion abundances of the quantitation ions for a given standard,

A_{rs} = sum of the integrated ion abundances of the quantitation ions for the appropriate recovery standard,

Q_{rs}, Q_{is} = quantities of, respectively, the recovery standard (rs) and a particular standard injected (pg),

RRF = relative response factor of a particular standard relative to an appropriate recovery standard, as determined from one injection, and

RRF = calculated mean relative response factor of a particular labeled standard relative to an appropriate recovery standard, as determined from the five initial calibration injections.

11.2. Acceptance Criteria for Initial Calibration - The criteria listed below for acceptable calibration must be met before the analysis is performed.

11.2.1. The percent relative standard deviations for the mean response factors [RRF (m)] from the 17 unlabeled standards must not exceed ± 20 percent, and those for the labeled reference compounds must not exceed ± 30 percent.

11.2.2. The S/N ratio for the GC signals present in every SICP (including the ones for the labeled standards) must be ≤ 10.

11.2.3. The isotopic ratios (Table 5) must be within the specified control limits.

NOTE: *If the criterion for acceptable calibration listed in Section 11.2 is met, the analyte specific RRF can then be considered independent of the analyte quantity for the calibration concentration range. The mean RRFs will be used for all calculations until the continuing calibration criteria (Section 11.4) are no longer met. At such time, new mean RRFs will be calculated from a new set of injections of the calibration solutions.*

11.3. Continuing Calibration Check (CONCAL) - Continuing calibrations must be performed at the beginning of a 12-hour period after successful mass resolution and RTCHK. A continuing calibration is also required at the end of a 12-hour shift.

11.3.1. Inject 2 µL of the CONCAL solution HRCC-4 standard (Table 1). By using the same HRGC/HRMS conditions as used in Table 2, determine and document an acceptable calibration as provided in Section 11.4.

11.4. Acceptance Criteria for Continuing Calibration - The following criteria must be met before further analysis is performed.

11.4.1. The measured RRFs [for the unlabeled standards] obtained during the continuing calibration runs must be within ± 20 percent of the mean values established during the initial calibration (Section 11.1).

11.4.2. The measured RRFs [for the labeled standards] obtained during the routine calibration runs must be within ± 30 percent of the mean values established during the initial calibration (Section 11.1).

11.4.3. The ion-abundance ratios (Table 5) must be within the allowed control limits.

11.4.4. If either one of the criteria in Sections 11.4.1 and 11.4.2 is not satisfied, repeat one more time. If these criteria are still not satisfied, the entire continuing calibration process (Section 11.3) must be reviewed.

11.4.5. If an acceptable CONCAL cannot be achieved, the GC/MS system must be evaluated. If the instrument is determined fit to run, an acceptable initial calibration must be obtained (section 11.1 above) before any sample may be analyzed.

12.0 PROCEDURE:

12.1. Remove the sample extract (blown to dryness) or blank from storage. Add 10 µL of recovery standard and mix thoroughly

NOTE: A final volume of 20 µL or more should be used whenever possible. A 10 µL final volume is difficult to handle, and injection of 2 µL out of 10 µL leaves little sample for confirmations and repeat injections, and for archiving.

12.2. Inject a 2 µL aliquot of the extract into the GC, operated under the GC conditions that have produced acceptable CONCAL and RTCHK results.

12.3. Acquire SIM data using the same acquisition and mass spectrometer operating conditions previously used to determine the relative response factors. Ions characteristic for polychlorinated diphenyl ethers are included in the descriptors listed in Table 3.

NOTE: The acquisition period must at least encompass the PCDD/PCDF overall retention time window previously determined. Selected ion current profiles (SICP) for the lock-mass ions (one per mass descriptor) must also be recorded and included in the data package. These SICPs must be true representations of the evolution of the lock-mass ions amplitudes during the HRGC/HRMS run. The analyst may be required to monitor a PFK ion, not as a lock mass, but as a QC ion, in order to meet this requirement. It is recommended to examine the QC ion or lock-mass ion SICP for obvious basic sensitivity and stability changes of the instrument during the GC/MS run that could affect the measurements [Tondeur et al., 1984, 1987]. Report any discrepancies in the case narrative.

12.4. Identification Criteria - For a gas chromatographic peak to be identified as a PCDD or PCDF, it must meet all of the following criteria:

12.4.1. Retention Times

12.4.1.1. For 2,3,7,8-substituted congeners, which have an isotopically labeled internal or recovery standard present in the sample extract (this represents a total of 10 congeners including OCDD; Table 1), the retention time (RRT; at maximum peak height) of the sample components (i.e., the two ions used for quantitation purposes listed in Table 3), must be within -1 to +3 seconds of the isotopically labeled standard.

12.4.1.2. For 2,3,7,8-substituted compounds that do not have an isotopically labeled internal standard present in the sample extract (this represents a total of eight congeners;

Table 3), the retention time must fall within 0.005 retention time units of the relative retention times measured in the continuing calibration. Identification of OCDF is based on its retention time relative to $^{13}C_{12}$-OCDD as determined from the 12 hour continuing calibration results.

12.4.1.3. For non-2,3,7,8-substituted compounds (tetra through octa; totaling 119 congeners), the retention time must be within the corresponding homologous retention time windows established by analyzing the RTCHK solution.

12.4.1.4. The ion current responses for both ions used in quantitation (e.g., for TCDDs: m/z 319.8965 and 321.8936) must reach maximum simultaneously (± 2 seconds).

12.4.1.5. The ion current responses for both ions used for the labeled standards (e.g., for $^{13}C_{12}$-TCDD: m/z 331.9368 and m/z 333.9339) must reach maximum simultaneously (± 2 seconds).

NOTE: *The analyst is required to ensure that the acquisition windows are set to acquire all 119 congeners properly.*

12.4.2. Ion Abundance Ratios

12.4.2.1. The integrated ion current for the two ions used for quantitation purposes must have a ratio between the lower and upper limits established for the homologous series to which the peak is assigned (Table 5).

12.4.3. Signal-to-Noise Ratio

12.4.3.1. All ion current intensities must be ≥ 2.5 times noise level for positive identification of a PCDD/PCDF compound or a group of coeluting isomers.

12.4.4. Polychlorinated Diphenyl Ether Interferences

12.4.4.1. In addition to the above criteria, the identification of a GC peak as a PCDF can only be made if no signal having a S/N ≥ 2.5 is detected, at the same retention time (± 2 seconds), in the corresponding polychlorinated diphenyl ether (PCDPE, Table 3) channel or if the PCDPE signal is less than 10% of the PCDF signal.

13.0 CALCULATIONS:

13.1. For gas chromatographic peaks that have met specified criteria calculate the concentration of the PCDD or PCDF compounds using the formula:

$$Cx = \frac{A_x \times Q_{is}}{A_{is} \times W \times RRF\,(n)}$$

Where:

C_x = concentration of unlabeled PCDD/PCDF congeners (or group of coeluting

isomers within an homologous series) in pg/g,

A_x = sum of the integrated ion abundances of the quantitation ions (Table 3) for unlabeled PCDDs/PCDFs,

A_{is} = sum of the integrated ion abundances of the quantitation ions (Table 3) for the labeled internal standards,

Q_{is} = quantity, in pg, of the internal standard added to the sample before extraction,

W = weight, in g, of the sample (solid or liquid), and

RRF = calculated mean relative response factor for the analyte.

13.2. Calculate the percent recovery of the nine internal standards measured in the sample extract, using the formula:

$$\text{Internal standard percent recovery} = \frac{A_{is} \times Q_{rs} \times 100}{Q_{is} \times A_{rs} \times RRF\,(m)}$$

Where:

A_{is} = sum of the integrated ion abundances of the quantitation ions (Table 3) for the labeled internal standard,

A_{rs} = sum of the integrated ion abundances of the quantitation ions (Table 3) for the labeled recovery standard; the selection of the recovery standard depends on the type of congeners (Table 7),

Q_{is} = quantity, in pg, of the internal standard added to the sample before extraction,

Q_{rs} = quantity, in pg, of the recovery standard added to the cleaned-up sample residue before HRGC/HRMS analysis, and

RRF (m) = calculated mean relative response factor for the labeled internal standard relative to the appropriate (see Table 7) recovery standard.

13.3. If the concentration in the final extract of any of the fifteen 2,3,7,8-substituted PCDD/PCDF compound exceeds the dynamic range of the instrument (i.e., saturation in a mass channel) then solvent will be added to the extract to bring the signal level into the instrument's dynamic range. The project manager is notified of all instances of saturation so the client can be notified.

13.3.1. The total concentration for each homologous series of PCDD and PCDF is calculated by summing up the concentrations of all positively identified isomers of each homologous series. Therefore, the total should also include the 2,3,7,8-substituted congeners. The total number of GC signals included in the homologous total concentration value must be specified in the report.

13.3.2. Sample Specific Estimated Detection Limit - The sample specific estimated detection limit (EDL) is the concentration of a given analyte required to produce a signal with a peak height of at least 2.5 times the background signal level. An EDL is calculated for each 2,3,7,8-substituted congener that is not identified, regardless of whether or not other non-2,3,7,8-substituted isomers are present. Two methods of calculation can be used, depending on the type of response produced during the analysis of a particular sample.

13.3.3. Samples giving a response for at least one quantitation ion that is less than 2.5 times the background level.

13.3.3.1. For determination of EDLs:

- Use the expression for EDL (specific 2,3,7,8- substituted PCDD/PCDF) below to calculate an EDL for each absent 2,3,7,8- substituted PCDD/PCDF (i.e., S/N < 2.5). The background level is determined by measuring the range of the noise (peak to peak) for the two quantitation ions (Table 6) of a particular 2,3,7,8-substituted isomer within an homologous series, in the region of the SICP trace corresponding to the elution of the internal standard (if the congener possesses an internal standard) or in the region of the SICP where the congener is expected to elute by comparison with the routine calibration data (for those congeners that do not have a ^{13}C-labeled standard), multiplying that noise height by 2.5, and relating the product to an estimated concentration that ould produce that peak height.

  Use the formula:

$$\text{EDL (specific 2,3,7,8-Subst. PCDD/PCDF)} = \frac{2.5 \times H_x \times Q_{is}}{H_{is} \times W \times \overline{RF_n}}$$

  Where:

  EDL = estimated detection limit for homologous 2,3,7,8- substituted PCDDs/PCDFs.

  H_x = sum of the height of the noise level for each quantitation ion (Table 6) for the unlabeled PCDDs/PCDFs, measured as shown in Figure 6.

  H_{is} = sum of the height of the noise level for each quantitation ion (Table 6) for the labeled internal standard, measured as shown in Figure 6.

  W, RF_n, and Q_{is} retain the same meanings as defined in Section 13.1.

13.3.3.2. Samples characterized by a response above the background level with a S/M of at least 2.5 for both quantitation ions (Tables 6 and 9):

- When the response of a signal having the same retention time as a 2,3,7,8-substituted congener has a S/N in excess of 235 and does not meet any of the other qualitative identification criteria listed in Section 12.4, calculate the "Estimated Maximum Possible Concentration" (EMPC) according to the expression shown in Section. 13.1, except that A_x in Section 13.1 should represent the sum of the area under the smaller peak and of the other peak area calculated using the theoretical chlorine isotope ratio.

13.3.4. Samples characterized by a response above the background level with a S/N of at least 2.5 for both quantitation ions.

- When the response of a signal having the same retention time (Section 12.4.1) as a 2,3,7,8-substituted congener has a S/N in excess of 2.5 and does not meet ion ratio requirements (Section 12.4.2) (Table 5) calculate the "Estimated Maximum Possible Concentration" (EMPC) according to the expression shown in Section 13.1, except that Ax should represent the sum of the area under either peak and of the other peak area calculated using the theoretical chlorine isotope ratio giving the smallest calculated total peak area.

13.4. The relative percent difference (RPD) of any duplicate sample results are calculated as follows:

$$RPD = \frac{S_1 - S_2}{(S_1 + S_2)/2} \times 100$$

S_1 and S_2 represent sample and duplicate sample results.

13.5. Calculation of percent recovery

Accuracy is estimated from the recovery of spiked analytes from the matrix of interest. Laboratory performance in a clean matrix is estimated from the recovery of analytes in the LCS. Calculate the recovery of each spiked analyte in the matrix spike, matrix spike duplicate (if performed) and LCS according t othe following formula.

$$\text{Recovery} = \%R = \frac{C_s - C_u}{C_n} \times 100$$

Where:

C_s = Measured concentration of the spiked sample aliquot.
C_u = Measured concentration of the unspiked sample aliquot (use 0 for the LCS).
C_n = Nominal (theoretical) concentration increase that results from spiking the sample, or the nominal concentration of the spiked aliquot (for LCS).

13.6. The 2,3,7,8-TCDD toxicity equivalents (TE) of PCDDs and PCDFs present in the sample are calculated, if requested by the data user, according to the method recommended by the Chlorinated Dioxins Workgroup (CDWG) of the EPA and the Center for Disease Control (CDC). This method assigns a 2,3,7,8-TCDD toxicity equivalency factor (TEF) to each of the fifteen 2,3,7,8- substituted PCDDs and PCDFs (Table 3) and the OCDD and OCDF, as shown in Table 10. The 2,3,7,8-TCDD equivalent of the PCDDs and PCDFs present in the sample is calculated by summing the TEF times their concentration for each of the compounds or groups of compounds listed in Table 10.

14.0 POLLUTION PREVENTION AND WASTE MANAGEMENT:

14.1. All appropriate pollution prevention procedures and waste management procedures as described in the DAT Waste Management Plan must be utilized by all personnel involved in the preparation and analysis of samples.

15.0 OUT OF CONTROL OR UNNACCEPTABLE DATA:

15.1. Raw data that is reported under failed QC criteria must be flagged as such. The analyst must provide an explanation of the corrective actions attempted and/ or applied and the reason for the flagged data.

16.0 REFERENCES:

16.1. EPA Method 8290: Polychlorinated Dibenzodioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by High-Resolution Gas Chromatography/High-Resolution Mass Spectrometry (HRGC/HRMS), Revision 0, September 1994.

16.2. DAT, Quality Assurance Manual, Revision 4.3, May 2007.

16.3. DAT, Waste Management Plan, September 2001.

TABLE 1:
COMPOSITION OF THE INITIAL CALIBRATION SOLUTIONS

Compound	Concentrations (pg/μL) Sol. Number: 1	2	3	4	5
Unlabeled Analytes					
2,3,7,8-TCDD	0.5	1	5	50	100
2,3,7,8-TCDF	0.5	1	5	50	100
1,2,3,7,8-PeCDD	2.5	5	25	250	500
1,2,3,7,8-PeCDF	2.5	5	25	250	500
2,3,4,7,8-PeCDF	2.5	5	25	250	500
1,2,3,4,7,8-HxCDD	2.5	5	25	250	500
1,2,3,6,7,8-HxCDD	2.5	5	25	250	500
1,2,3,7,8,9-HxCDD	2.5	5	25	250	500
1,2,3,4,7,8-HxCDF	2.5	5	25	250	500
1,2,3,6,7,8-HxCDF	2.5	5	25	250	500
1,2,3,7,8,9-HxCDF	2.5	5	25	250	500
2,3,4,6,7,8-HxCDF	2.5	5	25	250	500
1,2,3,4,6,7,8-HpCDD	2.5	5	25	250	500
1,2,3,4,6,7,8-HpCDF	2.5	5	25	250	500
1,2,3,4,7,8,9-HpCDF	2.5	5	25	250	500
OCDD	5	10	50	500	1000
OCDF	5	10	50	500	1000
Internal Standards					
$^{13}C_{12}$-2,3,7,8-TCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	100	100	100	100

TABLE 1: CONTINUED

Compound	Concentrations (pg/µL) Sol. Number: 1	2	3	4	5
Internal Standards Continued					
$^{13}C_{12}$-OCDD	200	200	200	200	200
$^{13}C_{12}$-2,3,7,8-TCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8-PeCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	100	100	100	100
Surrogate Standards					
$^{37}Cl_4$-2,3,7,8-TCDD	60	80	100	120	140
$^{13}C_{12}$-2,3,4,7,8-PeCDF	60	80	100	120	140
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	60	80	100	120	140
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	60	80	100	120	140
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	60	80	100	120	140
Alternate Standard					
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	60	80	100	120	140
$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	60	80	100	120	140
Recover Standards					
$^{13}C_{12}$-1,2,3,4-TCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	100	100	100	100	100

TABLE 2:
GAS CHROMATOGRAPHY CONDITIONS

	Primary Column	Confirmation Column
Column type	DB-5	DB-Dioxin
Length (m)	60	60
i.d. (mm)	0.25	0.25
Film Thickness (um)	0.25	0.15
Carrier Gas	Helium	Helium
Carrier Gas Flow (mL/min)	1-2	1.0
Injection Mode	←splitles→	
Purge On	1.7 min.	1.7 min.
Initial Temperature (°C)	180	180
Initial Time	1 min.	1 min.
Flow Rate	2.5° per/min	2.5° per/min
Final Temperature	270°C	270°C
Final Time	3 min.	3 min.

TABLE 3: ELEMENTAL COMPOSITIONS AND EXACT MASSES OF THE IONS MONITORED BY HRGC/HRMS FOR PCDDs AND PCDFs

Descriptor Number	Accurate Mass[2]	Ion Type	Elemental Composition	Analyte
2	292.9825	LOCK	C_7F_{11}	PFK
	303.9016	M	$C_{12}H_4{}^{35}Cl_4O$	TCDF
	305.8987	M+2	$C_{12}H_4{}^{35}Cl_3{}^{37}ClO$	TCDF
	315.9419	M	$^{13}C_{12}H_4{}^{35}Cl_4O$	TCDF (S)
	317.9389	M+2	$^{13}C_{12}H_4{}^{35}Cl_3{}^{37}ClO$	TCDF (S)
	319.8965	M	$C^{12}H_4{}^{35}Cl_4O_2$	TCDD
	321.8936	M+2	$C_{12}H_4{}^{35}Cl_3{}^{37}ClO_2$	TCDD
	327.8847	M	$C_{12}H_4{}^{37}Cl_4O_2$	TCDD (S)
	330.9792	QC	C_7F_{13}	PFK
	331.9368	M	$^{13}C_{12}H_4{}^{35}Cl_4O_2$	TCDD (S)
	333.9339	M+2	$^{13}C_{12}H_4{}^{35}Cl_3{}^{37}ClO_2$	TCDD (S)
	375.8364	M+2	$C_{12}H_4{}^{35}Cl_5{}^{37}ClO$	HxCDPE
	339.8597	M+2	$C_{12}H_3{}^{35}Cl_4{}^{37}ClO$	PeCDF
	341.8567	M+2	$C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O$	PeCDF
	351.9000	M+4	$^{13}C_{12}H_3{}^{35}Cl_4{}^{37}ClO$	PeCDF (S)
	353.8970	M+2	$^{13}C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O$	PeCDF (S)
	355.8546	M+4	$C_{12}H_3{}^{35}Cl_4{}^{37}ClO$	PeCDD
	357.8516	M+2	$C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O_2$	PeCDD
	367.8949	M+4	$^{13}C_{12}H_3{}^{35}Cl_4{}^{37}ClO_2$	PeCDD (S)
	369.8919	M+2	$^{13}C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O_2$	PeCDD (S)
	409.7974	M+4	$C_{12}H_3{}^{35}Cl_6{}^{37}ClO$	HpCDPE
3	373.8208	M+2	$C_{12}H_2{}^{35}Cl_5{}^{37}ClO$	HxCDF
	375.8178	M+2	$C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O$	HxCDF
	383.8639	M	$^{13}C_{12}H_2{}^{35}Cl_6O$	HxCDF (S)

TABLE 3 CONTINUED

Descriptor Number	Accurate Mass[2]	Ion Type	Elemental Composition	Analyte
3 Continued	385.8610	M+2	$^{13}C_{12}H_2{}^{35}Cl_5{}^{37}ClO$	HxCDF (S)
	389.8157	M+2	$C_{12}H_2{}^{35}Cl_5{}^{37}ClO_2$	HxCDD
	391.8127	M+4	$C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O_2$	HxCDD
	392.976	LOCK	C_9F_{15}	PFK
	401.8559	M+2	$^{13}C_{12}H_2{}^{35}Cl_5{}^{37}ClO_2$	HxCDD (S)
	403.8529	M+4	$^{13}C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O_2$	HxCDD (S)
	445.7555	M+4	$C_{12}H_2{}^{35}Cl_6{}^{37}Cl_2O$	OCDPE
	430.9729	QC	C_9F_{13}	PFK
4	407.7818	M+2	$C_{12}H^{35}Cl_6{}^{37}ClO$	HpCDF
	409.7789	M+4	$C_{12}H^{35}Cl_5{}^{37}Cl_2O$	HpCDF
	417.8253	M	$^{13}C_{12}H^{35}Cl_7O$	HxCDF (S)
	419.8220	M+2	$^{13}C_{12}H^{35}Cl_6{}^{37}ClO$	HxCDF (S)
	423.7766	M+2	$C_{12}H^{35}Cl_6{}^{37}ClO_2$	HpCDD
	425.7737	M+4	$C_{12}H^{35}Cl_5{}^{37}Cl_2O_2$	HpCDD
	435.8169	M+2	$^{13}C_{12}H^{35}Cl_6{}^{37}ClO_2$	HpCDD (S)
	437.8140	M+4	$^{13}C_{12}H^{35}Cl_5{}^{37}Cl_2O_2$	HpCDD (S)
	479.7165	M+4	$C_{12}H^{35}Cl_7{}^{37}Cl_2O$	NCDPE
	430.9729	LOCK	C_9F_{17}	PFK
	441.7528	M+2	$C_{12}{}^{35}Cl_7{}^{37}ClO$	OCDF
	443.7399	M+4	$C_{12}{}^{35}Cl_6{}^{37}Cl_2O$	OCDF
	457.7377	M+2	$C_{12}{}^{35}Cl_7{}^{37}ClO_2$	OCDD
	459.7348	M+4	$C_{12}{}^{35}Cl_6{}^{37}Cl_2O_2$	OCDD
	469.7779	M+2	$^{13}C_{12}{}^{35}Cl_7{}^{37}ClO_2$	OCDD (S)
	471.7750	M+4	$^{13}C_{12}{}^{35}Cl_6{}^{37}Cl_2O_2$	OCDD (S)

TABLE 3 CONTINUED

Descriptor Number	Accurate Mass[2]	Ion Type	Elemental Composition	Analyte
4 Continued	513.6775	M+4	$C_{12}{}^{35}Cl_6{}^{37}Cl_2O$	DCDPE
	442.9728	QC	$C_{10}F_{17}$	PFK

a) The following nuclidic masses were used:

H	=	1.007825
C	=	12.000000
^{13}C	=	13.003355
F	=	18.9984
S	=	Labeled Standard
QC	=	Ion Selected for Monitoring the Instrument Stability During the GC/MS Analysis

b) Descriptor 1 contains mono-, di- and trichlorinated dibenzodioxins and dibenzofuranse that are not quantitated by method 8290.

TABLE 4: INITIAL AND CONTINUING CALIBRATION RESPONSE FACTOR MINIMUM REQUIREMENTS

Compound	Relative Response Factors		
	I-Call %RSD	Con-Cal % Delta Beginning	Con-Cal %Delta Ending
Unlabeled Analytes			
2,3,7,8-TCDD	20	20	25
2,3,7,8-TCDF	20	20	25
1,2,3,7,8-PeCDD	20	20	25
1,2,3,7,8-PeCDF	20	20	25
2,3,4,7,8-PeCDF	20	20	25
1,2,3,4,7,8-HxCDD	20	20	25
1,2,3,6,7,8-HxCDD	20	20	25
1,2,3,7,8,9-HxCDD	20	20	25
1,2,3,4,7,8-HxCDF	20	20	25
1,2,3,6,7,8-HxCDF	20	20	25
1,2,3,7,8,9-HxCDF	20	20	25
2,3,4,6,7,8-HxCDF	20	20	25
1,2,3,4,6,7,8-HpCDD	20	20	25
1,2,3,4,6,7,8-HpCDF	20	20	25
1,2,3,4,7,8,9-HpCDF	20	20	25
OCDD	20	20	25
OCDF	20	20	25
Internal Standards	30	30	35
$^{13}C_{12}$-2,3,7,8-TCDD	30	30	35
$^{13}C_{12}$-1,2,3,7,8-PeCDD	30	30	35
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	30	30	35

TABLE 4 CONTINUED

Compound	Relative Response Factors		
	I-Call %RSD	Con-Cal % Delta Beginning	Con-Cal %Delta Ending
Internal Standards Continued			
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	30	30	35
$^{13}C_{12}$-OCDD	30	30	35
$^{13}C_{12}$-2,3,7,8-TCDF	30	30	35
$^{13}C_{12}$-1,2,3,7,8-PeCDF	30	30	35
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	30	30	35
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	30	30	35
Surrogate Standards			
$^{37}Cl_4$-2,3,7,8-TCDD	30	30	35
$^{13}C_{12}$-2,3,4,7,8-PeCDF	30	30	35
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	30	30	35
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	30	30	35
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	30	30	35
Alternate Standard			
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	30	30	35
$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	30	30	35

TABLE 5: ION-ABUNDANCE RATIO ACCEPTABLE RANGES FOR PCDDs AND PCDFs

Number of Chlorine Atoms	Ion Type	Theoretical Ration	Control Limits	
			Lower	Upper
4	M/M+2	0.77	0.65	0.89
5	M+2/M+4	1.55	1.32	1.78
6	M+2/M+4	1.24	1.05	1.43
6a	M/M+2	0.51	0.43	0.59
7b	M/M+2	0.44	0.37	0.51
7	M+2/M+4	1.04	0.88	1.20
8	M+2/M+4	0.89	0.76	1.02

a) Used only for ^{13}C-HxCDF

b) Used only for ^{13}C-HpCDF

TABLE 6: UNLABELED ANALYTES QUANTITATION RELATIONSHIPS

Analyte	Standard Used During Quantitation
2,3,7,8-TCDD	$^{13}C_{12}$-2,3,7,8-TCDD
Other TCDDs	$^{13}C_{12}$-2,3,7,8-TCDD
1,2,3,7,8-PeCDD	$^{13}C_{12}$-1,2,3,7,8-PeCDD
Other PeCDDs	$^{13}C_{12}$-1,2,3,7,8-PeCDD
1,2,3,4,7,8-HxCDD	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD
1,2,3,6,7,8-HxCDD	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD
1,2,3,7,8,9-HxCDD	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD
Other HxCDDs	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD
1,2,3,4,6,7,8-HpCDD	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD
Other HpCDDs	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD
OCDD	$^{13}C_{12}$-OCDD
2,3,7,8-TCDF	$^{13}C_{12}$-2,3,7,8-TCDF
Other TCDFs	$^{13}C_{12}$-2,3,7,8-TCDF
1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,7,8-PeCDF
2,3,4,7,8-PeCDF	$^{13}C_{12}$-1,2,3,7,8-PeCDF
Other PeCDFs	$^{13}C_{12}$-1,2,3,7,8-PeCDF
1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,6,7,8-HxCDF
1,2,3,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,6,7,8-HxCDF
1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-1,2,3,6,7,8-HxCDF

TABLE 6 CONTINUED

Analyte	Standard Used During Quantitation
2,3,4,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,6,7,8-HxCDF
Other HxCDFs	$^{13}C_{12}$-1,2,3,6,7,8-HxCDF
1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF
1,2,3,4,7,8,9-HpCDF	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF
Other HpCDFs	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF
OCDF	$^{13}C_{12}$-OCDF

TABLE 7: INTERNAL STANDARDS QUANTITATION RELATIONSHIPS

Internal Standard	Standard Used During Percent Recovery Determination
$^{13}C_{12}$-2,3,7,8-TCDD	$^{13}C_{12}$-1,2,3,4-TCDD
$^{13}C_{12}$-1,2,3,7,8-PeCDD	$^{13}C_{12}$-1,2,3,4-TCDD
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-OCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-2,3,7,8-TCDF	$^{13}C_{12}$-1,2,3,4-TCDD
$^{13}C_{12}$-1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,4-TCDD
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD

TABLE 8: SURROGATE/ALTERNATE STANDARDS QUANTITATION RELATIONSHIPS

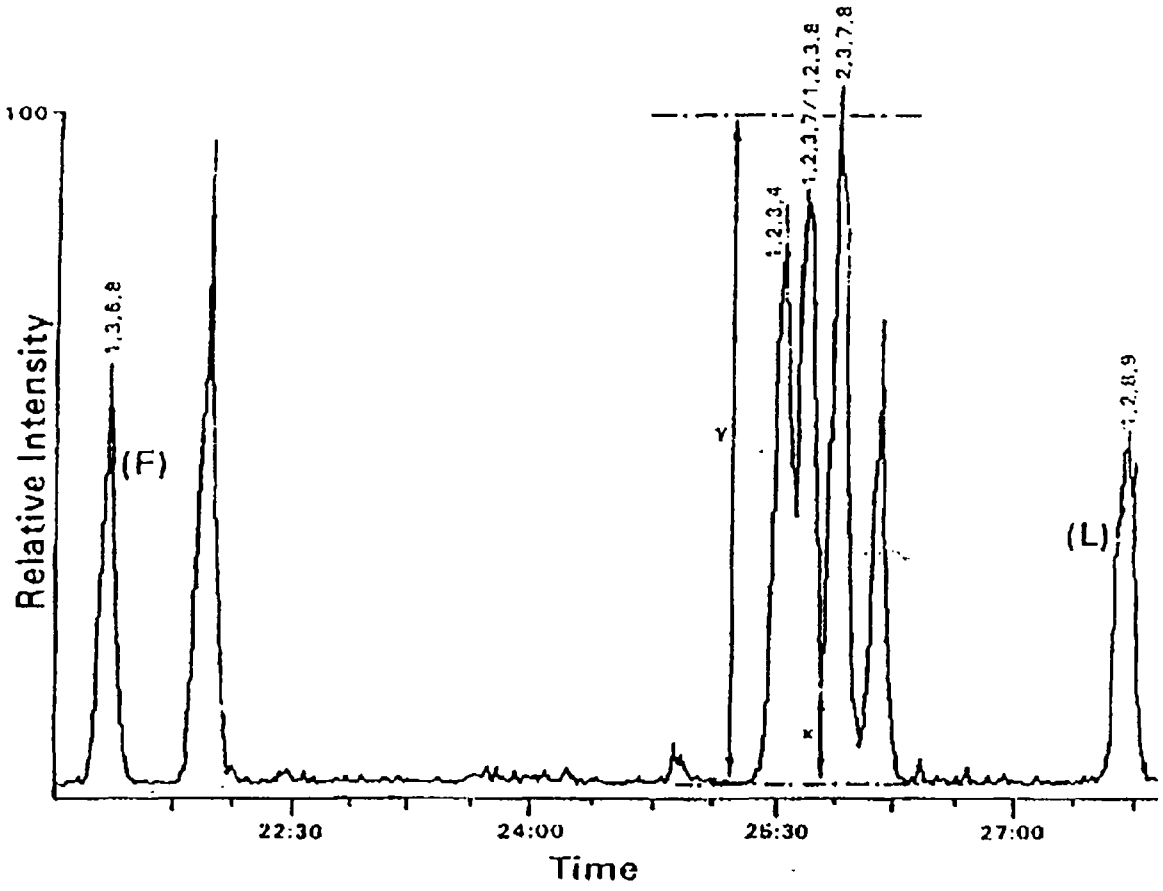
Surrogate/Alternate/Cleanup Standard	Standard Used During Percent Recovery Determination
$^{37}Cl_4$-2,3,7,8-TCDD	$^{13}C_{12}$-1,2,3,4-TCDD
$^{13}C_{12}$-2,3,4,7,8-PeCDF	$^{13}C_{12}$-1,2,3,4-TCDD
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-1,2,3,7,8,9-HpCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD

TABLE 9: TYPES OF MATRICES, SAMPLE SIZES AND 2,3,7,8-TCDD-BASED METHOD CALIBRATION LIMITS (Parts per Trillion)

	Water	Soil Sediment Paper Pulp	Fly Ash	Fish Tissue	Human Adipose Tissue	Sludges, Fuel Oil	Still-Bottom
Lower MCLa	0.01	1.0	1.0	1.0	1.0	5.0	10
Upper MCLa	2	200	200	200	200	1000	2000
Weight (g)	1000	10	10	10	10	2	1
IS Spiking Levels (ppt)	1	100	100	100	100	500	1000
Final Extr. Vol. (µL)	10-50	10-50	50	10-50	10-50	50	50

a = For other congeners multiply the values by 1 for TCDF/PeCDD/PeCDF, by 2.5 for HxCDD/HxCDF/HpCDD/HpCDF, and by 5 for OCDD/OCDF.

FIGURE 4



Selected ion current profile for m/z 322 (TCDDs) produced by MS analysis of the GC performance check solution on a 60 m DB-5 fused silica capillary column under the conditions listed in Sec. 7.6.

TABLE 10:
2,3,7,8-TCDD Toxicity Equivalency Factors (TEFs) for the Polychlorinated Dibenzodioxins and Dibenzofurans

Number	Compound (s)	TEF[a]
1	2,3,7,8-TCDD	1.00
2	1,2,3,7,8-PeCDD	0.50
3	1,2,3,6,7,8-HxCDD	0.10
4	1,2,3,7,8,9-HxCDD	0.10
5	1,2,3,4,7,8-HxCDD	0.10
6	1,2,3,4,6,7,8-HpCDD	0.01
7	1,2,3,4,6,7,8,9-OCDD	0.001
8	2,3,7,8-TCDF	0.1
9	1,2,3,7,8-PeCDF	0.05
10	2,3,4,7,8-PeCDF	0.5
11	1,2,3,6,7,8-HxCDF	0.1
12	1,2,3,7,8,9-HxCDF	0.1
13	1,2,3,4,7,8-HxCDF	0.1
14	2,3,4,6,7,8-HxCDF	0.1
15	1,2,3,4,6,7,8-HpCDF	0.01
16	1,2,3,4,7,8,9-HpCDF	0.01
17	1,2,3,4,6,7,8,9-OCDF	0.001

[a] Taken from "Interim Procedures for Estimating Risks Associated with Exposures to Mixtures of Chlorinated Dibenzo-p-Dioxin and -Dibenzofurans (CDDs and CDFs) and 1989 Update:, (EPA/625/3-89/016, March 1989).