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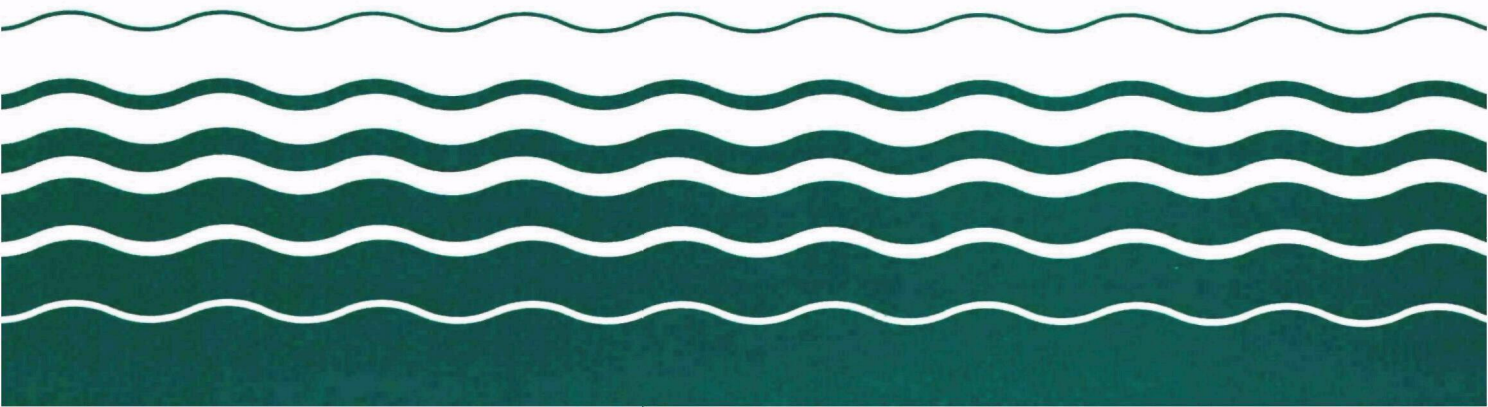
Method 1613: Tetra- through Octa- Chlorinated Dioxins and Furans by Isotope Dilution



Introduction

Method 1613 was developed by the Industrial Technology Division (ITD) within the United States Environmental Protection Agency's (USEPA) Office of Water Regulations and Standards (OWRS) to provide improved precision and accuracy of analysis of pollutants in aqueous and solid matrices. The ITD is responsible for development and promulgation of nationwide standards setting limits on pollutant levels in industrial discharges.

Method 1613 is a high resolution capillary column gas chromatography (HRGC)/high resolution mass spectrometry (HRMS) method for analysis of tetra- through octa- chlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) using isotope dilution. Specificity is provided for determination of the 2,3,7,8- substituted isomers of tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) and tetrachlorodibenzofuran (2,3,7,8-TCDF).

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Tetra- through Octa- Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS

1 SCOPE AND APPLICATION

1.1 This method is designed to meet the survey requirements of the USEPA ITD. The method is used to determine the tetra- through octa- chlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) associated with the Clean Water Act (as amended 1987); the Resource Conservation and Recovery Act (as amended 1986); and the Comprehensive Environmental Response, Compensation and Liability Act (as amended 1986); and other dioxin and furan compounds amenable to high resolution capillary column gas chromatography (HRGC)/high resolution mass spectrometry (HRMS). Specificity is provided for determination of the 2,3,7,8- substituted isomers of tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) and tetrachlorodibenzofuran (2,3,7,8-TCDF).

1.2 The method is based on EPA, industry, commercial laboratory, and academic methods (References 1 - 6).

1.3 The compounds listed in Table 1 may be determined in waters, soils, sludges, and other matrices by this method.

1.4 The detection limits of the method are usually dependent on the level of interferences rather than instrumental limitations. The levels in Table 2 typify the minimum quantities that can be detected with no interferences present.

1.5 The GCMS portions of the method are for use only by analysts experienced with HRGC/HRMS or under the close supervision of such qualified persons. Each laboratory that uses this method must demonstrate the ability to generate acceptable results using the procedure in Section 8.2.

2 SUMMARY OF METHOD

2.1 Stable isotopically labeled analogs of 16 of the PCDDs and PCDFs are added to each sample. Samples containing coarse solids are prepared for extraction by grinding or homogenization. Water samples are filtered and then extracted with methylene chloride using separatory funnel procedures; the particulates from the water samples, soils, and other finely divided solids are extracted using a combined Soxhlet extraction/Dean-Stark azeotropic distillation (Reference 7). Prior to cleanup and analysis, the extracts of the filtered water and the particulates are combined.

2.2 After extraction, $^{37}Cl_4$-labeled 2,3,7,8-TCDD is added to each extract to measure the efficiency of the cleanup process. Samples cleanup may include back extraction with acid and/or base, and gel permeation, alumina, silica gel, and activated carbon chromatography. High performance liquid chromatography (HPLC) can be used for further isolation of the 2,3,7,8- isomers or other specific isomers or congeners.

2.3 After cleanup, the extract is concentrated to near dryness. Immediately prior to injection, two internal standards are added to each extract, and a 1 uL aliquot of the extract is injected into the gas chromatograph. The analytes are separated by the GC and detected by a high resolution ($\geq$10,000) mass spectrometer. The labeled compounds serve to correct for the variability of the analytical technique.

2.4 Dioxins and furans are identified by comparing GC retention time ranges and the ion abundance ratios of the m/z's with the corresponding retention time ranges of authentic standards and the theoretical ion abundance ratios of the exact m/z's. Isomers and congeners are identified when the retention time ranges and m/z abundance ratios agree within pre-defined limits. By using a GC column or columns capable of resolving the 2,3,7,8-substituted isomers from all other tetra-isomers, the 2,3,7,8-substituted isomers are identified when the retention time and m/z abundance ratios agree within pre-defined limits of the retention times and exact m/z ratios of authentic standards.

2.5 Quantitative analysis is performed by GCMS using selected ion current profile (SICP) areas, in one of two ways: 1) For the

16 2,3,7,8-substituted isomers for which labeled analogs are available (see Table 1), the GCMS system is calibrated and the compound concentration is determined using an isotope dilution technique; 2) For non-2,3,7,8-substituted isomers and the total concentrations of all isomers within a level of chlorination (i.e., total TCDD), concentrations are determined assuming response factors from the calibration of labeled analogs at the same level of chlorination. Although a labeled analog of the octachlorinated dibenzofuran (OCDF) is available, using high resolution mass spectrometry, it produces an m/z that may interfere with the identification and quantitation of the native octachlorinated dibenzo-p-dioxin (OCDD). Therefore, this labeled analog has not been included in the calibration standards, and the native OCDF is quantitated against the labeled OCDD.

2.6 The quality of the analysis is assured through reproducible calibration and testing of the extraction, cleanup, and GCMS systems.

3 CONTAMINATION AND INTERFERENCES

3.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or elevated baselines causing misinterpretation of chromatograms (References 8 - 9). Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Where possible, reagents are cleaned by extraction or solvent rinse.

3.2 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.

3.2.1 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 s may aid in cleaning.

3.2.2 After detergent washing, glassware should be immediately rinsed first with methanol, then with hot tap water. The tap water rinse is followed by another methanol rinse, and then acetone, and methylene chloride.

3.2.3 Do not bake reusable glassware in an oven. Repeated baking of glassware may cause active sites on the glass surface that will irreversibly adsorb PCDDs/PCDFs.

3.2.4 Immediately prior to use, Soxhlet extraction glassware should be pre-extracted with toluene for approximately 3 hours. See Section 11.1.2.3. Separatory funnels should be shaken with methylene chloride for 2 minutes.

3.3 All materials used in the analysis shall be demonstrated to be free from interferences by running reference matrix blanks initially and with each sample set (samples started through the extraction process on a given 12-hour shift, to a maximum of 20). The reference matrix blank must simulate, as closely as possible, the sample matrix under test. Reagent water (Section 6.6.1) is used to simulate water samples; playground sand (Section 6.6.2) or white quartz sand (Section 6.5.4) can be used to simulate soils; filter paper (Section 6.6.3) is used to simulate papers and similar materials; other materials (Section 6.6.4) can be used to simulate other matrices.

3.4 Interferences coextracted 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, hydroxydiphenyl ethers, benzylphenyl 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 Section 12 can be used to reduce or eliminate these interferences and thereby permit reliable determination of the PCDDs and PCDFs the at levels shown in Table 2.

4 SAFETY

4.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level.

4.1.1 The 2,3,7,8-TCDD isomer has been found to be acnegenic, carcinogenic, and teratogenic in laboratory animal studies. It is soluble in water to approximately 200 parts-per-trillion and in organic solvents to 0.14 percent. On the basis of the available toxicological and physical properties of 2,3,7,8-TCDD, all of the PCDDs and PCDFs should be handled only by highly trained personnel thoroughly familiar with handling and cautionary procedures, and who understand the associated risks.

4.1.2 It is recommended that the laboratory purchase dilute standard solutions of the analytes in this method. However, if primary solutions are prepared, they shall be prepared in a hood, and a NIOSH/MESA approved toxic gas respirator shall be worn when high concentrations are handled.

4.2 The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of data handling sheets should also be made available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 10 - 13. The references and bibliography at the end of Reference 13 are particularly comprehensive in dealing with the general subject of laboratory safety.

4.3 The PCDDs and PCDFs and samples suspected to contain these compounds are handled using essentially the same techniques as those employed in handling radioactive or infectious materials. Well-ventilated, controlled access laboratories are required. Assistance in evaluating the health hazards of particular laboratory conditions may be obtained from certain consulting laboratories and from State Departments of Health or of Labor, many of which have an industrial health service. The PCDDs and PCDFs are extremely toxic to laboratory animals. However, they have been handled for years without injury in analytical and biological laboratories. Each laboratory must develop a strict safety program for handling the PCDDs and PCDFs. The following laboratory practices are recommended (References 2 and 14):

4.3.1 Facility -- When finely divided samples (dusts, soils, dry chemicals) are handled, all operations, including removal of samples from sample containers, weighing, transferring and mixing should be performed in a glove box demonstrated to be leak tight or fume hood demonstrated to have adequate air flow. Gross losses to the laboratory ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical and animal work presents no inhalation hazards except in the case of an accident.

4.3.2 Protective equipment -- Throwaway plastic gloves, apron or lab coat, safety glasses or mask, and a glove box or fume hood adequate for radioactive work. During analytical operations which may give rise to aerosols or dusts, personnel should wear respirators equipped with activated carbon filters. Eye protection equipment (preferably full face shields) must be worn while working with exposed samples or pure analytical standards. Latex gloves are commonly used to reduce exposure of the hands. When handling samples suspected or known to contain high concentrations of the PCDDs or PCDFs, an additional set of gloves can also be worn beneath the latex gloves.

4.3.3 Training -- Workers must be trained in the proper method of removing contaminated gloves and clothing without contacting the exterior surfaces.

4.3.4 Personal hygiene -- Thorough washing of hands and forearms after each manipulation and before breaks (coffee, lunch, and shift).

4.3.5 Confinement -- Isolated work area, posted with signs, segregated glassware and tools, plastic absorbent paper on bench tops.

4.3.6 Effluent vapors -- The effluents of sample splitters for the gas chromatograph and roughing pumps on the GC/MS should pass through either a column of activated charcoal or be bubbled through a trap containing oil or high-boiling alcohols.

4.3.7 Waste

4.3.7.1 Handling -- Good technique includes minimizing contaminated waste. Plastic bag liners should be used in waste cans. Janitors and other personnel must be trained in the safe handling of waste.

4.3.7.2 Disposal

4.3.7.2.1 The PCDDs and PCDFs decompose above 800 °C. Low-level waste such as absorbent paper, tissues, animal remains, and plastic gloves may be burned in an appropriate incinerator. Gross quantities (milligrams) should be packaged securely and disposed through commercial or governmental channels which are capable of handling extremely toxic wastes.

4.3.7.2.2 Liquid or soluble waste should be dissolved in methanol or ethanol and irradiated with ultraviolet light with a wavelength greater than 290 nm for several days. (Use F 40 BL lamps or equivalent.) Analyze liquid wastes and dispose of the solutions when the PCDDs and PCDFs can no longer be detected.

4.3.8 Decontamination

4.3.8.1 Personal decontamination -- Use any mild soap with plenty of scrubbing action.

4.3.8.2 Glassware, tools, and surfaces -- Chlorothene NU Solvent (Trademark of the Dow Chemical Company) is the least toxic solvent shown to be effective. Satisfactory cleaning may be accomplished by rinsing with Chlorothene, then washing with any detergent and water. If glassware is first rinsed with solvent, then the dish water may be disposed of in the sewer. Given the cost of disposal, it is prudent to minimize solvent wastes.

4.3.9 Laundry -- Clothing known to be contaminated should be collected in plastic bags. Persons who convey the bags and launder the clothing should be advised of the hazard and trained in proper handling. The clothing may be put into a washer without contact if the launderer knows of the potential problem. The washer should be run through a cycle before being used again for other clothing.

4.3.10 Wipe tests -- A useful method of determining cleanliness of work surfaces and tools is to wipe the surface with a piece of filter paper. Extraction and analysis by GC can achieve a limit of detection of 0.1 ug per wipe. Less than 0.1 ug per wipe indicates acceptable cleanliness; anything higher warrants further cleaning. More than 10 ug on a wipe constitutes an acute hazard and requires prompt cleaning before further use of the equipment or work space, and indicates that unacceptable work practices have been employed in the past.

4.3.11 Accidents -- Remove contaminated clothing immediately, taking precautions not to contaminate skin or other articles. Wash exposed skin vigorously and repeatedly until medical attention is obtained.

5 APPARATUS AND MATERIALS

5.1 Sampling equipment for discrete or composite sampling.

5.1.1 Sample bottles and caps

5.1.1.1 Liquid samples (waters, sludges and similar materials that contain less than five percent solids) -- Sample bottle, amber glass, 1.1 liters minimum, with screw cap.

5.1.1.2 Solid samples (soils, sediments, sludges, paper pulps, filter cake, compost, and similar materials that contain more than five percent solids) -- Sample bottle, wide mouth, amber glass, 500 mL minimum.

5.1.1.3 If amber bottles are not available, samples shall be protected from light.

5.1.1.4 Bottle caps -- Threaded to fit sample bottles. Caps shall be lined with Teflon.

5.1.1.5 Cleaning

5.1.1.5.1 Bottles are detergent water washed, then solvent rinsed before use.

5.1.1.5.2 Liners are detergent water washed, then rinsed with reagent water (Section 6.6.1) and then solvent, and baked at approximately 200 °C for one hour minimum prior to use.

5.1.2 Compositing equipment -- Automatic or manual compositing system incorporating glass containers cleaned per bottle cleaning procedure above. Glass or Teflon tubing only shall be used. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used in the pump only. Before use, the tubing shall be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize sample contamination. An integrating flow meter is used to collect proportional composite samples.

5.2 Equipment for glassware cleaning

5.2.1 Laboratory sink with overhead fume hood

5.3 Equipment for sample preparation

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

5.3.2 Glove box (optional)

5.3.3 Tissue homogenizer -- VirTis Model 45 Macro homogenizer (American Scientific Products H-3515, or equivalent) with stainless steel Macro-shaft and Turbo-shear blade.

5.3.4 Meat grinder -- Hobart, or equivalent, with 3 - 5 mm holes in inner plate.

5.3.5 Equipment for determining percent moisture

5.3.5.1 Oven, capable of maintaining a temperature of 110 ±5 °C.

5.3.5.2 Dessicator

5.3.6 Balances

5.3.6.1 Analytical -- Capable of weighing 0.1 mg.

5.3.6.2 Top loading -- Capable of weighing 10 mg.

5.4 Extraction apparatus

5.4.1 Water samples

5.4.1.1 pH meter, with combination glass electrode.

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

5.4.1.3 Graduated cylinder, 1 L capacity

5.4.1.4 1 L filtration flasks with side arm, for use in vacuum filtration of water samples.

5.4.1.5 Separatory funnels -- 250, 500, and 2000 mL, with Teflon stop cocks.

5.4.2 Soxhlet/Dean-Stark (SDS) extractor (Figure 1)

5.4.2.1 Soxhlet -- 50 mm i.d., 200 mL capacity with 500 mL flask (Cal-Glass LG-6900, or equivalent, except substitute 500 mL round bottom flask for 300 mL flat bottom flask).

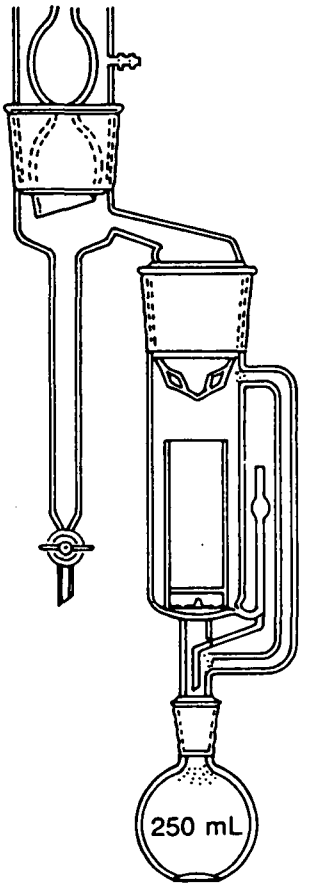


FIGURE 1 Soxhlet/Dean-Stark Extractor

5.4.2.2 Thimble -- 43 x 123 to fit Soxhlet (Cal-Glass LG-6901-122, or equivalent).

5.4.2.3 Moisture trap -- Dean Stark or Barret with Teflon stopcock, to fit Soxhlet.

5.4.2.4 Heating mantle -- Hemispherical, to fit 500 mL round bottom flask (Cal-Glass LG-8801-112, or equivalent).

5.4.2.5 Variable transformer -- Powerstat (or equivalent), 110 volt, 10 amp.

5.4.3 Beakers, 400 - 500 mL

5.4.4 Spatulas -- Stainless steel

5.5 Filtration apparatus

5.5.1 Pyrex glass wool -- Solvent extracted or baked at 450 °C for four hours minimum.

5.5.2 Glass funnel -- 125 - 250 mL

5.5.3 Glass fiber filter paper (Whatman GF/D, or equivalent)

5.5.4 Drying column -- 15 to 20 mm i.d. Pyrex chromatographic column equipped with coarse glass frit or glass wool plug.

5.5.5 Buchner funnel, 15 cm.

5.5.6 Glass fiber filter paper for above.

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

5.6 Centrifuge apparatus

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

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

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

5.7 Cleanup apparatus

5.7.1 Automated gel permeation chromatograph (Analytical Biochemical Labs, Inc, Columbia, MO, Model GPC Autoprep 1002, or equivalent).

5.7.1.1 Column -- 600 - 700 mm x 25 mm i.d., packed with 70 g of SX-3 Bio-beads (Bio-Rad Laboratories, Richmond, CA, or equivalent).

5.7.1.2 Syringe, 10 mL, with Luer fitting.

5.7.1.3 Syringe filter holder, stainless steel, and glass fiber or Teflon filters (Gelman 4310, or equivalent).

5.7.1.4 UV detectors -- 254-mu, preparative or semi-prep flow cell: (Isco, Inc., Type 6; Schmadzu, 5 mm path length; Beckman-Altex 152W, 8 uL micro-prep flow cell, 2 mm path; Pharmacia UV-1, 3 mm flow cell; LDC Milton-Roy UV-3, monitor #1203; or equivalent).

5.7.2 Reverse phase high performance liquid chromatograph

5.7.2.1 Column oven and detector -- Perkin-Elmer Model LC-65T (or equivalent) operated at 0.02 AUFS at 235 nm.

5.7.2.2 Injector -- Rheodyne 7120 (or equivalent) with 50 uL sample loop.

5.7.2.3 Column -- Two 6.2 x 250 mm Zorbax-ODS columns in series (DuPont Instruments Division, Wilmington, DE, or equivalent), operated at 50 °C with 2.0 mL/min methanol isocratic effluent.

5.7.2.4 Pump -- Altex 110A (or equivalent).

5.7.3 Pipets

5.7.3.1 Disposable, Pasteur, 150 mm x 5 mm i.d. (Fisher Sceintific 13-678-6A, or equivalent).

5.7.3.2 Disposable, serological, 10 mL (6 mm i.d.).

5.7.4 Chromatographic columns

5.7.4.1 150 mm x 8 mm i.d., (Kontes K-420155, or equivalent) with coarse glass frit or glass wool plug and 250 mL reservoir.

5.7.4.2 200 mm x 15 mm i.d., with coarse glass frit or glass wool plug and 250 mL reservoir.

5.7.5 Oven -- For storage of adsorbents, capable of maintaining a temperature of 130 ±5 °C.

5.8 Concentration apparatus

5.8.1 Rotary evaporator -- Buchi/Brinkman-American Scientific No. E5045-10 or equivalent, equipped with a variable temperature water bath.

5.8.1.1 A vacuum source is required for use of the rotary evaporator. It must be equipped with a shutoff valve at the evaporator, and preferably, have a vacuum gauge.

5.8.1.2 A recirculating water pump and chiller are recommended, as use of tap water for cooling the evaporator wastes large volumes of water and can lead to inconsistent performance as water temperatures and pressures vary.

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

5.8.2 Nitrogen blowdown apparatus -- Equipped with water bath controlled at 35 - 40 °C (N-Evap, Organomation Associates, Inc., South Berlin, MA, or equivalent), installed in a fume hood.

5.8.3 Sample vials -- Amber glass, 2 - 5 mL with Teflon-lined screw cap.

5.9 Gas chromatograph -- Shall have splitless or on-column injection port for capillary column, temperature program with isothermal hold, and shall meet all of the performance specifications in Section 14.

5.9.1 GC Column for PCDDs and PCDFs and for isomer specificity for 2,3,7,8-TCDD -- 60 ±5 m x 0.32 ±0.02 mm i.d.; 0.25 um 5% phenyl, 94% methyl, 1% vinyl silicone bonded phase fused silica capillary column (J & W DB-5, or equivalent).

5.9.2 GC Column for isomer specificity for 2,3,7,8-TCDF -- 30 ±5 m x 0.32 ±0.02 mm i.d.; 0.25 um bonded phase fused silica capillary column (J & W DB-225, or equivalent).

5.10 Mass spectrometer -- 28 - 40 eV electron impact ionization, shall repetitively selectively monitor 11 exact m/z's minimum at high resolution (≥10,000) during a period of approximately 1 second.

5.10.1 The groups of m/z's to be monitored are shown in Table 3. Each group or descriptor shall be monitored in succession as a function of GC retention time to ensure that all PCDDs and PCDFs are detected. The theoretical abundance ratios for the m/z's are given in Table 3A, along with the control limits of each ratio.

5.10.2 The mass spectrometer shall be operated in a mass drift correction mode, using perfluorokerosene (PFK) to provide lock masses. The lock mass for each group of m/z's is shown in Table 3. Each lock mass shall be monitored and shall not vary by more than ±10 percent throughout its respective retention time window. Variations of the lock mass by more than 10 percent indicate the presence of coeluting interferences that may significantly reduce the sensitivity of the mass spectrometer. Re-injection of another aliquot of the sample extract will not resolve the problem. Additional cleanup of the extract may be required to remove the interferences.

5.11 GC/MS interface -- The mass spectrometer shall be interfaced to the GC such that the end of the capillary column terminates within 1 cm of the ion source but does not intercept the electron or ion beams. All portions of the column which connect the GC to the ion source shall remain at or above the column temperature during analysis to preclude condensation of less volatile compounds.

5.12 Data system -- Shall collect and record and store MS data.

5.12.1 Data acquisition -- The signal at each exact m/z shall be collected repetitively throughout the monitoring period and stored on a mass storage device.

5.12.2 Response factors and multipoint calibrations -- The data system shall be used to record and maintain lists of response factors (response ratios for isotope dilution) and multi-point calibration curves. Computations of relative standard deviation (coefficient of variation) are used to test calibration linearity. Statistics on initial (Section 8.2) and ongoing (Section 14.5) performance shall be computed and maintained.

6 REAGENTS AND STANDARDS

6.1 pH adjustment and back extraction

6.1.1 Potassium hydroxide -- Dissolve 20 g reagent grade KOH in 100 mL reagent water.

6.1.2 Sulfuric acid -- Reagent grade (specific gravity 1.84).

6.1.3 Sodium chloride -- Reagent grade, prepare a five percent (w/v) solution in reagent water.

6.2 Solution drying and evaporation

6.2.1 Solution drying -- Sodium sulfate, reagent grade, granular anhydrous (Baker 3375, or equivalent), rinsed with methylene chloride (20 mL/g), baked at 400 °C for one hour minimum, cooled in a dessicator, and stored in a pre-cleaned glass bottle with screw cap that prevents moisture from entering.

6.2.2 Prepurified nitrogen

6.3 Solvents -- Acetone, toluene, cyclohexane, hexane, nonane, methanol, methylene chloride, and nonane: distilled-in-glass, pesticide quality, lot certified to be free of interferences.

6.4 GPC calibration solution -- Solution containing 300 mg/mL corn oil, 15 mg/mL bis(2-ethylhexyl) phthalate, 1.4 mg/mL pentachlorophenol, 0.1 mg/mL perylene, and 0.5 mg/mL sulfur

6.5 Adsorbents for sample cleanup

6.5.1 Silica gel

6.5.1.1 Activated silica gel -- Bio-Sil A, 100 - 200 mesh (Bio-Rad 131-1340, or equivalent), rinsed with methylene chloride, baked at 250 °C for one hour minimum, cooled in a dessicator, and stored in a pre-cleaned glass bottle with screw cap that prevents moisture from entering.

6.5.1.2 Acid silica gel (30 percent w/w) -- Thoroughly mix 4.4 g of concentrated sulfuric acid with 10.0 g activated silica gel. Break up aggregates with a stirring rod until a uniform mixture is obtained. Store in a screw-capped bottle with Teflon-lined cap.

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

6.5.2 Alumina

6.5.2.1 Neutral alumina -- Bio-Rad Laboratories 132-1140 Neutral Alumina Ag 7 (or equivalent). Heat to 600 °C for 24 hours minimum. Store at 130 °C in a covered flask. Use within five days of baking at 600 °C.

6.5.2.2 Acid alumina -- Bio-Rad Laboratories 132-1340 Acid Alumina AG 4 (or equivalent). Activate by heating to 130 °C for 12 hours minimum.

6.5.2.3 Basic alumina -- Bio-Rad Laboratories 132-1240 Basic Alumina AG 10 (or equivalent). Activate by heating to 600 °C for 24 hours minimum. Alternatively, activate by heating alumina in a tube furnace at 650 - 700 °C under an air flow of approximately 400 cc/min. To avoid melting the alumina, do not heat over 700 °C. Store at 130 °C in a covered flask. Use within five days of baking.

6.5.3 AX-21/Celite

6.5.3.1 Activated carbon -- AX-21 (Anderson Development Company, Adrian, MI, or equivalent). Prewash with methanol and dry in vacuo at 110 °C.

6.5.3.2 Celite 545 -- (Supelco 2-0199, or equivalent).

6.5.3.3 Thoroughly mix 5.35 g AX-21 and 62.0 g Celite 545 to produce a 7.9% w/w mixture. Activate the mixture at 130 °C for six hours minimum. Store in a dessicator.

6.5.4 White quartz sand, 60/70 mesh -- For Soxhlet/Dean-Stark extraction, (Aldrich Chemical Co, Milwaukee WI Cat No. 27,437-9, or equivalent). Bake at 450 °C for four hours minimum.

6.6 Reference matrices

6.6.1 Reagent water -- Water in which the PCDDs and PCDFs and interfering compounds are not detected by this method.

6.6.2 High solids reference matrix -- Playground sand or similar material in which the PCDDs and PCDFs and interfering compounds are not detected by this method. May be prepared by extraction with methylene chloride and/or baking at 450 °C for four hours minimum.

6.6.3 Filter paper -- Gelman type A (or equivalent) glass fiber filter paper in which the PCDDs and PCDFs and interfering compounds are not detected by this method. Cut the paper to simulate the surface area of the paper sample being tested.

6.6.4 Other matrices -- This method may be verified on any matrix by performing the tests given in Section 8.2. Ideally, the matrix should be free of the PCDDs and PCDFs, but in no case shall the background level of the PCDDs and PCDFs in the reference matrix exceed three times the minimum levels given in Table 2. If low background levels of the PCDDs and PCDFs are present in the reference matrix, the spike level of the analytes used in Section 8.2 should be increased to provide a spike-to-background ratio in the range of 1/1 to 5/1 (Reference 15).

6.7 Standard solutions -- Purchased as solutions or mixtures with certification to their purity, concentration, and authenticity, or prepared from materials of known purity and composition. If

compound purity is 98 percent or greater, the weight may be used without correction to compute the concentration of the standard. When not being used, standards are stored in the dark at room temperature in screw-capped vials with Teflon-lined caps. A mark is placed on the vial at the level of the solution so that solvent evaporation loss can be detected. If solvent loss has occurred, the solution should be replaced.

6.8 Stock solutions

6.8.1 Preparation -- Prepare in nonane per the steps below or purchase as dilute solutions (Cambridge Isotope Laboratories, Cambridge, MA, or equivalent). Observe the safety precautions in Section 4, and the recommendation in Section 4.1.2.

6.8.2 Dissolve an appropriate amount of assayed reference material in solvent. For example, weigh 1 - 2 mg of 2,3,7,8-TCDD to three significant figures in a 10 mL ground glass stoppered volumetric flask and fill to the mark with nonane. After the TCDD is completely dissolved, transfer the solution to a clean 15 mL vial with Teflon-lined cap.

6.8.3 Stock standard solutions should be checked for signs of degradation prior to the preparation of calibration or performance test standards. Reference standards that can be used to determine the accuracy of calibration standards are available from Cambridge Isotope Laboratories.

6.9 Secondary standard -- Using stock solutions (Section 6.8), prepare secondary standard solutions containing the compounds and concentrations shown in Table 4 in nonane.

6.10 Labeled compound spiking standard -- From stock standard solutions prepared as above, or from purchased mixtures, prepare this standard to contain the labeled compounds at the concentrations shown in Table 4 in nonane. This solution is diluted with acetone prior to use (Section 10.3.2).

6.11 Cleanup standard - Prepare $^{37}Cl_4$-2,3,7,8-TCDD at the concentration shown in Table 4 in nonane.

6.12 Internal standard -- Prepare at the concentration shown in Table 4 in nonane.

6.13 Calibration standards (CS1 through CS5) -- Combine the solutions in Sections 6.9, 6.10, 6.11, and 6.12 to produce the five calibration solutions shown in Table 4 in nonane. These solutions permit the relative response (labeled to unlabeled) and response factor to be measured as a function of concentration. The CS3 standard is used for calibration verification (VER).

6.14 Precision and recovery standard (PAR) -- Used for determination of initial (Section 8.2) and ongoing (Section 14.5) precision and recovery. This solution contains the analytes and labeled compounds at the concentrations listed in Table 4 in nonane. This solution is diluted with acetone prior to use (Section 10.3.4).

6.15 GC retention time window defining solutions -- Used to define the beginning and ending retention times for the dioxin and furan isomers.

6.15.1 DB-5 column window defining standard -- Cambridge Isotope Laboratories ED-1732-A, or equivalent, containing the compounds listed in Table 5.

6.16 Isomer specificity test standards -- Used to demonstrate isomer specificity for the 2,3,7,8-tetra- isomers of dioxin and furan.

6.16.1 Standards for the DB-5 column -- Cambridge Isotope Laboratories ED-908, ED-908-C, or ED-935, or equivalent, containing the compounds listed in Table 5.

6.16.2 Standards for the DB-225 column -- Cambridge Isotope Laboratories EF-937 or EF-938, or equivalent, containing the compounds listed in Table 5.

6.17 Stability of solutions -- Standard solutions used for quantitative purposes (Sections 6.9 - 6.14) shall be analyzed within 48 hours of preparation and on a monthly basis thereafter for signs of degradation. Standards will remain acceptable if the peak area at the quantitation m/z remains within ±15 percent of the area obtained in the initial analysis of the standard. Any standards failing to meet this criterion should be assayed against reference standards, as in Section 6.8.3., before further use.

7 CALIBRATION

7.1 Assemble the GCMS and establish the operating conditions necessary to meet the relative retention time specifications in Table 2.

7.1.1 The following GC operating conditions may be used for guidance and adjusted as needed to meet the relative retention time specifications in Table 2:

Injector temp: 270 °C
Interface temp: 290 °C
Initial temp and time: 200 °C, 2 min
Temp Program: 200-220 °C at 5 °C/min
220 °C for 16 min
220-235 °C at 5 °C/min
235 °C for 7 min
235-330 °C at 5 °C/min

7.1.2 Obtain a selected ion current profile of each analyte in Table 4 at the exact masses specified in Table 3 and at ≥10,000 resolving power by injecting an authentic standard of the PCDDs and PCDFs either singly or as part of a mixture in which there is no interference between closely eluted components, using the procedure in Section 13.

7.2 The ion abundance ratios, minimum levels, and absolute retention times -- Inject the CS1 calibration solution (Table 4) per the procedure in Section 13 and the conditions in Table 2.

7.2.1 Measure the selected ion current profile (SICP) areas for each analyte and compute the ion abundance ratios specified in Table 3. Compare the computed ratio to the theoretical ratio given in Table 3.

7.2.2 All PCDDs and PCDFs shall be within their respective ratios; otherwise, the mass spectrometer shall be adjusted and this test repeated until the m/z ratios fall within the limits specified. If the adjustment alters the resolution of the mass spectrometer, resolution shall be verified (Section 7.1) prior to repeat of the test.

7.2.3 Verify that the HRGC/HRMS instrument meets the minimum levels in Table 2; otherwise, the mass spectrometer shall be adjusted and this test repeated until the minimum levels in Table 2 are met.

7.2.4 The retention times of $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDF (the internal standards, Section 6.12) shall exceed 27 and 38 minutes, respectively, on the DB-5 column, and the retention time of $^{13}C_{12}$-1,2,3,4-TCDD shall exceed 17 minutes on the DB-225 column; otherwise, the GC temperature program shall be adjusted and this test repeated until the minimum retention time criteria are met.

7.3 Retention time windows -- Analyze the window defining mixtures (Section 6.15) using the procedure in Section 13 (Figures 2A - 2D).

7.4 Isomer specificity

7.4.1 Analyze the isomer specificity test standards (Section 6.16) using the procedure in Section 13.

7.4.2 Compute the percent valley between the GC peaks that elute most closely to the 2,3,7,8- TCDD and TCDF isomers, on their respective columns, per Figure 3.

7.4.3 Verify that the height of the valley between the most closely eluted isomers and the 2,3,7,8- isomers is less than 25 percent (computed as 100 x/y in Figure 3). If the valley exceeds 25 percent, adjust the analytical conditions and repeat the test or replace the GC column and recalibrate (Section 7.2 through 7.4).

7.5 Calibration with isotope dilution -- Isotope dilution is used when 1) labeled compounds are available, 2) interferences do not preclude its use, and 3) the SICP area for the analyte at the exact m/z (Table 3) is in the calibration range for the analyte. The reference compound for each native and labeled compound is shown in Table 6. Alternate labeled compounds and quantitation m/z's may be used based on availability. If any of the above conditions preclude isotope dilution, the internal standard method (Section 7.6) is used.

7.5.1 A calibration curve encompassing the concentration range is prepared for each compound to be determined. The relative response (native to labeled) vs concentration in standard solutions is plotted or computed using a linear regression. Relative response (RR) is determined according to the procedures described below. A minimum of five data points are employed for calibration.

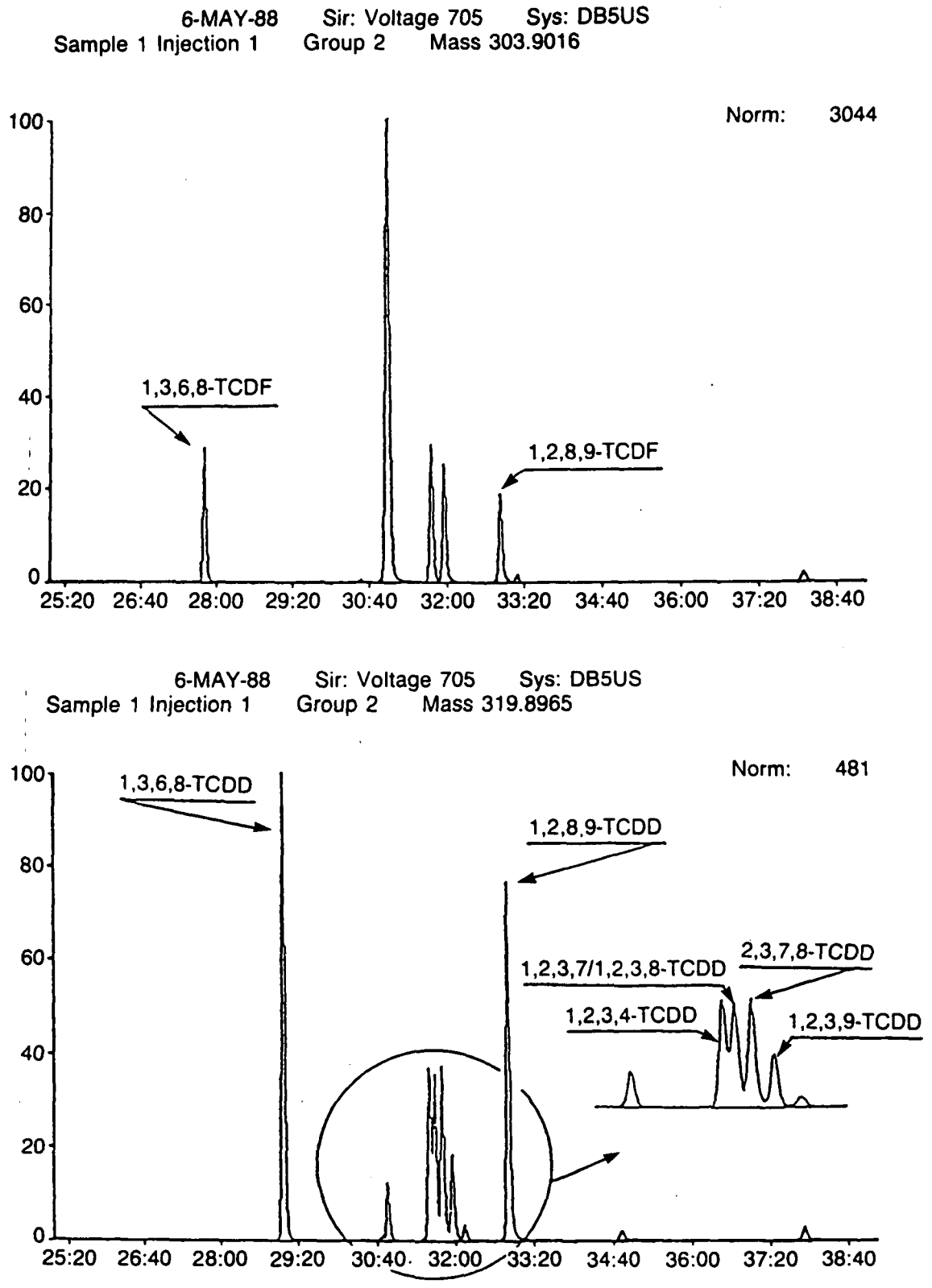
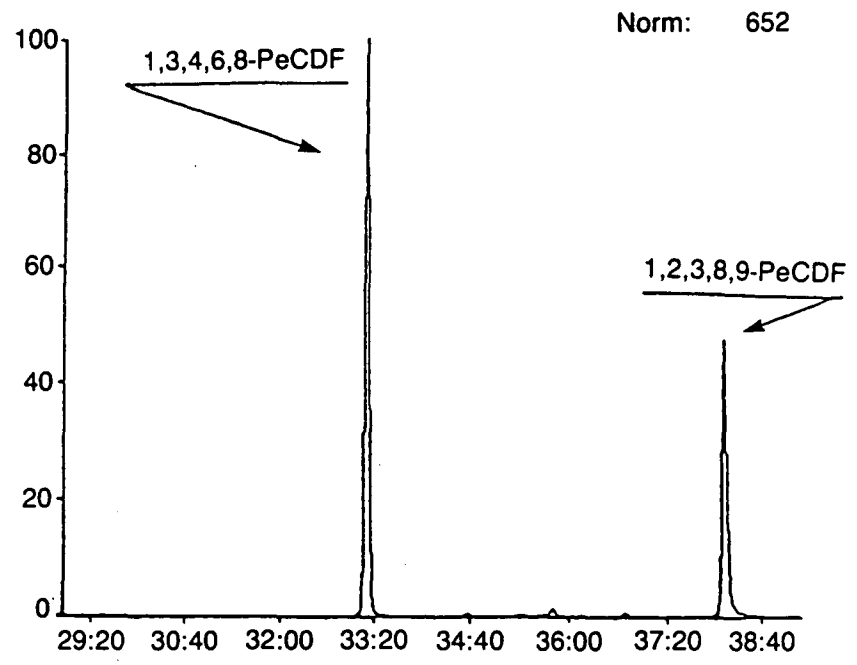


FIGURE 2A First and Last Eluted Tetra- Dioxin and Furan Isomers



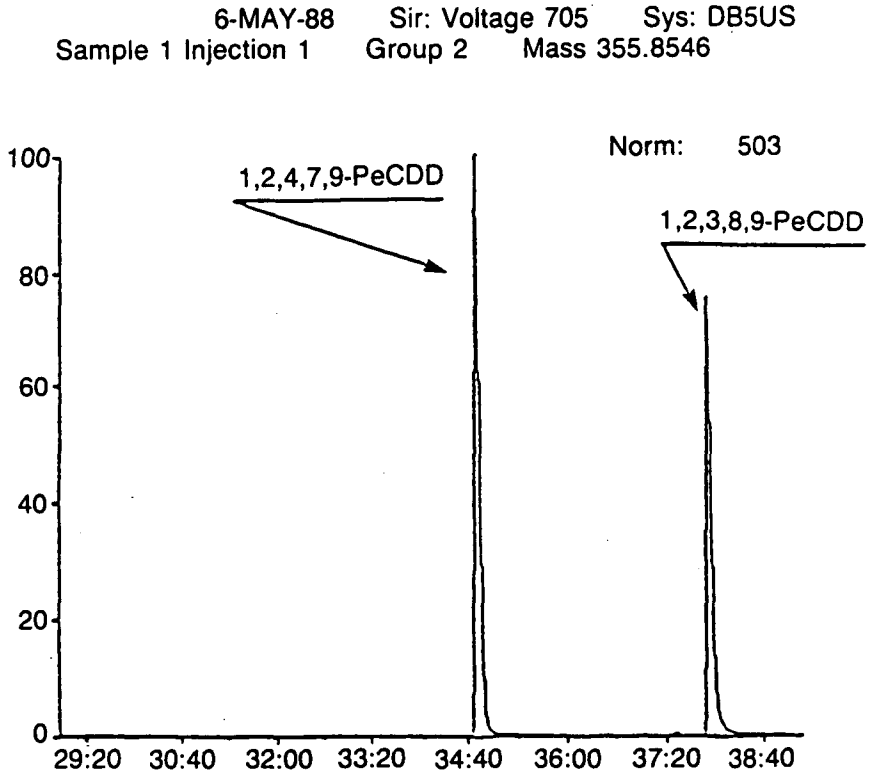
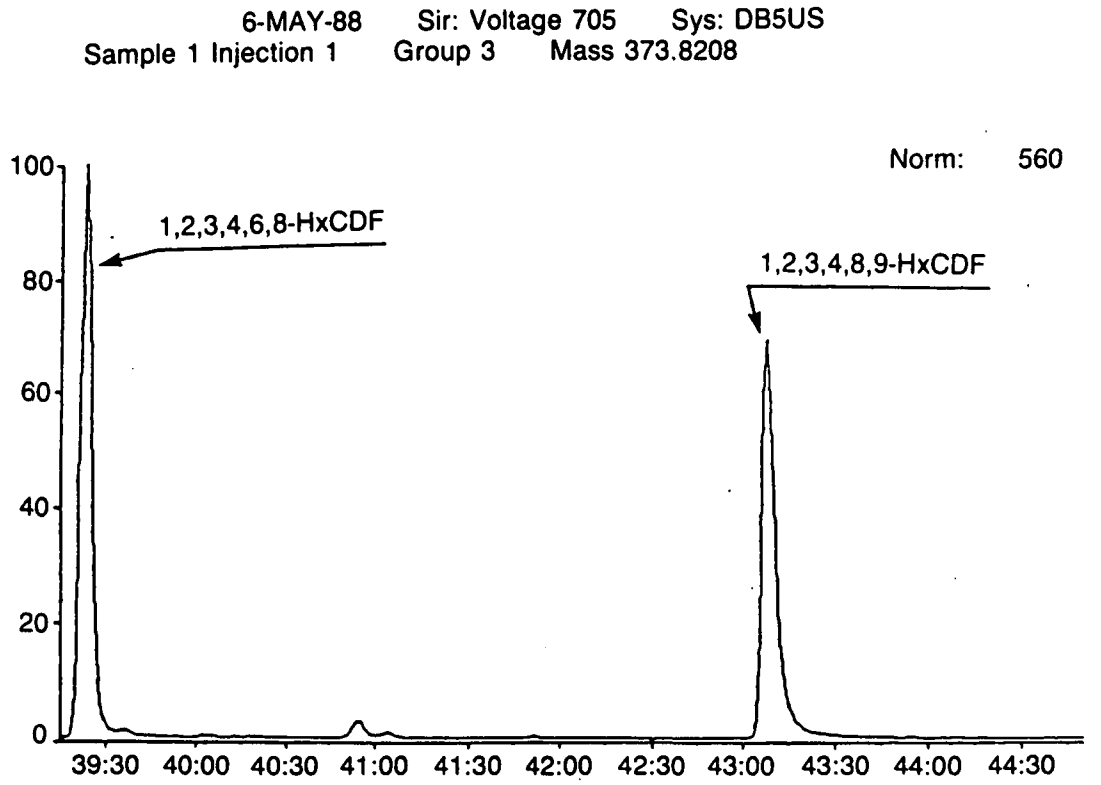


FIGURE 2B First and Last Eluted Penta- Dioxin and Furan Isomers



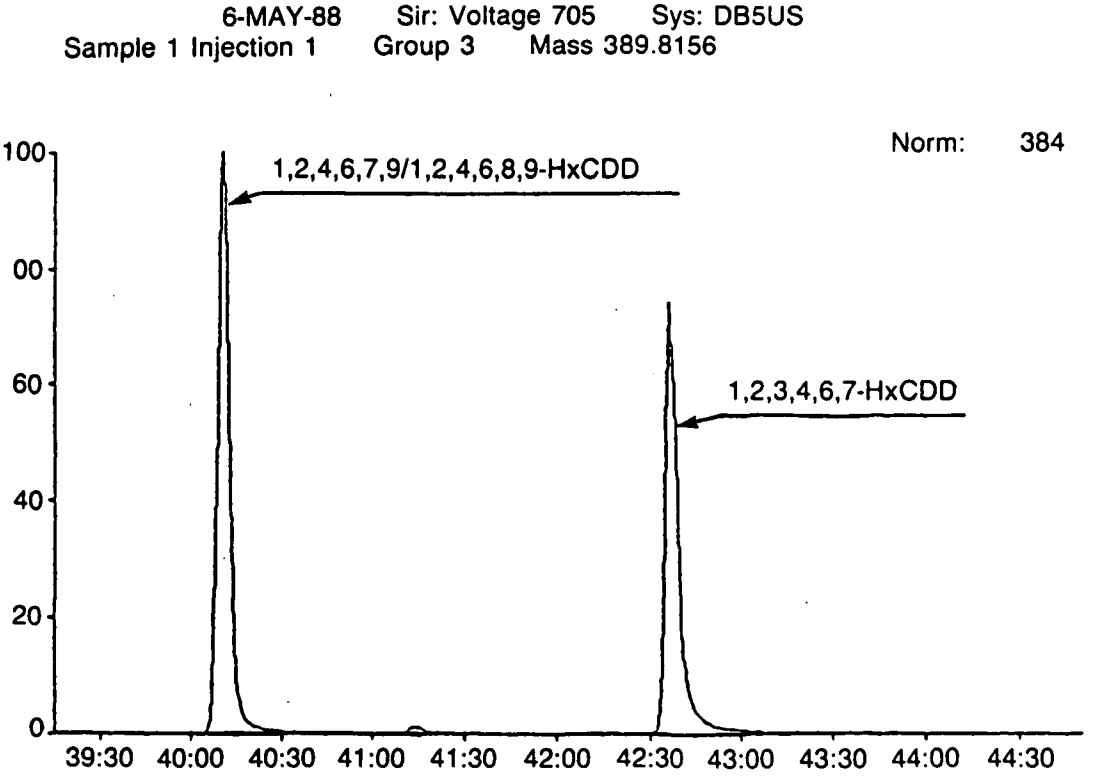


FIGURE 2C First and Last Eluted Hexa- Dioxin and Furan Isomers

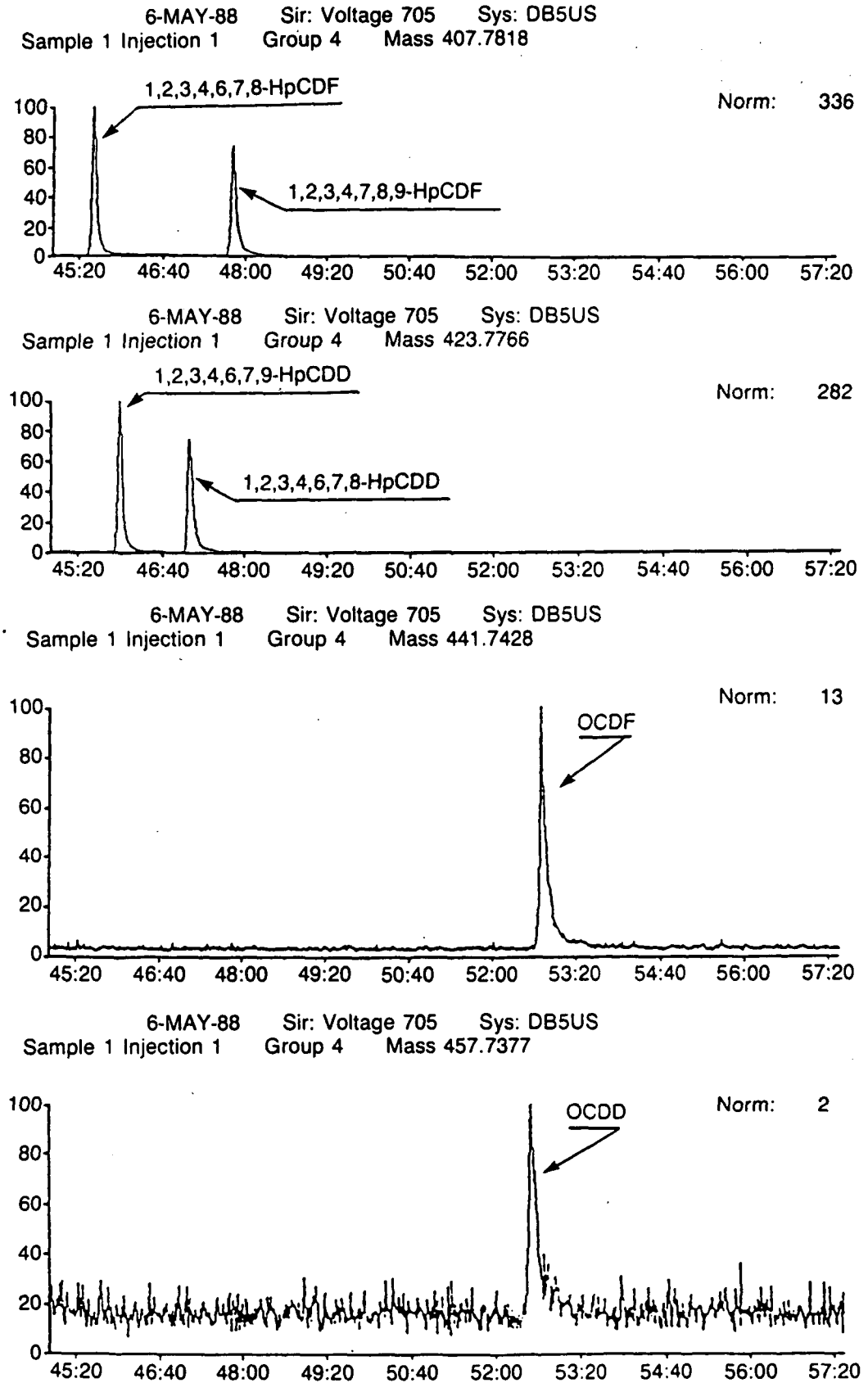


FIGURE 2D First and Last Eluted Hepta- Dioxin and Furan Isomers

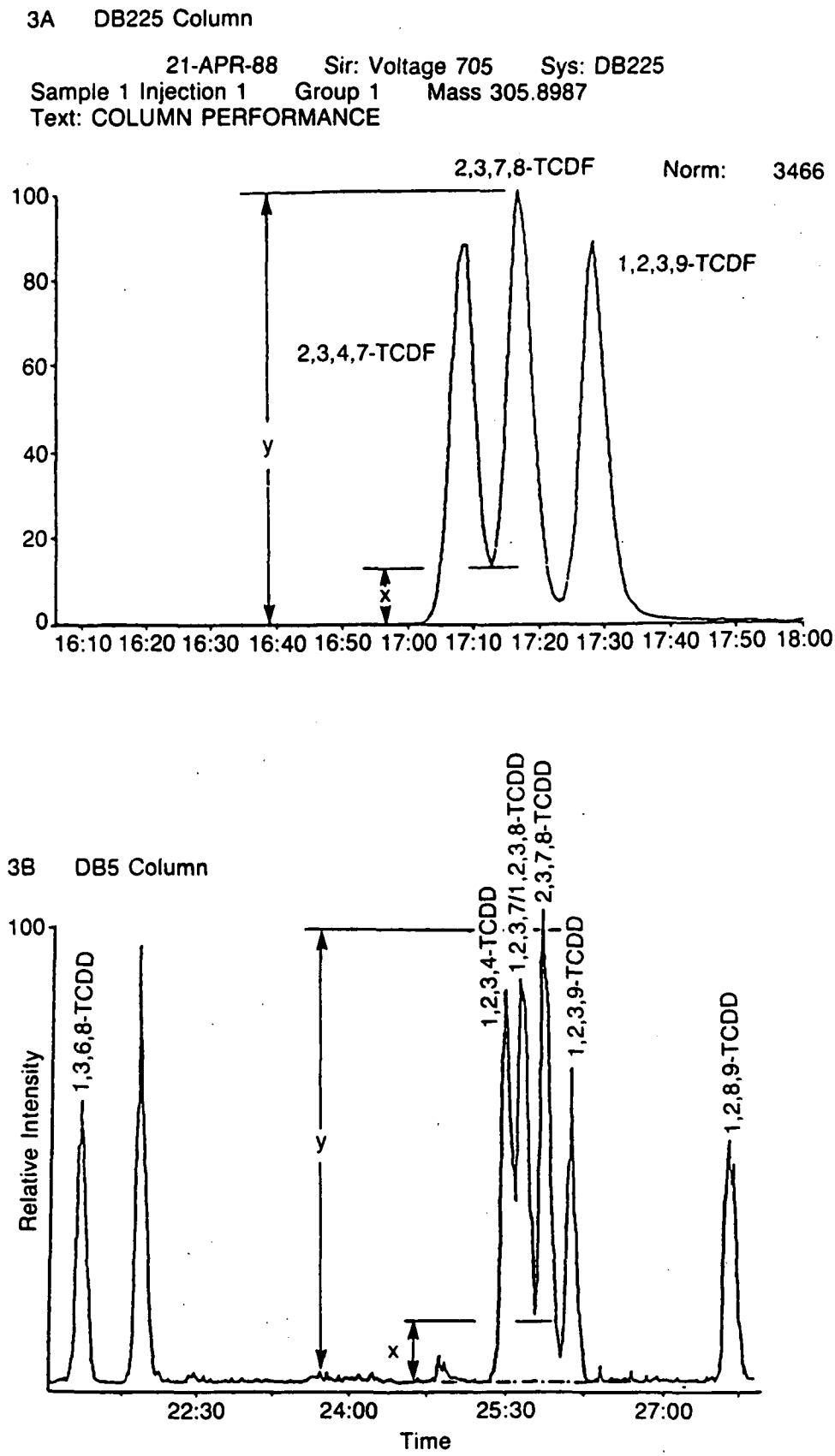


FIGURE 3 Valley between 2,3,7,8- Tetra Dioxin and Furan Isomers and Other Closely Eluted Isomers

7.5.2 The relative response of a PCDD or PCDF to its labeled analog is determined from isotope ratio values computed from acquired data. Three isotope ratios are used in this process:

Rx = the isotope ratio measured for the pure pollutant.

Ry = the isotope ratio measured for the labeled compound.

Rm = the isotope ratio of an analytical mixture of pollutant and labeled compounds.

The m/z's are selected such that Rx > Ry. If Rm is not between 2Ry and 0.5Rx, the method does not apply and the sample is analyzed by the internal standard method.

7.5.3 When there is no overlap between the GC peaks or the quantitation m/z's, as occurs with nearly all of the PCDDs and PCDFs and their respective labeled analogs, the RR is calculated per the following:

$$Rx = \frac{[\text{area m1/z}]}{1}$$

at the retention time of the native compound.

$$Ry = \frac{1}{[\text{area m2/z}]}$$

at the retention time of the labeled compound (RT2).

$$Rm = \frac{[\text{area at m1/z (at RT2)}]}{[\text{area at m2/z (at RT1)}]}$$

as measured in the mixture of the native and labeled compounds (Figure 4) (RT1).

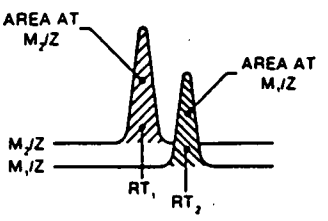


FIGURE 4 Selected Ion Current Profiles for Chromatographically Resolved Labeled (m_2/z) and Unlabeled (m_1/z) Pairs.

7.5.4 To calibrate the analytical system by isotope dilution, inject a 1.0 uL aliquot of calibration standards CS1 through CS5 (Section 6.13 and Table 4) using the procedure in Section 13 and the conditions in Table 2. Compute the RR at each concentration.

7.5.5 Linearity -- If the ratio of relative response to concentration for any compound is constant (less than 20 percent coefficient of variation) over the 5-point calibration range, an averaged relative response/concentration ratio may be used for that compound; otherwise, the complete calibration curve for that compound shall be used over the 5-point calibration range.

7.6 Calibration by internal standard -- The internal standard method is applied to determination of compounds having no labeled analog, and to measurement of labeled compounds for intra-laboratory statistics (Sections 8.4 and 14.5.4).

7.6.1 Response factors -- Calibration requires the determination of response factors (RF) defined by the following equation:

$$RF = \frac{(A_s \times C_{is})}{(A_{is} \times C_s)}$$

where,

A_s is the area of the exact m/z for the compound in the calibration standard.

A_{is} is the area of the exact m/z for the internal standard.

C_{is} is the concentration of the GCMS internal standard (Section 6.12 and Table 4) in pg/uL.

C_s is the concentration of the compound in the calibration standard in pg/uL.

7.6.1.1 The response factor is determined for at least five concentrations appropriate to the response of each compound (Section 6.13); nominally, 0.5, 2, 10, 40, and 200 ng/mL. The amount of internal standard added to each calibration solution and extract is the same (100 ng/mL) so that Cis remains constant. The RF is plotted vs concentration for each compound in the standard (Cs) to produce a calibration curve.

7.6.1.2 Linearity -- If the response factor (RF) for any compound is constant (less than 35

percent coefficient of variation) over the 5-point calibration range, an averaged response factor may be used for that compound; otherwise, the complete calibration curve for that compound shall be used over the 5-point range.

7.7 Combined calibration -- By using calibration solutions (Section 6.13 and Table 4) containing the unlabeled and labeled compounds, and the internal standards, a single set of analyses can be used to produce calibration curves for the isotope dilution and internal standard methods. These curves are verified each shift (Section 14.3) by analyzing the calibration verification standard (VER, Table 4). Recalibration is required if calibration verification criteria (Section 14.3.4) cannot be met.

8 QUALITY ASSURANCE/QUALITY CONTROL

8.1 Each laboratory that uses this method is required to operate a formal quality assurance program (Reference 16). The minimum requirements of this program consist of an initial demonstration of laboratory capability, analysis of samples spiked with labeled compounds to evaluate and document data quality, and analysis of standards and blanks as tests of continued performance. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance charac teristics of the method. If the method is to be applied routinely to samples containing high solids with very little moisture (e.g., soils, filter cake, compost) or to an alternate matrix, the high solids reference matrix (Section 6.6.2) or the alternate matrix (Section 6.6.4) is substituted for the reagent water matrix (Section 6.6.1) in all performance tests.

8.1.1 The analyst shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 The analyst is permitted to modify this method to improve separations or lower the costs of measurements, provided all performance specifications are met. Each time a modification is made to the method, the analyst is required to repeat the procedures in Sections 7.2 through 7.4 and Section 8.2 to demonstrate method performance.

8.1.3 Analyses of blanks are required to demonstrate freedom from contamination (Section 3.2). The procedures and criteria for analysis of a blank are described in Section 8.5.

8.1.4 The laboratory shall spike all samples with labeled compounds to monitor method performance. This test is described in Section 8.3. When results of these spikes indicate atypical method performance for samples, the samples are diluted to bring method performance within acceptable limits. Procedures for dilutions are given in Section 16.4.

8.1.5 The laboratory shall, on an ongoing basis, demonstrate through calibration verification and the analysis of the precision and recovery standard that the analytical system is in control. These procedures are described in Sections 14.1 through 14.5.

8.1.6 The laboratory shall maintain records to define the quality of data that is generated. Development of accuracy statements is described in Section 8.4.

8.2 Initial precision and accuracy -- To establish the ability to generate acceptable precision and accuracy, the analyst shall perform the following operations.

8.2.1 For low solids (aqueous samples), extract, concentrate, and analyze four 1-liter aliquots of reagent water spiked with the diluted precision and recovery standard (PAR) (Sections 6.14 and 10.3.4) according to the procedures in Sections 10 through 13. For an alternate sample matrix, four aliquots of the alternate matrix are used. All sample processing steps, including preparation (Section 10), extraction (Section 11), and cleanup (Section 12) that are to be used for processing samples shall be included in this test.

8.2.2 Using results of the set of four analyses, compute the average recovery (X) in ng/mL and the standard deviation of the recovery (s) in ng/mL for each compound, by isotope dilution for PCDDs and PCDFs with a labeled analog, and by internal standard for labeled compounds and PCDDs and PCDFs with no labeled analog.

8.2.3 For each compound, compare s and X with the corresponding limits for initial precision and accuracy in Table 7. If s and X for all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, any individual s exceeds the precision limit or any individual X falls outside the range for accuracy, system performance is unacceptable for that compound. Correct the problem and repeat the test (Section 8.2).

8.3 The laboratory shall spike all samples and QC aliquots with the diluted labeled compound spiking standard (Sections 6.10 and 10.3.2) to assess method performance on the sample matrix.

8.3.1 Analyze each sample according to the procedures in Sections 10 through 13.

8.3.2 Compute the percent recovery (P) of the labeled compounds in the labeled compound spiking standard and the cleanup standard using the internal standard method (Section 7.6).

8.3.3 Compare the labeled compound recovery for each compound with the corresponding limits in Table 7. If the recovery of any compound falls outside its limit, method performance is unacceptable for that compound in that sample. To overcome such difficulties, water samples are diluted and smaller amounts of soils, sludges, sediments and other matrices are reanalyzed per Section 17.

8.4 Method accuracy for samples shall be assessed and records shall be maintained.

8.4.1 After the analysis of five samples of a given matrix type (water, soil, sludge, pulp, etc) for which the labeled compound spiking standards pass the tests in Section 8.3, compute the average percent recovery (P) and the standard deviation of the percent recovery (sp) for the labeled compounds only. Express the accuracy assessment as a percent recovery interval from P - 2sp to P + 2sp for each matrix. For example, if P = 90% and sp = 10% for five analyses of pulp, the accuracy interval is expressed as 70 - 110%.

8.4.2 Update the accuracy assessment for each compound in each matrix on a regular basis (e.g., after each 5 - 10 new accuracy measurements).

8.5 Blanks -- Reference matrix blanks are analyzed to demonstrate freedom from contamination (Section 3.2).

8.5.1 Extract and concentrate a 1-liter reagent water blank (Section 6.6.1), high solids reference matrix blank (Section 6.6.2), paper matrix blank (Section 6.6.3) or alternate reference matrix blank (Section 6.6.4) with each sample set (samples started through the extraction process on the same 12-hour shift, to a maximum of 20 samples). Analyze the blank immediately after analysis of the precision and recovery standard (Section 14.5) to demonstrate freedom from contamination.

8.5.2 If any of the PCDDs or PCDFs (Table 1) or any potentially interfering compound is found in blank at greater than the minimum level (Table 2), assuming a response factor of 1 relative to the $^{13}C_{12}$-1,2,3,4-TCDD internal standard for compounds not listed in Table 1, analysis of samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination at this level.

8.6 The specifications contained in this method can be met if the apparatus used is calibrated properly and then maintained in a calibrated state. The standards used for calibration (Section 7), calibration verification (Section 14.3), and for initial (Section 8.2) and ongoing (Section 14.5) precision and recovery should be identical, so that the most precise results will be obtained. A GCMS instrument will provide the most reproducible results if dedicated to the settings and conditions required for the analyses of PCDDs and PCDFs by this method.

8.7 Depending on specific program requirements, field replicates may be collected to determine the precision of the sampling technique, and spiked samples may be required to determine the accuracy of the analysis when the internal standard method is used.

9 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

9.1 Collect samples in glass containers following conventional sampling practices (Reference 17). Aqueous samples which

flow freely are collected in refrigerated bottles using automatic sampling equipment. Solid samples are collected as grab samples using wide mouth jars.

9.2 Maintain samples at 0 - 4 °C from the time of collection until extraction. If residual chlorine is present in aqueous samples, add 80 mg sodium thiosulfate per liter of water. EPA Methods 330.4 and 330.5 may be used to measure residual chlorine (Reference 18).

9.3 Begin sample extraction within one year of collection, and analyze all extracts within 40 days of extraction.

10 SAMPLE PREPARATION

The sample preparation process involves modifying the physical form of the sample so that the PCDDs and PCDFs 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. Table 8 lists the phase(s) and quantity extracted for various sample matrices. Samples containing a solid phase and samples containing particle sizes larger than 1 mm require preparation prior to extraction. Because PCDDs/PCDFs are strongly associated with particulates, the preparation of aqueous samples is dependent on the solids content of the sample. Aqueous samples containing less than one percent solids are extracted in a separatory funnel. A smaller sample aliquot is used for aqueous samples containing one percent solids or more. For samples expected or known to contain high levels of the PCDDs and/or PCDFs, the smallest sample size representative of the entire sample should be used, and the sample extract should be diluted, if necessary, per Section 16.4.

10.1 Determine percent solids

10.1.1 Weigh 5 - 10 g of sample (to three significant figures) into a tared beaker. NOTE: This aliquot is used only for determining the solids content of the sample, not for analysis of PCDDs/PCDFs.

10.1.2 Dry overnight (12 hours minimum) at 110 ±5 °C, and cool in a dessicator.

10.1.3 Calculate percent solids as follows:

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

10.2 Determine particle size

10.2.1 Spread the dried sample from Section 10.1.2 on a piece of filter paper or aluminum foil in a fume hood or glove box.

10.2.2 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 1 mm or less prior to extraction.

10.3 Preparation of aqueous samples containing less than one percent solids -- The extraction procedure for aqueous samples containing less than one percent solids involves filtering the sample, extracting the particulate phase and the filtrate separately, and combining the extracts for analysis. The aqueous portion is extracted by shaking with methylene chloride in a separatory funnel. The particulate material is extracted using the SDS procedure.

10.3.1 Mark the original level of the sample on the sample bottle for reference. Weigh the sample in the bottle on a top loading balance to ±1 g.

10.3.2 Dilute a sufficient volume of the labeled compound spiking standard by a factor of 50 with acetone. 1.0 mL of the diluted solution is required for each sample, but no more solution should be prepared than can be used in one day. Spike 1.0 mL of the diluted solution into the sample bottle. Cap the bottle and mix the sample by careful shaking. Allow the sample to equilibrate for 1 - 2 hours, with occasional shaking.

10.3.3 For each sample or sample set (to a maximum of 20) to be extracted during the same 12-hour shift, place two 1.0 liter aliquots of reagent water in clean 2 liter separatory flasks.

10.3.4 Spike 1.0 mL of the diluted labeled compound spiking standard (Section 6.10) into one reagent water aliquot. This aliquot will serve as the blank. Dilute 20 uL of the precision and recovery standard (Section 6.14) to 1.0 mL with acetone. Spike 1.0 mL of the diluted precision and recovery standard into the

remaining reagent water aliquot. This aliquot will serve as the PAR (Section 14.5).

10.3.5 Assemble a Buchner funnel on top of a clean 1 L filtration flask. Apply a vacuum to the flask, and pour the entire contents of the sample bottle through a glass fiber filter (Section 5.5.4) in the Buchner funnel, swirling the sample remaining in the bottle to suspend any particulates.

10.3.6 Rinse the sample bottle twice with 5 mL of reagent water to transfer any remaining particulates onto the filter.

10.3.7 Rinse the any particulates off the sides of the Buchner funnel with small quantities of reagent water.

10.3.8 Weigh the empty sample bottle on a top-loading balance to ±1 g. Determine the weight of the sample by difference. Do not discard the bottle at this point.

10.3.9 Extract the filtrates using the procedures in Section 11.

10.3.10 Extract the particulates using the procedures in Section 11.

10.4 Preparation of samples containing greater than one percent solids

10.4.1 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 10.1.3) into a clean beaker or glass jar.

10.4.2 Spike 1.0 mL of the diluted labeled compound spiking solution (Section 10.3.2) into the sample aliquot(s).

10.4.3 For each sample or sample set (to a maximum of 20) to be extracted during the same 12-hour shift, weigh two 10 g aliquots of the appropriate reference matrix (Section 6.6) into clean beakers or glass jars.

10.4.4 Spike 1.0 mL of the diluted labeled compound spiking solution into one reference matrix aliquot. This aliquot will serve as the blank. Spike 1.0 mL of the diluted precision and recovery standard (Section 10.3.4) into the remaining reference matrix aliquot. This aliquot will serve as the PAR (Section 14.5).

10.4.5 Stir or tumble and equilibrate the aliquots for 1 - 2 hours.

10.4.6 Extract the aliquots using the procedures in Section 11.

10.5 Multi-phase samples

10.5.1 Pressure filter the sample, blank, and PAR aliquots through Whatman GF/D glass fiber filter paper. If necessary, centrifuge these aliquots for 30 minutes at greater than 5000 rpm prior to filtration.

10.5.2 Discard any aqueous phase (if present). Remove any non-aqueous liquid (if present) and reserve for recombination with the extract of the solid phase (Section 11.1.2.5). Prepare the filter papers of the sample and QC aliquots for particle size reduction and blending (Section 10.6).

10.6 Sample grinding, homogenization, or blending -- Samples with particle sizes greater than 1 mm (as determined by Section 10.2.2) 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 with a mortar and pestle. Softer particles can be reduced by grinding in a Wiley mill or meat grinder, by homogenization, or by blending.

10.6.1 Each size reducing preparation procedure on each matrix shall be verified by running the tests in Section 8.2 before the procedure is employed routinely.

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

10.6.3 Grinding -- Tissue samples, 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 10.4.5 or 10.5.2 in a clean grinder. Do not allow the sample temperature to exceed 50 °C. Grind the blank and reference matrix aliquots using a clean grinder.

10.6.4 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 sample, blank, and PAR aliquots from Section 10.4.5, 10.5.2, or 10.6.3.

10.6.5 Extract the aliquots using the procedures in Section 11.

11 EXTRACTION AND CONCENTRATION

11.1 Extraction

11.1.1 Extraction of filtrates -- extract the aqueous samples, blanks, and PAR aliquots according to the following procedures.

11.1.1.1 Pour filtered aqueous sample into a 2-L separatory funnel. Add 60 mL methylene chloride to the sample bottle, seal, and shake 60 seconds to rinse the inner surface.

11.1.1.2 Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting. 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, employ mechanical techniques to complete the phase separation (e.g. a glass stirring rod). Drain the methylene chloride extract into a 500-mL KD concentrator.

11.1.1.3 Extract the water sample two more times using 60 mL of fresh methylene chloride each time. Drain each extract into the KD concentrator. After the third extraction, rinse the separatory funnel with at least 30 mL of fresh methylene chloride.

11.1.2 Soxhlet/Dean-Stark extraction of solids -- Extract the solid samples, particulates, blanks, and PAR aliquots using the following procedure.

11.1.2.1 Charge a clean extraction thimble with 5.0 g of 100/200 mesh silica (Section 6.5.1.1) and 100 g of quartz sand (Section 6.5.4). NOTE: Do not disturb the silica layer throughout the extraction process.

11.1.2.2 Place the thimble in a clean extractor. Place 30 - 40 mL of toluene in the receiver and 200 - 250 mL in the flask.

11.1.2.3 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 condensor tip into the receiver. Extract the apparatus for 3 hours minimum.

11.1.2.4 After pre-extraction, cool and disassemble the apparatus. Rinse the thimble with toluene and allow to air dry.

11.1.2.5 Load the wet sample from Section 10.4.6, 10.5.2, 10.6.3, or 10.6.4, and any non-aqueous liquid from Section 10.5.2 into the thimble and manually mix into the sand layer with a clean metal spatula carefully breaking up any large lumps of sample.

11.1.2.6 Reassemble the pre-extracted SDS apparatus and add a fresh charge of toluene to the receiver and reflux flask.

11.1.2.7 Apply power to the heating mantle to begin refluxing. Adjust the reflux rate to match the rate of percolation through the sand and silica beds until water removal lessens the restriction to toluene flow. Check the apparatus for foaming frequently during the first 2 hours of extraction. If foaming occurs, reduce the reflux rate until foaming subsides.

11.1.2.8 Drain the water from the receiver at 1 - 2 hours and 8 - 9 hours, or sooner if the receiver fills with water. Reflux the sample for a total of 16 - 24 hours. Cool and disassemble the apparatus. Record the total volume of water collected.

11.1.2.9 Remove the distilling flask, estimate and record the volume of extract (to the nearest 100 mL), and pour the extract from the receiver and flask into a 500 mL separatory funnel. Rinse the receiver and flask with toluene and add to the separatory funnel. Proceed with back extraction per Section 11.1.3.

11.1.3 Back extraction with base and acid

11.1.3.1 Spike 1.0 mL of the cleanup standard (Section 6.11) into the separatory funnels containing the sample and QC extracts (Section 11.1.1.3 or 11.1.2.9).

11.1.3.2 Partition the extract against 50 mL of potassium hydroxide solution (Section 6.1.1). Shake for 2 minutes with periodic venting into a hood. Remove and discard the aqueous layer. Repeat the base washing until no color is visible in the aqueous layer, to a maximum of four washings. Minimize contact time between the extract and the base to prevent degradation of the PCDDs and PCDFs.

11.1.3.3 Partition the extract against 50 mL of sodium chloride solution (Section 6.1.3) in the same way as with base. Discard the aqueous layer.

11.1.3.4 Partition the extract against 50 mL of sulfuric acid (Section 6.1.2) in the same way as with base. Repeat the acid washing until no color is visible in the aqueous layer, to a maximum of four washings.

11.1.3.5 Repeat the partitioning against sodium chloride solution and discard the aqueous layer.

11.1.3.6 Pour each extract through a drying column containing 7 to 10 cm of anhydrous sodium sulfate. Rinse the separatory funnel with 30 - 50 mL of toluene and pour through the drying column. Collect each extract in a 500 mL round bottom flask. Concentrate and clean up the samples and QC aliquots per Sections 11.2 and 12.

11.2 Concentration

11.2.1 Macro-concentration -- Concentrate the extracts in separate 500 mL round bottom flasks on a rotary evaporator.

11.2.1.1 Assemble the rotary evaporator according to manufacturer's instructions, and warm the water bath to 45 °C. On a daily basis, preclean 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 contamination check if necessary. Between samples, three 2 - 3 mL aliquots of toluene should be rinsed down the feed tube into a waste beaker.

11.2.1.2 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.

11.2.1.3 Lower the flask into the water bath and adjust the speed of rotation and the temperature as required to complete the concentration in 15 - 20 minutes. 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.

11.2.1.4 When the liquid in the concentration flask has reached an apparent volume of 2 mL, remove the flask from the water bath and stop the rotation. Slowly and carefully, admit air into the system. Be sure not to open the valve so quickly that the sample is blown out of the flask. Rinse the feed tube with approximately 2 mL of hexane.

11.2.1.5 Transfer the extract to a vial using three 2 - 3 mL rinses of hexane. Proceed with micro-concentration and solvent exchange.

11.2.1.6 The extracts of the filtered aqueous sample and its particulates must be combined prior to cleanup and analysis. Transfer the concentrated extract of the aqueous sample to the flask containing the concentrated particulate extract. Rinse the flask twice with 5 mL toluene, and add these rinses to the flask with the combined extracts. Reattach the flask to the rotary evaporator and continue to concentrate the combined extract until the volume is approximately 2 mL. Proceed with micro-concentration and solvent exchange.

11.2.2 Micro-concentration and solvent exchange

11.2.2.1 Toluene extracts to be subjected to GPC cleanup are exchanged into methylene chloride. Extracts that are to be cleaned up using silica gel, alumina, and/or AX-21/Celite are exchanged into hexane. Extracts to be subjected to HPLC are exchanged into nonane.

11.2.2.2 Transfer the vial containing the sample extract to a nitrogen evaporation device. Adjust the flow of nitrogen so that the surface of the solvent is just visibly disturbed. NOTE: A large vortex in the solvent may cause analyte loss.

11.2.2.3 Lower the vial into a 45 °C water bath and continue concentrating.

11.2.2.4 When the volume of the liquid is approximately 100 uL, add 2 - 3 mL of the desired solvent (methylene chloride or hexane) and continue concentration to approximately 100 uL. Repeat the addition of solvent and concentrate once more.

11.2.2.5 If the extract is to be cleaned up by GPC, adjust the volume of the extract to 5.0 mL with methylene chloride. Proceed with GPC cleanup (Section 12.2).

11.2.2.6 If the extract is to be cleaned up by column chromatography (alumina, silica gel, AX-21/Celite), bring the final volume to 1.0 mL with hexane. Proceed with column cleanups (Sections 12.3 - 12.5).

11.2.2.7 For extracts to be concentrated for injection into the HPLC or GCMS -- add 10 uL of nonane to the vial. Evaporate the solvent to the level of the nonane. Evaporate the hexane in the vial to the level of the nonane.

11.2.2.8 Seal the vial and label with the sample number. Store in the dark at room temperature until ready for HPLC or GCMS.

12 EXTRACT CLEANUP

12.1 Cleanup may not be necessary for relatively clean samples (e.g., treated effluents, groundwater, drinking water). If particular circumstances require the use of a cleanup procedure, the analyst may use any or all of the procedures below or any other appropriate procedure. Before using a cleanup procedure, the analyst must demonstrate that the requirements of Section 8.2 can be met using the cleanup procedure.

12.1.1 Gel permeation chromatography (Section 12.2) removes many high molecular weight interferences that cause GC column performance to degrade. It may be used for all soil and sediment extracts and may be used for water extracts that are expected to contain high molecular weight organic compounds (e.g., polymeric materials, humic acids).

12.1.2 Acid, neutral, and basic silica gel, and alumina (Sections 12.3 and 12.4) are used to remove nonpolar and polar interferences.

12.1.3 AX-21/Celite (Section 12.5) is used to remove nonpolar interferences.

12.1.4 HPLC (Section 12.6) is used to provide specificity for the 2,3,7,8-substituted and other PCDD and PCDF isomers.

12.2 Gel permeation chromatography (GPC)

12.2.1 Column packing

12.2.1.1 Place 70 - 75 g of SX-3 Bio-beads in a 400 - 500 mL beaker.

12.2.1.2 Cover the beads with methylene chloride and allow to swell overnight (12 hours minimum).

12.2.1.3 Transfer the swelled beads to the column and pump solvent through the column, from bottom to top, at 4.5 - 5.5 mL/min prior to connecting the column to the detector.

12.2.1.4 After purging the column with solvent for 1 - 2 hours, adjust the column head pressure to 7 - 10 psig and purge for 4 - 5 hours to remove air. Maintain a head pressure of 7 - 10 psig. Connect the column to the detector.

12.2.2 Column calibration

12.2.2.1 Load 5 mL of the calibration solution (Section 6.4) into the sample loop.

12.2.2.2 Inject the calibration solution and record the signal from the detector. The elution pattern will be corn oil, bis(2-ethyl hexyl) phthalate, pentachlorophenol, perylene, and sulfur.

12.2.2.3 Set the "dump time" to allow >85 percent removal of the corn oil and >85 percent collection of the phthalate.

12.2.2.4 Set the "collect time" to the peak minimum between perylene and sulfur.

12.2.2.5 Verify the calibration with the calibration solution after every 20 extracts. Calibration is verified if the recovery of the pentachlorophenol is greater than 85 percent. If calibration is not verified, the system shall be recalibrated using the calibration solution, and the previous 20 samples shall be re-extracted and cleaned up using the calibrated GPC system.

12.2.3 Extract cleanup -- GPC requires that the column not be overloaded. The column specified in this method is designed to handle a maximum of 0.5 g of high molecular weight material in a 5 mL

extract. If the extract is known or expected to contain more than 0.5 g, the extract is split into aliquots for GPC and the aliquots are combined after elution from the column. The solids content of the extract may be obtained gravimetrically by evaporating the solvent from a 50 uL aliquot.

12.2.3.1 Filter the extract or load through the filter holder to remove particulates. Load the 5.0 mL extract onto the column.

12.2.3.2 Elute the extract using the calibration data determined in Section 12.2.2. Collect the eluate in a clean 400 - 500 mL beaker.

12.2.3.3 Rinse the sample loading tube thoroughly with methylene chloride between extracts to prepare for the next sample.

12.2.3.4 If a particularly dirty extract is encountered, a 5.0 mL methylene chloride blank shall be run through the system to check for carry-over.

12.2.3.5 Concentrate the eluate per Section 11.2.1, 11.2.2, and 11.3.1 or 11.3.2 for further cleanup or for injection into the GCMS.

12.3 Silica gel cleanup

12.3.1 Place a glass wool plug in a 15 mm i.d. chromatography column. Pack the column in the following order (bottom to top): 1 g silica gel (Section 6.5.1.1), four g basic silica gel (Section 6.5.1.3), 1 g silica gel, 8 g acid silica gel (Section 6.5.1.2), 2 g silica gel, 1 g sodium sulfate (Section 6.2.1). Tap the column to settle the adsorbents.

12.3.2 Pre-rinse the column with 50 - 100 mL of hexane. Close the stopcock when the hexane is within 1 mm of the sodium sulfate. Discard the eluate. Check the column for channeling. If channeling is present, discard the column and prepare another.

12.3.3 Apply the concentrated extract to the column. Open the stopcock until the extract is within 1 mm of the sodium sulfate.

12.3.4 Rinse the receiver twice with 1 mL portions of hexane and apply separately to the column. Elute the PCDDs/PCDFs with 100 mL hexane and collect the eluate.

12.3.5 Concentrate the eluate per Section 11.2.1 or 11.2.2 for further cleanup or for injection into the HPLC or GCMS.

12.4 Alumina cleanup

12.4.1 Place a glass wool plug in a 15 mm i.d. chromatography column.

12.4.2 Pack the column in the following order (bottom to top): 1 g neutral alumina (Section 6.5.2.1), 3 g basic alumina (Section 6.5.2.2), 1 g neutral alumina, 6 g acid alumina (Section 6.5.2.3), 2 g neutral alumina, 1 g sodium sulfate (Section 6.2.1). Tap the column to settle the adsorbents.

12.4.3 Pre-rinse the column with 50 - 100 mL of hexane. Close the stopcock when the hexane is within 1 mm of the sodium sulfate.

12.4.4 Discard the eluate. Check the column for channeling. If channeling is present, discard the column and prepare another.

12.4.5 Apply the concentrated extract to the column. Open the stopcock until the extract is within 1 mm of the sodium sulfate.

12.4.6 Rinse the receiver twice with 1 mL portions of hexane and apply separately to the column. Elute the interfering compounds with 100 mL hexane and discard the eluate.

12.4.7 Elute the PCDDs and PCDFs with 20 mL of methylene chloride:hexane (1:1 v/v).

12.4.8 Concentrate the eluate per Section 11.2.1 or 11.2.2 for further cleanup or for injection into the HPLC or GCMS.

12.5 AX-21/Celite

12.5.1 Cut both ends from a 10 mL disposable serological pipet to produce a 10 cm column. Fire polish both ends and flare both ends if desired. Insert a glass wool plug at one end, then pack the column with 1 g of the activated AX-21/Celite to form a 2 cm long adsorbent bed. Insert a glass wool plug on top of the bed to hold the adsorbent in place.

12.5.2 Pre-rinse the column with five mL of toluene followed by 2 mL methylene chloride:methanol:toluene (15:4:1 v/v), 1 mL methylene chloride:cyclohexane (1:1

v/v), and five mL hexane. If the flow rate of eluate exceeds 0.5 mL per min, discard the column.

12.5.3 When the solvent is within 1 mm of the column packing, apply the sample extract to the column. Rinse the sample container twice with 1 mL portions of hexane and apply separately to the column. Apply 2 mL of hexane to complete the transfer.

12.5.4 Elute the interfering compounds with 2 mL of hexane, 2 mL of methylene chloride:cyclohexane (1:1 v/v), and 2 mL of methylene chloride:methanol:toluene (15:4:1 v/v). Discard the eluate.

12.5.5 Invert the column and elute the PCDDs and PCDFs with 20 mL of toluene. If carbon particles are present in the eluate, filter through glass fiber filter paper.

12.5.6 Concentrate the eluate per Section 11.2.1 or 11.2.2 for further cleanup or for injection into the HPLC or GCMS.

12.6 HPLC (Reference 6)

12.6.1 Column calibration

12.6.1.1 Prepare a calibration standard containing the 2,3,7,8- isomers and/or other isomers of interest at a concentration of approximately 500 pg/uL in chloroform.

12.6.1.2 Inject 30 uL of the calibration solution into the HPLC and record the signal from the detector. Collect the eluant for re-use. The elution order will be the tetra- through octa-isomers.

12.6.1.3 Establish the collect time for the tetra-isomers and for the other isomers of interest. Following calibration, flush the injection system with copious quantities of chloroform, including a minimum of five 50-uL injections while the detector is monitored, to ensure that residual PCDDs and PCDFs are removed from the system.

12.6.1.4 Verify the calibration with the calibration solution after every 20 extracts. Calibration is verified if the recovery of the PCDDs and PCDFs from the calibration standard (Section 12.6.1.1) is 75 - 125 percent compared to the calibration (Section 12.6.1.2). If calibration is not verified, the system shall be recalibrated using the calibration solution, and the previous 20 samples shall be re-extracted and cleaned up using the calibrated system.

12.6.2 Extract cleanup -- HPLC requires that the column not be overloaded. The column specified in this method is designed to handle a maximum of 30 uL of extract. If the extract cannot be concentrated to less than 30 uL, it is split into fractions and the fractions are combined after elution from the column.

12.6.2.1 Rinse the sides of the vial twice with 30 uL of chloroform and reduce to the level of the nonane with the blowdown apparatus. Rinse the sides of the vial with 20 uL of chloroform to bring the extract volume to 30 uL.

12.6.2.2 Inject the 30 uL extract into the HPLC.

12.6.2.3 Elute the extract using the calibration data determined in 12.6.1. Collect the fraction(s) in a clean 20 mL concentrator tube containing 5 mL of hexane:acetone (1:1 v/v).

12.6.2.4 If an extract containing greater than 100 ng/mL of total PCDD or PCDF is encountered, a 30 uL chloroform blank shall be run through the system to check for carry-over.

12.6.2.5 Concentrate the eluate per Section 11.2.2 for injection into the GCMS.

13 HRGC/HRMS ANALYSIS

13.1 Establish the operating conditions given in Section 7.1.

13.2 Add 10 uL of the internal standard solution (Section 6.12) to the sample extract immediately prior to injection to minimize the possibility of loss by evaporation, adsorption, or reaction. If an extract is to be reanalyzed, do not add more instrument internal standard solution. Rather, bring the extract back to its previous volume (e.g., 19 uL) with pure nonane only.

13.3 Inject 1.0 uL of the concentrated extract containing the internal standatd solution, using on-column or splitless injection. Start the GC column initial isothermal hold upon injection. Start MS data collection after the solvent peak elutes. Stop data collection after the octachloro-

dioxin and furan have eluted. Return the column to the initial temperature for analysis of the next extract or standard.

14 SYSTEM AND LABORATORY PERFORMANCE

14.1 At the beginning of each 12-hour shift during which analyses are performed, GCMS system performance and calibration are verified for all native and labeled compounds. For these tests, analysis of the CS3 calibration verification (VER) standard (Section 6.13 and Table 4) and the isomer specificity test standards (Sections 6.16 and Table 5) shall be used to verify all performance criteria. Adjustment and/or recalibration (per Section 7) shall be performed until all performance criteria are met. Only after all performance criteria are met may samples, blanks, and precision and recovery standards be analyzed.

14.2 Mass spectrometer resolution -- A static resolving power of at least 10,000 (10 percent valley definition) 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 shift. Corrective actions must be implemented whenever the resolving power does not meet the requirement.

14.2.1 The analysis time for PCDDs and PCDFs may exceed the long-term mass stability of the mass spectrometer. Because the instrument is operated in the high-resolution mode, mass drifts of a few ppm (e.g., 5 ppm in mass) can have serious adverse effects on instrument performance. Therefore, a mass-drift correction is mandatory. A lock-mass ion from the reference compound (PFK) is used for tuning the mass spectrometer. The lock-mass ion is dependent on the masses of the ions monitored within each descriptor, as shown in Table 3. The level of the reference compound (PFK) metered into the ion chamber during HRGC/HRMS analyses should be adjusted so that the amplitude of the most intense selected lock-mass ion signal (regardless of the descriptor number) does not exceed 10 percent of the full-scale deflection for a given set of detector parameters. Under those conditions, sensitivity changes that might occur during the analysis can be more effectively monitored. NOTE: Excessive PFK (or any other reference substance) may cause noise problems and contamination of the ion source resulting in an increase in time lost in cleaning the source.

14.2.2 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 the peak matching unit and the PFK reference peak, verify that the exact mass of m/z 380.9760 (PFK) is within 5 ppm of the required value.

14.3 Calibration verification

14.3.1 Inject the VER standard using the procedure in Section 13.

14.3.2 The m/z abundance ratios for all PCDDs and PCDFs shall be within the limits in Table 3A; otherwise, the mass spectrometer shall be adjusted until the m/z abundance ratios fall within the limits specified, and the verification test (Section 14.3.1) repeated. If the adjustment alters the resolution of the mass spectrometer, resolution shall be verified (Section 14.2) prior to repeat of the verification test.

14.3.3 Compute the concentration of each native compound by isotope dilution (Section 7.5) for those compounds that have labeled analogs (Table 1). Compute the concentration of the labeled compounds by the internal standard method. These concentrations are computed based on the averaged relative response and averaged response factor from the calibration data in Section 7.

14.3.4 For each compound, compare the concentration with the calibration verification limit in Table 7. If all compounds meet the acceptance criteria, calibration has been verified. If, however, any compound fails, the measurement system is not performing properly for that compound. In this event, prepare a fresh calibration standard or correct the problem causing the failure and repeat the resolution (Section 14.2) and verification (Section 14.3.1) tests, or recalibrate (Section 7).

14.4 Retention times and GC resolution

14.4.1 Retention times

14.4.1.1 Absolute -- The absolute retention times of the $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDF GCMS internal standards shall be within ±15 seconds of the retention times obtained during calibration (Section 7.2.4).

14.4.1.2 Relative -- The relative retention times of native and labeled PCDDs and PCDFs shall be within the limits given in Table 2.

14.4.2 GC resolution

14.4.2.1 Inject the isomer specificity standards (Section 6.16) on their respective columns.

14.4.2.2 The valley height between 2,3,7,8-TCDD and the other tetra- dioxin isomers at m/z 319.8965, and between 2,3,7,8-TCDF and the other tetra- furan isomers at m/z 303.9016 shall not exceed 25 percent on their respective columns (Figure 3).

14.4.3 If the absolute or relative retention time of any compound is not within the limits specified or the 2,3,7,8- isomers are not resolved, the GC is not performing properly. In this event, adjust the GC and repeat the verification test (Section 14.3.1) or recalibrate (Section 7).

14.5 Ongoing precision and accuracy

14.5.1 Analyze the extract of the precision and recovery standard (PAR) (Section 10.3.4 or 10.4.4) prior to analysis of samples from the same set.

14.5.2 Compute the concentration of each PCDD or PCDF by isotope dilution (Section 7.5) for those compounds that have labeled analogs. Compute the concentration of the labeled compounds by the internal standard method.

14.5.3 For each compound, compare the concentration with the limits for ongoing accuracy in Table 7. If all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may proceed. If, however, any individual concentration falls outside of the range given, the extraction/concentration processes are not being performed properly for that compound. In this event, correct the problem, re-extract the sample set (Section 10) and repeat the ongoing precision and recovery test (Section 14.5).

14.5.4 Add results which pass the specifications in Section 14.5.3 to initial and previous ongoing data for each compound in each matrix. Update QC charts to form a graphic representation of continued laboratory performance. Develop a statement of laboratory accuracy for each PCDD and PCDF in each matrix type by calculating the average percent recovery (R) and the standard deviation of percent recovery (sr). Express the accuracy as a recovery interval from R - 2sr to R + 2sr. For example, if R = 95% and sr = 5%, the accuracy is 85 - 105%.

15 QUALITATIVE DETERMINATION

Identification is accomplished by comparison of data from analysis of a sample or blank with data for authentic standards. For compounds for which the relative retention times are known, identification is confirmed per Sections 15.1 and 15.2.

15.1 Labeled compounds and native PCDDs and PCDFs having no labeled analog

15.1.1 The signals for the exact m/z's being monitored (Table 3A) shall be present and shall maximize within the same two consecutive scans.

15.1.2 Either (1) the ratio of the background corrected exact SICP areas, or (2) the corrected relative intensities of the exact m/z's at the GC peak maximum shall be within the limits in Table 3A.

15.1.3 For the individual labeled compounds and individual PCDDs and PCDFs, the relative retention time shall be within the limits specified in Table 2.

15.2 PCDDs and PCDFs having a labeled analog

15.2.1 The signals for the exact m/z's being monitored (Table 3) shall be present and shall maximize within the same two consecutive scans.

15.2.2 The ratio of the ion abundances of the exact m/z's at the GC peak maximum shall agree within the limits in Table 3.

15.2.3 The relative retention time between the native compound and its labeled analog shall be within the windows specified in Table 2.

15.3 If identification is ambiguous, an experienced spectrometrist (Section 1.5) is to determine the presence or absence of the compound.

16 QUANTITATIVE DETERMINATION

16.1 Isotope dilution -- By adding a known amount of a labeled compound to every sample prior to extraction, correction for recovery of the native compound can be made because the native compound and its labeled analog exhibit the same effects upon extraction, concentration, and gas chromatography. Relative response (RR) values for sample mixtures are used in conjunction with calibration data described in Section 7.5 to determine concentrations directly, so long as labeled compound spiking levels are constant.

16.1.1 Because of a potential interference, the labeled analog of OCDF is not added to the sample. Therefore, this native analyte is quantitated against the labeled OCDD.

16.1.2 Because the labeled analog of 1,2,3,7,8,9-HxCDD is used as an internal standard (i.e., not added before extraction of the sample), it cannot be used to quantitate the native compound. Therefore, the native 1,2,3,7,8,9-HxCDD is quantitated using the average of the responses of the labeled analogs of the other two 2,3,7,8-substituted HxCDD's, 1,2,3,4,7,8-HxCDD and 1,2,3,6,7,8-HxCDD.

16.1.3 Any peaks representing non-2,3,7,8-substituted dioxins or furans are quantitated using an average of the response factors from all the labeled 2,3,7,8- isomers in the same level of chlorination.

16.2 Internal standard -- Compute the concentrations of the labeled analogs and the cleanup standard in the extract using the response factors determined from calibration data (Section 7.6) and the following equation:

$$C_{ex}\ (ng/mL) = \frac{(A_s \times C_{is})}{(A_{is} \times RF)}$$

where C_{ex} is the concentration of the compound in the extract and the other terms are as defined in Section 7.6.1.

16.3 The concentration of the native compound in the solid phase of the sample is computed using the concentration of the compound in the extract and the weight of the solids (Section 10), as follows:

$$\text{Concentration in solid (ng/kg)} = \frac{(C_{ex} \times V_{ex})}{W_s}$$

where,

V_{ex} is the extract volume in mL.

W_s is the sample weight in Kg.

16.4 If the SICP area at the quantitation m/z for any compound exceeds the calibration range of the system, a smaller sample aliquot is extracted.

16.4.1 For aqueous samples containing one percent solids or less, dilute 100 mL, 10 mL, etc., of sample to 1 liter with reagent water and extract per Section 11.

16.4.2 For samples containing greater than one percent solids, extract an amount of sample equal to 1/10, 1/100, etc of the amount determined in 10.1.3. Extract per Section 10.4.

16.4.3 If a smaller sample size will not be representative of the entire sample, dilute the sample extract by a factor of 10, adjust the concentration of the instrument internal standard to 100 pg/uL in the extract, and analyze an aliquot of this diluted extract by the internal standard method.

16.5 Results are reported to three significant figures for the native and labeled isomers found in all standards, blanks, and samples. For aqueous samples, the units are ng/L; for samples containing one percent or greater solids (soils, sediments, filter, cake, compost), the units are ng/kg, based on the dry weight of the sample.

16.5.1 Results for samples which have been diluted are reported at the least dilute level at which the area at the quantitation m/z is within the calibration range (Section 16.4).

16.5.2 For native compounds having a labeled analog, results are reported at the least dilute level at which the area at the quantitation m/z is within the calibration range (Section 16.4) and the labeled compound recovery is within the normal range for the method (Section 17.4).

16.5.3 Additionally, the total concentrations of all isomers in an individual level of chlorination (i.e. total TCDD, total PeCDD, etc.) are reported to three significant figures in units of ng/L, for both dioxins and furans. The total or ng/kg concentration in each level of chlorination is the sum of the concentrations of all isomers identified in that level, including any non-2,3,7,8-substituted isomers.

17 ANALYSIS OF COMPLEX SAMPLES

17.1 Some samples may contain high levels (>10 ng/L; >1000 ng/kg) of the compounds of interest, interfering compounds, and/or polymeric materials. Some extracts will not concentrate to 10 uL (Section 11); others may overload the GC column and/or mass spectrometer.

17.2 Analyze a smaller aliquot of the sample (Section 16.4) when the extract will not concentrate to 20 uL after all cleanup procedures have been exhausted.

17.3 Interferences at the primary m/z -- If an interference occurs at the primary quantitation m/z (Table 3) for any native or labeled compound, the alternate m/z is used for quantitation.

17.4 Recovery of labeled compound spiking standards -- In most samples, recoveries of the labeled compound spiking standards will be similar to those from reagent water or from the alternate matrix (Section 6.6). If recovery is outside of the limits given in Table 7, a diluted sample (Section 16.4) is analyzed. If the recoveries of the labeled compound spiking standards in the diluted sample are outside of the limits (per the criteria above), then the verification standard (Section 14.3) shall be analyzed and calibration verified (Section 14.3.4). If the calibration cannot be verified, a new calibration must be performed and the original sample extract reanalyzed. If the calibration is verified and the diluted sample does not meet the limits for labeled compound recovery, then the method does not apply to the sample being analyzed and the result may not be reported for regulatory compliance purposes.

18 METHOD PERFORMANCE

EPA is in the process of developing performance data for this draft method. When these tests are complete, the specifications in this method will be modified based on these data, and the supporting documents will be referenced in this section.

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2 "Measurement of 2,3,7,8-Tetrachlorinated Dibenzo-p-dioxin (TCDD) and 2,3,7,8-Tetrachlorinated Dibenzofuran (TCDF) in Pulp, Sludges, Process Samples and Wastewaters from Pulp and Paper Mills", Wright State University, Dayton OH 45435, June 1988.

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Table 1

POLYCHLORINATED DIBENZODIOXINS AND FURANS DETERMINED BY ISOTOPE DILUTION AND INTERNAL STANDARD HIGH RESOLUTION GAS CHROMATOGRAPHY (HRGC)/HIGH RESOLUTION MASS SPECTROMETRY (HRMS)

PCDDs/PCDFs (1) Isomer/Congener	CAS Registry	Labeled Analog	CAS Registry
2,3,7,8-TCDD	1746-01-6	$^{13}C_{12}$-2,3,7,8-TCDD	76523-40-5
		$^{37}Cl_4$-2,3,7,8-TCDD	85508-50-5
Total-TCDD	41903-57-5		
2,3,7,8-TCDF	51207-31-9	$^{13}C_{12}$-2,3,7,8-TCDF	89059-46-1
Total-TCDF	55722-27-5		
1,2,3,7,8-PeCDD	40321-76-4	$^{13}C_{12}$-1,2,3,7,8-PeCDD	109719-79-1
Total-PeCDD	36088-22-9		
1,2,3,7,8-PeCDF	57117-41-6	$^{13}C_{12}$-1,2,3,7,8-PeCDF	109719-77-9
2,3,4,7,8-PeCDF	57117-31-4	$^{13}C_{12}$-2,3,4,7,8-PeCDF	116843-02-8
Total-PeCDF	30402-15-4		
1,2,3,4,7,8-HxCDD	39227-28-6	$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	109719-80-4
1,2,3,6,7,8-HxCDD	57653-85-7	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	109719-81-5
1,2,3,7,8,9-HxCDD	19408-74-3	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD(2)	109719-82-6
Total-HxCDD	34465-4608		
1,2,3,4,7,8-HxCDF	70648-26-9	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	114423-98-2
1,2,3,6,7,8-HxCDF	57117-44-9	$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	116843-03-9
1,2,3,7,8,9-HxCDF	72918-21-9	$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	116843-04-0
2,3,4,6,7,8-HxCDF	60851-34-5	$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	116843-05-1
Total-HxCDF			
1,2,3,4,6,7,8-HpCDD	35822-46-9	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	109719-83-7
Total-HpCDD	37871-00-4		
1,2,3,4,6,7,8-HpCDF	67562-39-4	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	109719-84-8
1,2,3,4,7,8,9-HpCDF	55673-89-7	$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	109719-94-0
Total-HpCDF	38998-75-3		
OCDD	3268-87-9	$^{13}C_{12}$-OCDD	114423-97-1
OCDF	39001-02-0		

(1) Polychlorinated dioxins and furans

TCDD	=	Tetrachlorodibenzo-p-dioxin	TCDF	=	Tetrachlorodibenzofuran
PeCDD	=	Pentachlorodibenzo-p-dioxin	PeCDF	=	Pentachlorodibenzofuran
HxCDD	=	Hexachlorodibenzo-p-dioxin	HxCDF	=	Hexachlorodibenzofuran
HpCDD	=	Heptachlorodibenzo-p-dioxin	HpCDF	=	Heptachlorodibenzofuran
OCDD	=	Octachlorodibenzo-p-dioxin	OCDF	=	Octachlorodibenzofuran

(2) Labeled analog is used as an internal standard and therefore cannot be used for quantitation by isotope dilution.

Table 2

RETENTION TIMES AND MINIMUM LEVELS FOR PCDDs AND PCDFs

Compound	Absolute Retention Time (Minutes)	Retention Time Reference	Relative Retention Time (1)	Minimum Level (2) Water pg/L ppq	Solid ng/kg ppt	Extract pg/uL ppb
Compounds using $^{13}C_{12}$-1,2,3,4-TCDD as internal standard						
Native Compounds						
2,3,7,8-TCDF	26.35	$^{13}C_{12}$-2,3,7,8-TCDF	0.999 - 1.001	10	1	0.5
2,3,7,8-TCDD	27.24	$^{13}C_{12}$-2,3,7,8-TCDD	0.999 - 1.001	10	1	0.5
1,2,3,7,8-PeCDF	31.16	$^{13}C_{12}$-1,2,3,7,8-PeCDF	0.999 - 1.001	50	5	2.5
2,3,4,7,8-PeCDF	32.16	$^{13}C_{12}$-2,3,4,7,8-PeCDF	0.999 - 1.001	50	5	2.5
1,2,3,7,8-PeCDD	32.45	$^{13}C_{12}$-1,2,3,7,8-PeCDD	0.999 - 1.001	50	5	2.5
Labeled Compounds						
$^{13}C_{12}$-2,3,7,8-TCDF	26.35	$^{13}C_{12}$-1,2,3,4-TCDD	0.970 - 0.980			
$^{13}C_{12}$-1,2,3,4-TCDD	27.03	$^{13}C_{12}$-1,2,3,4-TCDD	1.000 - 1.000			
$^{13}C_{12}$-2,3,7,8-TCDD	27.22	$^{13}C_{12}$-1,2,3,4-TCDD	1.002 - 1.012			
$^{37}Cl_4$-2,3,7,8-TCDD	27.23	$^{13}C_{12}$-1,2,3,4-TCDD	1.002 - 1.013			
$^{13}C_{12}$-1,2,3,7,8-PeCDF	31.16	$^{13}C_{12}$-1,2,3,4-TCDF	1.147 - 1.159			
$^{13}C_{12}$-2,3,4,7,8-PeCDF	32.15	$^{13}C_{12}$-1,2,3,4-TCDD	1.183 - 1.196			
$^{13}C_{12}$-1,2,3,7,8-PeCDD	32.44	$^{13}C_{12}$-1,2,3,4-TCDD	1.194 - 1.206			
Compounds using $^{13}C_{12}$-1,2,3,7,8,9-HxCDD as internal standard						
Native Compounds						
1,2,3,4,7,8-HxCDF	36.19	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	0.999 - 1.001	50	5	2.5
1,2,3,6,7,8-HxCDF	36.29	$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	0.999 - 1.001	50	5	2.5
2,3,4,6,7,8-HxCDF	37.19	$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	0.999 - 1.001	50	5	2.5
1,2,3,4,7,8-HxCDD	37.30	$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	0.999 - 1.001	50	5	2.5
1,2,3,6,7,8-HxCDD	37.36	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	0.999 - 1.001	50	5	2.5
1,2,3,7,8,9-HxCDD	38.07	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	0.999 - 1.001	50	5	2.5
1,2,3,7,8,9-HxCDF	38.23	$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	0.999 - 1.001	50	5	2.5
1,2,3,4,6,7,8-HpCDF	40.55	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	0.999 - 1.001	50	5	2.5
1,2,3,4,6,7,8-HpCDD	42.27	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	0.999 - 1.001	50	5	2.5
1,2,3,4,7,8,9-HpCDF	43.01	$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	0.999 - 1.001	50	5	2.5
OCDD	46.56	$^{13}C_{12}$-OCDD	0.999 - 1.001	100	10	5.0
OCDF	47.05	$^{13}C_{12}$-OCDD	1.007 - 1.013	100	10	5.0
Labeled Compounds						
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	36.18	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.946 - 0.956			
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	36.27	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.948 - 0.958			
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	37.29	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.975 - 0.985			
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	37.38	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.977 - 0.987			
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	38.06	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1.000 - 1.000			
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	38.23	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0.999 - 1.010			
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	40.54	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1.060 - 1.071			
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	42.27	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1.105 - 1.116			
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	43.01	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1.124 - 1.136			
$^{13}C_{12}$-OCDD	46.55	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1.217 - 1.230			
$^{13}C_{12}$-OCDF	47.04	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1.229 - 1.242			

(1) Initial specifications are estimated based on isotope dilution and internal standard data from Method 1625. These specifications may be revised when further data have been collected by EPA using Method 1613.

(2) Level at which the analytical system will give acceptable SICP and calibration.

Table 3

DESCRIPTORS, MASSES, M/Z TYPES, AND ELEMENTAL COMPOSITIONS OF THE CDDs AND CDFs (1)

Descriptor Number	Accurate m/z (2)	m/z Type	Elemental Composition	Compound (3)	Primary m/z?
1	292.9825	Lock	C_7F_{11}	PFK	
	303.9016	M	$C_{12}H_4{}^{37}Cl_4O$	TCDF	Yes
	305.8987	M+2	$C_{12}H_4{}^{37}Cl_4O$	TCDF	
	315.9419	M	$^{13}C_{12}H_4{}^{35}Cl_4O$	TCDF(4)	Yes
	317.9389	M+2	$^{13}C_{12}H_4{}^{35}Cl_3{}^{37}ClO$	TCDF(4)	
	319.8965	M	$C_{12}H_4{}^{35}Cl_4O_2$	TCDD	Yes
	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(4)	
	330.9792	QC	C_7F_{13}	PFK	
	331.9368	M	$^{13}C_{12}H_4{}^{35}Cl_4O_2$	TCDD(4)	Yes
	333.9339	M+2	$^{13}C_{12}H_4{}^{35}Cl_3{}^{37}ClO_2$	TCDD(4)	
	375.8364	M+2	$C_{12}H_4{}^{35}Cl_5{}^{37}ClO$	HxCDPE	
2	339.8597	M+2	$C_{12}H_3{}^{35}Cl_4{}^{37}ClO$	PeCDF	Yes
	341.8567	M+4	$C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O$	PeCDF	
	351.9000	M+2	$^{13}C_{12}H_3{}^{35}Cl_4{}^{37}ClO$	PeCDF(4)	Yes
	353.8970	M+4	$^{13}C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O$	PeCDF(4)	
	354.9792	Lock	C_9F_{13}	PFK	
	355.8546	M+2	$C_{12}H_3{}^{35}Cl_4{}^{37}ClO_2$	PeCDD	Yes
	357.8516	M+4	$C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O_2$	PeCDD	
	367.8949	M+2	$^{13}C_{12}H_3{}^{35}Cl_4{}^{37}ClO_2$	PeCDD(4)	Yes
	369.8919	M+4	$^{13}C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O_2$	PeCDD(4)	
	409.7974	M+2	$C_{12}H_3{}^{35}Cl_6{}^{37}ClO$	HpCDPE	
3	373.8208	M+2	$C_{12}H_2{}^{35}Cl_5{}^{37}ClO$	HxCDF	Yes
	375.8178	M+4	$C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O$	HxCDF	
	383.8639	M	$^{13}C_{12}H_2{}^{35}Cl_6O$	HxCDF(4)	Yes
	385.8610	M+2	$^{13}C_{12}H_2{}^{35}Cl_5{}^{37}ClO$	HxCDF(4)	
	389.8157	M+2	$C_{12}H_2{}^{35}Cl_5{}^{37}ClO_2$	HxCDD	Yes
	391.8127	M+4	$C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O_2$	HxCDD	
	392.9760	Lock	C_9F_{15}	PFK	
	401.8559	M+2	$^{13}C_{12}H_2{}^{35}Cl_5{}^{37}ClO_2$	HxCDD(4)	Yes
	403.8529	M+4	$^{13}C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O_2$	HxCDD(4)	
	430.9729	QC	C_9F_{13}	PFK	
	445.7555	M+4	$C_{12}H_2{}^{35}Cl_6{}^{37}Cl_2O$	OCDPE	

Table 3 (continued)

DESCRIPTORS, MASSES, M/Z TYPES, AND ELEMENTAL COMPOSITIONS OF THE CDDs AND CDFs (1)

Descriptor Number	Accurate m/z (2)	m/z Type	Elemental Composition	Compound (3)	Primary m/z?
4	407.7818	M+2	$C_{12}\,H\,{}^{35}Cl_6\,{}^{37}Cl\,O$	HpCDF	Yes
	409.7789	M+4	$C_{12}\,H\,{}^{35}Cl_5\,{}^{37}Cl_2\,O$	HpCDF	
	417.8253	M	${}^{13}C_{12}\,H\,{}^{35}Cl_7\,O$	HpCDF(4)	Yes
	419.8220	M+2	${}^{13}C_{12}\,H\,{}^{35}Cl_6\,{}^{37}Cl\,O$	HpCDF(4)	
	423.7766	M+2	$C_{12}\,H\,{}^{35}Cl_6\,{}^{37}Cl\,O_2$	HpCDD	Yes
	425.7737	M+4	$C_{12}\,H\,{}^{35}Cl_5\,{}^{37}Cl_2\,O_2$	HpCDD	
	430.9729	Lock	$C_9\,F_{17}$	PFK	
	435.8169	M+2	${}^{13}C_{12}\,H\,{}^{35}Cl_6\,{}^{37}Cl\,O_2$	HpCDD(4)	Yes
	437.8140	M+4	${}^{13}C_{12}\,H\,{}^{35}Cl_5\,{}^{37}Cl_2\,O_2$	HpCDD(4)	
	479.7165	M+4	$C_{12}\,H\,{}^{35}Cl_7\,{}^{37}Cl_2\,O$	NCDPE	
5	441.7428	M+2	$C_{12}\,{}^{35}Cl_7\,{}^{37}Cl\,O$	OCDF	Yes
	442.9728	Lock	$C_{10}\,F_{17}$	PFK	
	443.7399	M+4	$C_{12}\,{}^{35}Cl_6\,{}^{37}Cl_2\,O$	OCDF	
	457.7377	M+2	$C_{12}\,{}^{35}Cl_7\,{}^{37}Cl\,O_2$	OCDD	Yes
	459.7348	M+4	$C_{12}\,{}^{35}Cl_6\,{}^{37}Cl_2\,O_2$	OCDD	
	469.7779	M+2	$13C_{12}\,{}^{35}Cl_7\,{}^{37}Cl\,O_2$	OCDD(4)	Yes
	471.7750	M+4	${}^{13}C_{12}\,{}^{35}Cl_6\,{}^{37}Cl_2\,O_2$	OCDD(4)	
	513.6775	M+4	$C_{12}\,{}^{35}Cl_8\,{}^{37}Cl_2\,O$	DCDPE	

(1) From Reference 5

(2) Nuclidic masses used:

H = 1.007825 C = 12.00000 ^{13}C = 13.003355 F = 18.9984
O = 15.994915 ^{35}Cl = 34.968853 ^{37}Cl = 36.965903

(3) Compound abbreviations:

Chlorinated dibenzo-p-dioxins
- TCDD = Tetrachlorodibenzo-p-dioxin
- PeCDD = Pentachlorodibenzo-p-dioxin
- HxCDD = Hexachlorodibenzo-p-dioxin
- HpCDD = Heptachlorodibenzo-p-dioxin
- OCDD = Octachlorodibenzo-p-dioxin

Chlorinated dibenzofurans
- TCDF = Tetrachlorodibenzofuran
- PeCDF = Pentachlorodibenzofuran
- HxCDF = Hexachlorodibenzofuran
- HpCDF = Heptachlorodibenzofuran

Chlorinated diphenyl ethers
- HxCDPE = Hexachlorodiphenyl ether
- HpCDPE = Heptachlorodiphenyl ether
- OCDPE = Octachlorodiphenyl ether
- NCDPE = Nonachlorodiphenyl ether
- DCDPE = Decachlorodiphenyl ether

Lock mass and QC compound
- PFK = Perfluorokerosene

(4) Labeled compound

Table 3A
THEORETICAL M/Z RATIOS AND CONTROL LIMITS

No. of Chlorine Atoms	m/z's Forming Ratio	Theoretical Ratio	Control Limits(1) 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
6 (2)	M/M+2	0.51	0.43	0.59
7	M+2/M+4	1.05	0.88	1.20
7 (3)	M/M+2	0.44	0.37	0.51
8	M+2/M+4	0.89	0.76	1.02

(1) Represent ± 15% windows around the theoretical ion abundance ratios.

(2) Used for ^{13}C-HxCDF only.

(3) Used for ^{13}C-HpCDF only.

Table 4

CONCENTRATIONS OF SOLUTIONS CONTAINING LABELED AND UNLABELED CDDS AND CDFS

Compound	Stock Solution (1) ng/mL	Spike Solutions (2) ng/mL	Calibration and Verification Solutions ng/mL CS1	CS2	VER(3) CS3	CS4	CS5	PAR(4) ng/mL
Native CDDs and CDFs								
2,3,7,8-TCDD	-	-	0.5	2	10	40	200	40
2,3,7,8-TCDF	-	-	0.5	2	10	40	200	40
1,2,3,7,8-PeCDD	-	-	2.5	10	50	200	1000	200
1,2,3,7,8-PeCDF	-	-	2.5	10	50	200	1000	200
2,3,4,7,8-PeCDF	-	-	2.5	10	50	200	1000	200
1,2,3,4,7,8-HxCDD	-	-	2.5	10	50	200	1000	200
1,2,3,6,7,8-HxCDD	-	-	2.5	10	50	200	1000	200
1,2,3,7,8,9-HxCDD	-	-	2.5	10	50	200	1000	200
1,2,3,4,7,8-HxCDF	-	-	2.5	10	50	200	1000	200
1,2,3,6,7,8-HxCDF	-	-	2.5	10	50	200	1000	200
1,2,3,7,8,9-HxCDF	-	-	2.5	10	50	200	1000	200
2,3,4,6,7,8-HxCDF	-	-	2.5	10	50	200	1000	200
1,2,3,4,6,7,8-HpCDD	-	-	2.5	10	50	200	1000	200
1,2,3,4,6,7,8-HpCDF	-	-	2.5	10	50	200	1000	200
1,2,3,4,7,8,9-HpCDF	-	-	2.5	10	50	200	1000	200
OCDD	-	-	5.0	20	100	400	2000	400
OCDF	-	-	5.0	20	100	400	2000	400
Labeled Compound Spiking Standards								
$^{13}C^{12}$-2,3,7,8-TCDD	100	2	100	100	100	100	100	-
$^{13}C^{12}$-2,3,7,8-TCDF	100	2	100	100	100	100	100	-
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	2	100	100	100	100	100	-
$^{13}C_{12}$-1,2,3,7,8-PeCDF	100	2	100	100	100	100	100	-
$^{13}C_{12}$-2,3,4,7,8-PeCDF	100	2	100	100	100	100	100	-
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	2	100	100	100	100	100	-
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	100	2	100	100	100	100	100	-
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	100	2	100	100	100	100	100	-
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	2	100	100	100	100	100	-
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	100	2	100	100	100	100	100	-
$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	100	2	100	100	100	100	100	-
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	2	100	100	100	100	100	-
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	2	100	100	100	100	100	-
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	100	2	100	100	100	100	100	-
$^{13}C_{12}$-OCDD	200	4	200	200	200	200	200	-
Cleanup Standard								
$^{37}Cl_4$-2,3,7,8-TCDD	-	0.8	0.5	2	10	40	200	-
Internal Standards								
$^{13}C_{12}$-1,2,3,4-TCDD	-	200	100	100	100	100	100	-
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	-	200	100	100	100	100	100	-

(1) Stock solution (Section 6.10) - Prepared in nonane, and diluted daily with acetone to prepare the spiking solution (Section 10.3.2).

(2) Spiking solutions (Sections 6.11, 6.12, 8.3, 10.3.2, and 10.4.2).

(3) Calibration verification solution (Section 14.3).

(4) Precision and recovery standard (Section 6.14) - Prepared in nonane, and diluted daily with acetone to prepare the spiking solution (Section 10.3.4).

Table 5

GC RETENTION TIME WINDOW DEFINING MIXTURES AND ISOMER SPECIFICITY TEST MIXTURES

DB-5 Column GC Retention Time Window Defining Standard (Section 6.15)

Congener	First Eluted	Last Eluted
TCDF	1,3,6,8-	1,2,8,9-
TCDD	1,3,6,8-	1,2,8,9-
PeCDF	1,3,4,6,8-	1,2,3,8,9-
PeCDD	1,2,4,7,9-	1,2,3,8,9-
HxXCDF	1,2,3,4,6,8-	1,2,3,4,8,9-
HxCDD	1,2,4,6,7,9-	1,2,3,4,6,7-
HpCDF	1,2,3,4,6,7,8-	1,2,3,4,7,8,9-
HpCDD	1,2,3,4,6,7,9-	1,2,3,4,6,7,8-

DB-5 TCDD Isomer Specificity Test Standard (Section 6.16.1)

1,2,3,4-TCDD	1,2,3,7-TCDD
1,2,7,8-TCDD	1,2,3,8-TCDD
1,4,7,8-TCDD	2,3,7,8-TCDD

DB-225 Column TCDF Isomer Specificity Test Standard (Section 6.16.2)

2,3,4,7-TCDF
2,3,7,8-TCDF
1,2,3,9-TCDF

Table 6
REFERENCE COMPOUNDS FOR NATIVE AND LABELED PCDDS AND PCDFS

Native PCDDs and PCDFs	Reference Compound	Labeled PCDDs and PCDFs	Reference Compound
2,3,7,8-TCDD	$^{13}C_{12}$-2,3,7,8-TCDD	$^{13}C_{12}$-2,3,7,8-TCDD	$^{13}C_{12}$-1,2,3,4-TCDD
2,3,7,8-TCDF	$^{13}C_{12}$-2,3,7,8-TCDF	$^{13}C_{12}$-2,3,7,8-TCDF	$^{13}C_{12}$-1,2,3,4-TCDD
1,2,3,7,8-PeCDD	$^{13}C_{12}$-1,2,3,7,8-PeCDD	$^{13}C_{12}$-1,2,3,7,8-PeCDD	$^{13}C_{12}$-1,2,3,4-TCDD
1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,4-TCDD
2,3,4,7,8-PeCDF	$^{13}C_{12}$-2,3,4,7,8-PeCDF	$^{13}C_{12}$-2,3,4,7,8-PeCDF	$^{13}C_{12}$-1,2,3,4-TCDD
1,2,3,4,7,8-HxCDD	$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
1,2,3,6,7,8-HxCDD	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
1,2,3,7,8,9-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
1,2,3,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
2,3,4,6,7,8-HxCDF	$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
1,2,3,4,6,7,8-HpCDD	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
1,2,3,4,7,8,9-HpCDF	$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
OCDD	$^{13}C_{12}$-OCDD	$^{13}C_{12}$-OCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
OCDF	$^{13}C_{12}$-OCDD	$^{37}Cl_4$-2,3,7,8-TCDD	$^{13}C_{12}$-1,2,3,4-TCDD

Table 7
ACCEPTANCE CRITERIA FOR PERFORMANCE TESTS (1)

Compound	Test Concentration (ng/mL)	Initial Precision and Accuracy Sec 8.2.3 s	Initial Precision and Accuracy Sec 8.2.3 X	Labeled Compound Recovery Sec 8.3 and 16.2 P (%)	Calibration Verification Sec 14.5 (ug/mL)	Ongoing Accuracy Sec 14.6 R (%)
PCDDs/PCDFs by internal standard						
^{13}C-tetra-hepta CDD and CDF	100	32	60 - 145	25 - 150	65 - 140	55 - 150
^{37}Cl-tetra CDD	40	13	24 - 58	25 - 150	26 - 56	22 - 60
^{13}C-octa CDD	200	64	120 - 290	25 - 150	130 - 280	110 - 300
PCDDs/PCDFs by isotope dilution						
tetra CDD and CDF	40	9	30 - 52	25 - 150	30 - 52	28 - 56
penta - hepta CDD and CDF	200	45	150 - 260	25 - 150	150 - 260	140 - 280
octa CDD and CDF	400	90	300 - 520	25 - 150	300 - 520	280 - 560

(1) Based on data from Method 1625.

Table 8
SAMPLE PHASE AND QUANTITY EXTRACTED FOR VARIOUS MATRICES

Sample Matrix (1)	Example	Percent Solids	Phase	Quantity Extracted
SINGLE PHASE				
Aqueous	Drinking water Groundwater Treated wastewater	<1	(2)	1000 mL
Solid	Dry soil Compost Ash	>20	Solid	10 g
Organic	Waste solvent Waste oil Organic polymer	<1	Organic	10 g
MULTIPHASE				
Liquid/Solid				
Aqueous/solid	Wet soil Untreated effluent Digested municipal sludge Filter cake Paper pulp Tissue	1-30	Solid	10 g
Organic/solid	Industrial sludge Oily waste	1-100	Both	10 g
Liquid/Liquid				
Aqueous/organic	In-process effluent Untreated effluent Drum waste	<1	Organic	10 g
Aqueous/organic/solid	Untreated effluent Drum waste	>1	Organic & solid	10 g

(1) The exact matrix may be vague for some samples. In general, when the CDDs and CDFs are in contact with a multiphase system in which one of the phases is water, they will be preferentially dispersed in or adsorbed on the alternate phase, because of their low solubility in water.

(2) Aqueous samples are filtered after spiking with labeled analogs. The filtrate and the material trapped on the filter are extracted separately, and then the extracts are combined for analysis.