`Title: Preparation of Samples for Analysis of Polychlorinated Dioxins and Furans for Analysis HRGC/HRMS

[Methods 8290, 8290A & TO-9A]

Approvals (Signature/Date):

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1. SCOPE AND APPLICATION

1.1. This method provides procedures for the preparation of samples prior to the analysis of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), polychlorinated dibenzo-p-dioxins (tetra- through octachlorinated homologs; PCDDs), and polychlorinated dibenzofurans (tetra- through octachlorinated homologs; PCDFs) in a variety of environmental matrices at part-per-trillion (ppt) concentrations by SW 846 Method 8290. The analytical method calls for the use of high-resolution gas chromatography and high-resolution mass spectrometry (HRGC/HRMS) on purified sample extracts. Refer to Table 1 for the list of analytes. Analysis is by SOP WS-ID-0005.

1.2. The sensitivity of this method is dependent upon the level of interferences within a given matrix.

1.3. This method is designed for use by analysts who are experienced with residue analysis.

1.4. Samples containing concentrations of specific congeners (PCDDs and PCDFs) that are greater than the calibration limit should be analyzed by a protocol designed for such concentrations, such as 8280A/B.

2. SUMMARY OF METHOD

2.1. This procedure uses matrix-specific extraction and analyte-specific cleanup techniques.

2.2. A specified amount (see Table 1) of soil, sediment, fly ash, water, sludge (including paper pulp), still-bottom, fuel oil, chemical reactor residue, air sample (QFF, PUF or XAD media) or fish tissue, is spiked with a solution containing specified amounts of each of nine isotopically (^{13}C) labeled PCDDs/PCDFs listed in Table 2. The sample is then extracted according to a matrix-specified extraction procedure. The extraction procedures are: a) toluene Soxhlet (or equivalent) extraction, for soil, sediment, fly ash samples, aqueous sludges, and solid air matrices (XAD, QFF, PUF); b) methylene chloride liquid-liquid extraction or solid phase extraction for water samples; c) dilution of a small sample aliquot in solvent for wastes/chemical products; and d) toluene (or hexane/methylene chloride) Soxhlet (or equivalent) extraction for fish tissue. This method can also use solid phase extraction (SPE), however, Test America West Sacramento is in the developmental stages for this extraction type and is not currently certified for its use.

2.3. If interferences are present, extracts may be cleaned as described below. The extracts are submitted to an acid and/or base washing treatment and dried. Following a solvent exchange step, the residue is cleaned up by column chromatography on acid/base silica, acid alumina and carbon on silica. The preparation of the final extract for HRGC/HRMS analysis is accomplished by adding 20 µL of a tetradecane solution containing 100 pg/µL of each of the two recovery standards $^{13}C_{12}$-1,2,3,4-TCDD and

$^{13}C_{12}$ -1,2,3,7,8,9-HxCDD (Table 2) to the concentrated eluate. The former is used to determine the percent recoveries of tetra- and penta-chlorinated PCDD/PCDF internal standards while the latter is used for the determination of hexa-, hepta- and octa-chlorinated PCDD/PCDF internal standard percent recoveries. Upon client approval, less final volume can be used to decrease detection limit and more final volume can be used to decrease severe interferences.

3. DEFINITIONS

3.1. Definitions of terms used in this SOP may be found in the glossary of the Quality Assurance Manual (QAM).

3.2. Data qualifiers are defined on each data report. Commonly used data qualifiers are defined in the QAM.

3.3. Internal Standard: An internal standard is a ^{13}C-labeled analog of a congener chosen from the compounds listed in Table 2. Internal standards are added to all samples including method blanks and quality control samples before extraction, and they are used to quantitate the concentration of the analytes. Nine internal standards are used in this method. There is one for each of the dioxin and furan homologs (except for OCDF) with the degree of chlorination ranging from four to eight. Additional internal standards may be added to act as retention time references, but they are not used for quantitation.

3.4. Recovery Standard: Two recovery standards are used to determine the percent recoveries for the internal standards. The $^{13}C_{12}$-1,2,3,4-TCDD is used to measure the percent recoveries of the tetra- and pentachlorinated internal standards while $^{13}C_{12}$-1,2,3,7,8,9-HxCDD is used to determine the recovery of the hexa-hepta- and octachlorinated internal standards. $^{13}C_{12}$-1,2,3,7,8,9-HxCDD also acts as a retention time reference for the unlabeled analog present in sample extracts. They are added to the final sample extract before HRGC/HRMS instrument analysis.

3.5. Cleanup Recovery Standard (CRS): A $^{37}Cl_4$-2,3,7,8-TCDD analog that is added to each sample following extraction to measure the efficiency of the cleanup process.

4. 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. All of these materials must be demonstrated to be free from interferents under the conditions of analysis by running laboratory method blanks. Analysts shall not use 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. 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 glassware surface.

4.3.1. Glassware should be rinsed with solvent and washed with a detergent solution as soon after use as is practical. Sonication of glassware containing a detergent solution for approximately 30 seconds may aid in cleaning. Glassware with removable parts, particularly separatory funnels with Teflon stopcocks, must be disassembled prior to detergent washing.

4.3.2. After detergent washing, glassware should be immediately rinsed with acetone, toluene, hexane, and then methylene chloride.

4.3.3. Do not kiln reusable glassware in an oven as a routine part of cleaning. Kilning may be warranted after particularly dirty samples are encountered, but should be minimized, as repeated kilning of glassware may cause the formation of active sites on the glass surface that will irreversibly adsorb PCDDs/ PCDFs.

4.3.4. Immediately prior to use, Soxhlet (or equivalent) extraction glassware should be pre-extracted with toluene for a minimum of 3 hours. Note: Accelerated extractors such as the Soxtherm can use a shorter cleaning cycle which exhibits subsequent extractions free of cross contamination and interferences.

Note: Re-use of glassware should be minimized to avoid the risk of contamination. All glassware that is re-used must be scrupulously cleaned as soon as possible after use, applying the following procedure:

4.4. Interferences co-extracted from samples will vary considerably from source to source, depending on the diversity of the site being sampled. Interfering compounds may be present at concentrations several orders of magnitude higher than the PCDDs and PCDFs. The most frequently encountered interferences are chlorinated-biphenyls, methoxy biphenyls, hydroxy biphenyl ethers, benzyl phenyl ethers, polynuclear aromatics, and pesticides. Because very low levels of PCDDs and PCDFs are measured by this method, the elimination of interferences is essential. The cleanup steps given in Sections 11.12 thru 11.16 can be used to reduce or eliminate these interferences.

4.4.1. If South Carolina samples show diphenyl ethers at levels that could contribute to positive furan hits, a subsequent clean-up to remove them must be performed.

5. SAFETY

Employees must abide by the policies and procedures in the Corporate Environmental Health and Safety Manual (CW-E-M-001), the West Sacramento Addendum to the Corporate EH&S Manual (WS-PEHS-002) and this document. This procedure may involve hazardous material, operations and equipment. This SOP does not purport to address all of the safety problems associated with its use. It is the responsibility of the user of the method to follow appropriate safety, waste disposal and health practices under the assumption that all samples and reagents are potentially hazardous. Safety glasses, gloves, lab coats and closed-toes, nonabsorbent shoes are a minimum.

5.1. Specific Safety Concerns or Requirements

5.1.1. Hearing protection must be worn when using mechanical systems to grind fish, tissue, or paper/pulp samples.

5.1.2. Finely divided dry soils contaminated with PCDDs and PCDFs are particularly hazardous because of the potential for inhalation and ingestion. Such samples are to be processed in a confined environment, such as a hood or a glove box.

5.1.3. Assembly and disassembly of glassware creates a risk of breakage and cuts. All staff members shall wear Kevlar or MAPA blue latex cut-resistant gloves over chemically resistant gloves when assembling and disassembling glassware.

5.1.4. Eye protection that satisfies ANSI Z87.1, laboratory coat, and chemically resistant gloves must be worn while samples, standards, solvents, and reagents are being handled. Latex and vinyl gloves provide no protection against most of the organic solvents used in this method. Nitrile or similar gloves must be used. Latex gloves may be used for methanol.

5.1.5. Exposure to chemicals must be maintained as low as reasonably achievable, therefore all samples must be opened, transferred and prepared in a fume hood. Solvent and waste containers will be kept closed unless transfers are being made.

5.1.6. Laboratory procedures such as repetitive use of pipets, repetitive transferring of extracts, and manipulation of filled separatory funnels and other glassware represent a significant potential for repetitive motion or other ergonomic injuries. Laboratory associates performing these procedures are in the best position to realize when they are at risk for these types of injuries. Whenever a situation is found in which an employee is performing the same repetitive motion, the employee shall immediately bring this to the attention of their supervisor, manager, or the EH&S staff. The task will be analyzed to determine a better means of accomplishing it.

5.1.7. The use of separatory funnels to extract aqueous samples with methylene chloride creates excessive pressure very rapidly. The use of separatory funnels during the partition and back extraction of sample extracts can also create excessive pressure. Initial venting should be done immediately after the sample container has been sealed and inverted. Vent the funnel into the hood away from people and other samples. This is considered a high-risk activity, and a face shield must be worn over safety glasses or goggles when it is performed. Alternately, the extraction can be performed behind a closed fume hood sash on a mechanical shaker.

5.1.8. When Dean-Stark/Soxhlet clean-ups or extractions are performed overnight or unattended, special precautions must be taken. Open the chiller valves to the system about 15 minutes before the heating elements are turned on, and check every condenser to ensure that it is cold and functioning properly before turning the heating elements on. Check every condenser again about 15 minutes after turning the heating elements on to ensure that they are still cold and functioning properly. If the system is left operating overnight or unattended for an extended period, the first chemist to come back into the lab must again check every condenser to ensure that it is still cold and functioning properly.

5.2. Primary Materials Used

The following is a list of the materials used in this method, which have a serious or significant hazard rating. **NOTE: This list does not include all materials used in the method. The table contains a summary of the primary hazards listed in the MSDS for each of the materials listed in the table.** A complete list of materials used in the method can be found in the reagents and materials section. Employees must review the information in the MSDS for each material before using it for the first time or when there are major changes to the MSDS.

Material (1)	Hazards	Exposure Limit (2)	Signs and symptoms of exposure
Acetone	Flammable	1000 ppm-TWA	Inhalation of vapors irritates the respiratory tract. May cause coughing, dizziness, dullness, and headache.
Benzene	Flammable Toxic Carcinogen	PEL: 1 ppm TWA ; 5 ppm 15 MIN. STEL	Causes skin irritation. Toxic if absorbed through skin. Causes severe eye irritation. Toxic if inhaled. Vapor or mist causes irritation to mucous membranes and upper respiratory tract. Exposure can cause narcotic effect. Inhalation at high concentrations may have an initial stimulatory effect on the central nervous system characterized by exhilaration, nervous excitation and/or giddiness, depression, drowsiness or fatigue. Victim may experience tightness in the chest, breathlessness, and loss of consciousness.
Cyclohexane	Flammable Irritant	300 ppm TWA	Inhalation of vapors causes irritation to the respiratory tract. Symptoms may include coughing, shortness of breath. High concentrations have a narcotic effect.

Material (1)	Hazards	Exposure Limit (2)	Signs and symptoms of exposure
Hexane	Flammable Irritant	500 ppm-TWA	Inhalation of vapors irritates the respiratory tract. Overexposure may cause lightheadedness, nausea, headache, and blurred vision. Vapors may cause irritation to the skin and eyes.
Isooctane	Flammable Irritant	None established	Inhalation of vapors may cause nausea, headache, dizziness, loss of consciousness, irritation to upper respiratory tract, pain in throat and nose, coughing, wheezing, shortness of breath.
Methanol	Flammable Poison Irritant	200 ppm-TWA	A slight irritant to the mucous membranes. Toxic effects exerted upon nervous system, particularly the optic nerve. Symptoms of overexposure may include headache, drowsiness and dizziness. Methyl alcohol is a defatting agent and may cause skin to become dry and cracked. Skin absorption can occur; symptoms may parallel inhalation exposure. Irritant to the eyes.
Methylene Chloride	Carcinogen Irritant	25 ppm-TWA 125 ppm-STEL	Causes irritation to respiratory tract. Has a strong narcotic effect with symptoms of mental confusion, light-headedness, fatigue, nausea, vomiting and headache. Causes irritation, redness and pain to the skin and eyes. Prolonged contact can cause burns. Liquid degreases the skin. May be absorbed through skin.
Sodium Hydroxide	Corrosive Poison	2 ppm, 5 mg/m^3	This material will cause burns if comes into contact with the skin or eyes. Inhalation of Sodium Hydroxide dust will cause irritation of the nasal and respiratory system.
Sulfuric Acid (1)	Corrosive Oxidizer Dehydra-dator	1 mg/m^3	This material will cause burns if comes into contact with the skin or eyes. Inhalation of vapors will cause irritation of the nasal and respiratory system.
Tetradecane	Irritant	None established	Inhalation of vapors may cause difficulty breathing, headache, intoxication and central nervous system damage.
Toluene	Flammable Poison Irritant	200 ppm-TWA 300 ppm-Ceiling	Inhalation may cause irritation of the upper respiratory tract. Symptoms of overexposure may include fatigue, confusion, headache, dizziness and drowsiness. Peculiar skin sensations (e. g. pins and needles) or numbness may be produced. Causes severe eye and skin irritation with redness and pain. May be absorbed through the skin.
1 – Always add acid to water to prevent violent reactions.			
2 – Exposure limit refers to the OSHA regulatory exposure limit.			

6. EQUIPMENT AND SUPPLIES

The following list of items does not necessarily constitute an exhaustive compendium of the equipment needed for this analytical method.

6.1. Nitrogen evaporation apparatus with variable flow rate.

6.2. Balances capable of accurately weighing to 0.01 g and 0.0001 g.

6.3. Centrifuge.

6.4. Water bath, equipped with concentric ring covers and capable of maintaining temperature control within ± 2ºC.

6.5. Stainless steel or glass containers large enough to hold contents of one-pint sample containers.

6.6. Drying oven.

6.7. Stainless steel spoons and spatulas.

6.8. Pipettes, disposable, Pasteur, 150 mm long x 5 mm ID.

6.9. Pipettes, disposable, serological, 10 mL, for the preparation of the carbon column specified in Section 7.1.

6.10. Reacti-vial, 2 mL, silanized clear glass.

6.11. Stainless steel meat grinder with a 3- to 5-mm hole size inner plate.

6.12. Separatory funnels, 250 mL.

6.13. Separatory funnels, 1000 mL.

6.14. Teflon® boiling chips (or equivalent) washed with methylene chloride before use.

6.15. Chromatographic column, glass, 300 mm x 10.5 mm, fitted with Teflon® stopcock.

6.16. Adapters for concentrator tubes.

6.17. Glass fiber filters, Whatman GF-D, GF-F, GMF150, or equivalent.

6.18. Solid phase extraction discs, 3M 90mm C18, or equivalent.

6.19. Dean-Stark trap, 5 or 10 mL, with T-joints, condenser and 125 mL flask.

6.20. Continuous liquid-liquid extractor.

6.21. All-glass Soxhlet apparatus, 500 mL flask.

6.22. Soxtherm extraction apparatus (or equivalent), including glass thimble holders, glass beakers, and gaskets.

6.23. Glass funnels, sized to hold 170 mL of liquid.

6.24. Desiccator.

6.25. Turbo evaporator

6.26. Rotary evaporator with a temperature controlled water bath.

6.27. High speed tissue homogenizer, equipped with an EN-8 probe or equivalent.

6.28. Glass wool, extracted with methylene chloride, dried and stored in a clean glass jar.

6.29. Vacuum extraction device for solid phase extraction, 1 Liter glass funnel with 90mm filter disc holder with a vacuum source, Kontes or equivalent.

7. REAGENTS AND STANDARDS

7.1. Column Chromatography Reagents

7.1.1. Silica Gel - Kieselgel 60 or equivalent, activate for 1 hour at 184°C before use. Store at 130°C in covered flask.

7.1.2. Acid Alumina - ICN or equivalent, activated as necessary.

7.1.3. Basic Alumina - ICN or equivalent. No activation required.

7.1.4. Granular carbon/silica gel - Mix 3.6 g granular carbon and 16.4 g activated silica gel; (alternatively, prepare carbon/silica gel (5%/95%); i.e., combine 5 g precleaned carbon with 95 g silica gel). Store at room temperature in a Teflon ® lined covered jar. The first LCS prepared with a new batch of column packing material is the quality control check of the packing materials. Refer to historical control limits before accepting the new batch of material.

7.1.5. 44% H_2SO_4 /silica gel - Mix 24 mL conc. H_2SO_4 and 56 g activated silica gel. Stir and shake until free flowing. Store at room temperature.

7.1.6. 33% NaOH/silica gel - Mix 34 mL 1N NaOH and 67 g activated silica gel. Stir and shake until free flowing. Store at room temperature.

7.2. Acid Alumina Activity Assessment

Alumina activity may vary with the matrix or environmental conditions. Monitor internal standard and cleanup recovery standard recoveries in extract analysis. Low recoveries of cleanup recovery standard (CRS) may indicate loss of alumina activity. Assess stability of alumina activity and apply corrective action as appropriate (reactivate and reprofile).

Note: a column profile should be done to show elution of all 2,3,7,8 substituted analogs so problems can be readily identified.

7.2.1. Profile each vendor lot of activated alumina as corrective action for low internal standard and CRS recoveries dictate. If necessary, proceed as follows:

7.2.1.1. Set up and label 3 acid alumina columns.

7.2.1.2. Pre-rinse with 20 mL hexane.

7.2.1.3. Add 2 mL hexane spiked with internal standards and natives (spike amounts equivalent to those for LCS) with 2X2 mL hexane rinse of fractions.

7.2.1.4. Elute each column with 20 mL hexane. Collect and label these fractions.

7.2.1.5. Elute each column with 5 x10 mL methylene chloride/hexane at the appropriate v/v percent. Collect and label these fractions separately.

7.2.1.6. Elute each column with 10 mL of 100% methylene chloride. Collect and label these fractions. Reduce all fractions to final volume and add recovery standard.

7.2.2. Review data and select an elution scheme. Group the fraction from each solvent system as follows:

7.2.2.1. Pre-analyte fraction - consists of all eluent prior to elution of first target analytes.

7.2.2.2. Analyte fraction - consists of all that contain detectable levels of target analytes.

7.2.2.3. Post-analyte fraction - consists of all eluents after elution of the last target analyte.

7.2.3. Select the solvent system which best meets the following two conditions:

7.2.3.1. Pre-analyte fraction consists of 20mL hexane and no more than 20 mL mixed solvent.

7.2.3.2. Analyte fraction consists of no more than 20mL of mixed solvent and contains greater than 90% of all target analytes and greater than 80% of all internal standards.

7.2.4. After selection of the appropriate solvent system and fractionation pattern, perform triplicate acid alumina cleanups on spiked hexane to ensure reproducibility of the fractionation pattern. Document each elution scheme.

7.2.5. Each subsequent batch of acid alumina used in the lab (from the same vendor lot) must be checked for stable activity.

7.3. Reagents

7.3.1. Sulfuric acid, concentrated, ACS grade, specific gravity 1.84.

7.3.2. Distilled water demonstrated to be free of interferents

7.3.3. 1 N HCl.

7.3.4. Silica gel.

7.3.5. Solution for breaking emulsions: Slowly add 1.0L of reagent grade NaOH solution to a 2.0L NaOH container, containing 1.0L of DI H2O, and leave the container in secondary containment with the lid off.

Warning: The solution will begin to heat so let the solution stand until equilibrium is met and the solution is at room temperature.

When this process is complete, the solution will then be ready for use in the samples.

7.3.6. Precleaned Sodium Sulfate.

7.3.7. Canola Oil (for tissue extraction only), or other suitable oil.

7.4. Desiccating Agent

7.4.1. Sodium sulfate, granular, anhydrous.

7.5. Solvents

7.5.1. High-purity, distilled-in-glass or highest available purity: Methylene chloride, hexane, methanol, tetradecane, isooctane, toluene, cyclohexane, and acetone.

7.6. All daily internal standard, daily clean up recovery standards, and daily spiking solutions are stable for one year from preparation. After 1 year, solutions may be re-verified. The re-verified solution may be used for an additional year, or until there is evidence of compound degradation or concentration. The re-verification must be performed using an unexpired, not previously re-verified solution from a second lot or second vendor.

7.6.1. Sealed ampules may be used until the manufacturer's expiration date is exceeded. If no expiration date is provided, then the expiration date will be 10 years from the date the ampule is opened. The solvent level should be monitored prior to each use to assure there has been no concentration of the standard over time.

7.6.2. Standards for method 8290A require storage at ≤ 6ºC.

7.7. Field Surrogate Solution (air matrices)

This solution contains one ^{37}Cl labeled analog (for Method TO-9/TO-9A) or one ^{37}Cl and four ^{13}C labeled analogs (for Method 0023) at the nominal concentration indicated in Table 2. It is used to assess sample collection and recovery procedures.

7.8. Internal Standard

This isooctane solution contains the nine internal standards at the nominal concentrations that are listed in Table 2. The solution contains at least one carbon-labeled standard for each homologous series, and it is used to measure the concentrations of the native substances. (Note that $^{13}C_{12}$ -OCDF is not present in the solution.)

7.9. Native Spike Standard

Also known as the Matrix Spike or Native Spike solution. Contains all the 2,3,7,8-substituted unlabeled analytes listed in Table 2. Prepare using the appropriate standards to yield a spiking solution with a concentration of 4.0 ng/ml for the tetra-CDDs/CDFs, 20 ng/ml for the penta-, hexa-, and hepta- CDDs/CDFs, and 40 ng/ml for the octa- CDD/CDF.

7.10. Recovery Standard Solution

This tetradecane solution contains two recovery standards ($^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$ -1,2,3,7,8,HxCDD). An appropriate volume of this solution is spiked into each sample extract before the final concentration step.

7.11. Cleanup Recovery Standard Solution (CRS)

Prepare $^{37}Cl_4$-2,3,7,8-TCDD at the concentration shown in Table 2, in isooctane (or toluene).

7.12. Preparation and QC of PUF material

7.12.1. The PUF material is purchased pre-cut.

7.12.2. The PUFs are rinsed by Soxhlet with acetone (or other appropriate solvent) for a minimum of 16 hours and air dried for a minimum of 2 hours in a contaminant-free area.

7.12.3. One PUF from the rinsed batch is randomly selected to be the QC sample for the batch.

7.12.4. The PUF is loaded into a pre-cleaned Soxhlet extractor charged with toluene.

7.12.5. The 1613/8290 daily internal standard solution is spiked into the PUF and it

is extracted for a minimum of 16 hours.

7.12.6. The Soxhlet extract is recovered and processed according to Section 11.4.

7.12.7. The batch of PUF is considered acceptable if no target analytes are detected at or above the laboratory or project specific reporting limit.

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1. The sample collection, shipping, handling, and chain-of-custody procedures are not described in this document. Sample collection personnel will, to the extent possible, homogenize samples in the field before filling the sample containers. This should minimize or eliminate the necessity for sample homogenization in the laboratory. The analyst should make a judgment, based on the appearance of the sample, regarding the necessity for additional mixing. If the sample is clearly non-homogeneous, the entire contents should be transferred to a glass or stainless steel pan for mixing with a stainless steel spoon or spatula before removal of a sample portion for analysis.

8.2. Grab and composite samples must be collected in glass containers.

8.3. Ambient air samples are collected on a Quartz Fiber Filter followed by a glass sleeve containing a polyurethane foam plug.

8.4. Samples from stationary sources are collected on glass or quartz fiber filters and XAD-2 Resin. (See WS-ID-0009 for sample preparation procedures).

8.5. Conventional sampling practices must be followed. Do not rinse the bottle with sample before collection. Sampling equipment must be free of potential sources of contamination.

8.6. Grinding or blending of fish samples.

If not otherwise specified by the client, the whole fish (frozen) should be blended or ground to provide a homogeneous 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 by the client. If so requested by the client, the above whole fish requirement is superseded. More detail can be found in "Tissue Sampling and Handling for a variety of Methods" (WS-WI-0018).

Warning: Hearing protection must be worn when grinding samples.

8.7. With the exception of the fish tissues, which must be stored at - 20°C, all samples should be stored at 4°C ± 2, extracted within 30 days and completely analyzed within 45 days of collection. The 30 day hold time is recommended. PCDDs and PCDFs have demonstrated stability for greater than one year.

8.8. All extracts must be stored capped, in the dark, at room temperature (approximately 21°C to 28°C). All extracts for method 8290A must be stored capped at ≤ 6ºC.

8.9. For moisture determinations refer to SOP WS-OP-0013.

9. QUALITY CONTROL

9.1. One method blank (MB) must be extracted with every process batch of similar matrix, not to exceed twenty (20) samples. The method blank is an aliquot of laboratory matrix (reagent water, sodium sulfate, PUF, XAD, filter, etc.) processed in the same manner and at the same time as the associated samples. Corrective actions must be documented on a Non-Conformance memo, then implemented when target analytes are detected in the method blank above the reporting limit or when surrogate recoveries are outside control limits. Re-extraction of the blank, other batch QC, and the affected samples are required when the method blank is deemed unacceptable. The method blank contains a PUF plug, XAD, or filter prepared from the same batch as the field samples whenever possible for air samples.

Certain programs, such as DOD, may require a more stringent evaluation of the method blank, for instance, that the blank not contain any analytes of interest at a concentration greater than ½ the lower calibration limit.

Note: Re-extraction of the blank, QC and affected samples for the air matrices (PUF, XAD, and filter) is not generally possible because the entire sample is consumed in the initial extraction.

9.1.1. If the accompanying samples are aqueous, use distilled water as a matrix. Take the method blank through all steps detailed in the analytical procedure.

9.1.2. Use sodium sulfate as the method laboratory matrix when solids are extracted. Use a mixture of sodium sulfate and canola oil as the matrix when tissues are extracted. Take the method blank through all steps detailed in the analytical procedure.

9.1.3. The method blank must be spiked prior to extraction with the same amount of ^{13}C -labeled internal standards as added to samples.

9.1.4. If method blank contamination is present, check solvents, reagents, fortification solutions, apparatus and glassware to locate and eliminate the source of contamination before any further samples are extracted and analyzed. The presence of any analyte in the method blank ate concentrations greater than the reporting limit (RL) is cause for corrective action.

9.1.4.1. OCDD is a ubiquitous laboratory contaminant. A method blank and the associated samples are deemed acceptable if the OCDD

concentration is <5x the specified reporting limit. Flag data appropriately. The analyst is expected to investigate and eliminate potential sources of systematic contamination.

9.1.4.2. If a target analyte is detected in the blank but the associated samples are ND (not detected), then the data may be reported, unless otherwise directed by the client. Note the action in the narrative.

9.1.4.3. If a target analyte is detected in the blank, but the concentration of the contaminant in the samples >10x the blank concentration, then the data may be reported, unless otherwise directed by the client. Note the action in the narrative.

9.1.4.4. If one of the conditions above is not met then the sample associated with a contaminated method blank must be re-extracted.

9.1.5. If new batches of reagents or solvents contain interfering contaminants, purify or discard them.

9.2. A Laboratory Control Sample (LCS) must be extracted with every process batch of similar matrix, not to exceed twenty (20) samples. The LCS is an aliquot of laboratory matrix (e.g. water, sodium sulfate, PUF, XAD, etc.) spiked with analytes of known identity and concentration. The LCS must be processed in the same manner and at the same time as the associated samples. Corrective actions must be documented on a Non-Conformance memo, then implemented when recoveries of any spiked analyte is outside control limits provided on the LIMS or by the client. Re-extraction of the blank, other batch QC and all associated samples are required if the LCS is deemed unacceptable. See policy WS-PQA-003 for specific acceptance criteria. When associated with PUF samples, the LCS should contain a PUF plug prepared from the same batch as the field samples whenever possible.

Note: Re-extraction of the blank, QC and affected samples for the air matrices (PUF, XAD, and filter) is not generally possible because the entire sample is consumed in the initial extraction.

9.2.1. A LCS is deemed acceptable if control analytes are above control limits and the associated samples are ND, unless otherwise specified by the client. Note any actions in the narrative.

9.3. The assessment of matrix effects on method performance, as required by NELAP, is met in Method 8290 and 8290A, as in all isotope dilution techniques, with the use of isotopically labeled compounds. These isotopically labeled compounds are analogs of target analytes and are spiked into each sample. Therefore, matrix effects on method performance may be judged by the recovery of these analogs. Sample analysis

acceptance is controlled by the performance of these analogs in each sample. A Matrix Spike/Matrix Spike Duplicate (MS/MSD or MS/SD) pair are extracted at the client's request only. Method 8290A does not address analysis of MS/MSD. An exception to this rule is a batch containing South Carolina samples for Method 8290. These batches must have an MS/MSD prepared. However, South Carolina requires Method 8290A after December 31, 2008. An MS/MSD pair are aliquots of a selected field sample spiked with analytes of known identity and concentration. When requested by the client, the MS/MSD pair shall be processed in the same manner and at the same time as the associated samples. Corrective actions must be documented on a Non-Conformance memo, then implemented when recoveries of any spike analyte is outside control limits provided on the LIMS or by the client. Re-extraction of the blank, the LCS, the selected field sample, and the MS/MSD may be required after evaluation and review. Matrix Spike/ Matrix Spike Duplicates are not generally applicable for air samples due to the difficulty in collecting identical or representative samples. An LCS/LCSD may be extracted to show precision of the extraction and analysis process.

9.3.1. Matrix Spike (MS): A sample, which is spiked with a known amount of the matrix spike fortification solution prior to the extraction step. The recoveries of the matrix spike compounds are determined; they are used to estimate the effect of the sample matrix upon the analytical methodology.

9.3.2. Matrix Spike Duplicate (MSD): A second portion of the same sample as used in the matrix spike analysis and which is treated like the matrix spike sample.

9.3.3. Locate the sample for the MS and MSD analyses (the sample may be labeled "double volume").

9.3.4. Add an appropriate volume of the matrix spike fortification solution, adjusting the fortification level as specified in Table 1, under IS Spiking Levels.

9.3.5. The results obtained from the MS and MSD samples (percent recovery and concentrations of 2,3,7,8-substituted PCDDs/PCDFs) should agree within 20 percent relative difference. Report all results and flag outliers.

9.3.6. Internal standard recoveries are flagged if they are outside the recovery goals. Re-extraction of affected samples should be performed if signal-to-noise for any internal standard is less than 10:1.

9.4. Duplicates

9.4.1. Upon client request, duplicates may be processed. Locate the sample specified for duplicate analysis, and prepare and analyze a second 10-g soil or sediment sample portion or 1 L water sample, or an appropriate amount of

the type of matrix under consideration. Duplicate samples are not generally applicable for air samples due to the difficulty in collecting identical or representative samples. A duplicate injection of a sample extract may be performed to display instrument precision.

9.4.1.1. 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. Report all results and flag outliers.

9.4.2. Internal standard recoveries are flagged if they are outside the recovery goals. Re-extraction of affected samples should be performed if signal-to-noise for any internal standard is less than 10:1.

9.5. Field Blanks

9.5.1. Each batch of samples may contain a field blank sample of nominally uncontaminated soil, sediment or water that is to be processed for analysis.

9.5.1.1. Weigh a 10-g portion or use 1 L (for aqueous samples) of the specified field blank sample and add the appropriate amount of internal standard to yield 100 pg/µL in the final extract.

9.5.1.2. Extract by using the procedures described in Section 11. As applicable, add the appropriate amount of recovery standard to yield 100 pg/µL in the final extract. Analyze a 1-2 µL aliquot of the concentrated extract using SOP WS-ID-0005.

9.6. Rinsate Samples

9.6.1. In addition to the field blank, a batch of samples may include a rinsate, which is a portion of the solvent (usually trichloroethylene) that was used to rinse sampling equipment. The rinsate is analyzed to assure that the samples were not contaminated by the sampling equipment.

9.6.2. The rinsate sample must be processed like a regular sample.

Take a 100-mL (± 0.5 mL) portion of the sampling equipment rinse solvent (rinsate sample), filter, if necessary, and add the appropriate amount of internal standard to yield 100 pg/µL in the final extract.

9.6.3. Using appropriate methods, concentrate to approximately 10 mL.

9.6.4. Just before analysis, add the appropriate amount of recovery standard to yield 100 pg/µL in the final extract. Reduce the volume to a final volume of 20 µL, as necessary. No column chromatography is required.

9.6.5. Analyze an aliquot following the same procedures used to analyze samples.

9.7. Surrogate/Clean Up Recovery Standard

A surrogate compound may be spiked into all air media samples prior to collection. For all other matrices, a clean up recovery standard is spiked following extraction and just prior to cleanup, in order to monitor relative loss of internal standard during both extraction and cleanup.

9.8. Internal Standards

An internal standard is a ^{13}C -labeled analog of a PCDD/PCDF congener. Internal standards are added to all samples including method blanks and quality control samples before extraction, and they are used to quantitate the concentration of the analytes. Nine internal standards are used in this method. There is one for each of the dioxin and furan homologs (except for OCDF) with the degree of chlorination ranging from four to eight. Additional internal standards may be added to act as retention time references, but they are not used for quantitation.

9.8.1. A 2000 pg aliquot of the internal standard mixture is added to all samples, regardless of sample size. As an example, for $^{13}C_{12}$ -2,3,7,8-TCDD, a 10-g soil sample requires the addition of 2000 pg of $^{13}C_{12}$ -2,3,7,8-TCDD to give the requisite fortification level.

9.8.2. Internal standards must be spiked into all samples, QC samples, and included in all calibrations.

9.8.3. For each sample and QC aliquot, calculate the percent recovery. The percent recovery should be between 40 percent and 135 percent for all nine internal standards.

9.8.4. A low or high percent recovery for a blank does not require discarding the analytical data but it may indicate a potential problem with future analytical data. Internal standard recoveries are flagged if they are outside the recovery goals. Re-extraction of affected samples should be performed if signal-to-noise for any internal standard is less than 10:1.

9.9. Recovery Standard: Two recovery standards are used to determine the percent recoveries for the internal standards. The $^{13}C_{12}$ -1,2,3,4-TCDD is used to measure the percent recoveries of the tetra- and pentachlorinated internal standards while $^{13}C_{12}$ -1,2,3,7,8,9-HxCDD is used to determine the recovery of the hexa-hepta- and octachlorinated internal standards. $^{13}C_{12}$ -1,2,3,7,8,9-HxCDD also acts as a retention time reference for the unlabeled analog present in sample extracts. They are added to the final sample extract before HRGC/HRMS instrument analysis.

9.10. Recommended Corrective Actions and Troubleshooting Steps

- Verify satisfactory instrument performance.
- If possible, verify that no error was made while weighing the sample aliquots.
- Review the analytical procedures with the performing laboratory personnel.

10. CALIBRATION

10.1. On a daily basis, calibrate any balance to be used in accordance with SOP WS-QA-0041.

10.2. On a monthly basis, calibrate any autopipettor to be used in accordance with SOP WS-QA-0004.

11. PROCEDURE

11.1. One time procedural variations are allowed only if deemed necessary in the professional judgment of the supervisor to accommodate variation in sample matrix, radioactivity, chemistry, sample size, or other parameters. Any variation in procedure shall be completely documented using a Nonconformance Memo and is approved by a Technical Specialist and QA Manager. If contractually required, the client shall be notified. The Nonconformance Memo shall be filed in the project file.

Any unauthorized deviations from this procedure must also be documented as a nonconformance, with a cause and corrective action described.

11.2. Refer to SOP WS-ID-0009 for the preparation of stationary source samples.

11.3. Sample Pre-Treatment

11.3.1. Paper Pulp Sludges are generally air-dried and ground prior to extraction following Section 11.5. Because of the drying procedure, a Dean-Stark water separator is optional for extraction.

11.3.2. Fly Ash — Fly ash samples are pretreated with HCl prior to extraction by both soxhlet and separatory funnel techniques.

11.3.2.1. Weigh 2-10g of sample aliquot into a clean glass jar.

11.3.2.2. Add 1.0mL of the internal standard mixture with 2 mL of acetone.

11.3.2.3. Add 150 mL of 1N hydrochloric acid and shake for 4 hours.

11.3.2.4. If the sample reacts violently with acid, then allow the sample to equilibrate for 4 hours with no shaking.

11.3.2.5. Filter the contents of the jar through a glass fiber filter.

11.3.2.6. Extract the solids as per Section 11.5, omitting the daily internal standard spike for the samples.

11.3.2.7. Extract the aqueous filtrate as per Section 11.8, using 100 mL of toluene for the first shake, and 100 mL of hexane for subsequent shakes.

11.3.2.8. Concentrate the combined toluene solutions to near dryness on a rotary evaporator at 50°C. Proceed with Section 11.12 as necessary.

Note: As an option, a Soxhlet/Dean Stark extractor system may be used, with toluene as the solvent. No sodium sulfate is added when using this option.

11.4. Waste Dilution (Still-Bottom/Fuel Oil, and other solvent-miscible materials).

11.4.1. Weigh 1 g of the waste (organic liquids, fuel oils, and solids that will dissolve in a solvent) into a vial.

11.4.2. Add 40 mL of toluene (or other solvent if the material is not miscible/soluble in toluene). Shake gently to dissolve.

11.4.3. Remove a 4.0 mL aliquot (0.1g sample equivalent) and place in a culture tube. Add 1.0 mL of daily internal standard and 1.0 mL of cleanup recovery standard, and proceed to Section 11.12.

11.5. Soxhlet Extraction (Solids, Tissues, Sludges, Wipes)

11.5.1. Pre-extract the glassware by heating the flask until the toluene is boiling. When properly adjusted, 1-2 drops of toluene per second will fall from the condenser tip into the receiver. Extract the apparatus for a minimum of four hours.

WARNING: Open the chiller supply valves about 15 minutes before turning on the heating element and ensure that all of the condensors are cold before you turn the heating element on. Check all of the condensors about 15 minutes after starting the heating process to ensure that they are still cold and functioning properly. If this cleaning cycle is to be left unattended (e.g., overnight) the first chemist to arrive the next morning is to check all condensers to ensure that they are still cold and functioning properly.

11.5.2. After pre-extraction, cool and disassemble the apparatus.

11.5.3. If tissues requiring % Lipids are to be extracted, for each sample weigh the concentration vessel with label and boiling chips. Record the mass on the benchsheet. Refer to SOP WS-QA-0018 “Subsampling”, for instructions on how to homogenize and subsample the container of sample.

11.5.4. Weigh a well-mixed aliquot of each sample (10 g, unless otherwise specified) into a clean Soxhlet thimble. Record the mass to the nearest 0.01g. Use sodium sulfate for the batch QC (MB, LCS) for solids, and a mixture of 9 g sodium sulfate and 1 g canola oil for the batch QC for tissue matrices.

11.5.4.1. In the case of wipes, place the entire wipe sample into the Soxhlet apparatus (no thimble needed), including any liquid present with the sample. Use pre-cleaned wipes for the batch QC samples.

11.5.5. Place the thimble into a Soxhlet apparatus equipped with a Dean-Stark water separator.

11.5.6. Spike all samples with 1.0 mL of internal standard solution (2 pg/µL), for a final concentration of 200 pg/g (based on a 10 g sample).

11.5.7. Spike the LCS (and MS/MSD, if present) with 50 uL of native spike.

11.5.8. Reassemble the pre-extracted apparatus and add a fresh charge (250-300 mL) of toluene to the receiver and reflux flask.

11.5.9. Reflux 16 hours, with the solvent cycling at least 5 times per hour.

WARNING: Open the chiller supply valves about 15 minutes before turning on the heating element and ensure that all of the condensors are cold before you turn the heating element on. Check all of the condensors about 15 minutes after starting the heating process to ensure that they are still cold and functioning properly. If this cleaning cycle is to be left unattended (e.g., overnight) the first chemist to arrive the next morning is to check all condensers to ensure that they are still cold and functioning properly.

11.5.10. Drain the water from the receiver if the receiver fills with water. Check and drain when necessary.

Note: If the receiver holds 10 mL of liquid, and 20 g of an approximately 10% solid sample is being extracted, then approximately 9 mL of water will end up in the receiver. In this case, the receiver will not need to be emptied (insufficient liquid to overflow), but it should be checked. If the sample amount is 50, and the percent solids is still 10%, then 45 mL of water will end up in the receiver. In this case, frequent checking is required, and the receiver will need to be emptied at least 5 times.

11.5.11. After refluxing, allow the apparatus to cool.

11.5.12. If samples DO NOT require % lipids add 100 µL of tetradecane as a keeper to the round bottom flask.

11.5.13. Proceed to Section 11.17.

11.6. SoxTherm Extraction (Solids, Tissues, Sludges,Wipes)

11.6.1. Prior to loading samples, run the system through 2 cleaning cycles (approximately 1 hour each).

11.6.2. After pre-extraction, cool and disassemble the apparatus.

11.6.3. Weigh a well-mixed aliquot of each sample (10 g, unless otherwise specified) into a clean Soxhlet thimble. Record the mass to the nearest 0.01g. Use sodium sulfate for the batch QC (MB, LCS) for solids, and a mixture of 9 g sodium sulfate and 1 g canola oil for the batch QC for tissue matrices.

11.6.3.1. In the case of wipes, place the entire wipe sample into the Soxhlet apparatus (no thimble needed), including any liquid present with the sample. Use pre-cleaned wipes for the batch QC samples.

11.6.4. Place the thimble into the Soxtherm apparatus.

11.6.5. Spike all samples with 1.0 mL of internal standard solution (2 pg/µL), for a final concentration of 200 pg/g (based on a 10 g sample).

11.6.6. Spike the LCS (and MS/MSD, if present) with 50 uL of native spike.

11.6.7. Reassemble the pre-extracted apparatus and add a fresh charge (150 mL) of toluene to the apparatus.

11.6.8. Program the system to boil for 1 hour, and reduce the toluene volume by 70-90 mL (volume < volume of the thimble).

11.6.9. Continue the extraction for one hour fifteen minutes, reducing the toluene volume by another 15 mL.

11.6.10. After refluxing, allow the apparatus to cool.

11.6.11. Pour the samples into round bottom flasks, and if samples DO NOT require % lipids add 100 µL of tetradecane as a keeper to the round bottom flask.

11.6.12. Proceed to Section 11.17.

11.7. Extract Splitting (Wipes)

Wipe extracts prepared using either Soxhlet or shaking techniques are split prior to further workup, to permit an archive aliquot, or analysis by an additional method.

Once the extract has been concentrated using the rotovap or Turbovap, proceed as follows:

11.7.1. Add approximately 1 mL of hexane or toluene to rinse the sides of the round bottom flask. Using a pipette, withdraw the sample from the round bottom flask and transfer the liquid into a test-tube. Use additional amounts of solvents to rinse the flask. Transfer all the liquid into the test-tube. Ensure that all traces of sample in the round bottom flask have been thoroughly rinsed from all surfaces. Bring the sample volume to 8.0 mL or 10.0 mL (or appropriate volume) with the addition of rinse solvent.

11.7.2. Upon completion of the rinsing, cap the test tube and shake vigorously. Take ½ of each sample (or an appropriate amount as instructed by the client, program manager or department manager) and transfer to a culture tube. Archive the remaining sample for future use.

11.7.2.1. If only one analysis is required, then ½ of the sample is archived and the other half is analyzed.

11.7.2.2. If "N" analyses are required, then the extract is divided into "N+1" equal portions, so that one portion is archived, and a portion is used for each test.

11.8. Aqueous Samples (liquid/liquid extraction).

11.8.1. When setting up the glassware for a batch, for each sample label one separatory funnel and one 500 mL round-bottom flask with the sample ID.

11.8.2. Weigh the sample in the bottle on the top loading balance to the nearest centigram (0.01g), and record the mass.

11.8.3. For each sample, add 1 mL of daily internal standard solution into 2 mL of acetone. Add this solution to the sample in the separatory funnel. Each aliquot of spike mixture is added similarly.

11.8.4. Dissolve 50µL of the target analyte into acetone and add this mixture into the LCS container.

11.8.5. Pour the entire sample (approximately 1L) into a 2L separatory funnel that is labeled with the sample ID.

11.8.6. Add 100 mL methylene chloride to the sample bottle, seal, and shake for 30 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel.

11.8.7. Create a blank and LCS by adding 1 L of laboratory reagent water to 2

additional separatory funnels. Add 100 mL methylene choride to each funnel.

11.8.8. To the LCS, add 50 µL of the precision and recovery standard dissolved into 2 mL of acetone.

11.8.9. Extract the samples by shaking each funnel for two minutes with periodic venting.

Warning: Separatory funnel extraction with methylene chloride is a high-risk activity. Pressure may build rapidly in the funnel. It should be vented after several seconds of shaking, and often enough to prevent build-up of pressure. Chemist performing separatory funnel extraction must wear a face shield over their safety glasses/goggles. Alternatively, the extraction can be performed behind a closed fume hood sash.

11.8.10. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation.

11.8.11. Repeat the extraction two additional times with methylene chloride.

11.8.12. Determine the original sample volume by re-weighing the sample bottle. Record the sample volume to the nearest centigram (0.01g).

11.8.13. Dry extract with sodium sulfate: Place glass wool in a precleaned filter funnel. Rinse glass wool with methylene chloride and load funnel with Na_2SO_4. Pour extract through Na_2SO_4 to remove water. Rinse Na_2SO with fresh methylene chloride and collect in round bottom flask.

11.8.14. Transfer the extract to a 500 mL round-bottom previously labeled with the sample ID, then add approximately 100 µL of tetradecane and concentrate on a rotary evaporator or TurboVap.

11.8.15. Perform macro-concentration as detailed in Section 11.17.

11.9. Aqueous Samples (solid phase extraction).

11.9.1. Weigh the sample in the bottle on the top loading balance to the nearest centigram (0.01g), and record the mass.

11.9.2. Create a blank and LCS by adding 1L of laboratory reagent water to 2 additional 1L bottles.

11.9.3. For each sample, add 1mL of daily internal standard solution in acetone.

Add this solution to the sample in the bottles. Each aliquot of spike mixture is added similarly.

11.9.4. To the LCS, add 50µL of the precision and recovery standard in acetone.

11.9.5. Prepare the C18 extraction discs by first soaking them in toluene for at least 5 minutes.

11.9.6. Assemble the filter holder and vacuum filtration flask and place the extraction disc onto the filter holder. Place a GF-F filter on top of the extraction disc. If the sample has a large amount of particulates a GF-D filter can be placed on top of the GF-F filter. Alternatively, a GMF-150 filter can be used in place of the two filters.

11.9.7. Place the filtering funnel onto the disc holder and clamp it in place.

11.9.8. Rinse the filter and discs with approximately 15mL of toluene and allow it to soak for about a minute. Apply vacuum and draw the toluene through the discs. Repeat the wash step using about 15mL of acetone. Apply vacuum and draw the acetone through the discs.

11.9.9. Rinse the filter and discs with approximately 15mL of methanol and allow it to soak for about a minute. Apply vacuum and draw the methanol through the discs, but **DO NOT ALLOW THE DISCS TO GO DRY**. If they do go dry, simply repeat the methanol rinse step, leaving a 1 – 2mm layer of solvent on top of the discs.

11.9.10. Rinse twice with about 50mL of reagent water, leaving a 1 – 2mm layer of water on the surface of the discs.

11.9.11. Pour the spiked method blank, LCS or sample into the reservoir and apply vacuum to begin the extraction. Adjust the vacuum such that the extraction takes approximately 10 minutes. Samples with large amounts of particulates may take much longer.

11.9.12. After most of the sample has been pulled through the discs, rinse the sample bottle with a few mLs of reagent water and add the rinse to the funnel. Rinse down the sides of the funnel with reagent water as well.

11.9.13. Allow the discs to dry, remove them from the holder and extract by soxhlet (11.5) or soxtherm (11.6) and proceed with cleanups.

11.9.14. Determine the original sample volume by re-weighing the sample bottle. Record the sample volume to the nearest centigram (0.01g).

11.10. Breaking Emulsions

There are several useful methods to decrease or eliminate emulsion in aqueous samples when extracting with methylene chloride. These methods may include stirring with a pipette to manually breakup the emulsions or to transfer the sample into centrifuge tubes and centrifuge at approximately 3000 RPM. The most useful method is to use a 10:1 $NaOH/H_2O$ solution to change the pH enough to disrupt the emulsion phase, which works 90% of the time. See Section 7.3.5 for reagent preparation.

11.10.1. Check the pH of the sample to verify that the pH is between 3 and 7. If the pH is greater than 7, consult the supervisor and client for instructions.

11.10.2. Pour approximately 100 mL of the 10:1 $NaOH/H_2O$ into a 1 L amber glass bottle (AGB).

11.10.3. Drain the sample with the emulsion from the 2 L separatory funnel into the 1 L AGB and let it stand.

11.10.4. Empty the aqueous waste into the LLE waste drum.

11.10.5. Pour the solution with methylene chloride back into the same 2 L separatory funnel and drain the methylene chloride phase through Na_2SO_4 into a 500 mL round-bottom flask.

11.10.6. Empty the aqueous waste into the LLE waste drum.

11.10.7. Proceed with macro-concentration (Section 11.17).

11.11. Filter/PUF Samples

11.11.1. Place the glass sleeve containing the PUF and the Quartz Fiber Filter into the pre-cleaned Soxhlet extractor charged with toluene.

11.11.2. Add 2 mL (4000 pg) of 1613/8290 daily Internal Standard solution to all samples and QC.

11.11.3. Add 50 uL of 1613/8290 Native Spike to the LCS.

11.11.4. Extract the samples and QC for a minimum of 16 hours.

11.11.5. Concentrate the extract from the round bottom flask with hexane and adjust the volume.

11.11.6. Transfer the extract from the round bottom flask with hexane and adjust the volume.

11.11.7. Split the extract 50:50 for analysis and archive.

11.11.8. Proceed to Section 11.12.

11.12. Extract Clean-Up

11.12.1. For all samples that are not air media, spike 1.0 mL of the Cleanup Recovery Standard (CRS) prior to any cleanup into the round bottom flasks containing the samples and QC Extracts (See also Section 9.7).

11.12.2. Proceed with further cleanups as dictated by the sample matrix and extract color. The "Option C" cleanup (Section 11.13) and the IFB Upper Column cleanup (Section 11.14) are applied to samples with high levels of interferences. The IFB column cleanup (Section 11.15) is applied to all samples.

11.13. Acid Partitioning ("Option C")

11.13.1. Use this clean up as needed on samples with high levels of interferences. Consult with a lead chemist or department manager to determine applicability.

11.13.2. Partition the extract in 50-125 mL of hexane against 40 mL concentrated H_2SO_4 in a separatory funnel. Shake for two minutes. Remove and discard the H_2SO_4 layer (bottom). Repeat the acid washing until no color is visible in the acid layer (perform a maximum of four acid washings).

Warning: Shaking with a concentrated caustic is a high-risk activity. Analyst must wear a face shield over safety glasses/goggles, or the shaking must take behind a closed hood sash.

11.13.3. Partition the extract against 50 mL of distilled H_2O. Shake for two minutes. Remove and discard the aqueous layer (bottom). Dry the extract by pouring it through a funnel containing anhydrous sodium sulfate and collect it in a round-bottom flask. Rinse the sodium sulfate with two 15 mL portions of hexane, add the rinsates to the flask, and concentrate the hexane solution to near dryness on a rotary evaporator (35°C water bath), making sure all traces of toluene (when applicable) are removed. (Use of blow-down with an inert gas to concentrate the extract is also permitted.) The DI H_20 partition is applied only as samples warrant it at the discretion of the analyst.

11.14. IFB Upper Column Cleanup

11.14.1. Use this clean up as needed on samples with high levels of interferences. Consult with a lead chemist or department manager to determine applicability.

11.14.2. Set up the upper of the two chromatography columns as depicted in Figure 2.

The column (20 mm diameter) is packed in this order: a glass wool plug, 2 g activated silica gel, 4 g Acid silica gel, 2 g activated silica gel, and 1 g sodium sulfate.

11.14.3. Pre-rinse the column with 20 mL hexane, and discard the rinsate.

11.14.4. Add extract to the column. Rinse extract vessel 2 times with 1 mL each of hexane and add to column.

11.14.5. Elute 60 mL hexane directly onto acid silica column (upper column).

11.14.6. Collect the eluate, and concentrate before proceeding with the IFB cleanup (Section 11.15).

11.15. IFB Column Cleanup

Most samples will undergo this cleanup, either direction following concentration on the rotovap, or following the cleanup in Section 11.13 (Option C) or Section 11.14 (IFB Upper Column).

11.15.1. Set up two chromatography columns as depicted in Figure 2. The upper column (20 mm diameter) is packed in this order: a glass wool plug, 2 g activated silica gel, 4 g Acid silica gel, 2 g activated silica gel, and 1 g sodium sulfate. The lower column (15 mm diameter) is packed in this order: a glass wool plug, 6 g acid alumina, and 1 g sodium sulfate.

11.15.2. Pre-rinse each column with 20 mL hexane, and discard the rinsate.

11.15.3. Put one column above the other.

11.15.4. Add extract to the top column (silica column). Rinse extract vessel 2 times with 1 mL each of hexane and add to column.

11.15.5. Elute 60 mL hexane directly onto acid silica column (upper column).

11.15.6. Discard upper column.

11.15.7. Elute lower column with 10 mL of 20% methylene chloride/hexane. Discard in proper waste stream.

11.15.8. Elute lower column with 30 mL of 65% methylene chloride/hexane. Save and collect in culture tube.

11.15.9. Proceed with additional cleanups as necessary.

11.16. Carbon Column Clean-up (D2 Column)

Prepare an activated Carbon & Silica Gel column as described in below. Refer to the diagram in Figure 3 as well.

11.16.1. Push a glasswool plug down to the 3 inch mark in a pre-cut D2 column.

11.16.2. Add 1 g of 5% activated carbon/silica. Top with a glasswool plug.

11.16.3. With the column oriented with "A" on the top (and the carbon on the lower end of the column), pre-elute with 5 mL 1:1 methylene chloride :cyclohexane.

11.16.4. Discard pre-eluates.

11.16.5. Invert the column so that the column is oriented with the "B" on the top and pre-elute with 3 mL of 1:1 methylene chloride.

11.16.6. Dilute the extract to 1 mL with hexane and transfer to the column (still oriented in the "B" direction).

11.16.7. Rinse sample vial onto the column with 2 x 2 mL 1:1 methylene chloride:cyclohexane.

11.16.8. Elute with 6 mL 1:1 methylene chloride :cyclohexane

11.16.9. Elute with 5 mL 75:25 methylene chloride:methanol

11.16.10. Discard eluates.

11.16.11. Turn the column over (so that the "A" end is on top), and elute with 30 mL of toluene. Collect this eluate.

11.16.12. Concentrate to NEAR dryness using the Rotovap (Section 11.17) or Turbovap (Section 11.18), then proceed to the recovery standard step (Section 11.19).

11.17. Macro-concentration (Rotary Evaporator)

Concentrate the extracts in separate round bottom flasks on rotary evaporator.

11.17.1. Assemble the rotary evaporator according to manufacture's instructions, and warm the water bath. On a daily basis, preclean the rotary evaporator by solvent rinsing. Between samples, 2-3 mL rinses of toluene followed by a 2-3 mL rinse of hexane should be rinsed down the feed tube into a waste beaker.

Rotovap Conditions		
Solvent	**Bath Temperature (C)**	**Vacuum Setting (PSI)**
Toluene	80	25
Hexane	65	15
Methylene Chloride	70	No vacuum applied

11.17.2. Attach the round bottom flask containing the sample extract to the rotary evaporator. Slowly apply vacuum to the system, and begin rotating the sample flask.

11.17.3. Lower the flask into the water bath and adjust the speed of rotation and the temperature as required. At the proper rate of concentration, the flow of solvent into the receiving flask will be steady, but no bumping or visible boiling of the extract will occur.

NOTE: If the rate of concentration is too fast, analyte loss may occur.

11.17.4. For samples requiring % Lipids analysis:

11.17.4.1. Concentrate until the toluene has been completely removed. Add approximately 25 mL hexane and concentrate to ensure that only the lipids are present.

11.17.4.2. Dry the concentration vessel and let stand at room temperature. Weigh the vessel and record on the benchsheet.

11.17.4.3. Calculate % lipids as follows:

$$\%\ \text{Lipids} = \frac{\text{Final Vessel Mass - Initial Vessel Mass}}{\text{Sample Size}} \times 100\%$$

11.17.5. Proceed to extract cleanups, or transfer to a micro concentration vial for the recovery standard step (Section 11.19).

11.18. Micro-concentration (Turbovap)

Concentrate the extracts in 35 mL culture tubes in a turbo-evaporator. The turbo-evaporator model that the laboratory uses can hold up to 50-35 mL culture tubes. Other turbo-evaporator models can be used that may or may not have the same culture tube sizes and/or capacity. Adjust temperature according to solvent (65ºC for toluene and 45ºC for hexane or hexane/ methylene chloride mixtures)

11.18.1. The evaporating times are dependent on sample volume and solvent. The following are examples and can change from sample to sample. Each sample should be checked in intermittent intervals to make sure samples do not go dry.

11.18.2. When evaporating 30 mL toluene, it will normally take approximately 30-50

minutes with the temperature setting described above.

11.18.3. When evaporating 30 mL hexane/ methylene chloride, it will normally take approximately 20-30 minutes with the temperature setting described above.

11.18.4. For samples requiring % Lipids analysis refer to Section 11.17.4.

11.18.5. Proceed to extract cleanups, or transfer to a micro concentration vial for the recovery standard step (Section 11.19).

11.19. Recovery Standard

11.19.1. Transfer extracts to a micro concentration vial (test tubes and other small vessels may also be used)

11.19.2. With a stream of dry, purified nitrogen, reduce the extract volume to approximately 100 µL.

11.19.3. Add 20 µL of the recovery standard solution (Table 2).

11.19.4. With a stream of dry, purified nitrogen, reduce the extract volume to 20 µL.

11.19.5. Transfer the extract to an autoinjection vial and store in the dark at room temperature.

11.19.6. A smaller final volume can be used to decrease the detection limit upon client approval.

11.19.7. A larger final volume can be use to decrease potential matrix interferences, if the column and acid cleanups were unsuccessful.

11.20. Sample Dilution Procedure

11.20.1. Simple dilutions: Dilutions from 2X to 50X can be achieved without respiking the final extract. The calculation to determine the final extract concentration is as follows:

$$\text{Final Conc. of Extract} = \frac{(\text{Conc. of original extract}) \text{ x } (\text{Amount of aliquot taken})}{(\text{Volume of diluted extract})}$$

Ex:
$$\frac{(10\,\text{g}) \text{ x } (2\,\mu\text{L})}{(20\,\mu\text{L}) \text{ x } (100\,\mu\text{L})} = \frac{1\,\text{g}}{100\,\mu\text{L}} \text{ FV}$$

Record the final sample concentration on the extract label.

11.20.2. Complex dilution requiring respiking of IS and RS:

Dilutions greater than 50x must be done by diluting and respiking the extract with IS and RS. This procedure may require serial dilution to be performed. If this procedure is done, then the sample size must be adjusted to reflect the aliquot taken.

Ex. 100X dilution (original sample with 10 g/20 µL final volume)

Take a 2 µL aliquot (1/10 of original sample) and add 18 µL of solvent keeper. Take a 2 µL aliquot of the dilution (1/100 of the original sample), respike with 1 mL IS and 20 µL RS, reduced to 20 µL FV.

Record the final sample concentration of the extract label.

12. CALCULATIONS/DATA REDUCTION

12.1. Not applicable

13. METHOD PERFORMANCE

It must be documented that all applicable system performance criteria specified were met before analysis of any sample is performed. .

13.1. The group/team leader has the responsibility to ensure that this procedure is performed by an associate who has been properly trained in its use and has the required expertise.

13.2. Method Detection Limit

The laboratory must generate a valid method detection limit for each analyte of interest. The MDL must be below the reporting limit for each analyte. The procedure for determination of the method detection limit is given in 40 CFR Part 136, Appendix B, and further defined in SOP WS-QA-0006. MDLs are available in the Quality Assurance Department.

13.3. Initial Demonstration

The laboratory must make an initial demonstration of capability for each individual method. Demonstration of capability for both soil and water matrices is required. This requires analysis of QC check samples containing all of the standard analytes for the method. For some tests it may be necessary to use more than one QC check mix to cover all analytes of interest.

13.3.1. Four aliquots of the QC check sample are analyzed using the same procedures used to analyze samples, including sample preparation. The concentration of the QC check sample should be less than or equivalent to the LCS samples.

13.3.2. Calculate the average recovery and standard deviation of the recovery for each analyte of interest. Compare these to the laboratory generated QC Limits.

13.4. If any analyte does not meet the acceptance criteria the test must be repeated. Only those analytes that did not meet criteria in the first test need to be evaluated. Repeated failure for any analyte indicates the need for the laboratory to evaluate the analytical procedure and take corrective action.

14. POLLUTION CONTROL

It is TestAmerica's policy to evaluate each method and look for opportunities to minimize waste generated (i.e., examine recycling options, ordering chemicals based on quantity needed, preparation of reagents based on anticipated usage and reagent stability). Employees must abide by the policies in Section 13 of the Corporate Environmental Health and Safety Manual (CW-E-M-001) for "Waste Management and Pollution Prevention."

14.1. The use of Roto-vaps and Turbo-vaps rather than Kuderna-Danish reduction allows extraction solvents to be collected and disposed of rather than released to the atmosphere.

14.2. Toluene, which is a less hazardous solvent, has been substituted for benzene as an extraction solvent.

14.3. The use of SoxTherm extraction rather than soxhlet extraction, when appropriate, reduces the volume of solvent used.

14.4. Standards should be prepared in volumes consistent with laboratory use to minimize the volume of expired standards that must be discarded.

14.5. All waste will be disposed of in accordance with Federal, State, and Local regulations. Where reasonably feasible, technological changes have been implemented to minimize the potential for pollution of the environment.

14.6. Do not allow waste solvent to vent into the hoods. All solvent waste is stored in capped containers unless they are being filled.

14.7. Transfer waste solvent from collection cups (tri-pour and similar containers) to jugs and/or carboys as quickly as possible to minimize evaporation.

15. WASTE MANAGEMENT

Waste management practices are conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes are disposed of in an accepted manner. Waste description rules and land disposal restrictions are followed. Waste disposal procedures are incorporated by reference to SOP WS-EHS-0001. The following waste streams are produced when this method is carried out.

15.1. Extracted aqueous/leachate samples contaminated with methylene chloride are collected at the fume hood in a 5-gallon or smaller carboy. If the samples are not at a

neutral pH, add small quantities of sodium bicarbonate to bring the waste to neutral. Stir well. Once neutralized, immediately pour the carboy contents into a blue plastic LLE drum in the H3 closet. When full to between two and six inches of the top, or after no more than 75 days, move the LLE drum to the waste collection area for shipment.

15.2. Extracted soil samples and thimbles, extracted PUF filters, XAD-2 resin, paper funnel filters, glass wool, sodium sulfate, assorted disposable glassware, fish/crawfish or similar materials, silica gel, alumina, and carbon from column clean-ups, contaminated with various solvents and eluates. Dump the materials into a orange contaminated lab trash bucket. When the bucket is full or at the end of the day, tie the plastic bag liner shut and put the lab trash into the steel collection drum in the H3 closet. When the drum is full or after no more than 75 days, move it to the waste collection area for shipment.

15.3. Flammable solvent and methylene chloride waste generated during glassware and sodium sulfate cleaning. Solvent waste collected during roto-vap/turbo-vap reduction of extracted samples. Collect the waste solvents in tripours during use. Empty the tripours into a 1-liter to 4-liter carboy at the fume hood. When the carboy is full, or at the end of your shift, whichever comes first, empty the carboy into the steel solvent drum in the H3 closet. When full to between two and six inches of the top, or after no more than 75 days, move the steel drum to the waste collection area for shipment.

15.4. Assorted flammable solvents and methylene chloride waste generated during quartz fiber filter preparation, PUF adsorbent preparation, XAD-2 resin preparation, PUF/XAD-2 cartridge preparation, glassware rinsing and sodium sulfate pre-rinsing.. Waste solvents and methylene chloride collected during roto-rap/turbo-vap reduction of extracted samples. Collect the waste solvents in tripours during use. Empty the tripours into a 1-liter to 4-liter carboy at the fume hood. When the carboy is full, or at the end of your shift, whichever comes first, empty the carboy into the steel drum in the H3 closet. When the drum is full to between two and six inches of the top, or after no more than 75 days, move the steel drum to the waste collection area for shipment.

15.5. Contaminated sulfuric acid used during extract cleanup. Collect the used sulfuric acid in empty, 2.5-liter, plastic coated jars. When full or after one year, whichever comes first, transfer these jars to the waste collection area for shipment.

15.6. Contaminated distilled water used during extract cleanup. Collect the contaminated water in a 1-liter to 4-liter carboy at the fume hood. When the carboy is full, or at the end of your shift, whichever comes first, empty the carboy into the plastic LLE drum in the H3 closet. When full to between two and six inches of the top, or after no more than 75 days, move the plastic drum to the waste collection area for shipment.

16. REFERENCES/CROSS REFERENCES

16.1. SW846, Test Methods for Evaluating Solid Waste, Third edition, Update IV. Method 8290A Polychlorinated Dibenzodioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by high-Resolution Mass Spectrometry February 2007.

16.2. SW846, Test Methods for Evaluating Solid Waste, Third edition, Update III. Method 8290 Polychlorinated Dibenzodioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by high-Resolution Mass Spectrometry September 1994.

16.3. SW846, Test Methods for Evaluating Solid Waste, Third edition, Update III. Method 0023A, Sampling Method for Polychlorinated Dibenzo-p-dioxins and Polychlorinated Dibenzofurans Emissions from Stationary Sources. December 1996.

16.4. Compendium Method TO-9A “Determination of Polychlorinated, Polybrominated, and Brominated, Cholorinated Dibenxo-p-dioxins and Dibenzofurans in Ambient Air”, EPA compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air, second edition, January 1997.

16.5. Protocol for the Analysis of 2,3,7,8-TCDD by HRGC/HRMS". J. S. Stanley and T. M. Sack, EPA 600/4-86-004.

16.6. “Safety in Academic Chemistry Laboratories", American Chemical Society Publication, Committee on Chemical Safety (3rd Edition, 1979.)

16.7. “Carcinogens - Working with Carcinogens". Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control. National Institute for Occupational Safety and Health. Publication No. 77-206, August 1977.

16.8. "OSHA Safety and Health Standards, General Industry", (29 CFR 1910) Occupational Safety and Health Administration, OSHA 2206 (revised January 1976).

17. METHOD MODIFICATIONS

17.1. Deviations from EPA 8290 and 8290A.

17.1.1. Tetradecane instead of nonane is used as the final solvent to increase the stability of extracts and standards. Tetradecane is less volatile than nonane. Loss of analyte as a result of solvent incompatibility is monitored through recovery checks and calibration acceptance criteria.

17.1.2. Extract clean-ups are performed at the discretion of the analyst when interferences are observed. Then, the analyst should select the clean-up procedure appropriate to the interferent.

17.1.3. Section 7.4.6.4 of Method 8290 indicates that extracts should be transferred with hexane, then toluene. Toluene is used to transfer extracts to maintain compound solubility and minimize analyte loss.

17.1.4. Section 7.5.1.2 of Method 8290 specifies that a NaCl solution should be used for partitioning. Instead, the laboratory uses laboratory water only. NaCl is used to break up emulsions that may form. An analyst may use NaCl, NaOH, or any mechanical means to break up an emulsion.

17.1.5. Section 7.5.3 of Method 8290 specifies that hexane is used as a column elution solvent. The laboratory uses cyclohexane to achieve better and more reproducible separation of the target analyte from the interferent.

17.1.6. Carbon columns are packed with silica gel in place of celite. Elution solvents are changed accordingly. (SOP Section 11.4; Method 8290 Section 7.5.3.2, 8290A Section 7.3.6.).

17.2. Modifications from TO-9A method

17.2.1. Quartz Fiber Filters are cleaned by Soxhlet extraction with methylene chloride, not baked at 400 degrees C for 5 hours.

17.2.2. The PUF material may be pre-cleaned with methylene chloride or other appropriate solvent. The PUFs are not reused.

17.2.3. The $^{37}Cl_4$-2,3,7,8-TCDD surrogate is present at varying levels in the calibration curve (0.5-200 pg/ µL).

17.2.4. Samples are extracted with toluene not benzene.

17.2.5. Concentration is performed by rotary evaporation not Kuderna-Danish.

17.2.6. All cleanup procedures are optional and applied based on the analyst's discretion.

17.2.7. The laboratory uses 2 labeled recovery standard for the quantitation of labeled internal standards.

17.2.8. The final volume is adjusted to 20 µL in tetradecane.

17.2.9. Calibration and quantitation are performed in accordance to this SOP.

18. ATTACHMENTS

18.1. Table 1 - Types of Matrices

18.2. Table 2 - Composition of Sample Fortification and Recovery Standard Solutions.

18.3. Table 3 - The Seventeen 2,3,7,8-Substituted PCDD and PCDF Congeners

18.4. Figure 1 - Analysis Flowchart

18.5. Figure 2 — IFB column cleanup

18.6. Figure 3 — D2 Column cleanup

18.7. Appendix A - Periodic Wipe Test Performance

19. REVISION HISTORY

19.1. WS-IDP-0005, Revision 1.5, Effective 12/21/2012

19.1.1. Clarified extraction procedure by revising Section(s) 11.8.1- 11.8.4 and adding an extra extraction step (Section 11.8.3).

19.1.2. Editorial revisions. .

19.2. WS-IDP-0005, Revision 1.4, Effective 03/20/2012

19.2.1. Appended to Section 2.2: “This method can also use solid phase extraction (SPE), however, Test America West Sacramento is in the developmental stages for this extraction type and is not currently certified for its use.”

19.2.2. Editorial changes.

19.3. WS-IDP-0005, Revision 1.3., Effective 06/10/2011

19.3.1. Added Section 11.9: Aqueous Samples (Solid Phase Extraction).

19.3.2. Editorial revisions.

19.4. WS-IDP-0005, Revision 1.2, Effective 2/11/2011

19.4.1. Added benzene to Section 5.2 Table..

19.4.2. Editorial revisions.

19.5. WS-IDP-0005, Revision 1.1, Effective 2/12/2010

19.5.1. Section 11.2 – updated SOP reference from SAC-ID-0009 to WS-ID-0009.

19.5.2. Section 11.6.1 – changed: “Prior to loading samples, run the system through

a cleaning cycle (approximately 3 hours)" to "(approximately 1 hour)."

19.5.3. Section 11.6.8 – changed "…fresh charge (140 mL) of toluene…" to "…fresh charge (150 mL) of toluene….".

19.5.4. Section 11.16.1 – inserted in Table "No vacuum applied" under vacuum setting (PSI) for solvent Methylene chloride.

19.6. WS-IDP-0005, Revision 1, Effective 10/2/2008

19.6.1. Added 8290A references.

19.6.1.1. Extract and standard storage.

19.6.1.2. Removal of MS/MSD.

19.6.2. Updated to TestAmerica format.

19.6.3. Separated the analytical steps from the preparation steps, this SOP is concerned only with the sample preparation.

19.7. WS-ID-0005, Revision 6.7, Effective 8/21/2008

19.7.1. Changed the word "toluene" to "acetone" in 7.11.2.

19.8. WS-ID-0005, Revision 6.6, Effective 4/9/2008

19.8.1. Added South Carolina rule to prepare an MS/MSD with every batch.

19.8.2. Modified to include extraction and analysis of ambient air samples collected in filter/PUF material.

TABLE 1

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	Human/ Fish Tissue	Adipose Tissue	Sludges, Fuel Oil	Still-Bottom	Ambient or Source Samples
Lower MCL(a)	0.01	1.0	2.0	1.0	2.0	10	20	40
Upper MCL(a)	4.0	400	400	400	400	2000	4000	8000
Weight (g)	1000	10	10	10	10	2.0	1.0	1 sample
IS Spiking Levels (ng)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	4.0
Final Extract Volume (μL)	20	20	20	20	20	20	20	20

(a) For other congeners, multiply the values by 1 for TCDF, by 5 for PeCDD/PeCDF/HxCDD/HxCDF/HpCDD/HpCDF, and by 10 for OCDD/OCDF.

TABLE 2

Composition of the Sample Fortification and Recovery Standard Solutions

Analyte	Semple Fortification Solution Concentration pg/µL; Solvent: Isooctane	Recovery Standard Solution Concentration pg/µL; Solvent: Tetradecane
$^{13}C_{12}$ -2,3,7,8-TCDD	2[a], 100[c]	--
$^{13}C_{12}$ -2,3,7,8-TCDF	2[a], 100[c]	--
$^{13}C_{12}$ -1,2,3,4-TCDD	--	100
$^{13}C_{12}$ -1,2,3,7,8-PeCDD	2[a], 100[c]	--
$^{113}C_{12}$ -1,2,3,7,8-PeCDF	2[a], 100[c]	--
$^{13}C_{12}$ -1,2,3,6,7,8-HxCDD	2[a], 100[c]	--
$^{13}C_{12}$ -1,2,3,4,7,8-HxCDF[d]	2[a], 100[c]	--
$^{113}C_{12}$ -1,2,3,7,8,9-HxCDD	--	100
$^{13}C_{12}$-2,3,7,8-TCDD[b][c]	0.8[b],100[c]	
	100[c]	
$^{13}C_{12}$ -2,3,4,7,8-PeCDF[c]	100[c]	
$^{13}C_{12}$ -1,2,3,6,7,8-HxCDF[c][d]	100[c]	
$^{13}C_{12}$ -1,2,3,4,7,8-HxCDD[c]	100[c]	
$^{13}C_{12}$ -1,2,3,4,7,8,9-HpCDD[c]	100[c]	
$^{13}C_{12}$ -1,2,3,4,6,7,8-HpCDD	2[a], 100[c]	--
$^{13}C_{12}$ -1,2,3,4,6,7,8-HpCDF	2[a], 100[c]	--
$^{13}C_{12}$ -OCDD	4[a], 200[c]	--

(a) Standard 8290, Method 23, Method 0023A, TO9 and TO9A Sample Fortification Solution concentrations
(b) Method TO9 and TO9A surrogate concentrations
(c) Method 23 and Method 0023A surrogate concentrations
(d) $^{13}C_{12}$ -1,2,3,6,7,8-HxCDF is used as a Sample Fortification Solution and $^{13}C_{12}$-1,2,3,4,7,8-HxCDF is used as a surrogate solution in Method 23 and Method 0023A

TABLE 3

The Seventeen 2,3,7,8-Substituted PCDD and PCDF Congeners

PCDD	PCDF
2,3,7,8-TCDD(*)	2,3,7,8-TCDF(*)
1,2,3,7,8-PeCDD(*)	1,2,3,7,8-PeCDD(*)
1,2,3,6,7,8-HxCDD(*)	2,3,4,7,8-PeCDF
1,2,3,4,7,8-HxCDD	1,2,3,6,7,8-HxCDF
1,2,3,7,8,9-HxCDD(+)	1,2,3,7,8,9-HxCDF
1,2,3,4,6,7,8-HpCDD(*)	1,2,3,4,7,8-HxCDF(*)
1,2,3,4,5,6,7,8-OCDD(*)	2,3,4,6,7,8-HxCDF
	1,2,3,4,6,7,8-HpCDF(*)
	1,2,3,4,7,8,9-HpCDF
	1,2,3,4,5,6,7,8-OCDF

(*)The ^{13}C -labeled analog is used as an internal standard.
(+)The ^{13}C -labeled analog is used as a recovery standard.

FIGURE 1 — Flowchart of Process

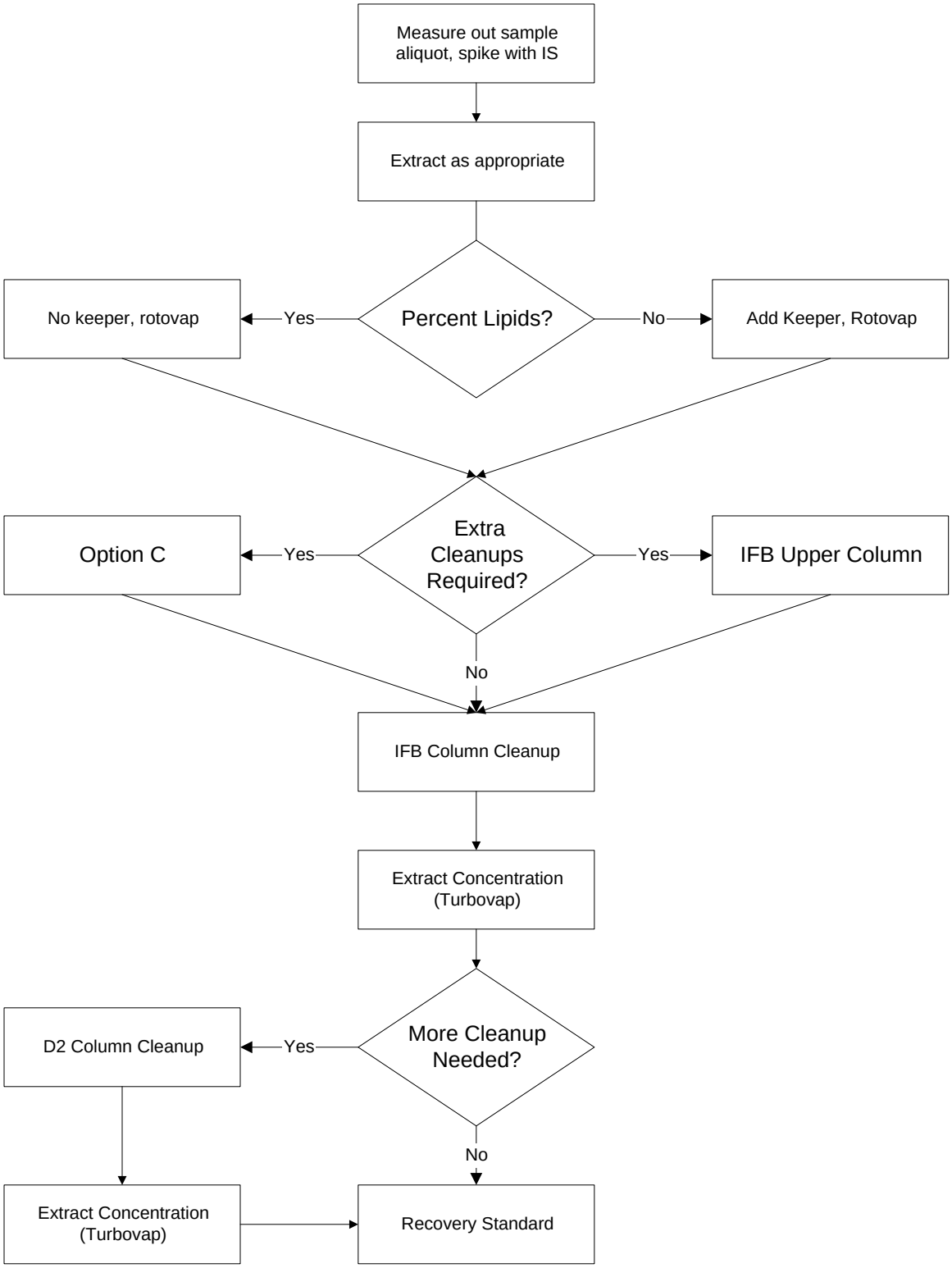


Figure 2 – Diagram of IFB Column Cleanup

Use 20 mm column for top column (IFB Column)
Use 16 mm column for bottom column* (Acid Alumina)
Note: Upper and lower columns are piggy backed for IFB cleanup, upper column only can be used for additional cleaning.

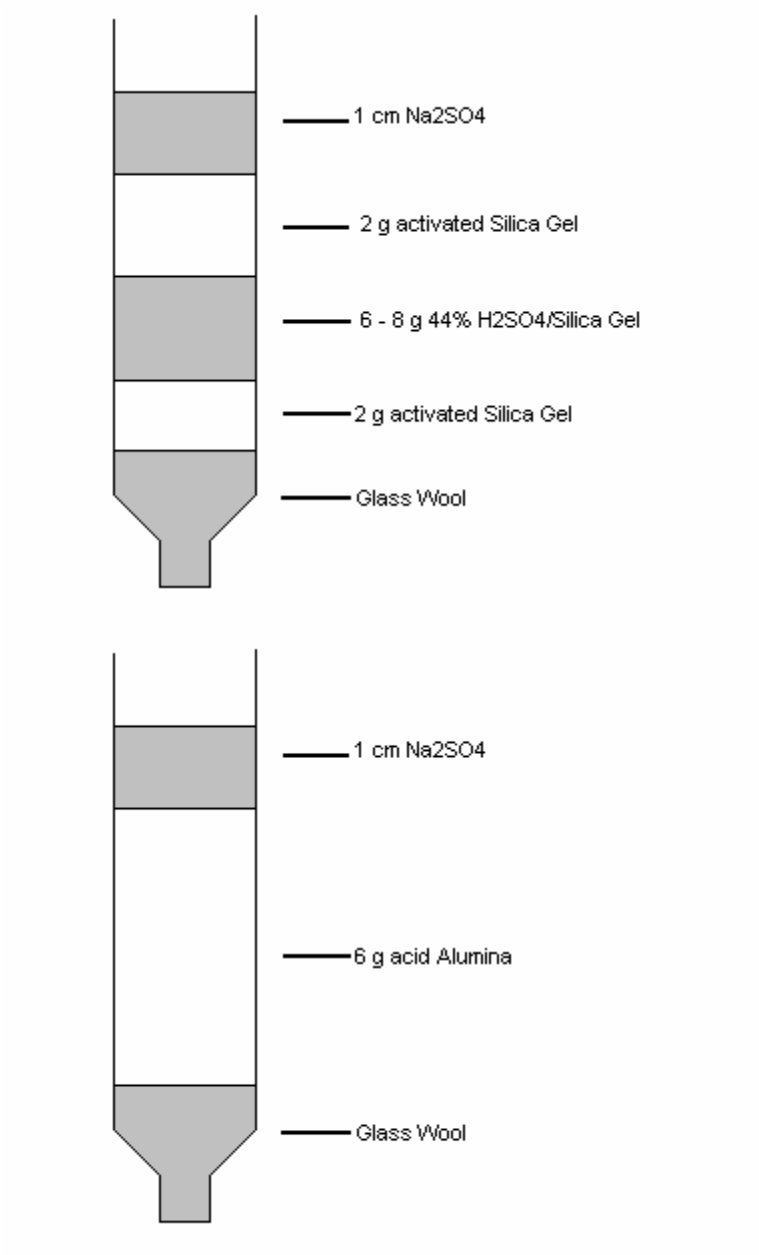
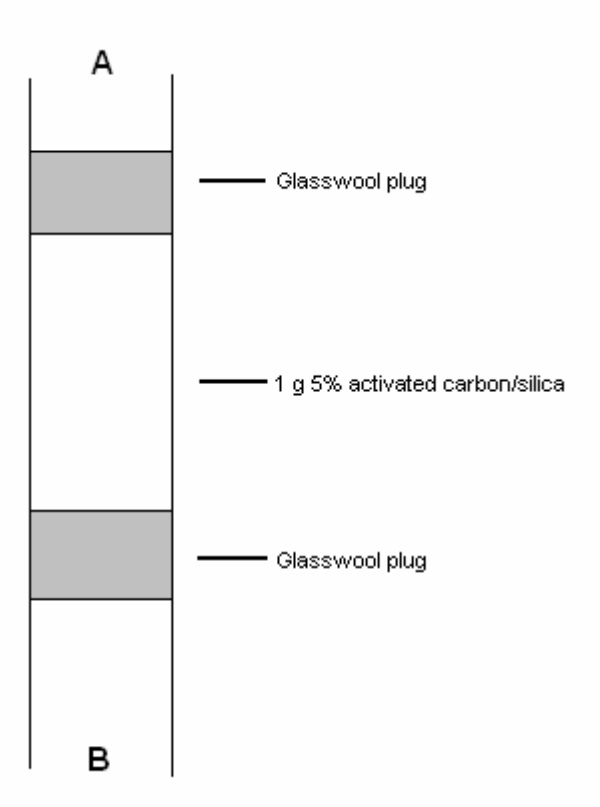


Figure 3— D2 Carbon Column:



APPENDIX A — Screening the Laboratory for 2,3,7,8 Congeners

This procedure is designed for the periodic evaluation of potential contamination by 2,3,7,8-substituted PCDD/PCDF congeners of the working areas inside the laboratory.

PERFORMING WIPE TEST
Perform the wipe tests on surface areas of two inches by one foot with laboratory wipers saturated with distilled-in-glass acetone or appropriate solvent using a pair of clean stainless steel forceps. Use one wiper for each of the designated areas. Combine the wipers to one composite sample in an extraction jar containing 200 mL distilled-in-glass hexane. Place an equal number of unused wipers in 200 mL hexane and use this as a control.

SAMPLE PREPARATION
Close the jar containing the wipes and 200 mL hexane and extract for 20 minutes using a wrist-action shaker. Use an appropriate means to reduce the volume to approximately 1.0 mL. Put through an alumina column to clean up potential interfering compounds. Add appropriate amount of recovery standard.

EXTRACT ANALYSIS
Concentrate the contents of the vial to a final volume of 20 µL (either in a minivial or in a capillary tube). Inject 2 µL of each extract (wipe and control) onto a capillary column and analyze for 2,3,7,8-substituted PCDDs/PCDFs as specified in the analytical method Section 11 (this exhibit). Perform calculations according to Section 12 (this exhibit).

REPORTING FORMAT
Report the presence of 2,3,7,8-substituted PCDDs and PCDFs as a quantity (pg or ng) per wipe test experiment (WTE). Under the conditions outlined in this analytical protocol, a lower limit of calibration of 25 pg/WTE is expected for 2,3,7,8-TCDD. A positive response for the blank (control) is defined as a signal in the TCDD retention time window at any of the masses monitored which is equivalent to or above 8 pg of 2,3,7,8-TCDD per WTE. For other congeners, use the multiplication factors listed in Table 1, footnote (a) (e.g., for OCDD, the lower MCL is 25 x 5 = 125 pg/WTE and the positive response for the blank would be 8 x 5 = 40 pg). Also, report the recoveries of the internal standards during the simplified cleanup procedure.

FREQUENCY OF WIPE TESTS
Wipe tests should be performed when there is evidence of contamination in the method blanks.

CORRECTIVE ACTION
An upper limit of 25 pg per TCDD isomer and per wipe test experiment is allowed. (Use multiplication factors listed in footnote (a) from Table 1 for other congeners.) This value corresponds to the lower calibration limit of the analytical method. Steps to correct the contamination must be taken whenever these levels are exceeded. To that effect, first vacuum the working places (hoods, benches, sink) using a vacuum cleaner equipped with a high-efficiency

particulate absorbent (HEPA) filter and then wash with a detergent. A new set of wipes should be analyzed before anyone is allowed to work in the dioxin area of the laboratory.

The test results and the decontamination procedure must be reviewed with EH&S.