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Monday  
September 15, 1997

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**Part III**

**Environmental  
Protection Agency**

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40 CFR Part 136  
Guidelines Establishing Test Procedures  
for the Analysis of Pollutants; EPA  
Method 1613; Final Rule

**ENVIRONMENTAL PROTECTION AGENCY**

**40 CFR Part 136**

[FRL-5889-3]

RIN 2040-AC64

**Guidelines Establishing Test Procedures for the Analysis of Pollutants; EPA Method 1613**

**AGENCY:** Environmental Protection Agency (EPA).

**ACTION:** Final rule.

**SUMMARY:** Today's final regulation amends the "Guidelines Establishing Test Procedures for the Analysis of Pollutants" under section 304(h) of the Clean Water Act to approve EPA Method 1613 for determination of tetra-through octa-chlorinated, 2,3,7,8-substituted, dibenzo-*p*-dioxins and dibenzofurans (CDDs/CDFs) by high resolution gas chromatography (HRGC) coupled with high resolution mass spectrometry (HRMS). This regulation makes available at 40 CFR part 136 an additional, more sensitive test procedure for CDDs/CDFs. Method 1613 is the most sensitive analytical test procedure approved under the Clean Water Act for the analysis of CDDs/CDFs because it measures into the low part-per-quadrillion (ppq) range. Use of approved test procedures is required whenever the discharge constituent specified is required to be measured for: a National Pollutant Discharge Elimination System (NPDES) permit application; discharge monitoring reports; state certification; and other requests from the permitting authority for quantitative or qualitative effluent data. Use of approved test procedures also is required for the expression of pollutant amounts, characteristics, or properties in effluent limitations guidelines and standards of performance and pretreatment standards, unless otherwise specifically noted or defined.

**EFFECTIVE DATE:** This regulation is effective October 15, 1997. In accordance with 40 CFR 23.2, this rule shall be considered issued for the purposes of judicial review September 29, 1997, at 1 p.m. eastern daylight time. Under section 509(b)(1) of the Clean Water Act, judicial review of these amendments can be obtained only by filing a petition for review in the United States Court of Appeals within 120 days after they are considered issued for the purposes of judicial review. Under section 509(b)(2) of the Clean Water Act, the requirements of these amendments may not be challenged later in civil or

criminal proceedings to enforce these requirements.

**ADDRESSES:** Documents that support this final rule are in the Water Docket and are available for public inspection from 9 a.m. to 4 p.m. in Room M2616, 401 M Street, SW., Washington, D.C. 20460, phone: (202) 260-3027. The Docket staff request that interested parties call for an appointment before visiting the Docket. The EPA regulations at 40 CFR Part 2 provide that a reasonable fee may be charged for copying.

**FOR FURTHER INFORMATION CONTACT:** Mr. Ben Honaker at (202) 260-2272, USEPA Office of Science and Technology, Engineering and Analysis Division (4303), 401 M Street, SW., Washington, DC 20460.

**SUPPLEMENTARY INFORMATION:**

**Regulated Entities**

This action approves a test procedure for the determination of tetra- through octa-chlorinated, 2,3,7,8-substituted, CDDs/CDFs in wastewater by HRGC/HRMS. Regulatory authorities may, at their discretion, require use of this method in NPDES permits. Entities potentially regulated by this action are listed in the table below.

Category	Examples of regulated entities
Public ...	Government laboratories that develop or employ analytical methods for use in demonstrating compliance with the CWA.
Private ..	Commercial laboratories, consensus methods organizations, instrument manufacturers, vendors, and other entities that develop or employ analytical methods for use in demonstrating compliance with the CWA.

This table is not intended to be exhaustive, but rather provides a guide for readers regarding entities likely to be regulated by this action. This table lists the types of entities that EPA is now aware could potentially be regulated by this action. Other types of entities not listed in the table could also be regulated. To determine whether your organization is regulated by this action, you should carefully examine the applicability language of today's rule at § 136.3. If you have questions regarding the applicability of this action to a particular entity, consult the person listed in the preceding **FOR FURTHER INFORMATION CONTACT** section.

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  - B. Unfunded Mandates Reform Act
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  - D. Paperwork Reduction Act
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**I. Authority**

Today's final rule is promulgated under the authority of sections 301, 304(h), 307, 308 and 501(a) of the Clean Water Act (CWA), 33 U.S.C. 1251 *et seq.* (the Federal Water Pollution Control Act Amendments of 1972 as amended by the Clean Water Act of 1977 and the Water Quality Act of 1987), 33 U.S.C. 1311, 1314(h), 1328, 1329, 1361(a); 86 Stat.

816, Pub. L. 92-500; 91 Stat. 1567, Pub. L. 95-217; 100 Stat. 7, Pub. L. 100-4 (the "Act"). Section 301 of the Act prohibits the discharge of any pollutant into navigable waters unless the discharge complies with an NPDES permit issued under section 402 of the Act. Section 301 also specifies levels of pollutant reductions to be achieved by certain dates. Section 304(h) of the Act requires the EPA Administrator to "promulgate guidelines establishing test procedures for the analysis of pollutants that shall include the factors which must be provided in any certification pursuant to section 401 of this Act or permit applications pursuant to section 402 of this Act." These test procedures for the analysis of pollutants also assist in the implementation of section 301. Section 501(a) of the Act authorizes the Administrator to prescribe such regulations as are necessary to carry out her function under the Act.

The Administrator has also made these test procedures (methods) applicable to monitoring and reporting of NPDES permit applications and permits (40 CFR part 122, §§ 122.21, 122.41, 122.44, 122.48, and 123.25), and implementation of the pretreatment standards issued under section 307 of CWA (40 CFR part 403, §§ 403.10 and 402.12).

## II. Background and History

### A. Analytical Methods Under 40 CFR Part 136, Including Method 1613

The Agency provided a history of analytical methods under 40 CFR part 136 on February 7, 1991 (56 FR 5090) when EPA proposed the rule being promulgated today. The preamble to today's final rule updates that history with technical changes to EPA Method 1613 between proposal and promulgation. These technical changes are described below in Section III.B., "Summary of Improvements Since Proposal."

### B. Promulgation of Method 1613 Under EPA's Drinking Water Rules

Under the Safe Drinking Water Act, EPA proposed Method 1613 for the measurement of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), also known as dioxin, in support of the National Primary Drinking Water Regulation for that contaminant. See 55 FR 30426 (July 25, 1990). EPA also discussed plans to conduct an interlaboratory method validation study to determine whether the detection and quantitation values derived by EPA for Method 1613 represented a reasonable expectation for different laboratories. EPA solicited comments on the

appropriate level to be set the maximum contaminant level (MCL) for the drinking water rule. EPA further discussed Method 1613 for determination of dioxin in drinking water in a "Notice of Availability with Request for Comment" on November 29, 1991, at 56 FR 60949.

On December 5, 1994, EPA promulgated Method 1613 for measurement of dioxin in drinking water at 40 CFR parts 141 and 142 (59 FR 62455). In section I.B.3.b of the preamble to that rulemaking, EPA responded to general and specific comments on the application of EPA Method 1613 to drinking water. EPA stated in the preamble that the Agency had previously solicited and received comments on the proposal of Method 1613 for application to wastewater, that some of these same comments had been received in response to the proposal of Method 1613 for application to drinking water, and that EPA would restrict its responses to general issues covering the application of Method 1613 to both drinking water and wastewater and to issues specific to drinking water. In today's preamble, EPA is responding to all comments received on the proposal of Method 1613 for application to wastewater (56 FR 5090), including general comments that were duplicated in comments received on the drinking water notice (56 FR 60949).

The December 5, 1994, revision to Method 1613 (for application to drinking water) is consistent with the version of the Method in today's rule. Therefore, with today's rulemaking, the same version of EPA Method 1613 applies to analysis of wastewater and drinking water.

### C. Proposal of Method 1613 for Monitoring in Pulp, Paper, and Paperboard Industry Wastewaters

On December 17, 1993, EPA proposed national effluent limitations and guidelines, pretreatment standards, and new source performance standards for the Pulp, Paper, and Paperboard industrial point source category. See 58 FR 66078. In the proposal, EPA referenced a compendium titled "Analytical Methods for the Determination of Pollutants in Pulp and Paper Industry Wastewater." This compendium contained methods that had not been promulgated at 40 CFR part 136, but would be applicable for monitoring compliance with the numerical limitations and standards proposed in the Pulp, Paper, and Paperboard rule. These methods were proposed for promulgation at 40 CFR part 430 to support the proposed regulation and were included in the

docket for the proposed pulp and paper rule.

The methods proposed for monitoring under the proposed pulp and paper rule included an earlier version of Method 1613 than the version EPA is promulgating today. To further conform analytical methods, NPDES permits issued after the effective date of today's rule will require use of today's promulgated revision of Method 1613 for determining compliance with the final rule for the Pulp, Paper, and Paperboard category.

## III. Summary of the Final Rule Amending Part 136

### A. Purpose

This rule allows the use of Method 1613 for determination of seventeen tetra-through octa-chlorinated, 2,3,7,8-substituted dibenzo-p-dioxins and dibenzofurans (CDDs/CDFs) in effluent samples by isotope dilution high resolution gas chromatography (HRGC) combined with high resolution mass spectrometry (HRMS). Method 1613 was developed to lower the measurable range of minimum levels for the CDDs/CDFs, specifically, into the low part per quadrillion (ppq) range for aqueous samples and into the low part-per-trillion (ppt) range for solid and semi-solid sample matrices. EPA believes Method 1613 is adequate and applicable for the measurement of solid and semi-solid sample matrices, such as biosolids and fish tissue, but today's rule does not amend test procedures for sewage sludge regulations at 40 CFR 503.8 and does not constitute rulemaking for measurement of fish tissue. Today's rulemaking at 40 CFR part 136 applies for measurement of aqueous samples.

The promulgation of Method 1613 provides a test procedure (analytical method) for compliance monitoring under the National Pollutant Discharge Elimination System (CWA section 402) and CWA section 401 certifications. Method 1613 is also available for: Development of and monitoring compliance with effluent limitations guidelines, pretreatment standards, and new source performance standards in EPA's water programs; ambient water quality monitoring; and general laboratory use. By today's action, however, EPA is not withdrawing approval of the existing method, Method 613, which also measures 2,3,7,8-TCDD, albeit with limited sensitivity. Method 613 is still applicable for those NPDES permits that require that this method be used and thus existing permits do not need to be modified prior to expiration. In addition, Method 613 remains available

for screening purposes. However, NPDES permits issued after promulgation of today's rule must include Method 1613 if the permit contains effluent limitations for dioxin.

#### B. Summary of Improvements Since Proposal

EPA proposed Method 1613 on February 7, 1991. See 56 FR 5090. At the time of proposal, EPA had initiated (but had not completed) an Interlaboratory Method Validation Study (IMVS) and was considering other improvements to Method 1613 to increase the utility of the Method and make the Method more efficient and cost-effective. EPA proceeded with proposal of Method 1613 before completion of the IMVS because:

- Method 1613 had been validated in single-laboratory studies and in data gathering by EPA. The data gathering consisted of over 500 analyses of real-world environmental samples to support regulation development in EPA's effluent guidelines and other programs.

- EPA desired to make Method 1613 available for reporting of CDDs/CDFs under the NPDES permit regulations at 40 CFR parts 122 and 123, and the pretreatment regulations at 40 CFR part 403. At that time, the only method approved for the determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) under 40 CFR part 136 was Method 613. Method 613 is 200 times less sensitive than Method 1613 for 2,3,7,8-TCDD and does not measure other CDDs/CDFs.

- EPA was developing regulations for the Pulp, Paper, and Paperboard industrial category. A high sensitivity method for 2,3,7,8-TCDD and 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF) was required for development of these regulations.

- EPA desired to collect comments on proposed Method 1613 to improve the Method and learn of deficiencies that needed to be addressed before promulgation.

Since proposal, EPA has received a considerable number of suggestions on improving the utility of Method 1613, both as described below in Section IV, "Public Participation and Response to Comments," and in technical meetings and informal and formal discussions with laboratories, academicians, and the regulated industry. Based on the IMVS and these discussions, EPA has made technical revisions to Method 1613 to improve the usability of the method for water and other sample matrices. This section of the preamble describes how EPA developed some of these

improvements in response to public comment.

#### 1. Development of Improved Quality Control Acceptance Criteria

As proposed, Method 1613 contained performance specifications in the form of quality control (QC) acceptance criteria that were based upon data gathered by EPA during the development of Method 1613 between 1988 and 1991. EPA developed improved QC acceptance criteria using data from EPA's IMVS and data from the paper industry and EPA's Pulp and Paper Long-term Variability Study (LTVS). EPA has revised the QC acceptance criteria in the version of Method 1613 being promulgated today. The IMVS and LTVS studies are described below. A more detailed description of the IMVS and development of the revised QC acceptance criteria is given in the report titled "Results of the International Interlaboratory Validation Study of USEPA Method 1613" (1613 Report). The 1613 Report is included in the docket for today's final rule.

(a) *Interlaboratory Method Validation Study.* In February 1990, EPA began its interlaboratory validation of Method 1613 for the determination of CDDs/CDFs by HRGC/HRMS. The study was international in scope, ultimately involving receipt of data from 20 laboratories in five countries. The purpose of the study was to further characterize Method 1613 and to gather additional data to support today's promulgation.

Details of the IMVS study design are given in the "Study Plan for the Evaluation of Method 1613" (Study Plan). The Study Plan was included in the docket at proposal, and the results of the study are summarized in the 1613 Report included in the docket for this final rule. The pertinent specifics of the IMVS are summarized below.

#### (i) Simulated Sample Extracts

Each laboratory participating in the IMVS received two concentrated extracts prepared from a large-volume sample of industrial wastewater. This large-volume sample was extracted with benzene, and the benzene extract was concentrated. The concentrate was highly colored and contained small amounts of solids derived from the bulk extraction of the original sample.

The extract concentrate was split into three portions: low, medium, and high. The low concentration extract was not fortified with any additional CDDs/CDFs, and contained 2,3,7,8-TCDD and 2,3,7,8-TCDF at approximately 60 and 300 ppq, respectively. The medium

extract was fortified with most of the CDDs/CDFs not already present at concentrations in the 100- to 500-ppq range. The high extract was fortified with most of the CDDs/CDFs in the 250- to 1000-ppq range. After spiking, each of the three portions was further split and sealed into glass ampules.

Two ampules of the same concentration were submitted to each laboratory as a single blind duplicate sample, i.e., the laboratory did not know which, if any, CDDs/CDFs were in the ampules and did not know the concentrations of the CDDs/CDFs that were present in the ampules. The ampules were shipped to the laboratories over a period of four months, as additional participants joined the study.

The study design formed an incomplete block, i.e., not all laboratories were sent each of the three different concentrates. Under the incomplete block design used in this study, eight laboratories were sent two low-concentration ampules each, seven laboratories were sent two medium-concentration samples each, and the seven remaining laboratories were sent two high-concentration ampules each. At each laboratory, each concentrate was withdrawn from its ampule, further concentrated, and solvent-exchanged to acetone to ensure that the extract would be water miscible. Each acetone solution was then spiked into a one-liter volume of reagent water to produce a simulated effluent sample.

#### (ii) Sample Processing

Each simulated effluent sample was processed through the sample extraction procedure in the proposed version of Method 1613. Although all but one of the laboratories were experienced in performing CDD/CDF analyses using HRGC/HRMS, less than one-third of the 22 laboratories had direct experience with Method 1613. Therefore, laboratories were given time to familiarize themselves with the details of the Method, and each laboratory was required to demonstrate its general proficiency with the Method through the analysis of four initial precision and recovery (IPR) aliquots, as described in the Method.

In addition to demonstrating method proficiency and analyzing the simulated effluent samples according to Method 1613, the participating laboratories were required to perform all other QC procedures described in the Method. These QC requirements were described in Section III.D. of the proposal (56 FR 5092-5093).

For each sample and quality control analysis, the laboratories were to

provide the concentration of each analyte detected and the recovery of each labeled standard. All supporting raw data, including selected ion current profiles, were to be reported for all analyses.

(iii) Data Submission by Laboratories

A total of 22 laboratories in 6 countries agreed to participate in the study on a voluntary basis. The list of laboratories is given in the 1613 Report. After two years, data were received from a total of 20 laboratories in 5 countries. Data from each laboratory were reviewed thoroughly and, after resolution of data problems with the laboratories, the data were entered into a data set and combined with data from the LTVS to construct the final QC acceptance criteria for Method 1613 being promulgated today. EPA wishes to publicly thank the laboratories that participated in the study, particularly those that took the time to submit additional data and suggestions for improvement of Method 1613.

(b) *Data from the Pulp and Paper Long-term Variability Study.* Data gathering in the LTVS is described in detail in Section 7.5.2 of the Technical Support Document for the rule proposed for the Pulp, Paper, and Paperboard category (58 FR 66078). The procedures for validation of these data were developed in discussions between EPA and representatives of the paper industry. These validation procedures included detailed examination of all QC data associated with each field sample result. Specifically, the QC data were used to determine if the field sample results should be included in or excluded from the LTVS database that was used during development of the proposed pulp and paper industry effluent limitations guidelines and standards. Both the QC and the field sample data were maintained by EPA in a separate database intended for method development purposes. This included QC data for Method 1613, which were used to develop the final QC acceptance criteria in the version of the Method being promulgated today. The statistical procedures used to develop these final acceptance criteria are summarized below.

(c) *Statistical Analysis.* QC limits were calculated by constructing statistical prediction intervals for future observations of a quantity of interest using statistical estimates from data from the IMVS and LTVS. The statistical methods used are the same as those used to develop QC limits for EPA Method 1625 (49 FR 43234).

In other EPA method validation studies, compound-specific

performance specifications usually have been determined at individual test levels with a probability of 0.05 (i.e., based on 95 percent confidence limits for a single future observation). Using such specifications, each compound measured would have a five percent chance of falling outside its QC limit. Because of the large number of compounds simultaneously tested in the quality control tests for Method 1613, it would be extremely likely that one or more criteria on each test would be failed simply by random chance if the tests were all performed at individual test levels of  $p = .05$ . It was deemed desirable, instead, to specify test limits such that the global test level (i.e., the chance of failing on any one or more of the CDDs/CDFs out of the whole list) was held to five percent. This was done by adjusting the significance level used on each compound such that the overall Type I error rate would be 0.05 for each test situation. Details of the binomial calculations for these considerations are given in appendix A to the 1613 Report.

QC acceptance criteria were developed for tests of calibration linearity, calibration verification (VER), precision of relative retention time (RRT), IPR, ongoing precision and recovery (OPR), and labeled compound recovery in field samples and blanks.

Separate QC acceptance criteria were developed for the instances in which 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDF) are determined independently of the other CDDs/CDFs. The purpose of creating these separate criteria is to support those regulations, such as drinking water rules and the proposed rule for the Pulp, Paper, and Paperboard category, in which only TCDD and/or TCDF are regulated.

2. Procedures for Fish and Other Tissues

Procedures for the homogenization, preparation, extraction, and cleanup of fish and other tissues have been included in the revision of Method 1613 being promulgated today to increase the applicability of Method 1613 to these sample matrices. EPA is including these tissue extraction procedures based on tissue sample data gathered by EPA's Duluth laboratory, Dow Chemical Company, and commercial laboratories performing tests for EPA and other entities. See the docket for today's rule for references 21 through 28 cited in section 22.0 of Method 1613.

(a) *Extraction Procedures.* Two extraction procedures are in common use for the extraction of the CDDs/CDFs from tissue: Dehydration and Soxhlet Extraction, and Hydrochloric Acid

Digestion and Extraction. Both of these procedures have been incorporated into the version of Method 1613 that is being promulgated today.

(i) Dehydration and Soxhlet Extraction

In this procedure, a 10-gram aliquot of homogenized tissue is mixed with powdered, anhydrous sodium sulfate and allowed to stand for 12–24 hours so that the sodium sulfate can adsorb most of the moisture in the tissue. After re-mixing, the tissue is placed in a Soxhlet extractor and extracted for 18–24 hours with methylene chloride:hexane (1:1). The organic extract containing the CDDs/CDFs and lipids is evaporated to dryness, and the lipid content of the residue is determined. The residue is dissolved in hexane and subjected to one of the two bulk lipid removal procedures associated with the Soxhlet extraction that are described below.

(ii) Hydrochloric Acid Digestion and Extraction

In this procedure, a 10-gram aliquot of homogenized tissue is digested with hydrochloric acid and simultaneously extracted with methylene chloride:hexane (1:1) in a glass bottle. The organic extract containing the CDDs/CDFs and lipids is decanted and evaporated to dryness, and the lipid content of the residue is determined. The residue is dissolved in hexane, and lipids are removed using the sulfuric acid back-extraction procedure described below.

(b) *Bulk Lipid Removal Procedures for Soxhlet Extracts.* Two procedures are in common use for removal of lipids from extracts produced by the Soxhlet extraction procedure. Both of these procedures have been incorporated into the version of Method 1613 that is being promulgated today.

(i) Anthropogenic Isolation Column

This column contains, in order from bottom to top, neutral silica gel, potassium silicate, anhydrous sodium sulfate, acid silica gel, and anhydrous sodium sulfate. The column is pre-eluted with hexane, and the extract from the Soxhlet extraction is placed on the column and eluted with 200 mL of hexane. Fats, lipids, and other materials are retained on the column while the CDDs/CDFs elute in the hexane.

(ii) Acidified Silica Gel

In this bulk cleanup procedure, 30–100 grams of acidified silica gel are stirred for 2–3 hours with the extract from the Soxhlet extraction. After stirring, the solution is filtered to remove the silica gel. Fats, lipids, and other materials are retained on the silica

gel while the CDDs/CDFs remain in solution in the hexane.

(c) *Sulfuric Acid Back-extraction for HCl-digested Extracts.* In this cleanup procedure, the re-dissolved residue from the hydrochloric acid digestion is back-extracted with concentrated sulfuric acid for a maximum exposure time of 45 seconds. The sulfuric acid severs the bonds in the lipidic material during this period but there is insufficient contact time for the acid to attack the CDDs/CDFs. After back-extraction with sulfuric acid, the extract is further back-extracted with potassium hydroxide solution to remove residual lipidic material and to neutralize any residual acid that may be present.

(d) *Further Cleanup of Tissue Extracts.* After each of the procedures for extraction and bulk cleanup described above, the extract is further cleaned up using any or all of the cleanup procedures in Method 1613. For further cleanup of tissues (and for general use), a Florisil® cleanup procedure has been added to the revision of Method 1613 being promulgated today. The Florisil® cleanup is intended primarily for removal of chlorodiphenylethers, a common contaminant in tissues. Though Florisil® is a trade name for a specific adsorbent, EPA does not endorse any specific product or manufacturer; equivalent products may be substituted.

After cleanup, the extract is reconcentrated, internal standards are added, and an aliquot is injected into the HRGC/HRMS, as in the proposed version of Method 1613.

### 3. Solid-phase Extraction of Aqueous Samples

An optional solid-phase extraction (SPE) procedure has been added to the revision of Method 1613 being promulgated today. This SPE procedure allows laboratories to minimize solvent usage and is therefore consistent with EPA's objectives for source reduction of pollutants and pollution prevention. The SPE procedure is for use with water samples containing less than one percent suspended solids and is therefore applicable to drinking water, river water, ocean water, and relatively clean wastewaters.

In this optional SPE procedure, an SPE disk is placed on a fritted glass disk on top of a vacuum flask. A glass-fiber filter is placed on top of the SPE disk, and a glass container is placed on top of the stack of disks. The assembly is clamped to prevent leakage.

Particles in a 1-L aqueous sample are allowed to settle. The disk is wetted with organic solvents and water, and is

kept wet during the extraction. The aqueous sample is poured through the disks. Vacuum is used to increase the flow rate of sample through the disks, if desired. The particles remaining in the bottle are poured in last to minimize plugging of the disks. The sample bottle is rinsed and the rinsate is added to the container on top of the disks.

After all of the sample has been processed through the disks, the disks are extracted using the SDS procedures given in Method 1613 and described at proposal (56 FR 5094).

### 4. Sample Preservation and Holding Times

Dechlorination, pH reduction below pH=9, and refrigeration or freezing (depending on the sample matrix) are the only techniques required to stabilize the CDDs/CDFs against degradation during storage.

There are no demonstrated maximum holding times associated with CDDs/CDFs in aqueous, solid, semi-solid, tissue, or other sample matrices. If stored in the dark at 0–4°C and preserved as described above, aqueous samples may be stored for up to one year. Similarly, if stored in the dark at <–10°C, solid, semi-solid, and tissue samples may be stored for up to one year. Sample extracts are stored in the dark at <–10°C until analyzed. If stored in the dark at <–10°C, sample extracts may be stored for up to one year.

The version of Method 1613 that is being promulgated today reflects these findings. In addition, today's rule revises Table II of 40 CFR part 136 to reflect the changes in sample preservation and holding times in Method 1613 being promulgated today.

### 5. Other Improvements

Other significant improvements include: Addition of an optional rotary evaporation procedure for concentration of extracts; simplification of test solutions for demonstration of isomer-specific separation of 2,3,7,8-TCDD and 2,3,7,8-TCDF; and the addition of flow charts to illustrate procedures for aqueous, solid, tissue, and multi-phase samples.

With the improvements described above, EPA believes that the flexibility within Method 1613 has been increased and the costs of performing analyses using Method 1613 have potentially been reduced, thereby resulting in an overall benefit to the regulated and analytical communities.

### C. Method Detection Limit (MDL) studies

At the time of proposal, EPA had conducted an initial "Method Detection

Limit" (MDL) study and determined that Method 1613 could achieve an MDL of 5.6 ppq for 2,3,7,8-TCDD. EPA used this MDL to support a minimum level (ML) of 10 ppq in Method 1613. In Section IV, "Public Participation and Response to Comments," in this preamble, EPA responds to comments about this initial MDL study.

Since proposal, EPA has conducted four additional MDL studies to confirm the MDL for 2,3,7,8-TCDD (TCDD) and to measure MDLs and confirm MLs for the other CDDs/CDFs. The four studies were conducted in reagent water and in final effluent and bleach plant effluent from a pulp and paper industry facility. The studies of reagent water resulted in MDLs of 5.1 and 1.0 ppq for TCDD and MDLs for the other CDDs/CDFs that are consistent with the MLs in Method 1613. For the final effluent, the MDL for TCDD was 4.2 ppq and the MDLs for the other CDDs/CDFs were consistent with the MLs in Method 1613, except for one hexachloro-dioxin, one heptachlorofuran, heptachlorodioxin, and OCDD, which were higher than expected. For the bleach plant effluent, the MDLs were consistent with the MLs in Method 1613 except for 2,3,7,8-TCDD and 2,3,7,8-TCDF, which did not meet the MDL procedure criteria because of high background concentrations of these compounds in the sample.

The results of the four MDL studies are included in the docket for this final rule. Collectively, the four MDL studies demonstrate that the MDLs and MLs for the CDDs/CDFs can be attained in reagent water and in wastewaters from a pulp and paper industry facility.

## IV. Public Participation and Response to Comments

Condensed significant comments and responses are presented below. The full text of summarized comments and responses are contained in the docket in the document titled "Detailed Responses to Comments on Proposal of Method 1613." Comments and responses are presented by the following subject areas:

- A. Duplication of Methods
- B. Method Flexibility
- C. Feasibility—Instrumentation and Cost Issues
  - 1. Waste
  - 2. Instrumentation
- D. Insufficient Validation—General Comments
- E. Insufficient Validation of the Matrices Specified in the **Federal Register** Notice
- F. Interlaboratory Study
- G. Method Detection Limit Studies
- H. Detection/Quantitation Levels
- I. Quality Assurance/Quality Control
- J. Miscellaneous
- K. Technical Comments

### A. Duplication of Methods

*Comment:* Proposed Method 1613 and Office of Solid Waste SW-846 Method 8290 are significantly different. Another commenter stated that Methods 1613 and 8290 are similar and that the efforts by OW and OSW are duplicative.

*Response:* EPA agrees that the two methods are different in exact technical detail, but asserts that the principle of the two methods is the same. EPA is in the process of consolidating methods for dioxin measurement in air, water, and solid waste. However, the Agency's intention for such a merger for all of these matrices should not preclude prompt development and promulgation of this method for the water matrix. Method 1613 is a test method specifically designed to support revisions of the effluent guidelines for the Pulp, Paper, and Paperboard category under the CWA. EPA used Method 1613 in the development of those regulations, specifically for the water matrix. Therefore, EPA is promulgating Method 1613 for evaluation of matrices regulated under the CWA, notwithstanding any technical differences in the method used to evaluate matrices evaluated under the Resource Conservation and Recovery Act. EPA also notes that today's action does not promulgate a test method for measurement of dioxin in sewage sludge, even though the Agency used Method 1613 to measure dioxin concentrations in the "National Sewage Sludge Survey." In the future, EPA intends to propose and invite comment on the use of Method 1613 (or the consolidated multi-matrix method) for the measurement of dioxin in sewage sludge.

### B. Method Flexibility

*Comment:* Flexibility in sample preparation and tailoring of the procedure to the matrix type are desirable, but allowing the analyst the flexibility to modify the Method may adversely affect method performance on real-world samples.

*Response:* Flexibility is permitted only in discretionary elements of the test procedures indicated by use of the terms "may" and "can." All data generated must meet all performance criteria (quality control acceptance criteria) in the Method. Applicability of the QC performance criteria will preclude adverse effects of any modifications allowable under the flexibility in the method.

### C. Feasibility—Instrumentation and Cost Issues

#### 1. Waste

*Comment:* Substantial volumes of CDD/CDF-contaminated lab wastes will be created that cannot be disposed of or treated. The use of isotope dilution instead of external standard techniques will result in the generation of more hazardous waste because each sample is spiked with labeled analogs.

*Response:* Any analytical method that employs analytical standards for calibration and quality control (QC) purposes will generate a certain amount of laboratory waste. EPA believes that there are environmental benefits associated with using isotope dilution techniques, namely better monitoring and regulation of CDDs/CDFs at very low levels. These benefits outweigh any possible disadvantage of creating relatively small amounts of laboratory waste.

#### 2. Instrumentation

*Comment:* High resolution mass spectrometer (HRMS) instruments are expensive and there are no U.S. manufacturers.

*Response:* HRMS instrumentation represents state-of-the-art technology that allows detection of CDD/CDF compounds at far lower levels in less time and with greater certainty than LRMS instrumentation and is therefore worth the added cost. Currently, there are several U.S. manufacturers of HRMS instruments. Moreover, Method 1613 will not be the only applicable method for dioxin in all instances; approval of Method 613 is not being withdrawn by today's action.

*Comment:* Method 1613 is not very practical since it requires at least two separate analytical runs on two different GC columns, resulting in considerable instrument down-time to switch columns and data collection criteria.

*Response:* EPA disagrees with the commenters' conclusion that the separate analytical runs will be required in all circumstances. The use of a second GC column is routinely used in many analytical laboratories for confirmatory purposes. An analytical run on the second column is not required unless 2,3,7,8-TCDF is found or if ambiguities exist about the identification of other CDD/CDF congeners on the first column.

*Comment:* The Soxhlet/Dean-Stark (SDS) extraction procedure for solids has only been tested to a limited extent on one municipal sludge.

*Response:* Since proposal of Method 1613, EPA, NCASI, and others have extracted many samples using the SDS

technique. Although some data show that some of the higher isomers and congeners of dioxin may not be extracted as efficiently with the SDS technique as with other extraction techniques, EPA has not yet confirmed these results. The originators of the application of the SDS technique at the Dow Chemical Company tested the technique on many samples prior to the time that EPA adopted the technique and showed that the technique was able to extract certain CDDs/CDFs from samples believed to contain non-detectable levels of these compounds. In one of the single-laboratory tests, EPA confirmed Dow's findings that certain isomers/congeners were extracted more efficiently with the SDS procedure than with the Soxhlet extractor. EPA reported the results of its SDS extraction study in its proposal of Method 1613 (56 FR 5094). EPA therefore believes that the SDS extractor represents the best available technique for a diversity of sample matrices. Most importantly, however, by today's rulemaking, EPA is not promulgating a test procedure for measurement of solid matrix samples, only waste water samples.

*Comment:* The procedures proposed for extraction of solids are inappropriate for use on process pulps, dried pulps, or fiber-containing sludges.

*Response:* EPA is aware that dried pulp and similar samples present a formidable extraction problem. Pulp swells when wet, allowing exchange of the extraction solvent with the water in the interstices of the pulp. Low molecular weight alcohols also seem to swell the pulp fibers and are an alternative to the use of nonpolar solvents for the extraction of CDDs/CDFs from dry pulp. EPA believes that if the dry pulp or similar material is completely swollen in reagent water, however, the SDS extractor will reliably extract CDDs/CDFs from this matrix. EPA has included instructions for dealing with dried pulp and similar materials in the version of Method 1613 being promulgated today.

### D. Insufficient Validation—General Comments

*Comment:* EPA is premature in proposing Method 1613 under section 304(h) of CWA since it was not completely and thoroughly subjected to intra- and interlaboratory testing according to accepted scientific standards.

*Response:* Prior to proposal of EPA Method 1613, EPA had completed a single-laboratory validation of the SDS extraction technique in municipal sewage sludge and a single-laboratory method detection limit (MDL) study.

Since proposal, EPA has completed a total of four additional MDL studies and the IMVS described in this preamble and in greater detail in the 1613 Report that is included in the docket for today's rule. The four additional MDL studies were performed in reagent water and in bleach plant effluent and final effluent from a pulp and paper industry facility. EPA conducted the international IMVS with the express purpose of further characterizing Method 1613 and developing QC acceptance criteria. EPA believes that the results of these studies provide more than sufficient validation to confirm that Method 1613 is suitable for use as a test procedure in accordance with the requirements of the Clean Water Act. These data confirmed EPA's conclusions about achievable MDLs, which were based on intralaboratory validation studies. Therefore, EPA does not believe it is premature to promulgate Method 1613 at this time because the interlaboratory validation data merely confirms EPA's earlier conclusions.

*Comment:* EPA has failed to publish performance information for Method 1613, whereas such performance information has been published for the organic methods already incorporated into 40 CFR part 136, appendix A. This commenter urges EPA to include interlaboratory and intralaboratory performance data in any final rule it publishes for Method 1613.

*Response:* EPA has included performance information in the 1613 Report and in the results of the MDL studies conducted between proposal and this promulgation. These performance data are included in the docket that supports today's final rule.

#### *E. Insufficient Validation of the Matrices Specified in the Federal Register Document*

*Comment:* There has been insufficient intralaboratory testing and validation using the sample matrices for which EPA claims applicability for Method 1613.

*Response:* EPA has collected single-laboratory data on several matrices, including reagent water, treated and untreated wastewater, paper pulp, sludge, soil, and fish tissue, but has not undertaken complete intra- and interlaboratory validation of each matrix. EPA will perform intra- and interlaboratory validations of Method 1613 and other methods on those matrices for which the Agency believes that such validation is necessary and appropriate. However, EPA believes that it is unnecessary to perform extensive validation studies of Method 1613 or any other method on every sample

matrix to which the method is to be applied. For example, EPA regulates more than 600 subcategories of wastewater discharge. EPA believes that interlaboratory validation of Method 1613 on each discharge not only would be costly and impose an enormous administrative burden, but would not be likely to yield any more improvements in the Method than would be gained by single-laboratory tests on a few such representative discharges, particularly for aqueous samples from every conceivable type of industrial facility. Most importantly, however, though EPA believes that Method 1613 is adequate and applicable for the measurement of solid matrices, such as sewage sludge and fish tissue, today's action does not promulgate a method for measurement of those solid matrices. In the future, EPA does intend to propose and invite public comment on use of Method 1613 for measurement of dioxin in sewage sludge.

#### *F. Interlaboratory Study*

*Comment:* Several commenters stated that EPA had not completed its interlaboratory study at time of proposal and that EPA is premature in proposing Method 1613 without validating it first.

*Response:* The international IMVS has been completed and data from the study were combined with data from the pulp and paper LTVS to produce the final QC acceptance criteria in Method 1613 being promulgated today.

*Comment:* The use of extracts rather than real-world mill effluents in the interlaboratory study will not provide the necessary validation of Method 1613.

*Response:* EPA used extracts of real-world samples because the Agency felt that domestic and international shipment of large volumes of dioxin-containing water would create too great a risk to human health and the environment. The Agency also felt that it would be too difficult to produce a homogeneous mixture of CDDs/CDFs in such large water volumes.

#### *G. Method Detection Limit Studies*

*Comment:* The MDLs in Method 1613 have not been demonstrated and it is not possible for even the best laboratories to attain the MDL developed by EPA. The 5 ppq MDL for 2,3,7,8-TCDD in Method 1613 was calculated from a single-shot experiment that was not conducted properly and does not represent a real-world estimate of the MDL because it was not conducted in pulp and paper industry wastewater.

*Response:* EPA disagrees. EPA had demonstrated an MDL of 5 ppq using

Method 1613, as described at proposal. EPA conducted the iterative MDL procedure according to the procedures specified in 40 CFR part 136 appendix B. Since proposal, EPA has conducted a total of four additional MDL studies in reagent water and in in-process and final effluents from the pulp and paper industry. The results of these MDL studies confirm results from the reagent water MDL study described in the Method proposal.

#### *H. Detection/Quantitation Levels*

*Comment:* Method 1613 will not ensure, or even come close to ensuring, that dioxin concentrations at or below EPA's water quality criterion will be achieved. The proposed Method will not be capable of detecting effluent dioxin levels that exceed the in-stream water quality criterion, yet are less than 10 ppq.

*Response:* EPA agrees. EPA's water quality criterion for 2,3,7,8-TCDD is 13 parts per quintillion (ppqt), while the Method 1613 Minimum Level is 10 ppq. Method 1613 is the product of an extensive method development effort to produce a method that utilizes state-of-the-art technology to reliably achieve the lowest level of detection possible with one-liter water samples. While Method 1613 is not capable of achieving the water quality criterion of 13 ppqt, it is at least 200 times more sensitive than the only currently approved 304(h) dioxin method, Method 613. EPA will continue to explore new measurement techniques to develop methods that yield MDLs that will allow determination of 2,3,7,8-TCDD at the ambient criteria level. In the meantime, however, EPA must regulate effluent discharges at levels lower than those in Method 613, and therefore must move forward with promulgation of Method 1613 for such purposes.

*Comment:* The term "minimum level" (ML) as defined in the proposed rule is inconsistent with previous EPA definitions of ML. EPA equates the ML with the American Chemical Society's limit of quantitation (LOQ), which is different from other EPA documents in which EPA appears to equate the ML to a limit of detection not a limit of quantitation. EPA also stated that the ML is to be calculated based on interlaboratory analyses of the analyte in the matrix of concern. EPA should develop scientifically sound conventions for limits of detection and quantitation, allow public review and comment, and apply those limits consistently to avoid confusion in the interpretation of test data.

*Response:* EPA believes that the definitions of the ML in methods are



consistent. EPA agrees, however, that there is a need for greater consensus on the definition of terms among methods from all EPA offices and Federal and State analytical programs. EPA is currently addressing these issues through internal communications and meetings with stakeholders. The MLs for Method 1613 have been verified in every laboratory that uses the method by requiring calibration at the ML. MLs can be verified by single laboratory studies or by use since laboratories must calibrate at the ML. EPA will continue to examine the issues of detection and quantitation and will involve the public on these issues when an improved concept is developed.

#### *I. Quality Assurance/Quality Control (QA/QC)*

*Comment:* The instrument calibration procedure outlined in Method 1613 is much more involved than procedures for the 600 series methods. It would be extremely difficult to meet the 12-hour calibration requirements after running a few "dirty" samples.

*Response:* EPA agrees that the calibration procedure in Method 1613 may be somewhat more complicated than the procedures in the 600 series methods. However, the calibration procedure in Method 1613 is virtually identical to the procedures in Method 1624 and 1625, the isotope dilution variants of Methods 624 and 625.

As to the statement that it would be extremely difficult to meet the 12-hour calibration requirements after running a few dirty samples, laboratories under contract to EPA have not reported that verifying calibration is a problem. These laboratories have analyzed in excess of 1,000 samples for EPA using Method 1613.

*Comment:* No other method in 40 CFR part 136 has a requirement for initial demonstration of laboratory capability (IPRs, Section 8.2 of Method 1613) and Method 1613 should not either. The existing methods approved for measurement under the CWA and the SDWA already require determination of MDLs in accordance with 40 CFR part 136, which should be sufficient for Method 1613.

*Response:* The use of IPR analyses, which are also referred to as the start-up test, are not new requirements in CWA and SDWA methods. All 600 and 1600 series methods promulgated at 40 CFR part 136 appendix A include a requirement for demonstration of analyst/laboratory capability. These IPR tests are not equivalent to MDL determinations, nor are they intended to be. Although many of the CWA and SDWA methods specify MDLs, few

require determination of these MDLs as proofs of performance.

*Comment:* Method 1613 calls for instrument calibration to be verified at a high level, but calibration should be verified instead at the ML because of uncertainties at that level.

*Response:* EPA disagrees that calibration should be verified at the ML. In Method 1613, calibration is verified at the mid-point of the analytical range. This verification is common and accepted practice for analytical methods.

*Comment:* Method 1613 relies on the use of reagent water for tests to determine initial precision and recovery (IPR) and ongoing precision and recovery (OPR). This practice is inappropriate for methods that must rely on extensive cleanup.

*Response:* EPA disagrees that reagent water is inappropriate for use in the determination of IPR, OPR, and other tests because the primary purpose of these tests is to demonstrate laboratory performance rather than performance on a sample matrix. In addition, Method 1613 requires that if the method is to be applied to a sample matrix other than water (e.g., soil, filter cake, compost, tissue), the most appropriate alternate matrix is substituted for the reagent water matrix in these performance tests. Alternate matrices are listed in Section 7 of Method 1613. Further, Method 1613 requires that all steps used for processing samples, including preparation, extraction, and cleanup, shall be included in the performance tests. This requirement assures that performance problems will be found prior to application of the method to analysis of an environmental sample.

#### *J. Miscellaneous*

*Comment:* For samples containing less than one percent solids, the sample preparation procedure in Method 1613 (which is designed for liquids and solids) could take twice as long as the Method 613 preparation procedure (which is designed for liquids only), and for samples with more than one percent solids, it could take 3–4 times as long as the Method 613 preparation procedure.

*Response:* EPA agrees that the sample preparation procedures in Method 1613 will be more time-consuming than those in Method 613. Since CDDs/CDFs are known to be strongly associated with any particles in the sample, EPA believes that the additional filtration and extraction steps are necessary to accurately measure CDDs/CDFs in environmental samples at low concentrations.

To reduce the time required for extraction of aqueous samples containing less than 1 percent solids, and to reduce costs and the amount of solvent used in extraction in the interest of pollution prevention, EPA has added a procedure for solid-phase extraction (SPE) to the version of Method 1613 being promulgated today. EPA believes that this procedure will reduce the time required for extraction to levels commensurate with those required for extraction using Method 613.

*Comment:* The proposed rulemaking provides an insufficient basis for a thorough discussion and consideration of wet weight/dry weight issues for permits.

*Response:* Nothing in the promulgation of this Method requires the use of dry weight values in establishing effluent limitations in NPDES permits.

*Comment:* The proposal does not require the use of Method 1613 for any NPDES permits, but permittees should not presume that the NPDES permitting authority would not require use of Method 1613 if the authority determines that pollutants of concern in the effluent can only be measured at the level of concern by Method 1613.

*Response:* EPA agrees and intends for Method 1613 to be specified in NPDES permits at the discretion of the NPDES permitting authority.

#### *K. Technical Comments*

*Comment:* Table 3 should have one additional chlorinated diphenyl ether monitored (PeCDPE, HxCDEPE, HpCDPE, OCDPE, and NCPDPE). The commenter suggested a specific modification to sections 15.1 through 15.4 in those cases when a chlorodiphenyl ether may interfere with the determination of certain CDDs and CDFs.

*Response:* EPA agrees in principle with the commenter's suggestion but instead has incorporated requirements that meet the spirit of the suggestion into Section 18.3 of Method 1613. The method states that if chromatographic peaks are detected at the retention time of CDDs/CDFs in any of the m/z channels being monitored for the chlorodiphenyl ethers, cleanup procedures must be employed until these interferences are removed. This statement encompasses all the chlorodiphenyl ethers that may interfere in the analysis.

*Comment:* Methylene chloride is a poor extraction solvent because the solubility of CDDs/CDFs in it is less than that of other readily available solvents. Benzene or toluene should be used instead.

*Response:* EPA believes that methylene chloride is the solvent of choice for the aqueous filtrates because its higher than water density simplifies the extraction procedure. Similarly, EPA believes that toluene is most suitable for the SDS extraction of particulate sample matter. Finally, EPA believes that safety concerns over the use of a carcinogen such as benzene preclude the use of this traditional solvent in new analytical methods.

*Comment:* EPA is correct in pointing out the significant importance of handling particulates from aqueous samples, but further study of the methodology is needed to demonstrate that it can produce true quantitative and accurate values which can be used for compliance monitoring.

*Response:* The SDS extraction technique that is used in Method 1613 is based on widely published uses of the technique. Ample data to support its use are available in the open literature. For example, see references 6 and 7 cited in section 22.0 of Method 613. Further, EPA has now tested the SDS procedure on hundreds of aqueous environmental samples containing particulates (e.g., the databases for the IMVS and LTVS) and believes that SDS is the preferred procedure for such samples.

*Comment:* The Method should include a statement that indicates the expected analytical range of the Method.

*Response:* EPA agrees in principle with the comment, however, the analytical range is constrained on the low end by the calibration range, the sample size, and the ability to take a representative aliquot of a sample. The analytical range is not constrained on the upper end because the sample may be diluted to bring the concentrations of CDDs/CDFs within the calibration range, as described in Sections 17.5 and 18.2 of Method 1613.

*Comment:* NCASI included with its comments approximately 40 pages of suggested technical modifications to Method 1613 to improve the reliability of the Method.

*Response:* EPA appreciates NCASI's suggestions. NCASI has participated in EPA's validation studies, conducted validation studies of its own, scrutinized the details of Method 1613, and provided many valuable suggestions for improvements to the Method. EPA has adopted most of these suggestions, as well as the suggestions of others, as described in the "Detailed Responses to Comments on the Proposal of EPA Method 1613" included in the docket for today's rule. EPA will continue to work with all interested parties to ensure that Method 1613 and

other analytical methods are as state-of-the-art as possible.

## V. Regulatory Analysis

### A. Executive Order 12866

Under Executive Order 12866, 58 FR 51,735 (Oct. 4, 1993), the Agency must determine whether the regulatory action is "significant" and therefore subject to OMB review and the requirements of the Executive Order. The Order defines "significant regulatory action" as one that is likely to result in a rule that may: (1) Have an annual effect on the economy of \$100 million or more or adversely affect in a material way the economy, a sector of the economy, productivity, competition, jobs, the environment, public health or safety, or State, local, or tribal governments or communities; (2) create a serious inconsistency or otherwise interfere with an action taken or planned by another agency; (3) materially alter the budgetary impact of entitlements, grants, user fees, or loan programs or the rights and obligations of recipients thereof; or (4) raise novel legal or policy issues arising out of legal mandates, the President's priorities, or the principles set forth in the Executive Order."

This regulation is not major because it approves a testing procedure for use in compliance monitoring and data gathering but does not itself require these activities. Therefore, this regulation would not result in a cost to the economy of \$100 million or more; would not result in a major increase in costs or prices for consumers or individual industries; and would not have significant adverse effects on competition, investment, innovation, or international trade.

It has been determined that this rule is not a "significant regulatory action" under the terms of Executive Order 12866 and is therefore not subject to OMB review.

### B. Unfunded Mandates Reform Act

Title II of the Unfunded Mandates Reform Act of 1995 (UMRA), Pub. L. 104-4, establishes requirements for Federal agencies to assess the effects of their regulatory actions on State, local, and tribal governments and the private sector. Under section 202 of the UMRA, EPA generally must prepare a written statement, including a cost-benefit analysis, for proposed and final rules with "Federal mandates" that may result in expenditures to State, local, and tribal governments, in the aggregate, or to the private sector, of \$100 million or more in any one year. Before promulgating an EPA rule for which a written statement is needed, section 205

of the UMRA generally requires EPA to identify and consider a reasonable number of regulatory alternatives and adopt the least costly, most cost-effective or least burdensome alternative that achieves the objectives of the rule. The provisions of section 205 do not apply when they are inconsistent with applicable law. Moreover, section 205 allows EPA to adopt an alternative other than the least costly, most cost-effective or least burdensome alternative if the Administrator publishes with the final rule an explanation why that alternative was not adopted. Before EPA establishes any regulatory requirements that may significantly or uniquely affect small governments, including tribal governments, it must have developed under section 203 of the UMRA a small government agency plan. The plan must provide for notifying potentially affected small governments, enabling officials of affected small governments to have meaningful and timely input in the development of EPA regulatory proposals with significant Federal intergovernmental mandates, and informing, educating, and advising small governments on compliance with the regulatory requirements.

EPA has determined that this rule does not contain a Federal mandate that may result in expenditures of \$100 million or more for State, local, and tribal governments, in the aggregate, or the private sector in any one year. This rule makes available a testing procedure for use in compliance monitoring and data gathering but does not require these activities. Thus, today's rule is not subject to the requirements of sections 202 and 205 of the UMRA.

EPA has determined that this rule contains no regulatory requirements that might significantly or uniquely affect small governments. This rule simply approves a test procedure to be available for use by testing laboratories.

### C. Regulatory Flexibility Act

Pursuant to section 605(b) of the Regulatory Flexibility Act, 5 U.S.C. 605(b), the Administrator certifies that this rule will not have a significant economic impact on a substantial number of small entities. This regulation simply approves a test procedure to be available for use by testing laboratories.

### D. Paperwork Reduction Act

In accordance with the Paperwork Reduction Act of 1980, 44 U.S.C. 3501 *et seq.*, EPA must submit a copy of any rule that contains a collection-of-information requirement to the Director of the Office of Management and Budget (OMB) for review and approval. This

rule contains no additional information collection requirements beyond those already required by 40 CFR parts 122, 141, 142, 403, and 425, and approved by OMB (40 CFR part 9). The relevant OMB control numbers are 2040-0086, 2040-0170, 2040-0068, 2040-0110, 2040-0004, 2040-0090, and 2040-0009. Therefore, preparation of an information collection request to accompany this rule is unnecessary.

*E. Submission to Congress and the General Accounting Office*

Under 5 U.S.C. 801(a)(1)(A) as added by the Small Business Regulatory Enforcement Fairness Act of 1996, EPA submitted a report containing this rule and other required information to the U.S. Senate, the U.S. House of Representatives, and the Comptroller

General of the General Accounting Office, prior to publication of the rule in today's **Federal Register**. This rule is not a "major rule" as defined by 5 U.S.C. 804(2).

**List of Subjects in 40 CFR Part 136**

Environmental protection, Reporting and recordkeeping requirements, Water pollution control.

Dated: September 2, 1997.

**Carol M. Browner,**  
*Administrator.*

In consideration of the preceding, USEPA amends 40 CFR Part 136 as set forth below.

**PART 136—[AMENDED]**

1. The authority citation for part 136 continues to read as follows:

**Authority:** Secs. 301, 304(h), 307, and 501(a) Pub. L. 95-217, Stat. 1566, *et seq.* (33 U.S.C. 1251, *et seq.*) (The Federal Water Pollution Control Act Amendments of 1972 as amended by the Clean Water Act of 1977 and the Water Quality Act of 1987), 33 U.S.C. 1314 and 1361; 86 Stat. 816, Pub. L. 92-500; 91 Stat. 1567, Pub. L. 92-217; Stat. 7, Pub. L. 100-4 (The "Act").

2. In § 136.3(a), Table 1C.—List of Approved Test Procedures for Non-pesticide Organic Compounds, is amended by revising entries 60 through 97, by adding new entries 60 through 113, and by revising Table IC Notes <sup>1</sup> and <sup>2</sup> as follows:

**§ 136.3 Identification of test procedures.**

\* \* \* \* \*

TABLE 1C.—LIST OF APPROVED TEST PROCEDURES FOR NON-PESTICIDE ORGANIC COMPOUNDS

Parameter <sup>1</sup>	GC	EPA method number <sup>2,7</sup>			ASTM	Other
		GC/MS	HPLC	Standard methods 18th ed.		
* * * * *						
60. 1,2,3,4,6,7,8-Heptachlorodibenzofuran.	.....	1613	.....	.....		
61. 1,2,3,4,7,8,9-Heptachlorodibenzofuran.	.....	1613	.....	.....		
62. 1,2,3,4,6,7,8-Heptachlorodibenzo- <i>p</i> -dioxin.	.....	1613	.....	.....		
63. Hexachlorobenzene	612	625, 1625	.....	6410 B		
64. Hexachlorobutadiene	612	625, 1625	.....	6410 B		
65. Hexachlorocyclopentadiene	612	625, 1625 <sup>5</sup>	.....	6410 B		
66. 1,2,3,4,7,8-Hexachlorodibenzofuran	.....	1613	.....	.....		
67. 1,2,3,6,7,8-Hexachlorodibenzofuran	.....	1613	.....	.....		
68. 1,2,3,7,8,9-Hexachlorodibenzofuran	.....	1613	.....	.....		
69. 2,3,4,6,7,8-Hexachlorodibenzofuran	.....	1613	.....	.....		
70. 1,2,3,4,7,8-Hexachlorodibenzo- <i>p</i> -dioxin.	.....	1613	.....	.....		
71. 1,2,3,6,7,8-Hexachlorodibenzo- <i>p</i> -dioxin.	.....	1613	.....	.....		
72. 1,2,3,7,8,9-Hexachlorodibenzo- <i>p</i> -dioxin.	.....	1613	.....	.....		
73. Hexachloroethane	616	625, 1625	.....	6410 B		
74. Ideno(1,2,3-cd)pyrene	610	625, 1625	610	6410 B, 6440 B	D4657-87	
75. Isophorone	609	625, 1625	.....	6410 B		
76. Methylene chloride	601	624, 1624	.....	6230 B		Note 3, p. 130.
77. 2-Methyl-4,6-dinitrophenol	604	625, 1625	.....	6420 B, 6410 B		
78. Naphthalene	610	625, 1625	610	6410 B, 6440 B		
79. Nitrobenzene	609	625, 1625	.....	6410 B	D4657-87	
80. 2-Nitrophenol	604	625, 1625	.....	6410 B, 6420 B		
81. 4-Nitrophenol	604	625, 1625	.....	6410 B, 6420 B		
82. N-Nitrosodimethylamine	607	625, 1625	.....	6410 B		
83. N-Nitrosodi-n-propylamine	607	625, 1625 <sup>5</sup>	.....	6410 B		
84. N-Nitrosodiphenylamine	607	625, 1625 <sup>5</sup>	.....	6410 B		
85. Octachlorodibenzofuran	.....	1613	.....	.....		
86. Octachlorodibenzo- <i>p</i> -dioxin	.....	1613	.....	.....		
87. 2,2-Oxybis(1-chloropropane)	611	625, 1625	.....	6410 B		
88. PCB-1016	608	625	.....	6410 B		Note 3, p. 43.
89. PCB-1221	608	625	.....	6410 B		Note 3, p. 43.
90. PCB-1232	608	625	.....	6410 B		Note 3, p. 43.
91. PCB 1242	608	625	.....	6410 B		Note 3, p. 43.
92. PCB-1248	608	625	.....	.....		
93. PCB-1254	608	625	.....	6410 B		Note 3, p. 43.
94. PCB-1260	608	625	.....	6410 B, 6630 B		Note 3, p. 43.
95. 1,2,3,7,8-Pentachlorodibenzofuran ..	.....	1613	.....	.....		
96. 2,3,4,7,8-Pentachlorodibenzofuran ..	.....	1613	.....	.....		

TABLE 1C.—LIST OF APPROVED TEST PROCEDURES FOR NON-PESTICIDE ORGANIC COMPOUNDS—Continued

Parameter <sup>1</sup>	GC	EPA method number <sup>2,7</sup>			ASTM	Other
		GC/MS	HPLC	Standard methods 18th ed.		
97. 1,2,3,7,8-Pentachlorodibenzo- <i>p</i> -dioxin.		1613				
98. Pentachlorophenol	604	625, 1625		6410 B, 6630 B		Note 3, p. 140.
99. Phenanthrene	610	625, 1625	610	6410 B, 6440 B	D4657-87	
100. Phenol	604	625, 1625		6420 B, 6410 B		
101. Pyrene	610	625, 1625	610	6410 B, 6440 B	D4657-87	
102. 2,3,7,8-Tetrachlorodibenzofuran		1613				
103. 2,3,7,8-Tetrachlorodibenzo- <i>p</i> -dioxin.		613, 1613 <sup>5</sup>				
104. 1,1,2,2-Tetrachloroethane	601	624, 1624		6230 B, 6210 B		Note 3, p. 130.
105. Tetrachloroethene	601	624, 1624		6230 B, 6410 B		Note 3, p. 130.
106. Toluene	602	624, 1624		6210 B, 6220 B		
107. 1,2,4-Trichlorobenzene	612	625, 1625		6410 B		Note 3, p. 130.
108. 1,1,1-Trichloroethane	601	624, 1624		6210 B, 6230 B		
109. 1,1,2-Trichloroethane	601	624, 1624		6210 B, 6230 B		Note 3, p. 130.
110. Trichloroethene	601	624, 1624		6210 B, 6230 B		
111. Trichlorofluoromethane	601	624		6210 B, 6230 B		
112. 2,4,6-Trichlorophenol	604	625, 1625		6410 B, 6240 B		
113. Vinyl chloride	601	624, 1624		6210 B, 6230 B		

<sup>1</sup> All parameters are expressed in micrograms per liter (µg/L) except for Method 1613 in which the parameters are expressed in picograms per liter (pg/L).

<sup>2</sup> The full text of Methods 601-613, 624, 625, 1624, and 1625, are given at Appendix A, "Test Procedures for Analysis of Organic Pollutants," of this Part 136. The full text of Method 1613 is incorporated by reference into this Part 136 and is available from the National Technical Information Services as stock number PB95-104774. The standardized test procedure to be used to determine the method detection limit (MDL) for these test procedures is given at Appendix B, "Definition and Procedures for the Determination of the Method Detection Limit," of this Part 136.

<sup>5</sup> <sup>5a</sup>, and <sup>7</sup> unchanged.

3. In § 136.3(b), the listing titled References, Sources, Costs, and Table Citations is amended by revising the first sentence of paragraph (1) to read as follows:

**§ 136.3 Identification of test procedures.**

\* \* \* \* \*

**References, Sources, Costs, and Table Citations**

(1) The full texts of Methods 601-613, 624, 625, 1613, 1624, and 1625 are

printed in appendix A of this part 136.

\* \* \*

4. In § 136.3(e), Table II—Required Containers, Preservation Techniques, and Holding Times, is amended by revising Table IC—Organic Tests to read as follows:

TABLE II.—REQUIRED CONTAINERS, PRESERVATION TECHNIQUES, AND HOLDING TIMES

Parameter No./name	Container <sup>1</sup>	Preservation <sup>2,3</sup>	Maximum holding time <sup>4</sup>
Table IC—Organic Tests <sup>8</sup>			
13, 18-20, 22, 24-28, 34-37, 39-43, 45-47, 56, 76, 104, 105, 108-111, 113. Purgeable Halocarbons.	G, Teflon-lined septum	Cool, 4°C, 0.008% Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> <sup>5</sup> .	14 days.
6, 57, 106. Purgeable aromatic hydrocarbons.	.....do	Cool, 4°C, 0.008% Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> <sup>5</sup> HCl to pH2 <sup>9</sup> .	Do.
3, 4. Acrolein and acrylonitrile	.....do	Cool, 4°C, 0.008% Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> <sup>5</sup> adjust pH to 4-5 <sup>10</sup> .	Do.
23, 30, 44, 49, 53, 77, 80, 81, 98, 100, 112. Phenols <sup>11</sup> .	G, Teflon-lined cap.	Cool, 4°C, 0.008% Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> <sup>5</sup>	7 days until extraction; 40 days after extraction.
7, 38. Benzidines <sup>11</sup>	.....do	.....do	7 days until extraction. <sup>13</sup>
14, 17, 48, 50-52. Phthalate esters <sup>11</sup> .	.....do	Cool, 4°C	7 days until extraction; 40 days after extraction.
82-84. Nitrosamines <sup>11,14</sup>	.....do	Cool, 4°C, 0.008% Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> <sup>5</sup> store in dark.	Do.
88-94. PCBs <sup>11</sup>	.....do	Cool, 4°C	Do.
54, 55, 75, 79. Nitroaromatics and isophorone <sup>11</sup> .	.....do	Cool, 4°C, 0.008% Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> <sup>5</sup> store in dark.	Do.
1, 2, 5, 8-12, 32, 33, 58, 59, 74, 78, 99, 101. Polynuclear aromatic hydrocarbons <sup>11</sup> .	.....do	.....do	Do.
15, 16, 21, 31, 87. Haloethers <sup>11</sup>	.....do	Cool, 4°C, 0.008% Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> <sup>5</sup>	Do.

TABLE II.—REQUIRED CONTAINERS, PRESERVATION TECHNIQUES, AND HOLDING TIMES—Continued

Parameter No./name	Container <sup>1</sup>	Preservation <sup>2,3</sup>	Maximum holding time <sup>4</sup>
29, 35–37, 63–65, 73, 107. Chlorinated hydrocarbons <sup>11</sup> .	do	Cool, 4°C	Do.
60–62, 66–72, 85, 86, 95–97, 102, 103. CDDs/CDFs <sup>11</sup>			
aqueous: field and lab preservation..	G	Cool, 0–4°C, pH<9, 0.008% Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> <sup>5</sup> ..	1 year.
Solids, mixed phase, and tissue: field preservation..	do	Cool, <4°C	7 days.
Solids, mixed phase, and tissue: lab preservation.	do	Freeze, <–10°C	1 year.
*	*	*	*
*	*	*	*

Note: The footnotes remain unchanged.

4. In part 136, appendix A is amended by adding Method 1613 to read as follows:

**Method 1613, Revision B**

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

**1.0 Scope and Application**

1.1 This method is for determination of tetra- through octa-chlorinated dibenzo-p-dioxins (CDDs) and dibenzofurans (CDFs) in water, soil, sediment, sludge, tissue, and other sample matrices by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS). The method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act. The method is based on a compilation of EPA, industry, commercial laboratory, and academic methods (References 1–6).

1.2 The seventeen 2,3,7,8-substituted CDDs/CDFs listed in Table 1 may be determined by this method. Specifications are also provided for separate determination of 2,3,7,8-tetrachloro-dibenzo-p-dioxin (2,3,7,8-TCDD) and 2,3,7,8-tetrachloro-dibenzofuran (2,3,7,8-TCDF).

1.3 The detection limits and quantitation levels in this method are usually dependent on the level of interferences rather than instrumental limitations. The minimum levels (MLs) in Table 2 are the levels at which the CDDs/CDFs can be determined with no interferences present. The Method Detection Limit (MDL) for 2,3,7,8-TCDD has been determined as 4.4 pg/L (parts-per-quadrillion) using this method.

1.4 The GC/MS portions of this 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 9.2.

1.5 This method is "performance-based". The analyst is permitted to modify the method to overcome interferences or lower the cost of measurements, provided that all performance criteria in this method are met. The requirements for establishing method equivalency are given in Section 9.1.2.

1.6 Any modification of this method, beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

**2.0 Summary of Method**

Flow charts that summarize procedures for sample preparation, extraction, and analysis are given in Figure 1 for aqueous and solid samples, Figure 2 for multi-phase samples, and Figure 3 for tissue samples.

**2.1 Extraction.**

2.1.1 Aqueous samples (samples containing less than 1% solids)—Stable isotopically labeled analogs of 15 of the 2,3,7,8-substituted CDDs/CDFs are spiked into a 1 L sample, and the sample is extracted by one of three procedures:

2.1.1.1 Samples containing no visible particles are extracted with methylene chloride in a separatory funnel or by the solid-phase extraction technique summarized in Section 2.1.1.3. The extract is concentrated for cleanup.

2.1.1.2 Samples containing visible particles are vacuum filtered through a glass-fiber filter. The filter is extracted in a Soxhlet/Dean-Stark (SDS) extractor (Reference 7), and the filtrate is extracted with methylene chloride in a

separatory funnel. The methylene chloride extract is concentrated and combined with the SDS extract prior to cleanup.

2.1.1.3 The sample is vacuum filtered through a glass-fiber filter on top of a solid-phase extraction (SPE) disk. The filter and disk are extracted in an SDS extractor, and the extract is concentrated for cleanup.

2.1.2 Solid, semi-solid, and multi-phase samples (but not tissue)—The labeled compounds are spiked into a sample containing 10 g (dry weight) of solids. Samples containing multiple phases are pressure filtered and any aqueous liquid is discarded. Coarse solids are ground or homogenized. Any non-aqueous liquid from multi-phase samples is combined with the solids and extracted in an SDS extractor. The extract is concentrated for cleanup.

2.1.3 Fish and other tissue—The sample is extracted by one of two procedures:

2.1.3.1 Soxhlet or SDS extraction—A 20 g aliquot of sample is homogenized, and a 10 g aliquot is spiked with the labeled compounds. The sample is mixed with sodium sulfate, allowed to dry for 12–24 hours, and extracted for 18–24 hours using methylene chloride:hexane (1:1) in a Soxhlet extractor. The extract is evaporated to dryness, and the lipid content is determined.

2.1.3.2 HCl digestion—A 20 g aliquot is homogenized, and a 10 g aliquot is placed in a bottle and spiked with the labeled compounds. After equilibration, 200 mL of hydrochloric acid and 200 mL of methylene chloride:hexane (1:1) are added, and the bottle is agitated for 12–24 hours. The extract is evaporated to dryness, and the lipid content is determined.

2.2 After extraction, <sup>37</sup>Cl<sub>4</sub>-labeled 2,3,7,8-TCDD is added to each extract to measure the efficiency of the cleanup process. Sample cleanups may include

back-extraction with acid and/or base, and gel permeation, alumina, silica gel, Florisil 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. Prior to the cleanup procedures cited above, tissue extracts are cleaned up using an anthropogenic isolation column, a batch silica gel adsorption, or sulfuric acid and base back-extraction, depending on the tissue extraction procedure used.

2.3 After cleanup, the extract is concentrated to near dryness. Immediately prior to injection, internal standards are added to each extract, and an 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. Two exact  $m/z$ 's are monitored for each analyte.

2.4 An individual CDD/CDF is identified by comparing the GC retention time and ion-abundance ratio of two exact  $m/z$ 's with the corresponding retention time of an authentic standard and the theoretical or acquired ion-abundance ratio of the two exact  $m/z$ 's. The non-2,3,7,8 substituted isomers and congeners are identified when retention times and ion-abundance ratios agree within predefined limits. Isomer specificity for 2,3,7,8-TCDD and 2,3,7,8-TCDF is achieved using GC columns that resolve these isomers from the other tetra-isomers.

2.5 Quantitative analysis is performed using selected ion current profile (SICP) areas, in one of three ways:

2.5.1 For the 15 2,3,7,8-substituted CDDs/CDFs with labeled analogs (see Table 1), the GC/MS system is calibrated, and the concentration of each compound is determined using the isotope dilution technique.

2.5.2 For 1,2,3,7,8,9-HxCDD, OCDF, and the labeled compounds, the GC/MS system is calibrated and the concentration of each compound is determined using the internal standard technique.

2.5.3 For non-2,3,7,8-substituted isomers and for all isomers at a given level of chlorination (i.e., total TCDD), concentrations are determined using response factors from calibration of the CDDs/CDFs at the same level of chlorination.

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

### 3.0 Definitions

Definitions are given in the glossary at the end of this method.

### 4.0 Contamination and Interferences

4.1 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.

4.2 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.

4.2.1 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 seconds may aid in cleaning. Glassware with removable parts, particularly separatory funnels with fluoropolymer stopcocks, must be disassembled prior to detergent washing.

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

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

4.2.4 Immediately prior to use, the Soxhlet apparatus should be pre-extracted with toluene for approximately three hours (see Sections 12.3.1 through 12.3.3). Separatory funnels should be shaken with methylene chloride/toluene (80/20 mixture) for two minutes, drained, and then shaken with pure methylene chloride for two minutes.

4.3 All materials used in the analysis shall be demonstrated to be free from interferences by running reference matrix method blanks initially and with each sample batch (samples started through the extraction process on a given 12-hour shift, to a maximum of 20 samples).

4.3.1 The reference matrix must simulate, as closely as possible, the

sample matrix under test. Ideally, the reference matrix should not contain the CDDs/CDFs in detectable amounts, but should contain potential interferences in the concentrations expected to be found in the samples to be analyzed. For example, a reference sample of human adipose tissue containing pentachloronaphthalene can be used to exercise the cleanup systems when samples containing pentachloronaphthalene are expected.

4.3.2 When a reference matrix that simulates the sample matrix under test is not available, reagent water (Section 7.6.1) can be used to simulate water samples; playground sand (Section 7.6.2) or white quartz sand (Section 7.3.2) can be used to simulate soils; filter paper (Section 7.6.3) can be used to simulate papers and similar materials; and corn oil (Section 7.6.4) can be used to simulate tissues.

4.4 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 CDDs/CDFs. The most frequently encountered interferences are chlorinated biphenyls, methoxy biphenyls, hydroxydiphenyl ethers, benzylphenyl ethers, polynuclear aromatics, and pesticides. Because very low levels of CDDs/CDFs are measured by this method, the elimination of interferences is essential. The cleanup steps given in Section 13 can be used to reduce or eliminate these interferences and thereby permit reliable determination of the CDDs/CDFs at the levels shown in Table 2.

4.5 Each piece of reusable glassware should be numbered to associate that glassware with the processing of a particular sample. This will assist the laboratory in tracking possible sources of contamination for individual samples, identifying glassware associated with highly contaminated samples that may require extra cleaning, and determining when glassware should be discarded.

4.6 Cleanup of tissue—The natural lipid content of tissue can interfere in the analysis of tissue samples for the CDDs/CDFs. The lipid contents of different species and portions of tissue can vary widely. Lipids are soluble to varying degrees in various organic solvents and may be present in sufficient quantity to overwhelm the column chromatographic cleanup procedures used for cleanup of sample extracts. Lipids must be removed by the lipid removal procedures in Section 13.7, followed by alumina (Section 13.4) or Florisil (Section 13.8), and carbon

(Section 13.5) as minimum additional cleanup steps. If chlorodiphenyl ethers are detected, as indicated by the presence of peaks at the exact m/z's monitored for these interferents, alumina and/or Florisil cleanup must be employed to eliminate these interferences.

## 5.0 Safety

5.1 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.

5.1.1 The 2,3,7,8-TCDD isomer has been found to be acrogenic, carcinogenic, and teratogenic in laboratory animal studies. It is soluble in water to approximately 200 ppt and in organic solvents to 0.14%. On the basis of the available toxicological and physical properties of 2,3,7,8-TCDD, all of the CDDs/CDFs should be handled only by highly trained personnel thoroughly familiar with handling and cautionary procedures and the associated risks.

5.1.2 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.

5.2 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 material safety data sheets (MSDSs) should also be made available to all personnel involved in these analyses. It is also suggested that the laboratory perform personal hygiene monitoring of each analyst who uses this method and that the results of this monitoring be made available to the analyst. 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.

5.3 The CDDs/CDFs and samples suspected to contain these compounds are handled using essentially the same techniques 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 Labor, many of which have an industrial health service. The CDDs/CDFs are extremely toxic to laboratory animals. Each laboratory must develop a strict safety program for handling these compounds. The practices in References 2 and 14 are highly recommended.

5.3.1 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 in a 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.

5.3.2 Protective equipment—Disposable plastic gloves, apron or lab coat, safety glasses or mask, and a glove box or fume hood adequate for radioactive work should be used. During analytical operations that 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 CDDs/CDFs, an additional set of gloves can also be worn beneath the latex gloves.

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

5.3.4 Personal hygiene—Hands and forearms should be washed thoroughly after each manipulation and before breaks (coffee, lunch, and shift).

5.3.5 Confinement—Isolated work areas posted with signs, segregated glassware and tools, and plastic absorbent paper on bench tops will aid in confining contamination.

5.3.6 Effluent vapors—The effluents of sample splitters from the gas chromatograph (GC) and from roughing pumps on the mass spectrometer (MS) should pass through either a column of activated charcoal or be bubbled through a trap containing oil or high-boiling alcohols to condense CDD/CDF vapors.

5.3.7 Waste 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.

### 5.3.8 Decontamination

5.3.8.1 Decontamination of personnel—Use any mild soap with plenty of scrubbing action.

5.3.8.2 Glassware, tools, and surfaces—Chlorothene NU Solvent 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.

5.3.9 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.

5.3.10 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 with an electron capture detector (ECD) can achieve a limit of detection of 0.1 µg per wipe; analysis using this method can achieve an even lower detection limit. Less than 0.1 µg per wipe indicates acceptable cleanliness; anything higher warrants further cleaning. More than 10 µg 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.

5.3.11 Table or wrist-action shaker—The use of a table or wrist-action shaker for extraction of tissues presents the possibility of breakage of the extraction bottle and spillage of acid and flammable organic solvent. A secondary containment system around the shaker is suggested to prevent the spread of acid and solvents in the event of such a breakage. The speed and intensity of shaking action should also be adjusted to minimize the possibility of breakage.

## 6.0 Apparatus and Materials

**Note:** Brand names, suppliers, and part numbers are for illustration purposes only and no endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here. Meeting the performance requirements of this method is the responsibility of the laboratory.

### 6.1 Sampling Equipment for Discrete or Composite Sampling

6.1.1 Sample bottles and caps  
6.1.1.1 Liquid samples (waters, sludges and similar materials containing 5% solids or less)—Sample bottle, amber glass, 1.1 L minimum, with screw cap.

6.1.1.2 Solid samples (soils, sediments, sludges, paper pulps, filter cake, compost, and similar materials that contain more than 5% solids)—Sample bottle, wide mouth, amber glass, 500 mL minimum.

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

6.1.1.4 Bottle caps—Threaded to fit sample bottles. Caps shall be lined with fluoropolymer.

6.1.1.5 Cleaning

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

6.1.1.5.2 Liners are detergent water washed, rinsed with reagent water (Section 7.6.1) followed by solvent, and baked at approximately 200°C for a minimum of 1 hour prior to use.

6.1.2 Compositing equipment—Automatic or manual compositing system incorporating glass containers cleaned per bottle cleaning procedure above. Only glass or fluoropolymer tubing 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 rinsing with reagent water to minimize sample contamination. An integrating flow meter is used to collect proportional composite samples.

6.2 Equipment for Glassware Cleaning—Laboratory sink with overhead fume hood.

6.3 Equipment for Sample Preparation

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

6.3.2 Glove box (optional).

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

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

6.3.5 Equipment for determining percent moisture

6.3.5.1 Oven—Capable of maintaining a temperature of 110 ±5°C.

6.3.5.2 Dessicator.

6.3.6 Balances

6.3.6.1 Analytical—Capable of weighing 0.1 mg.

6.3.6.2 Top loading—Capable of weighing 10 mg.

6.4 Extraction Apparatus

6.4.1 Water samples

6.4.1.1 pH meter, with combination glass electrode.

6.4.1.2 pH paper, wide range (Hydriion Papers, or equivalent).

6.4.1.3 Graduated cylinder, 1 L capacity.

6.4.1.4 Liquid/liquid extraction—Separatory funnels, 250 mL, 500 mL, and 2000 mL, with fluoropolymer stopcocks.

6.4.1.5 Solid-phase extraction

6.4.1.5.1 One liter filtration apparatus, including glass funnel, glass frit support, clamp, adapter, stopper, filtration flask, and vacuum tubing (Figure 4). For wastewater samples, the apparatus should accept 90 or 144 mm disks. For drinking water or other samples containing low solids, smaller disks may be used.

6.4.1.5.2 Vacuum source capable of maintaining 25 in. Hg, equipped with shutoff valve and vacuum gauge.

6.4.1.5.3 Glass-fiber filter—Whatman GMF 150 (or equivalent), 1 micron pore size, to fit filtration apparatus in Section 6.4.1.5.1.

6.4.1.5.4 Solid-phase extraction disk containing octadecyl (C<sub>18</sub>) bonded silica uniformly enmeshed in an inert matrix—Fisher Scientific 14-378F (or equivalent), to fit filtration apparatus in Section 6.4.1.5.1.

6.4.2 Soxhlet/Dean-Stark (SDS) extractor (Figure 5)—For filters and solid/sludge samples.

6.4.2.1 Soxhlet—50 mm ID, 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).

6.4.2.2 Thimble—43 × 123 to fit Soxhlet (Cal-Glass LG-6901-122, or equivalent).

6.4.2.3 Moisture trap—Dean Stark or Barret with fluoropolymer stopcock, to fit Soxhlet.

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

6.4.2.5 Variable transformer—Powerstat (or equivalent), 110 volt, 10 amp.

6.4.3 Apparatus for extraction of tissue.

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

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

6.4.3.3 Mechanical shaker—Wrist-action or platform-type rotary shaker that produces vigorous agitation (Sybron Thermolyne Model LE “Big Bill” rotator/shaker, or equivalent).

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

6.4.4 Beakers—400–500 mL.

6.4.5 Spatulas—Stainless steel.

6.5 Filtration Apparatus.

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

**Note:** Baking glass wool may cause active sites that will irreversibly adsorb CDDs/CDFs.

6.5.2 Glass funnel—125–250 mL.

6.5.3 Glass-fiber filter paper—Whatman GF/D (or equivalent), to fit glass funnel in Section 6.5.2.

6.5.4 Drying column—15–20 mm ID Pyrex chromatographic column equipped with coarse-glass frit or glass-wool plug.

6.5.5 Buchner funnel—15 cm.

6.5.6 Glass-fiber filter paper—to fit Buchner funnel in Section 6.5.5.

6.5.7 Filtration flasks—1.5–2.0 L, with side arm.

6.5.8 Pressure filtration apparatus—Millipore YT30 142 HW, or equivalent.

6.6 Centrifuge Apparatus.

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

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

6.6.3 Centrifuge tubes—12–15 mL, with screw-caps, to fit centrifuge.

6.7 Cleanup Apparatus.

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

6.7.1.1 Column—600–700 mm long × 25 mm ID, packed with 70 g of SX-3 Bio-beads (Bio-Rad Laboratories, Richmond, CA, or equivalent).

6.7.1.2 Syringe—10 mL, with Luer fitting.

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

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

6.7.2 Reverse-phase high-performance liquid chromatograph.

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

6.7.2.2 Injector—Rheodyne 7120 (or equivalent) with 50 μL sample loop.

6.7.2.3 Column—Two 6.2 mm × 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.

6.7.2.4 Pump—Altex 110A (or equivalent).

6.7.3 Pipets.

6.7.3.1 Disposable, pasteur—150 mm long × 5-mm ID (Fisher Scientific 13-678-6A, or equivalent).

6.7.3.2 Disposable, serological—10 mL (6 mm ID).

6.7.4 Glass chromatographic columns.

6.7.4.1 150 mm long × 8-mm ID, (Kontes K-420155, or equivalent) with coarse-glass frit or glass-wool plug and 250 mL reservoir.

6.7.4.2 200 mm long × 15 mm ID, with coarse-glass frit or glass-wool plug and 250 mL reservoir.

6.7.4.3 300 mm long × 25 mm ID, with 300 mL reservoir and glass or fluoropolymer stopcock.

6.7.5 Stirring apparatus for batch silica cleanup of tissue extracts.

6.7.5.1 Mechanical stirrer—Corning Model 320, or equivalent.

6.7.5.2 Bottle—500–600 mL wide-mouth clear glass.

6.7.6 Oven—For baking and storage of adsorbents, capable of maintaining a constant temperature ( $\pm 5^\circ\text{C}$ ) in the range of 105–250°C.

6.8 Concentration Apparatus.

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

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

6.8.1.2 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.

6.8.1.3 Round-bottom flask—100 mL and 500 mL or larger, with ground-glass fitting compatible with the rotary evaporator.

6.8.2 Kuderna-Danish (K-D) Concentrator.

6.8.2.1 Concentrator tube—10 mL, graduated (Kontes K-570050-1025, or equivalent) with calibration verified. Ground-glass stopper (size 19/22 joint) is used to prevent evaporation of extracts.

6.8.2.2 Evaporation flask—500 mL (Kontes K-570001-0500, or equivalent), attached to concentrator tube with springs (Kontes K-662750-0012 or equivalent).

6.8.2.3 Snyder column—Three-ball macro (Kontes K-503000-0232, or equivalent).

6.8.2.4 Boiling chips.

6.8.2.4.1 Glass or silicon carbide—Approximately 10/40 mesh, extracted with methylene chloride and baked at 450°C for one hour minimum.

6.8.2.4.2 Fluoropolymer (optional)—Extracted with methylene chloride.

6.8.2.5 Water bath—Heated, with concentric ring cover, capable of maintaining a temperature within  $\pm 2^\circ\text{C}$ , installed in a fume hood.

6.8.3 Nitrogen blowdown apparatus—Equipped with water bath controlled in the range of 30–60°C (N-Evap, Organomation Associates, Inc., South Berlin, MA, or equivalent), installed in a fume hood.

6.8.4 Sample vials.

6.8.4.1 Amber glass—2–5 mL with fluoropolymer-lined screw-cap.

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

6.9 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 10.

6.9.1 GC column for CDDs/CDFs and for isomer specificity for 2,3,7,8-TCDD— $60 \pm 5$  m long ×  $0.32 \pm 0.02$  mm ID; 0.25  $\mu\text{m}$  5% phenyl, 94% methyl, 1% vinyl silicone bonded-phase fused-silica capillary column (J&W DB-5, or equivalent).

6.9.2 GC column for isomer specificity for 2,3,7,8-TCDF— $30 \pm 5$  m long ×  $0.32 \pm 0.02$  mm ID; 0.25  $\mu\text{m}$  bonded-phase fused-silica capillary column (J&W DB-225, or equivalent).

6.10 Mass Spectrometer—28–40 eV electron impact ionization, shall be capable of repetitively selectively monitoring 12 exact m/z's minimum at high resolution ( $\geq 10,000$ ) during a period of approximately one second, and shall meet all of the performance specifications in Section 10.

6.11 GC/MS Interface—The mass spectrometer (MS) 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.

6.12 Data System—Capable of collecting, recording, and storing MS data.

## 7.0 Reagents and Standards

7.1 pH Adjustment and Back-Extraction.

7.1.1 Potassium hydroxide—Dissolve 20 g reagent grade KOH in 100 mL reagent water.

7.1.2 Sulfuric acid—Reagent grade (specific gravity 1.84).

7.1.3 Hydrochloric acid—Reagent grade, 6N.

7.1.4 Sodium chloride—Reagent grade, prepare at 5% (w/v) solution in reagent water.

7.2 Solution Drying and Evaporation.

7.2.1 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. If, after heating, the sodium sulfate develops a noticeable grayish cast (due to the presence of carbon in the crystal matrix), that batch of reagent is not suitable for use and should be discarded. Extraction with methylene chloride (as opposed to simple rinsing) and baking at a lower temperature may produce sodium sulfate that is suitable for use.

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

7.2.3 Prepurified nitrogen.

7.3 Extraction.

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

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

7.4 GPC Calibration Solution—Prepare a 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.

7.5 Adsorbents for Sample Cleanup.

7.5.1 Silica gel.

7.5.1.1 Activated silica gel—100–200 mesh, Supelco 1-3651 (or equivalent), rinsed with methylene chloride, baked at 180°C for a minimum of one hour, cooled in a dessicator, and stored in a pre-cleaned glass bottle with screw-cap that prevents moisture from entering.

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

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

7.5.1.4 Potassium silicate.

7.5.1.4.1 Dissolve 56 g of high purity potassium hydroxide (Aldrich, or

equivalent) in 300 mL of methanol in a 750–1000 mL flat-bottom flask.

7.5.1.4.2 Add 100 g of silica gel and a stirring bar, and stir on a hot plate at 60–70°C for one to two hours.

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

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

7.5.1.4.5 Activate overnight at 200–250°C.

7.5.2 Alumina—Either one of two types of alumina, acid or basic, may be used in the cleanup of sample extracts, provided that the laboratory can meet the performance specifications for the recovery of labeled compounds described in Section 9.3. The same type of alumina must be used for all samples, including those used to demonstrate initial precision and recovery (Section 9.2) and ongoing precision and recovery (Section 15.5).

7.5.2.1 Acid alumina—Supelco 19996–6C (or equivalent). Activate by heating to 130°C for a minimum of 12 hours.

7.5.2.2 Basic alumina—Supelco 19944–6C (or equivalent). Activate by heating to 600°C for a minimum of 24 hours. Alternatively, activate by heating in a tube furnace at 650–700°C under an air flow rate of approximately 400 cc/minute. Do not heat over 700°C, as this can lead to reduced capacity for retaining the analytes. Store at 130°C in a covered flask. Use within five days of baking.

7.5.3 Carbon.

7.5.3.1 Caropak C—(Supelco 1–0258, or equivalent).

7.5.3.2 Celite 545—(Supelco 2–0199, or equivalent).

7.5.3.3 Thoroughly mix 9.0 g Caropak C and 41.0 g Celite 545 to produce an 18% w/w mixture. Activate the mixture at 130°C for a minimum of six hours. Store in a desiccator.

7.5.4 Anthropogenic isolation column—Pack the column in Section 6.7.4.3 from bottom to top with the following:

7.5.4.1 2 g silica gel (Section 7.5.1.1).

7.5.4.2 2 g potassium silicate (Section 7.5.1.4).

7.5.4.3 2 g granular anhydrous sodium sulfate (Section 7.2.1).

7.5.4.4 10 g acid silica gel (Section 7.5.1.2).

7.5.4.5 2 g granular anhydrous sodium sulfate.

7.5.5 Florisil column.

7.5.5.1 Florisil—60–100 mesh, Floridin Corp (or equivalent). Soxhlet extract in 500 g portions for 24 hours.

7.5.5.2 Insert a glass wool plug into the tapered end of a graduated serological pipet (Section 6.7.3.2). Pack with 1.5 g (approx 2 mL) of Florisil topped with approx 1 mL of sodium sulfate (Section 7.2.1) and a glass wool plug.

7.5.5.3 Activate in an oven at 130–150°C for a minimum of 24 hours and cool for 30 minutes. Use within 90 minutes of cooling.

7.6 Reference Matrices—Matrices in which the CDDs/CDFs and interfering compounds are not detected by this method.

7.6.1 Reagent water—Bottled water purchased locally, or prepared by passage through activated carbon.

7.6.2 High-solids reference matrix—Playground sand or similar material. Prepared by extraction with methylene chloride and/or baking at 450°C for a minimum of four hours.

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

7.6.4 Tissue reference matrix—Corn or other vegetable oil. May be prepared by extraction with methylene chloride.

7.6.5 Other matrices—This method may be verified on any reference matrix by performing the tests given in Section 9.2. Ideally, the matrix should be free of the CDDs/CDFs, but in no case shall the background level of the CDDs/CDFs in the reference matrix exceed three times the minimum levels in Table 2. If low background levels of the CDDs/CDFs are present in the reference matrix, the spike level of the analytes used in Section 9.2 should be increased to provide a spike-to-background ratio in the range of 1:1 to 5:1 (Reference 15).

7.7 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 the chemical purity is 98% 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 fluoropolymer-lined caps. A mark is placed on the vial at the level of the solution so that solvent loss by evaporation can be detected. If solvent loss has occurred, the solution should be replaced.

7.8 Stock Solutions.

7.8.1 Preparation—Prepare in nonane per the steps below or purchase as dilute solutions (Cambridge Isotope Laboratories (CIL), Woburn, MA, or equivalent). Observe the safety

precautions in Section 5, and the recommendation in Section 5.1.2.

7.8.2 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 fluoropolymer-lined cap.

7.8.3 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 CIL and may be available from other vendors.

7.9 PAR Stock Solution

7.9.1 All CDDs/CDFs—Using the solutions in Section 7.8, prepare the PAR stock solution to contain the CDDs/CDFs at the concentrations shown in Table 3. When diluted, the solution will become the PAR (Section 7.14).

7.9.2 If only 2,3,7,8-TCDD and 2,3,7,8-TCDF are to be determined, prepare the PAR stock solution to contain these compounds only.

7.10 Labeled-Compound Spiking Solution.

7.10.1 All CDDs/CDFs—From stock solutions, or from purchased mixtures, prepare this solution to contain the labeled compounds in nonane at the concentrations shown in Table 3. This solution is diluted with acetone prior to use (Section 7.10.3).

7.10.2 If only 2,3,7,8-TCDD and 2,3,7,8-TCDF are to be determined, prepare the labeled-compound solution to contain these compounds only. This solution is diluted with acetone prior to use (Section 7.10.3).

7.10.3 Dilute a sufficient volume of the labeled compound solution (Section 7.10.1 or 7.10.2) by a factor of 50 with acetone to prepare a diluted spiking solution. Each sample requires 1.0 mL of the diluted solution, but no more solution should be prepared than can be used in one day.

7.11 Cleanup Standard—Prepare <sup>37</sup>C<sup>14</sup>-2,3,7,8-TCDD in nonane at the concentration shown in Table 3. The cleanup standard is added to all extracts prior to cleanup to measure the efficiency of the cleanup process.

7.12 Internal Standard(s).

7.12.1 All CDDs/CDFs—Prepare the internal standard solution to contain <sup>13</sup>C<sup>12</sup>-1,2,3,4-TCDD and <sup>13</sup>C<sup>12</sup>-1,2,3,7,8,9-HxCDD in nonane at the concentration shown in Table 3.

7.12.2 If only 2,3,7,8-TCDD and 2,3,7,8-TCDF are to be determined,

prepare the internal standard solution to contain  $^{13}\text{C}_{12}$ -1,2,3,4-TCDD only.

**7.13 Calibration Standards (CS1 through CS5)**—Combine the solutions in Sections 7.9 through 7.12 to produce the five calibration solutions shown in Table 4 in nonane. These solutions permit the relative response (labeled to native) and response factor to be measured as a function of concentration. The CS3 standard is used for calibration verification (VER). If only 2,3,7,8-TCDD and 2,3,7,8-TCDF are to be determined, combine the solutions appropriate to these compounds.

**7.14 Precision and Recovery (PAR) Standard**—Used for determination of initial (Section 9.2) and ongoing (Section 15.5) precision and recovery. Dilute 10  $\mu\text{L}$  of the precision and recovery standard (Section 7.9.1 or 7.9.2) to 2.0 mL with acetone for each sample matrix for each sample batch. One mL each are required for the blank and OPR with each matrix in each batch.

**7.15 GC Retention Time Window Defining Solution and Isomer Specificity Test Standard**—Used to define the beginning and ending retention times for the dioxin and furan isomers and to demonstrate isomer specificity of the GC columns employed for determination of 2,3,7,8-TCDD and 2,3,7,8-TCDF. The standard must contain the compounds listed in Table 5 (CIL EDF—4006, or equivalent), at a minimum. It is not necessary to monitor the window-defining compounds if only 2,3,7,8-TCDD and 2,3,7,8-TCDF are to be determined. In this case, an isomer-specificity test standard containing the most closely eluted isomers listed in Table 5 (CIL EDF-4033, or equivalent) may be used.

**7.16 QC Check Sample**—A QC Check Sample should be obtained from a source independent of the calibration standards. Ideally, this check sample would be a certified reference material containing the CDDs/CDFs in known concentrations in a sample matrix similar to the matrix under test.

**7.17 Stability of Solutions**—Standard solutions used for quantitative purposes (Sections 7.9 through 7.15) should be analyzed periodically, and should be assayed against reference standards (Section 7.8.3) before further use.

## **8.0 Sample Collection, Preservation, Storage, and Holding Times**

**8.1** Collect samples in amber glass containers following conventional sampling practices (Reference 16). Aqueous samples that flow freely are collected in refrigerated bottles using automatic sampling equipment. Solid

samples are collected as grab samples using wide-mouth jars.

**8.2** Maintain aqueous samples in the dark at 0–4°C from the time of collection until receipt at the laboratory. 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 17). If sample pH is greater than 9, adjust to pH 7–9 with sulfuric acid.

Maintain solid, semi-solid, oily, and mixed-phase samples in the dark at <4°C from the time of collection until receipt at the laboratory.

Store aqueous samples in the dark at 0–4°C. Store solid, semi-solid, oily, mixed-phase, and tissue samples in the dark at <–10°C.

### **8.3 Fish and Tissue Samples.**

**8.3.1** Fish may be cleaned, filleted, or processed in other ways in the field, such that the laboratory may expect to receive whole fish, fish fillets, or other tissues for analysis.

**8.3.2** Fish collected in the field should be wrapped in aluminum foil, and must be maintained at a temperature less than 4°C from the time of collection until receipt at the laboratory.

**8.3.3** Samples must be frozen upon receipt at the laboratory and maintained in the dark at <–10°C until prepared. Maintain unused sample in the dark at <–10°C.

### **8.4 Holding Times.**

**8.4.1** There are no demonstrated maximum holding times associated with CDDs/CDFs in aqueous, solid, semi-solid, tissues, or other sample matrices. If stored in the dark at 0–4°C and preserved as given above (if required), aqueous samples may be stored for up to one year. Similarly, if stored in the dark at <–10°C, solid, semi-solid, multi-phase, and tissue samples may be stored for up to one year.

**8.4.2** Store sample extracts in the dark at <–10°C until analyzed. If stored in the dark at <–10°C, sample extracts may be stored for up to one year.

## **9.0 Quality Assurance/Quality Control**

**9.1** Each laboratory that uses this method is required to operate a formal quality assurance program (Reference 18). 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 characteristics of the method.

If the method is to be applied to sample matrix other than water (e.g., soils, filter cake, compost, tissue) the most appropriate alternate matrix (Sections 7.6.2 through 7.6.5) is substituted for the reagent water matrix (Section 7.6.1) in all performance tests.

**9.1.1** 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 9.2.

**9.1.2** In recognition of advances that are occurring in analytical technology, and to allow the analyst to overcome sample matrix interferences, the analyst is permitted certain options to improve separations or lower the costs of measurements. These options include alternate extraction, concentration, cleanup procedures, and changes in columns and detectors. Alternate determinative techniques, such as the substitution of spectroscopic or immuno-assay techniques, and changes that degrade method performance, are not allowed. If an analytical technique other than the techniques specified in this method is used, that technique must have a specificity equal to or better than the specificity of the techniques in this method for the analytes of interest.

**9.1.2.1** Each time a modification is made to this method, the analyst is required to repeat the procedure in Section 9.2. If the detection limit of the method will be affected by the change, the laboratory is required to demonstrate that the MDL (40 CFR Part 136, Appendix B) is lower than one-third the regulatory compliance level or one-third the ML in this method, whichever is higher. If calibration will be affected by the change, the analyst must recalibrate the instrument per Section 10.

**9.1.2.2** The laboratory is required to maintain records of modifications made to this method. These records include the following, at a minimum:

**9.1.2.2.1** The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and of the quality control officer who witnessed and will verify the analyses and modifications.

**9.1.2.2.2** A listing of pollutant(s) measured, by name and CAS Registry number.

**9.1.2.2.3** A narrative stating reason(s) for the modifications.

**9.1.2.2.4** Results from all quality control (QC) tests comparing the modified method to this method, including:

(a) Calibration (Section 10.5 through 10.7).

(b) Calibration verification (Section 15.3).

(c) Initial precision and recovery (Section 9.2).

(d) Labeled compound recovery (Section 9.3).

(e) Analysis of blanks (Section 9.5).

(f) Accuracy assessment (Section 9.4).

9.1.2.2.5 Data that will allow an independent reviewer to validate each determination by tracing the instrument output (peak height, area, or other signal) to the final result. These data are to include:

(a) Sample numbers and other identifiers.

(b) Extraction dates.

(c) Analysis dates and times.

(d) Analysis sequence/run chronology.

(e) Sample weight or volume (Section 11).

(f) Extract volume prior to each cleanup step (Section 13).

(g) Extract volume after each cleanup step (Section 13).

(h) Final extract volume prior to injection (Section 14).

(i) Injection volume (Section 14.3).

(j) Dilution data, differentiating between dilution of a sample or extract (Section 17.5).

(k) Instrument and operating conditions.

(l) Column (dimensions, liquid phase, solid support, film thickness, etc.).

(m) Operating conditions (temperatures, temperature program, flow rates).

(n) Detector (type, operating conditions, etc.).

(o) Chromatograms, printer tapes, and other recordings of raw data.

(p) Quantitation reports, data system outputs, and other data to link the raw data to the results reported.

9.1.3 Analyses of method blanks are required to demonstrate freedom from contamination (Section 4.3). The procedures and criteria for analysis of a method blank are described in Sections 9.5 and 15.6.

9.1.4 The laboratory shall spike all samples with labeled compounds to monitor method performance. This test is described in Section 9.3. When results of these spikes indicate atypical method performance for samples, the samples are diluted to bring method performance within acceptable limits. Procedures for dilution are given in Section 17.5.

9.1.5 The laboratory shall, on an ongoing basis, demonstrate through calibration verification and the analysis of the ongoing precision and recovery aliquot that the analytical system is in

control. These procedures are described in Sections 15.1 through 15.5.

9.1.6 The laboratory shall maintain records to define the quality of data that is generated. Development of accuracy statements is described in Section 9.4.

9.2 Initial Precision and Recovery (IPR)—To establish the ability to generate acceptable precision and recovery, the analyst shall perform the following operations.

9.2.1 For low solids (aqueous) samples, extract, concentrate, and analyze four 1 L aliquots of reagent water spiked with the diluted labeled compound spiking solution (Section 7.10.3) and the precision and recovery standard (Section 7.14) according to the procedures in Sections 11 through 18. For an alternative sample matrix, four aliquots of the alternative reference matrix (Section 7.6) are used. All sample processing steps that are to be used for processing samples, including preparation (Section 11), extraction (Section 12), and cleanup (Section 13), shall be included in this test.

9.2.2 Using results of the set of four analyses, compute the average concentration (X) of the extracts in ng/mL and the standard deviation of the concentration (s) in ng/mL for each compound, by isotope dilution for CDDs/CDFs with a labeled analog, and by internal standard for 1,2,3,7,8,9-HxCDD, OCDF, and the labeled compounds.

9.2.3 For each CDD/CDF and labeled compound, compare s and X with the corresponding limits for initial precision and recovery in Table 6. If only 2,3,7,8-TCDD and 2,3,7,8-TCDF are to be determined, compare s and X with the corresponding limits for initial precision and recovery in Table 6a. 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 9.2).

9.3 The laboratory shall spike all samples with the diluted labeled compound spiking solution (Section 7.10.3) to assess method performance on the sample matrix.

9.3.1 Analyze each sample according to the procedures in Sections 11 through 18.

9.3.2 Compute the percent recovery of the labeled compounds and the cleanup standard using the internal standard method (Section 17.2).

9.3.3 The recovery of each labeled compound must be within the limits in

Table 7 when all 2,3,7,8-substituted CDDs/CDFs are determined, and within the limits in Table 7a when only 2,3,7,8-TCDD and 2,3,7,8-TCDF are determined. If the recovery of any compound falls outside of these limits, 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 18.4.

9.4 Recovery of labeled compounds from samples should be assessed and records should be maintained.

9.4.1 After the analysis of five samples of a given matrix type (water, soil, sludge, pulp, etc.) for which the labeled compounds pass the tests in Section 9.3, compute the average percent recovery (R) and the standard deviation of the percent recovery (SR) for the labeled compounds only. Express the assessment as a percent recovery interval from  $R - 2S_R$  to  $R + 2S_R$  for each matrix. For example, if  $R = 90\%$  and  $S_R = 10\%$  for five analyses of pulp, the recovery interval is expressed as 70–110%.

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

9.5 Method Blanks—Reference matrix method blanks are analyzed to demonstrate freedom from contamination (Section 4.3).

9.5.1 Prepare, extract, clean up, and concentrate a method blank with each sample batch (samples of the same matrix started through the extraction process on the same 12-hour shift, to a maximum of 20 samples). The matrix for the method blank shall be similar to sample matrix for the batch, e.g., a 1 L reagent water blank (Section 7.6.1), high-solids reference matrix blank (Section 7.6.2), paper matrix blank (Section 7.6.3); tissue blank (Section 7.6.4) or alternative reference matrix blank (Section 7.6.5). Analyze the blank immediately after analysis of the OPR (Section 15.5) to demonstrate freedom from contamination.

9.5.2 If any 2,3,7,8-substituted CDD/CDF (Table 1) is found in the blank at greater than the minimum level (Table 2) or one-third the regulatory compliance level, whichever is greater; or if any potentially interfering compound is found in the blank at the minimum level for each level of chlorination given in Table 2 (assuming a response factor of 1 relative to the  $^{13}\text{C}_{12}$ -1,2,3,4-TCDD internal standard for compounds not listed in Table 1), analysis of samples is halted until the blank associated with the sample batch shows no evidence of contamination at

this level. All samples must be associated with an uncontaminated method blank before the results for those samples may be reported for regulatory compliance purposes.

9.6 QC Check Sample—Analyze the QC Check Sample (Section 7.16) periodically to assure the accuracy of calibration standards and the overall reliability of the analytical process. It is suggested that the QC Check Sample be analyzed at least quarterly.

9.7 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 10), calibration verification (Section 15.3), and for initial (Section 9.2) and ongoing (Section 15.5) precision and recovery should be identical, so that the most precise results will be obtained. A GC/MS instrument will provide the most reproducible results if dedicated to the settings and conditions required for the analyses of CDDs/CDFs by this method.

9.8 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.

## 10.0 Calibration

10.1 Establish the operating conditions necessary to meet the minimum retention times for the internal standards in Section 10.2.4 and the relative retention times for the CDDs/CDFs in Table 2.

10.1.1 Suggested GC operating conditions:

Injector temperature: 270°C  
Interface temperature: 290°C  
Initial temperature: 200°C  
Initial time: Two minutes  
Temperature program:  
200–220°C, at 5°C/minute  
220°C for 16 minutes  
220–235°C, at 5°C/minute  
235°C for seven minutes  
235–330°C, at 5°C/minute

**Note:** All portions of the column that connect the GC to the ion source shall remain at or above the interface temperature specified above during analysis to preclude condensation of less volatile compounds.

Optimize GC conditions for compound separation and sensitivity. Once optimized, the same GC conditions must be used for the analysis of all standards, blanks, IPR and OPR aliquots, and samples.

10.1.2 Mass spectrometer (MS) resolution—Obtain a selected ion current profile (SICP) of each analyte in

Table 3 at the two exact  $m/z$ 's specified in Table 8 and at  $\geq 10,000$  resolving power by injecting an authentic standard of the CDDs/CDFs either singly or as part of a mixture in which there is no interference between closely eluted components.

10.1.2.1 The analysis time for CDDs/CDFs 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 and a lock-mass  $m/z$  from PFK is used for drift correction. The lock-mass  $m/z$  is dependent on the exact  $m/z$ 's monitored within each descriptor, as shown in Table 8. The level of PFK metered into the HRMS during analyses should be adjusted so that the amplitude of the most intense selected lock-mass  $m/z$  signal (regardless of the descriptor number) does not exceed 10% 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 necessitating increased frequency of source cleaning.

10.1.2.2 If the HRMS has the capability to monitor resolution during the analysis, it is acceptable to terminate the analysis when the resolution falls below 10,000 to save reanalysis time.

10.1.2.3 Using a PFK molecular leak, tune the instrument to meet the minimum required resolving power of 10,000 (10% valley) at  $m/z$  304.9824 (PFK) or any other reference signal close to  $m/z$  304 (from TCDF). For each descriptor (Table 8), monitor and record the resolution and exact  $m/z$ 's of three to five reference peaks covering the mass range of the descriptor. The resolution must be greater than or equal to 10,000, and the deviation between the exact  $m/z$  and the theoretical  $m/z$  (Table 8) for each exact  $m/z$  monitored must be less than 5 ppm.

10.2 Ion Abundance Ratios, Minimum Levels, Signal-to-Noise Ratios, and Absolute Retention Times—Choose an injection volume of either 1  $\mu\text{L}$  or 2  $\mu\text{L}$ , consistent with the capability of the HRGC/HRMS instrument. Inject a 1  $\mu\text{L}$  or 2  $\mu\text{L}$  aliquot of the CS1 calibration solution (Table 4) using the GC conditions from Section 10.1.1. If only 2,3,7,8-TCDD and 2,3,7,8-TCDF are to be determined, the operating conditions and specifications

below apply to analysis of those compounds only.

10.2.1 Measure the SICP areas for each analyte, and compute the ion abundance ratios at the exact  $m/z$ 's specified in Table 8. Compare the computed ratio to the theoretical ratio given in Table 9.

10.2.1.1 The exact  $m/z$ 's to be monitored in each descriptor are shown in Table 8. Each group or descriptor shall be monitored in succession as a function of GC retention time to ensure that all CDDs/CDFs are detected. Additional  $m/z$ 's may be monitored in each descriptor, and the  $m/z$ 's may be divided among more than the five descriptors listed in Table 8, provided that the laboratory is able to monitor the  $m/z$ 's of all the CDDs/CDFs that may elute from the GC in a given retention-time window. If only 2,3,7,8-TCDD and 2,3,7,8-TCDF are to be determined, the descriptors may be modified to include only the exact  $m/z$ 's for the tetra- and penta-isomers, the diphenyl ethers, and the lock  $m/z$ 's.

10.2.1.2 The mass spectrometer shall be operated in a mass-drift correction mode, using perfluorokerosene (PFK) to provide lock  $m/z$ 's. The lock-mass for each group of  $m/z$ 's is shown in Table 8. Each lock mass shall be monitored and shall not vary by more than  $\pm 20\%$  throughout its respective retention time window. Variations of the lock mass by more than 20% indicate the presence of coeluting interferences that may significantly reduce the sensitivity of the mass spectrometer. Reinjection of another aliquot of the sample extract will not resolve the problem. Additional cleanup of the extract may be required to remove the interferences.

10.2.2 All CDDs/CDFs and labeled compounds in the CS1 standard shall be within the QC limits in Table 9 for their respective ion abundance 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 10.1.2) prior to repeat of the test.

10.2.3 Verify that the HRGC/HRMS instrument meets the minimum levels in Table 2. The peaks representing the CDDs/CDFs and labeled compounds in the CS1 calibration standard must have signal-to-noise ratios (S/N) greater than or equal to 10.0. Otherwise, the mass spectrometer shall be adjusted and this test repeated until the minimum levels in Table 2 are met.

10.2.4 The absolute retention time of  $^{13}\text{C}_{12}$ -1,2,3,4-TCDD (Section 7.12) shall exceed 25.0 minutes on the DB-5 column, and the retention time of  $^{13}\text{C}_{12}$ -

1,2,3,4-TCDD shall exceed 15.0 minutes on the DB-225 column; otherwise, the GC temperature program shall be adjusted and this test repeated until the above-stated minimum retention time criteria are met.

10.3 Retention-Time Windows—Analyze the window defining mixtures (Section 7.15) using the optimized temperature program in Section 10.1. Table 5 gives the elution order (first/last) of the window-defining compounds. If 2,3,7,8-TCDD and 2,3,7,8-TCDF only are to be analyzed, this test is not required.

#### 10.4 Isomer Specificity.

10.4.1 Analyze the isomer specificity test standards (Section 7.15) using the procedure in Section 14 and the optimized conditions for sample analysis (Section 10.1.1).

10.4.2 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 Figures 6 and 7.

10.4.3 Verify that the height of the valley between the most closely eluted isomers and the 2,3,7,8-substituted isomers is less than 25% (computed as  $100 \times y/x$  in Figures 6 and 7). If the valley exceeds 25%, adjust the analytical conditions and repeat the test or replace the GC column and recalibrate (Sections 10.1.2 through 10.7).

10.5 Calibration by Isotope Dilution—Isotope dilution calibration is used for the 15 2,3,7,8-substituted CDDs/CDFs for which labeled compounds are added to samples prior to extraction. The reference compound for each CDD/CDF compound is shown in Table 2.

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

10.5.2 The response of each CDD/CDF relative to its labeled analog is determined using the area responses of both the primary and secondary exact m/z's specified in Table 8, for each calibration standard, as follows:

$$RR = \frac{(A_{1n} + A_{2n}) C_1}{(A_{1l} + A_{2l}) C_n}$$

Where:

$A_{1n}$  and  $A_{2n}$  = The areas of the primary and secondary m/z's for the CDD/CDF.

$A_{1l}$  and  $A_{2l}$  = The areas of the primary and secondary m/z's for the labeled compound.

$C_1$  = The concentration of the labeled compound in the calibration standard (Table 4).

$C_n$  = The concentration of the native compound in the calibration standard (Table 4).

10.5.3 To calibrate the analytical system by isotope dilution, inject a volume of calibration standards CS1 through CS5 (Section 7.13 and Table 4) identical to the volume chosen in Section 10.2, using the procedure in Section 14 and the conditions in Section 10.1.1 and Table 2. Compute the relative response (RR) at each concentration.

10.5.4 Linearity—If the relative response for any compound is constant (less than 20% coefficient of variation) over the five-point calibration range, an averaged relative response may be used for that compound; otherwise, the complete calibration curve for that compound shall be used over the five-point calibration range.

10.6 Calibration by Internal Standard—The internal standard method is applied to determination of 1,2,3,7,8,9-HxCDD (Section 17.1.2), OCDF (Section 17.1.1), the non 2,3,7,8-substituted compounds, and to the determination of labeled compounds for intralaboratory statistics (Sections 9.4 and 15.5.4).

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

$$RF = \frac{(A_{1s} + A_{2s}) C_{is}}{(A_{1is} + A_{2is}) C_s}$$

Where:

$A_{1s}$  and  $A_{2s}$  = The areas of the primary and secondary m/z's for the CDD/CDF.

$A_{1is}$  and  $A_{2is}$  = The areas of the primary and secondary m/z's for the internal standard.

$C_{is}$  = The concentration of the internal standard (Table 4).

$C_s$  = The concentration of the compound in the calibration standard (Table 4).

**Note:** There is only one m/z for  $^{37}\text{Cl}_4$ -2,3,7,8-TCDD. See Table 8.

10.6.2 To calibrate the analytical system by internal standard, inject 1.0  $\mu\text{L}$  or 2.0  $\mu\text{L}$  of calibration standards CS1 through CS5 (Section 7.13 and Table 4) using the procedure in Section 14 and the conditions in Section 10.1.1 and Table 2. Compute the response factor (RF) at each concentration.

10.6.3 Linearity—If the response factor (RF) for any compound is constant (less than 35% coefficient of variation) over the five-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 five-point range.

10.7 Combined Calibration—By using calibration solutions (Section 7.13 and Table 4) containing the CDDs/CDFs 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 15.3) by analyzing the calibration verification standard (VER, Table 4). Recalibration is required if any of the calibration verification criteria (Section 15.3) cannot be met.

10.8 Data Storage—MS data shall be collected, recorded, and stored.

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

10.8.2 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 multipoint calibration curves. Computations of relative standard deviation (coefficient of variation) shall be used to test calibration linearity. Statistics on initial performance (Section 9.2) and ongoing performance (Section 15.5) should be computed and maintained, either on the instrument data system, or on a separate computer system.

## 11.0 Sample Preparation

11.1 Sample preparation involves modifying the physical form of the sample so that the CDDs/CDFs can be extracted efficiently. In general, the samples must be in a liquid form or in the form of finely divided solids in order for efficient extraction to take place. Table 10 lists the phases and suggested quantities for extraction of various sample matrices.

For samples known or expected to contain high levels of the CDDs/CDFs, the smallest sample size representative of the entire sample should be used (see Section 17.5).

For all samples, the blank and IPR/OPR aliquots must be processed through the same steps as the sample to check for contamination and losses in the preparation processes.

11.1.1 For samples that contain particles, percent solids and particle size are determined using the procedures in Sections 11.2 and 11.3, respectively.

11.1.2 Aqueous samples—Because CDDs/CDFs may be bound to suspended

particles, the preparation of aqueous samples is dependent on the solids content of the sample.

11.1.2.1 Aqueous samples visibly absent particles are prepared per Section 11.4 and extracted directly using the separatory funnel or SPE techniques in Sections 12.1 or 12.2, respectively.

11.1.2.2 Aqueous samples containing visible particles and containing one percent suspended solids or less are prepared using the procedure in Section 11.4. After preparation, the sample is extracted directly using the SPE technique in 12.2 or filtered per Section 11.4.3. After filtration, the particles and filter are extracted using the SDS procedure in

Section 12.3 and the filtrate is extracted using the separatory funnel procedure in Section 12.1.

11.1.2.3 For aqueous samples containing greater than one percent solids, a sample aliquot sufficient to provide 10 g of dry solids is used, as described in Section 11.5.

11.1.3 Solid samples are prepared using the procedure described in Section 11.5 followed by extraction via the SDS procedure in Section 12.3.

11.1.4 Multiphase samples—The phase(s) containing the CDDs/CDFs is separated from the non-CDD/CDF phase using pressure filtration and centrifugation, as described in Section 11.6. The CDDs/CDFs will be in the organic phase in a multiphase sample in which an organic phase exists.

11.1.5 Procedures for grinding, homogenization, and blending of various sample phases are given in Section 11.7.

11.1.6 Tissue samples—Preparation procedures for fish and other tissues are given in Section 11.8.

11.2 Determination of Percent Suspended Solids.

**Note:** This aliquot is used for determining the solids content of the sample, not for determination of CDDs/CDFs.

11.2.1 Aqueous liquids and multiphase samples consisting of mainly an aqueous phase.

11.2.1.1 Dessicate and weigh a GF/D filter (Section 6.5.3) to three significant figures.

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

11.2.1.2 Filter 10.0±0.02 mL of well-mixed sample through the filter.

11.2.1.3 Dry the filter a minimum of 12 hours at 110±5Ψ ανδ ψοολ ιν α δεσσιψατορ.

11.2.1.4 Calculate percent solids as follows:

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

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

11.2.2.1 Weigh 5–10 g of sample to three significant figures in a tared beaker.

11.2.2.2 Dry a minimum of 12 hours at 110±5°C, and cool in a dessicator.

11.2.2.3 Calculate percent solids as follows:

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

11.3 Determination of Particle Size.  
11.3.1 Spread the dried sample from Section 11.2.2.2 on a piece of filter paper or aluminum foil in a fume hood or glove box.

11.3.2 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 using the procedures in Section 11.7.

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

11.4.1 Aqueous samples visibly absent particles are prepared per the procedure below and extracted directly using the separatory funnel or SPE techniques in Sections 12.1 or 12.2, respectively. Aqueous samples containing visible particles and one percent suspended solids or less are prepared using the procedure below and

extracted using either the SPE technique in Section 12.2 or further prepared using the filtration procedure in Section 11.4.3. The filtration procedure is followed by SDS extraction of the filter and particles (Section 12.3) and separatory funnel extraction of the filtrate (Section 12.1). The SPE procedure is followed by SDS extraction of the filter and disk.

11.4.2 Preparation of sample and QC aliquots.

11.4.2.1 Mark the original level of the sample on the sample bottle for reference. Weigh the sample plus bottle to ± 1.

11.4.2.2 Spike 1.0 mL of the diluted labeled-compound spiking solution (Section 7.10.3) into the sample bottle. Cap the bottle and mix the sample by careful shaking. Allow the sample to equilibrate for one to two hours, with occasional shaking.

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

11.4.2.4 Spike 1.0 mL of the diluted labeled-compound spiking solution (Section 7.10.3) into both reagent water aliquots. One of these aliquots will serve as the method blank.

11.4.2.5 Spike 1.0 mL of the PAR standard (Section 7.14) into the remaining reagent water aliquot. This aliquot will serve as the OPR (Section 15.5).

11.4.2.6 If SPE is to be used, add 5 mL of methanol to the sample, cap and shake the sample to mix thoroughly, and proceed to Section 12.2 for extraction. If SPE is not to be used, and the sample is visibly absent particles, proceed to Section 12.1 for extraction. If SPE is not to be used and the sample



contains visible particles, proceed to the following section for filtration of particles.

#### 11.4.3 Filtration of particles.

11.4.3.1 Assemble a Buchner funnel (Section 6.5.5) on top of a clean filtration flask. Apply vacuum to the flask, and pour the entire contents of the sample bottle through a glass-fiber filter (Section 6.5.6) in the Buchner funnel, swirling the sample remaining in the bottle to suspend any particles.

11.4.3.2 Rinse the sample bottle twice with approximately 5 mL portions of reagent water to transfer any remaining particles onto the filter.

11.4.3.3 Rinse any particles off the sides of the Buchner funnel with small quantities of reagent water.

11.4.3.4 Weigh the empty sample bottle to  $\pm 1$  g. Determine the weight of the sample by difference. Save the bottle for further use.

11.4.3.5 Extract the filtrate using the separatory funnel procedure in Section 12.1.

11.4.3.6 Extract the filter containing the particles using the SDS procedure in Section 12.3.

#### 11.5 Preparation of Samples Containing Greater Than 1% Solids.

11.5.1 Weigh a well-mixed aliquot of each sample (of the same matrix type) sufficient to provide 10 g of dry solids (based on the solids determination in Section 11.2) into a clean beaker or glass jar.

11.5.2 Spike 1.0 mL of the diluted labeled compound spiking solution (Section 7.10.3) into the sample.

11.5.3 For each sample or sample batch (to a maximum of 20 samples) to be extracted during the same 12-hour shift, weigh two 10 g aliquots of the appropriate reference matrix (Section 7.6) into clean beakers or glass jars.

11.5.4 Spike 1.0 mL of the diluted labeled compound spiking solution (Section 7.10.3) into each reference matrix aliquot. One aliquot will serve as the method blank. Spike 1.0 mL of the PAR standard (Section 7.14) into the other reference matrix aliquot. This aliquot will serve as the OPR (Section 15.5).

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

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

11.5.7 If particles  $>1$ mm are present in the sample (as determined in Section 11.3.2), spread the sample on clean aluminum foil in a hood. After the sample is dry, grind to reduce the particle size (Section 11.7).

11.5.8 Extract the sample and QC aliquots using the SDS procedure in Section 12.3.

#### 11.6 Multiphase Samples.

11.6.1 Using the percent solids determined in Section 11.2.1 or 11.2.2, determine the volume of sample that will provide 10 g of solids, up to 1 L of sample.

11.6.2 Pressure filter the amount of sample determined in Section 11.6.1 through Whatman GF/D glass-fiber filter paper (Section 6.5.3). Pressure filter the blank and OPR aliquots through GF/D papers also. If necessary to separate the phases and/or settle the solids, centrifuge these aliquots prior to filtration.

11.6.3 Discard any aqueous phase (if present). Remove any non-aqueous liquid present and reserve the maximum amount filtered from the sample (Section 11.6.1) or 10 g, whichever is less, for combination with the solid phase (Section 12.3.5).

11.6.4 If particles  $>1$ mm are present in the sample (as determined in Section 11.3.2) and the sample is capable of being dried, spread the sample and QC aliquots on clean aluminum foil in a hood. After the aliquots are dry or if the sample cannot be dried, reduce the particle size using the procedures in Section 11.7 and extract the reduced particles using the SDS procedure in Section 12.3. If particles  $>1$ mm are not present, extract the particles and filter in the sample and QC aliquots directly using the SDS procedure in Section 12.3.

11.7 Sample grinding, homogenization, or blending—Samples with particle sizes greater than 1 mm (as determined in Section 11.3.2) 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 in a blender.

11.7.1 Each size-reducing preparation procedure on each matrix shall be verified by running the tests in Section 9.2 before the procedure is employed routinely.

11.7.2 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.

11.7.3 Grinding—Certain papers and pulps, slurries, and amorphous solids can be ground in a Wiley mill or heavy duty meat grinder. In some cases, reducing the temperature of the sample to freezing or to dry ice or liquid nitrogen temperatures can aid in the grinding process. Grind the sample aliquots from Section 11.5.7 or 11.6.4 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.

11.7.4 Homogenization or blending—Particles that are not ground effectively, or particles greater than 1 mm in size after grinding, can often be reduced in size by high speed homogenization or blending. Homogenize and/or blend the particles or filter from Section 11.5.7 or 11.6.4 for the sample, blank, and OPR aliquots.

11.7.5 Extract the aliquots using the SDS procedure in Section 12.3.

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

#### 11.8.1 Homogenization.

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

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

11.8.1.3 Homogenize the sample in a tissue homogenizer (Section 6.3.3) or grind in a meat grinder (Section 6.3.4). Cut tissue too large to feed into the grinder into smaller pieces. To assure homogeneity, grind three times.

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

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

#### 11.8.2 QC aliquots.

11.8.2.1 Prepare a method blank by adding approximately 10 g of the oily liquid reference matrix (Section 7.6.4) to a 400–500 mL beaker. For the alternate HCl digestion/extraction, add the



reference matrix to a 500–600 mL wide-mouth bottle. Record the weight to the nearest 10 mg.

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

#### 11.8.3 Spiking

11.8.3.1 Spike 1.0 mL of the labeled compound spiking solution (Section 7.10.3) into the sample, blank, and OPR aliquot.

11.8.3.2 Spike 1.0 mL of the PAR standard (Section 7.14) into the OPR aliquot.

11.8.4 Extract the aliquots using the procedures in Section 12.4.

### 12.0 Extraction and Concentration

Extraction procedures include separatory funnel (Section 12.1) and solid phase (Section 12.2) for aqueous liquids; Soxhlet/Dean-Stark (Section 12.3) for solids, filters, and SPE disks; and Soxhlet extraction (Section 12.4.1) and HCl digestion (Section 12.4.2) for tissues. Acid/base back-extraction (Section 12.5) is used for initial cleanup of extracts.

Macro-concentration procedures include rotary evaporation (Section 12.6.1), heating mantle (Section 12.6.2), and Kuderna-Danish (K–D) evaporation (Section 12.6.3). Micro-concentration uses nitrogen blowdown (Section 12.7).

12.1 Separatory funnel extraction of filtrates and of aqueous samples visibly absent particles.

12.1.1 Pour the spiked sample (Section 11.4.2.2) or filtrate (Section 11.4.3.5) into a 2 L separatory funnel. Rinse the bottle or flask twice with 5 mL of reagent water and add these rinses to the separatory funnel.

12.1.2 Add 60 mL methylene chloride to the empty sample bottle (Section 12.1.1), seal, and shake 60 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel, and extract the sample by shaking the funnel for two minutes with periodic venting. Allow the organic layer to separate from the aqueous phase for a minimum of 10 minutes. If an emulsion forms and is more than one-third the volume of the solvent layer, employ mechanical techniques to complete the phase separation (see note below). Drain the methylene chloride extract through a solvent-rinsed glass funnel approximately one-half full of

granular anhydrous sodium sulfate (Section 7.2.1) supported on clean glass-fiber paper into a solvent-rinsed concentration device (Section 12.6).

**Note:** If an emulsion forms, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration through glass wool, use of phase separation paper, centrifugation, use of an ultrasonic bath with ice, addition of NaCl, or other physical methods. Alternatively, solid-phase or other extraction techniques may be used to prevent emulsion formation. Any alternative technique is acceptable so long as the requirements in Section 9 are met.

Experience with aqueous samples high in dissolved organic materials (e.g., paper mill effluents) has shown that acidification of the sample prior to extraction may reduce the formation of emulsions. Paper industry methods suggest that the addition of up to 400 mL of ethanol to a 1 L effluent sample may also reduce emulsion formation. However, studies by EPA suggest that the effect may be a result of sample dilution, and that the addition of reagent water may serve the same function. Mechanical techniques may still be necessary to complete the phase separation. If either acidification or addition of ethanol is utilized, the laboratory must perform the startup tests described in Section 9.2 using the same techniques.

12.1.3 Extract the water sample two more times with 60 mL portions of methylene chloride. Drain each portion through the sodium sulfate into the concentrator. After the third extraction, rinse the separatory funnel with at least 20 mL of methylene chloride, and drain this rinse through the sodium sulfate into the concentrator. Repeat this rinse at least twice. Set aside the funnel with sodium sulfate if the extract is to be combined with the extract from the particles.

12.1.4 Concentrate the extract using one of the macro-concentration procedures in Section 12.6.

12.1.4.1 If the extract is from a sample visibly absent particles (Section 11.1.2.1), adjust the final volume of the concentrated extract to approximately 10 mL with hexane, transfer to a 250 mL separatory funnel, and back-extract using the procedure in Section 12.5.

12.1.4.2 If the extract is from the aqueous filtrate (Section 11.4.3.5), set aside the concentration apparatus for addition of the SDS extract from the particles (Section 12.3.9.1.2).

12.2 SPE of Samples Containing Less Than 1% Solids (References 19–20).

12.2.1 Disk preparation.

12.2.1.1 Place an SPE disk on the base of the filter holder (Figure 4) and wet with toluene. While holding a GMF 150 filter above the SPE disk with tweezers, wet the filter with toluene and lay the filter on the SPE disk, making sure that air is not trapped between the filter and disk. Clamp the filter and SPE disk between the 1 L glass reservoir and the vacuum filtration flask.

12.2.1.2 Rinse the sides of the filtration flask with approx 15 mL of toluene using a squeeze bottle or syringe. Apply vacuum momentarily until a few drops appear at the drip tip. Release the vacuum and allow the filter/disk to soak for approx one minute. Apply vacuum and draw all of the toluene through the filter/disk. Repeat the wash step with approx 15 mL of acetone and allow the filter/disk to air dry.

12.2.1.3 Re-wet the filter/disk with approximately 15 mL of methanol, allowing the filter/disk to soak for approximately one minute. Pull the methanol through the filter/disk using the vacuum, but retain a layer of methanol approximately 1 mm thick on the filter. Do not allow the disk to go dry from this point until the end of the extraction.

12.2.1.4 Rinse the filter/disk with two 50-mL portions of reagent water by adding the water to the reservoir and pulling most through, leaving a layer of water on the surface of the filter.

12.2.2 Extraction.

12.2.2.1 Pour the spiked sample (Section 11.4.2.2), blank (Section 11.4.2.4), or IPR/OPR aliquot (Section 11.4.2.5) into the reservoir and turn on the vacuum to begin the extraction. Adjust the vacuum to complete the extraction in no less than 10 minutes. For samples containing a high concentration of particles (suspended solids), filtration times may be eight hours or longer.

12.2.2.2 Before all of the sample has been pulled through the filter/disk, rinse the sample bottle with approximately 50 mL of reagent water to remove any solids, and pour into the reservoir. Pull through the filter/disk. Use additional reagent water rinses until all visible solids are removed.

12.2.2.3 Before all of the sample and rinses have been pulled through the filter/disk, rinse the sides of the reservoir with small portions of reagent water.

12.2.2.4 Allow the filter/disk to dry, then remove the filter and disk and place in a glass Petri dish. Extract the filter and disk per Section 12.3.

12.3 SDS Extraction of Samples Containing Particles, and of Filters and/or Disks.

12.3.1 Charge a clean extraction thimble (Section 6.4.2.2) with 5.0 g of 100/200 mesh silica (Section 7.5.1.1) topped with 100 g of quartz sand (Section 7.3.2).

**Note:** Do not disturb the silica layer throughout the extraction process.

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

12.3.3 Pre-extract the glassware by heating the flask until the toluene is boiling. When properly adjusted, one to two drops of toluene will fall per second from the condenser tip into the receiver. Extract the apparatus for a minimum of three hours.

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

12.3.5 Load the wet sample, filter, and/or disk from Section 11.4.3.6, 11.5.8, 11.6.4, 11.7.3, 11.7.4, or 12.2.2.4 and any nonaqueous liquid from Section 11.6.3 into the thimble and manually mix into the sand layer with a clean metal spatula, carefully breaking up any large lumps of sample.

12.3.6 Reassemble the pre-extracted SDS apparatus, and add a fresh charge of toluene to the receiver and reflux flask. 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. Frequently check the apparatus for foaming during the first two hours of extraction. If foaming occurs, reduce the reflux rate until foaming subsides.

12.3.7 Drain the water from the receiver at one to two hours and eight to nine 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.

12.3.8 Remove the distilling flask. Drain the water from the Dean-Stark receiver and add any toluene in the receiver to the extract in the flask.

12.3.9 Concentrate the extract using one of the macro-concentration procedures in Section 12.6 per the following:

12.3.9.1 Extracts from the particles in an aqueous sample containing less than 1% solids (Section 11.4.3.6).

12.3.9.1.1 Concentrate the extract to approximately 5 mL using the rotary evaporator or heating mantle procedures in Section 12.6.1 or 12.6.2.

12.3.9.1.2 Quantitatively transfer the extract through the sodium sulfate (Section 12.1.3) into the apparatus that

was set aside (Section 12.1.4.2) and reconcentrate to the level of the toluene.

12.3.9.1.3 Adjust to approximately 10 mL with hexane, transfer to a 250 mL separatory funnel, and proceed with back-extraction (Section 12.5).

12.3.9.2 Extracts from particles (Sections 11.5 through 11.6) or from the SPE filter and disk (Section 12.2.2.4)—Concentrate to approximately 10 mL using the rotary evaporator or heating mantle (Section 12.6.1 or 12.6.2), transfer to a 250 mL separatory funnel, and proceed with back-extraction (Section 12.5).

12.4 Extraction of Tissue—Two procedures are provided for tissue extraction.

12.4.1 Soxhlet extraction (Reference 21).

12.4.1.1 Add 30–40 g of powdered anhydrous sodium sulfate to each of the beakers (Section 11.8.4) and mix thoroughly. Cover the beakers with aluminum foil and allow to equilibrate for 12–24 hours. Remix prior to extraction to prevent clumping.

12.4.1.2 Assemble and pre-extract the Soxhlet apparatus per Sections 12.3.1 through 12.3.4, except use the methylene chloride:hexane (1:1) mixture for the pre-extraction and rinsing and omit the quartz sand. The Dean-Stark moisture trap may also be omitted, if desired.

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

12.4.1.4 Transfer the sample/sodium sulfate mixture (Section 12.4.1.1) to the Soxhlet thimble, and install the thimble in the Soxhlet apparatus.

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

12.4.1.6 After extraction, cool and disassemble the apparatus.

12.4.1.7 Quantitatively transfer the extract to a macro-concentration device (Section 12.6), and concentrate to near dryness. Set aside the concentration apparatus for re-use.

12.4.1.8 Complete the removal of the solvent using the nitrogen blowdown procedure (Section 12.7) and a water bath temperature of 60°C. Weigh the receiver, record the weight, and return the receiver to the blowdown apparatus, concentrating the residue until a constant weight is obtained.

12.4.1.9 Percent lipid determination—The lipid content is determined by extraction of tissue with the same solvent system (methylene chloride:hexane) that was used in EPA's National Dioxin Study (Reference 22) so

that lipid contents are consistent with that study.

12.4.1.9.1 Redissolve the residue in the receiver in hexane and spike 1.0 mL of the cleanup standard (Section 7.11) into the solution.

12.4.1.9.2 Transfer the residue/hexane to the anthropogenic isolation column (Section 13.7.1) or bottle for the acidified silica gel batch cleanup (Section 13.7.2), retaining the boiling chips in the concentration apparatus. Use several rinses to assure that all material is transferred. If necessary, sonicate or heat the receiver slightly to assure that all material is re-dissolved. Allow the receiver to dry. Weigh the receiver and boiling chips.

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

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

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

12.4.2 HCl digestion/extraction and concentration (References 23–26).

12.4.2.1 Add 200 mL of 6 N HCl and 200 mL of methylene chloride:hexane (1:1) to the sample and QC aliquots (Section 11.8.4).

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

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

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

12.4.2.5 Decant the solvent through a glass funnel with glass-fiber filter (Sections 6.5.2 through 6.5.3) containing approximately 10 g of granular anhydrous sodium sulfate (Section 7.2.1) into a macro-concentration apparatus (Section 12.6). Rinse the contents of the bottle with two 25 mL portions of hexane and pour through the sodium sulfate into the apparatus.

12.4.2.6 Concentrate the solvent to near dryness using a macro-concentration procedure (Section 12.6).

12.4.2.7 Complete the removal of the solvent using the nitrogen blowdown apparatus (Section 12.7) and a water bath temperature of 60°C. Weigh the receiver, record the weight, and return the receiver to the blowdown apparatus, concentrating the residue until a constant weight is obtained.

12.4.2.8 Percent lipid determination—The lipid content is determined in the same solvent system [methylene chloride:hexane (1:1)] that was used in EPA's National Dioxin Study (Reference 22) so that lipid contents are consistent with that study.

12.4.2.8.1 Redissolve the residue in the receiver in hexane and spike 1.0 mL of the cleanup standard (Section 7.11) into the solution.

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

12.4.2.8.3 Calculate the percent lipid per Section 12.4.1.9.3. It is not necessary to determine the lipid content of the blank, IPR, or OPR aliquots.

12.4.2.9 Clean up the extract per Section 13.7.3.

12.5 Back-Extraction with Base and Acid.

12.5.1 Spike 1.0 mL of the cleanup standard (Section 7.11) into the separatory funnels containing the sample and QC extracts from Section 12.1.4.1, 12.3.9.1.3, or 12.3.9.2.

12.5.2 Partition the extract against 50 mL of potassium hydroxide solution (Section 7.1.1). Shake for two 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 CDDs/CDFs. Stronger potassium hydroxide solutions may be employed for back-extraction, provided that the laboratory meets the specifications for labeled compound recovery and demonstrates acceptable performance using the procedure in Section 9.2.

12.5.3 Partition the extract against 50 mL of sodium chloride solution (Section 7.1.4) in the same way as with base. Discard the aqueous layer.

12.5.4 Partition the extract against 50 mL of sulfuric acid (Section 7.1.2) 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.

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

12.5.6 Pour each extract through a drying column containing 7–10 cm of granular anhydrous sodium sulfate (Section 7.2.1). Rinse the separatory funnel with 30–50 mL of solvent, and pour through the drying column. Collect

each extract in a round-bottom flask. Re-concentrate the sample and QC aliquots per Sections 12.6 through 12.7, and clean up the samples and QC aliquots per Section 13.

12.6 Macro-Concentration—Extracts in toluene are concentrated using a rotary evaporator or a heating mantle; extracts in methylene chloride or hexane are concentrated using a rotary evaporator, heating mantle, or Kuderna-Danish apparatus.

12.6.1 Rotary evaporation—Concentrate the extracts in separate round-bottom flasks.

12.6.1.1 Assemble the rotary evaporator according to manufacturer's instructions, and warm the water bath to 45°C. On a daily basis, pre-clean the rotary evaporator by concentrating 100 mL of clean extraction solvent through the system. Archive both the concentrated solvent and the solvent in the catch flask for a contamination check if necessary. Between samples, three 2–3 mL aliquots of solvent should be rinsed down the feed tube into a waste beaker.

12.6.1.2 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.

12.6.1.3 Lower the flask into the water bath, and adjust the speed of rotation and the temperature as required to complete 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.

12.6.1.4 When the liquid in the concentration flask has reached an apparent volume of approximately 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 solvent.

12.6.1.5 Proceed to Section 12.6.4 for preparation for back-extraction or micro-concentration and solvent exchange.

12.6.2 Heating mantle—Concentrate the extracts in separate round-bottom flasks.

12.6.2.1 Add one or two clean boiling chips to the round-bottom flask, and attach a three-ball macro Snyder column. Prewet the column by adding approximately 1 mL of solvent through the top. Place the round-bottom flask in a heating mantle, and apply heat as

required to complete the concentration in 15–20 minutes. At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood.

12.6.2.2 When the liquid has reached an apparent volume of approximately 10 mL, remove the round-bottom flask from the heating mantle and allow the solvent to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the glass joint into the receiver with small portions of solvent.

12.6.2.3 Proceed to Section 12.6.4 for preparation for back-extraction or micro-concentration and solvent exchange.

12.6.3 Kuderna-Danish (K-D)—Concentrate the extracts in separate 500 mL K-D flasks equipped with 10 mL concentrator tubes. The K-D technique is used for solvents such as methylene chloride and hexane. Toluene is difficult to concentrate using the K-D technique unless a water bath fed by a steam generator is used.

12.6.3.1 Add one to two clean boiling chips to the receiver. Attach a three-ball macro Snyder column. Prewet the column by adding approximately 1 mL of solvent through the top. Place the K-D apparatus in a hot water bath so that the entire lower rounded surface of the flask is bathed with steam.

12.6.3.2 Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15–20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood.

12.6.3.3 When the liquid has reached an apparent volume of 1 mL, remove the K-D apparatus from the bath and allow the solvent to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1–2 mL of solvent. A 5 mL syringe is recommended for this operation.

12.6.3.4 Remove the three-ball Snyder column, add a fresh boiling chip, and attach a two-ball micro Snyder column to the concentrator tube. Prewet the column by adding approximately 0.5 mL of solvent through the top. Place the apparatus in the hot water bath.

12.6.3.5 Adjust the vertical position and the water temperature as required to complete the concentration in 5–10 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood.

12.6.3.6 When the liquid reaches an apparent volume of 0.5 mL, remove the

apparatus from the water bath and allow to drain and cool for at least 10 minutes.

12.6.3.7 Proceed to 12.6.4 for preparation for back-extraction or micro-concentration and solvent exchange.

12.6.4 Preparation for back-extraction or micro-concentration and solvent exchange.

12.6.4.1 For back-extraction (Section 12.5), transfer the extract to a 250 mL separatory funnel. Rinse the concentration vessel with small portions of hexane, adjust the hexane volume in the separatory funnel to 10–20 mL, and proceed to back-extraction (Section 12.5).

12.6.4.2 For determination of the weight of residue in the extract, or for clean-up procedures other than back-extraction, transfer the extract to a blowdown vial using two to three rinses of solvent. Proceed with micro-concentration and solvent exchange (Section 12.7).

12.7 Micro-Concentration and Solvent Exchange.

12.7.1 Extracts to be subjected to GPC or HPLC cleanup are exchanged into methylene chloride. Extracts to be cleaned up using silica gel, alumina, carbon, and/or Florisil are exchanged into hexane.

12.7.2 Transfer the vial containing the sample extract to a nitrogen blowdown 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.

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

12.7.3.1 If the extract is to be concentrated to dryness for weight determination (Sections 12.4.1.8, 12.4.2.7, and 13.7.1.4), blow dry until a constant weight is obtained.

12.7.3.2 If the extract is to be concentrated for injection into the GC/MS or the solvent is to be exchanged for extract cleanup, proceed as follows:

12.7.4 When the volume of the liquid is approximately 100 L, add 2–3 mL of the desired solvent (methylene chloride for GPC and HPLC, or hexane for the other cleanups) and continue concentration to approximately 100 µL. Repeat the addition of solvent and concentrate once more.

12.7.5 If the extract is to be cleaned up by GPC, adjust the volume of the extract to 5.0 mL with methylene chloride. If the extract is to be cleaned up by HPLC, further concentrate the extract to 30 µL. Proceed with GPC or HPLC cleanup (Section 13.2 or 13.6, respectively).

12.7.6 If the extract is to be cleaned up by column chromatography

(alumina, silica gel, Carbowax/Celite, or Florisil), bring the final volume to 1.0 mL with hexane. Proceed with column cleanups (Sections 13.3 through 13.5 and 13.8).

12.7.7 If the extract is to be concentrated for injection into the GC/MS (Section 14), quantitatively transfer the extract to a 0.3 mL conical vial for final concentration, rinsing the larger vial with hexane and adding the rinse to the conical vial. Reduce the volume to approximately 100 µL. Add 10 µL of nonane to the vial, and evaporate the solvent to the level of the nonane. Seal the vial and label with the sample number. Store in the dark at room temperature until ready for GC/MS analysis. If GC/MS analysis will not be performed on the same day, store the vial at <math>-10^{\circ}\text{C}</math>.

### 13.0 Extract Cleanup

13.1 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 9.2 can be met using the cleanup procedure. If only 2,3,7,8-TCDD and 2,3,7,8-TCDF are to be determined, the cleanup procedures may be optimized for isolation of these two compounds.

13.1.1 Gel permeation chromatography (Section 13.2) removes high molecular weight interferences that cause GC column performance to degrade. It should 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).

13.1.2 Acid, neutral, and basic silica gel (Section 13.3), alumina (Section 13.4), and Florisil (Section 13.8) are used to remove nonpolar and polar interferences. Alumina and Florisil are used to remove chlorodiphenyl ethers.

13.1.3 Carbowax/Celite (Section 13.5) is used to remove nonpolar interferences.

13.1.4 HPLC (Section 13.6) is used to provide specificity for the 2,3,7,8-substituted and other CDD and CDF isomers.

13.1.5 The anthropogenic isolation column (Section 13.7.1), acidified silica gel batch adsorption procedure (Section 13.7.2), and sulfuric acid and base back-extraction (Section 13.7.3) are used for removal of lipids from tissue samples.

13.2 Gel Permeation Chromatography (GPC).

13.2.1 Column packing.

13.2.1.1 Place 70–75 g of SX-3 Bio-beads (Section 6.7.1.1) in a 400–500 mL beaker.

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

13.2.1.3 Transfer the swelled beads to the column (Section 6.7.1.1) and pump solvent through the column, from bottom to top, at 4.5–5.5 mL/minute prior to connecting the column to the detector.

13.2.1.4 After purging the column with solvent for one to two hours, adjust the column head pressure to 7–10 psig and purge for four to five hours to remove air. Maintain a head pressure of 7–10 psig. Connect the column to the detector (Section 6.7.1.4).

13.2.2 Column calibration.

13.2.2.1 Load 5 mL of the calibration solution (Section 7.4) into the sample loop.

13.2.2.2 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.

13.2.2.3 Set the “dump time” to allow >85% removal of the corn oil and >85% collection of the phthalate.

13.2.2.4 Set the “collect time” to the peak minimum between perylene and sulfur.

13.2.2.5 Verify the calibration with the calibration solution after every 20 extracts. Calibration is verified if the recovery of the pentachlorophenol is greater than 85%. 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.

13.2.3 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 residue content of the extract may be obtained gravimetrically by evaporating the solvent from a 50 µL aliquot.

13.2.3.1 Filter the extract or load through the filter holder (Section 6.7.1.3) to remove the particles. Load the 5.0 mL extract onto the column.

13.2.3.2 Elute the extract using the calibration data determined in Section

13.2.2. Collect the eluate in a clean 400–500 mL beaker.

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

13.2.3.4 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.

13.2.3.5 Concentrate the eluate per Sections 12.6 and 12.7 for further cleanup or injection into the GC/MS.

### 13.3 Silica Gel Cleanup.

13.3.1 Place a glass-wool plug in a 15 mm ID chromatography column (Section 6.7.4.2). Pack the column bottom to top with: 1 g silica gel (Section 7.5.1.1), 4 g basic silica gel (Section 7.5.1.3), 1 g silica gel, 8 g acid silica gel (Section 7.5.1.2), 2 g silica gel, and 4 g granular anhydrous sodium sulfate (Section 7.2.1). Tap the column to settle the adsorbents.

13.3.2 Pre-elute 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.

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

13.3.4 Rinse the receiver twice with 1 mL portions of hexane, and apply separately to the column. Elute the CDDs/CDFs with 100 mL hexane, and collect the eluate.

13.3.5 Concentrate the eluate per Sections 12.6 and 12.7 for further cleanup or injection into the HPLC or GC/MS.

13.3.6 For extracts of samples known to contain large quantities of other organic compounds (such as paper mill effluents), it may be advisable to increase the capacity of the silica gel column. This may be accomplished by increasing the strengths of the acid and basic silica gels. The acid silica gel (Section 7.5.1.2) may be increased in strength to as much as 44% w/w (7.9 g sulfuric acid added to 10 g silica gel). The basic silica gel (Section 7.5.1.3) may be increased in strength to as much as 33% w/w (50 mL 1N NaOH added to 100 g silica gel), or the potassium silicate (Section 7.5.1.4) may be used.

**Note:** The use of stronger acid silica gel (44% w/w) may lead to charring of organic compounds in some extracts. The charred material may retain some of the analytes and lead to lower recoveries of CDDs/CDFs. Increasing the strengths of the acid and basic silica gel may also require different volumes of hexane than those specified above to elute

the analytes off the column. Therefore, the performance of the method after such modifications must be verified by the procedure in Section 9.2.

### 13.4 Alumina Cleanup.

13.4.1 Place a glass-wool plug in a 15 mm ID chromatography column (Section 6.7.4.2).

13.4.2 If using acid alumina, pack the column by adding 6 g acid alumina (Section 7.5.2.1). If using basic alumina, substitute 6 g basic alumina (Section 7.5.2.2). Tap the column to settle the adsorbents.

13.4.3 Pre-elute the column with 50–100 mL of hexane. Close the stopcock when the hexane is within 1 mm of the alumina.

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

13.4.5 Apply the concentrated extract to the column. Open the stopcock until the extract is within 1 mm of the alumina.

13.4.6 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.

13.4.7 The choice of eluting solvents will depend on the choice of alumina (acid or basic) made in Section 13.4.2.

13.4.7.1 If using acid alumina, elute the CDDs/CDFs from the column with 20 mL methylene chloride:hexane (20:80 v/v). Collect the eluate.

13.4.7.2 If using basic alumina, elute the CDDs/CDFs from the column with 20 mL methylene chloride:hexane (50:50 v/v). Collect the eluate.

13.4.8 Concentrate the eluate per Sections 12.6 and 12.7 for further cleanup or injection into the HPLC or GC/MS.

### 13.5 Carbon Column.

13.5.1 Cut both ends from a 10 mL disposable serological pipet (Section 6.7.3.2) to produce a 10 cm column. Fire-polish both ends and flare both ends if desired. Insert a glass-wool plug at one end, and pack the column with 0.55 g of Carboapak/Celite (Section 7.5.3.3) to form an adsorbent bed approximately 2 cm long. Insert a glass-wool plug on top of the bed to hold the adsorbent in place.

13.5.2 Pre-elute the column with 5 mL of toluene followed by 2 mL of methylene chloride: methanol:toluene (15:4:1 v/v), 1 mL of methylene chloride:cyclohexane (1:1 v/v), and 5 mL of hexane. If the flow rate of eluate exceeds 0.5 mL/minute, discard the column.

13.5.3 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.

13.5.4 Elute the interfering compounds with two 3 mL portions 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.

13.5.5 Invert the column, and elute the CDDs/CDFs with 20 mL of toluene. If carbon particles are present in the eluate, filter through glass-fiber filter paper.

13.5.6 Concentrate the eluate per Sections 12.6 and 12.7 for further cleanup or injection into the HPLC or GC/MS.

### 13.6 HPLC (Reference 6).

#### 13.6.1 Column calibration.

13.6.1.1 Prepare a calibration standard containing the 2,3,7,8-substituted isomers and/or other isomers of interest at a concentration of approximately 500 pg/μL in methylene chloride.

13.6.1.2 Inject 30 μL of the calibration solution into the HPLC and record the signal from the detector. Collect the eluant for reuse. The elution order will be the tetra- through octa-isomers.

13.6.1.3 Establish the collection time for the tetra-isomers and for the other isomers of interest. Following calibration, flush the injection system with copious quantities of methylene chloride, including a minimum of five 50 μL injections while the detector is monitored, to ensure that residual CDDs/CDFs are removed from the system.

13.6.1.4 Verify the calibration with the calibration solution after every 20 extracts. Calibration is verified if the recovery of the CDDs/CDFs from the calibration standard (Section 13.6.1.1) is 75–125% compared to the calibration (Section 13.6.1.2). 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.

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

13.6.2.1 Rinse the sides of the vial twice with 30 μL of methylene chloride and reduce to 30 μL with the evaporation apparatus (Section 12.7).

13.6.2.2 Inject the 30  $\mu$ L extract into the HPLC.

13.6.2.3 Elute the extract using the calibration data determined in Section 13.6.1. Collect the fraction(s) in a clean 20 mL concentrator tube containing 5 mL of hexane:acetone (1:1 v/v).

13.6.2.4 If an extract containing greater than 100 ng/mL of total CDD or CDF is encountered, a 30  $\mu$ L methylene chloride blank shall be run through the system to check for carry-over.

13.6.2.5 Concentrate the eluate per Section 12.7 for injection into the GC/MS.

13.7 Cleanup of Tissue Lipids—Lipids are removed from the Soxhlet extract using either the anthropogenic isolation column (Section 13.7.1) or acidified silica gel (Section 13.7.2), or are removed from the HCl digested extract using sulfuric acid and base back-extraction (Section 13.7.3).

13.7.1 Anthropogenic isolation column (References 22 and 27)—Used for removal of lipids from the Soxhlet/SDS extraction (Section 12.4.1).

13.7.1.1 Prepare the column as given in Section 7.5.4.

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

13.7.1.3 Load the sample and rinses (Section 12.4.1.9.2) onto the column by draining each portion to the top of the bed. Elute the CDDs/CDFs from the column into the apparatus used for concentration (Section 12.4.1.7) using 200 mL of hexane.

13.7.1.4 Concentrate the cleaned up extract (Sections 12.6 through 12.7) to constant weight per Section 12.7.3.1. If more than 500 mg of material remains, repeat the cleanup using a fresh anthropogenic isolation column.

13.7.1.5 Redissolve the extract in a solvent suitable for the additional cleanups to be used (Sections 13.2 through 13.6 and 13.8).

13.7.1.6 Spike 1.0 mL of the cleanup standard (Section 7.11) into the residue/solvent.

13.7.1.7 Clean up the extract using the procedures in Sections 13.2 through 13.6 and 13.8. Alumina (Section 13.4) or Florisil (Section 13.8) and carbon (Section 13.5) are recommended as minimum additional cleanup steps.

13.7.1.8 Following cleanup, concentrate the extract to 10  $\mu$ L as described in Section 12.7 and proceed with the analysis in Section 14.

13.7.2 Acidified silica gel (Reference 28)—Procedure alternate to the anthropogenic isolation column (Section 13.7.1) that is used for removal of lipids from the Soxhlet/SDS extraction (Section 12.4.1).

13.7.2.1 Adjust the volume of hexane in the bottle (Section 12.4.1.9.2) to approximately 200 mL.

13.7.2.2 Spike 1.0 mL of the cleanup standard (Section 7.11) into the residue/solvent.

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

13.7.2.4 Add 30–100 g of acid silica gel (Section 7.5.1.2) to the bottle while stirring, keeping the silica gel in motion. Stir for two to three hours.

**Note:** 30 grams of silica gel should be adequate for most samples and will minimize contamination from this source.

13.7.2.5 After stirring, pour the extract through approximately 10 g of granular anhydrous sodium sulfate (Section 7.2.1) contained in a funnel with glass-fiber filter into a macro concentration device (Section 12.6). Rinse the bottle and sodium sulfate with hexane to complete the transfer.

13.7.2.6 Concentrate the extract per Sections 12.6 through 12.7 and clean up the extract using the procedures in Sections 13.2 through 13.6 and 13.8. Alumina (Section 13.4) or Florisil (Section 13.8) and carbon (Section 13.5) are recommended as minimum additional cleanup steps.

13.7.3 Sulfuric acid and base back-extraction—Used with HCl digested extracts (Section 12.4.2).

13.7.3.1 Spike 1.0 mL of the cleanup standard (Section 7.11) into the residue/solvent (Section 12.4.2.8.2).

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

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

13.7.3.4 Back extract the solvent/residue with 50 mL of potassium hydroxide solution per Section 12.5.2, followed by two reagent water rinses.

13.7.3.5 Drain the extract through a filter funnel containing approximately 10 g of granular anhydrous sodium sulfate in a glass-fiber filter into a macro concentration device (Section 12.6).

13.7.3.6 Concentrate the cleaned up extract to a volume suitable for the additional cleanups given in Sections 13.2 through 13.6 and 13.8. Gel permeation chromatography (Section 13.2), alumina (Section 13.4) or Florisil (Section 13.8), and Carbowax/Celite (Section 13.5) are recommended as minimum additional cleanup steps.

13.7.3.7 Following cleanup, concentrate the extract to 10 L as described in Section 12.7 and proceed with analysis per Section 14.

13.8 Florisil Cleanup (Reference 29).  
13.8.1 Pre-elute the activated Florisil column (Section 7.5.3) with 10 mL of methylene chloride followed by 10 mL of hexane:methylene chloride (98:2 v/v) and discard the solvents.

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

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

13.8.4 Elute the CDDs/CDFs with 35 mL of methylene chloride and collect the eluate. Concentrate the eluate per Sections 12.6 through 12.7 for further cleanup or for injection into the HPLC or GC/MS.

#### 14.0 HRGC/HRMS Analysis

14.1 Establish the operating conditions given in Section 10.1.

14.2 Add 10  $\mu$ L of the appropriate internal standard solution (Section 7.12) 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 and evaporation has occurred, do not add more instrument internal standard solution. Rather, bring the extract back to its previous volume (e.g., 19 L) with pure nonane only (18 L if 2 L injections are used).

14.3 Inject 1.0  $\mu$ L or 2.0  $\mu$ L of the concentrated extract containing the internal standard solution, using on-column or splitless injection. The volume injected must be identical to the volume used for calibration (Section 10). Start the GC column initial isothermal hold upon injection. Start MS data collection after the solvent peak elutes. Stop data collection after the OCDD and OCDF have eluted. If only 2,3,7,8-TCDD and 2,3,7,8-TCDF are to be determined, stop data collection after elution of these compounds. Return the column to the initial temperature for analysis of the next extract or standard.

#### 15.0 System and Laboratory Performance

15.1 At the beginning of each 12-hour shift during which analyses are performed, GC/MS system performance and calibration are verified for all CDDs/CDFs and labeled compounds. For these tests, analysis of the CS3 calibration verification (VER) standard (Section

7.13 and Table 4) and the isomer specificity test standards (Section 7.15 and Table 5) shall be used to verify all performance criteria. Adjustment and/or recalibration (Section 10) shall be performed until all performance criteria are met. Only after all performance criteria are met may samples, blanks, IPRs, and OPRs be analyzed.

15.2 MS Resolution—A static resolving power of at least 10,000 (10% valley definition) must be demonstrated at the appropriate  $m/z$  before any analysis is performed. Static resolving power checks must be performed at the beginning and at the end of each 12-hour shift according to procedures in Section 10.1.2. Corrective actions must be implemented whenever the resolving power does not meet the requirement.

#### 15.3 Calibration Verification.

15.3.1 Inject the VER standard using the procedure in Section 14.

15.3.2 The  $m/z$  abundance ratios for all CDDs/CDFs shall be within the limits in Table 9; otherwise, the mass spectrometer shall be adjusted until the  $m/z$  abundance ratios fall within the limits specified, and the verification test shall be repeated. If the adjustment alters the resolution of the mass spectrometer, resolution shall be verified (Section 10.1.2) prior to repeat of the verification test.

15.3.3 The peaks representing each CDD/CDF and labeled compound in the VER standard must be present with  $S/N$  of at least 10; otherwise, the mass spectrometer shall be adjusted and the verification test repeated.

15.3.4 Compute the concentration of each CDD/CDF compound by isotope dilution (Section 10.5) for those compounds that have labeled analogs (Table 1). Compute the concentration of the labeled compounds by the internal standard method (Section 10.6). These concentrations are computed based on the calibration data in Section 10.

15.3.5 For each compound, compare the concentration with the calibration verification limit in Table 6. If only 2,3,7,8-TCDD and 2,3,7,8-TCDF are to be determined, compare the concentration to the limit in Table 6a. If all compounds meet the acceptance criteria, calibration has been verified and analysis of standards and sample extracts may proceed. If, however, any compound fails its respective limit, 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 15.2) and verification (Section 15.3) tests, or recalibrate (Section 10).

#### 15.4 Retention Times and GC Resolution.

##### 15.4.1 Retention times.

15.4.1.1 Absolute—The absolute retention times of the  $^{13}C_{12-1,2,3,4}$ -TCDD and  $^{13}C_{12-1,2,3,7,8,9}$ -HxCDD GCMS internal standards in the verification test (Section 15.3) shall be within  $\pm 15$  seconds of the retention times obtained during calibration (Sections 10.2.1 and 10.2.4).

15.4.1.2 Relative—The relative retention times of CDDs/CDFs and labeled compounds in the verification test (Section 15.3) shall be within the limits given in Table 2.

##### 15.4.2 GC resolution.

15.4.2.1 Inject the isomer specificity standards (Section 7.15) on their respective columns.

15.4.2.2 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% on their respective columns (Figures 6 and 7).

15.4.3 If the absolute retention time of any compound is not within the limits specified or if 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 15.3) or recalibrate (Section 10), or replace the GC column and either verify calibration or recalibrate.

#### 15.5 Ongoing Precision and Recovery.

15.5.1 Analyze the extract of the ongoing precision and recovery (OPR) aliquot (Section 11.4.2.5, 11.5.4, 11.6.2, 11.7.4, or 11.8.3.2) prior to analysis of samples from the same batch.

15.5.2 Compute the concentration of each CDD/CDF by isotope dilution for those compounds that have labeled analogs (Section 10.5). Compute the concentration of 1,2,3,7,8,9-HxCDD, OCDF, and each labeled compound by the internal standard method (Section 10.6).

15.5.3 For each CDD/CDF and labeled compound, compare the concentration to the OPR limits given in Table 6. If only 2,3,7,8-TCDD and 2,3,7,8-TCDF are to be determined, compare the concentration to the limits in Table 6a. 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-prepare, extract, and clean up the sample batch and repeat the ongoing precision and recovery test (Section 15.5).

15.5.4 Add results that pass the specifications in Section 15.5.3 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 CDD/CDF in each matrix type by calculating the average percent recovery ( $R$ ) and the standard deviation of percent recovery ( $S_R$ ). Express the accuracy as a recovery interval from  $R - 2S_R$  to  $R + 2S_R$ . For example, if  $R = 95\%$  and  $S_R = 5\%$ , the accuracy is 85–105%.

15.6 Blank—Analyze the method blank extracted with each sample batch immediately following analysis of the OPR aliquot to demonstrate freedom from contamination and freedom from carryover from the OPR analysis. The results of the analysis of the blank must meet the specifications in Section 9.5.2 before sample analyses may proceed.

## 16.0 Qualitative Determination

A CDD, CDF, or labeled compound is identified in a standard, blank, or sample when all of the criteria in Sections 16.1 through 16.4 are met.

16.1 The signals for the two exact  $m/z$ 's in Table 8 must be present and must maximize within the same two seconds.

16.2 The signal-to-noise ratio ( $S/N$ ) for the GC peak at each exact  $m/z$  must be greater than or equal to 2.5 for each CDD or CDF detected in a sample extract, and greater than or equal to 10 for all CDDs/CDFs in the calibration standard (Sections 10.2.3 and 15.3.3).

16.3 The ratio of the integrated areas of the two exact  $m/z$ 's specified in Table 8 must be within the limit in Table 9, or within  $\pm 10\%$  of the ratio in the midpoint (CS3) calibration or calibration verification (VER), whichever is most recent.

16.4 The relative retention time of the peak for a 2,3,7,8-substituted CDD or CDF must be within the limit in Table 2. The retention time of peaks representing non-2,3,7,8-substituted CDDs/CDFs must be within the retention time windows established in Section 10.3.

16.5 Confirmatory Analysis—Isomer specificity for 2,3,7,8-TCDF cannot be achieved on the DB-5 column. Therefore, any sample in which 2,3,7,8-TCDF is identified by analysis on a DB-5 column must have a confirmatory analysis performed on a DB-225, SP-2330, or equivalent GC column. The operating conditions in Section 10.1.1 may be adjusted to optimize the analysis on the second GC column, but the GC/MS must meet the mass resolution



and calibration specifications in Section 10.

16.6 If the criteria for identification in Sections 16.1 through 16.5 are not met, the CDD or CDF has not been identified and the results may not be reported for regulatory compliance purposes. If interferences preclude identification, a new aliquot of sample must be extracted, further cleaned up, and analyzed.

**17.0 Quantitative Determination**

17.1 Isotope Dilution Quantitation—By adding a known amount of a labeled compound to every sample prior to extraction, correction for recovery of the CDD/CDF can be made because the CDD/CDF and its labeled analog exhibit similar effects upon extraction, concentration, and gas chromatography. Relative response (RR) values are used in conjunction with the initial calibration data described in Section 10.5 to determine concentrations directly, so long as labeled compound spiking levels are constant, using the following equation:

$$C_{ex} \text{ (ng/mL)} = \frac{(A_{1n} + A_{2n}) C_1}{(A_{1l} + A_{2l}) RR}$$

Where:

Where:

$C_{ex}$  = The concentration of the compound in the extract.

$V_{ex}$  = The extract volume in mL.

$W_s$  = The sample weight (dry weight) in kg.

17.4 The concentration of a CDD/CDF in the aqueous phase of the sample is computed using the concentration of the compound in the extract and the volume of water extracted (Section 11.4 or 11.5), as follows:

$$\text{Recovery (\%)} = \frac{\text{Concentration found (\mu g/mL)}}{\text{Concentration spiked (\mu g/mL)}} \times 100$$

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

Where:

$C_{ex}$  = The concentration of the compound in the extract.

$V_{ex}$  = The extract volume in mL.

$V_s$  = The sample volume in liters.

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

$C_{ex}$  = The concentration of the CDD/CDF in the extract, and the other terms are as defined in Section 10.5.2.

17.1.1 Because of a potential interference, the labeled analog of OCDF is not added to the sample. Therefore, OCDF is quantitated against labeled OCDD. As a result, the concentration of OCDF is corrected for the recovery of the labeled OCDD. In instances where OCDD and OCDF behave differently during sample extraction, concentration, and cleanup procedures, this may decrease the accuracy of the OCDF results. However, given the low toxicity of this compound relative to the other dioxins and furans, the potential decrease in accuracy is not considered significant.

17.1.2 Because  $^{13}C_{12}$ -1,2,3,7,8,9-HxCDD is used as an instrument internal standard (i.e., not added before extraction of the sample), it cannot be used to quantitate the 1,2,3,7,8,9-HxCDD by strict isotope dilution procedures. Therefore, 1,2,3,7,8,9-HxCDD is quantitated using the averaged response 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. As a result, the concentration of 1,2,3,7,8,9-HxCDD is

corrected for the average recovery of the other two HxCDD's.

17.1.3 Any peaks representing non-2,3,7,8-substituted CDDs/CDFs are quantitated using an average of the response factors from all of the labeled 2,3,7,8-isomers at the same level of chlorination.

17.2 Internal Standard Quantitation and Labeled Compound Recovery.

17.2.1 Compute the concentrations of 1,2,3,7,8,9--HxCDD, OCDF, the  $^{13}C$ -labeled analogs and the  $^{37}C$ -labeled cleanup standard in the extract using the response factors determined from the initial calibration data (Section 10.6) and the following equation:

$$C_{ex} \text{ (ng/mL)} = \frac{(A_{1s} + A_{2s}) C_{is}}{(A_{1is} + A_{2is}) RF}$$

Where:

$C_{ex}$  = The concentration of the CDD/CDF in the extract, and the other terms are as defined in Section 10.6.1.

**Note:** There is only one m/z for the  $^{37}Cl$ -labeled standard.

17.2.2 Using the concentration in the extract determined above, compute the percent recovery of the  $^{13}C$ -labeled compounds and the  $^{37}C$ -labeled cleanup standard using the following equation:

17.5.1 For aqueous samples containing 1% solids or less, dilute 100 mL, 10 mL, etc., of sample to 1 L with reagent water and re-prepare, extract, clean up, and analyze per Sections 11 through 14.

17.5.2 For samples containing greater than 1% solids, extract an amount of sample equal to 1/10, 1/100, etc., of the amount used in Section

11.5.1. Re-prepare, extract, clean up, and analyze per Sections 11 through 14.

17.5.3 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/μL in the extract, and analyze an aliquot of this diluted extract by the internal standard method.



17.6 Results are reported to three significant figures for the CDDs/CDFs and labeled compounds found in all standards, blanks, and samples.

#### 17.6.1 Reporting units and levels.

17.6.1.1 Aqueous samples—Report results in pg/L (parts-per-quadrillion).

17.6.1.2 Samples containing greater than 1% solids (soils, sediments, filter cake, compost)—Report results in ng/kg based on the dry weight of the sample. Report the percent solids so that the result may be corrected.

17.6.1.3 Tissues—Report results in ng/kg of wet tissue, not on the basis of the lipid content of the sample. Report the percent lipid content, so that the data user can calculate the concentration on a lipid basis if desired.

#### 17.6.1.4 Reporting level.

17.6.1.4.1 Standards (VER, IPR, OPR) and samples—Report results at or above the minimum level (Table 2). Report results below the minimum level as not detected or as required by the regulatory authority.

17.6.1.4.2 Blanks—Report results above one-third the ML.

17.6.2 Results for CDDs/CDFs in samples that have been diluted are reported at the least dilute level at which the areas at the quantitation  $m/z$ 's are within the calibration range (Section 17.5).

17.6.3 For CDDs/CDFs 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 17.5) and the labeled compound recovery is within the normal range for the method (Section 9.3 and Tables 6, 6a, 7, and 7a).

17.6.4 Additionally, if requested, the total concentration of all isomers in an individual level of chlorination (i.e., total TCDD, total TCDF, total Paced, etc.) may be reported by summing the concentrations of all isomers identified in that level of chlorination, including both 2,3,7,8-substituted and non-2,3,7,8-substituted isomers.

## 18.0 Analysis of Complex Samples

18.1 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  $10 \mu\text{L}$  (Section 12.7); others may overload the GC column and/or mass spectrometer.

18.2 Analyze a smaller aliquot of the sample (Section 17.5) when the extract will not concentrate to  $10 \mu\text{L}$  after all cleanup procedures have been exhausted.

18.3 Chlorodiphenyl Ethers—If chromatographic peaks are detected at the retention time of any CDDs/CDFs in

any of the  $m/z$  channels being monitored for the chlorodiphenyl ethers (Table 8), cleanup procedures must be employed until these interferences are removed. Alumina (Section 13.4) and Florisil (Section 13.8) are recommended for removal of chlorodiphenyl ethers.

18.4 Recovery of Labeled Compounds—In most samples, recoveries of the labeled compounds will be similar to those from reagent water or from the alternate matrix (Section 7.6).

18.4.1 If the recovery of any of the labeled compounds is outside of the normal range (Table 7), a diluted sample shall be analyzed (Section 17.5).

18.4.2 If the recovery of any of the labeled compounds in the diluted sample is outside of normal range, the calibration verification standard (Section 7.13) shall be analyzed and calibration verified (Section 15.3).

18.4.3 If the calibration cannot be verified, a new calibration must be performed and the original sample extract reanalyzed.

18.4.4 If the calibration is verified and the diluted sample does not meet the limits for labeled compound recovery, the method does not apply to the sample being analyzed and the result may not be reported for regulatory compliance purposes. In this case, alternate extraction and cleanup procedures in this method must be employed to resolve the interference. If all cleanup procedures in this method have been employed and labeled compound recovery remains outside of the normal range, extraction and/or cleanup procedures that are beyond this scope of this method will be required to analyze these samples.

## 19.0 Pollution Prevention

19.1 The solvents used in this method pose little threat to the environment when managed properly. The solvent evaporation techniques used in this method are amenable to solvent recovery, and it is recommended that the laboratory recover solvents wherever feasible.

19.2 Standards should be prepared in volumes consistent with laboratory use to minimize disposal of standards.

## 20.0 Waste Management

20.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance is

also required with any sewage discharge permits and regulations.

20.2 Samples containing HCl to pH  $<2$  are hazardous and must be neutralized before being poured down a drain or must be handled as hazardous waste.

20.3 The CDDs/CDFs decompose above  $800^\circ\text{C}$ . 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 of through commercial or governmental channels that are capable of handling extremely toxic wastes.

20.4 Liquid or soluble waste should be dissolved in methanol or ethanol and irradiated with ultraviolet light with a wavelength shorter than  $290$  nm for several days. Use F40 BL or equivalent lamps. Analyze liquid wastes, and dispose of the solutions when the CDDs/CDFs can no longer be detected.

20.5 For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" and "Less is Better—Laboratory Chemical Management for Waste Reduction," available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

## 21.0 Method Performance

Method performance was validated and performance specifications were developed using data from EPA's international interlaboratory validation study (References 30–31) and the EPA/paper industry Long-Term Variability Study of discharges from the pulp and paper industry (58 FR 66078).

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**23.0 Tables and Figures**

TABLE 1.—CHLORINATED DIBENZO-P-DIOXINS AND FURANS DETERMINED BY ISOTOPE DILUTION AND INTERNAL STANDARD HIGH RESOLUTION GAS CHROMATOGRAPHY (HRGC)/HIGH RESOLUTION MASS SPECTROMETRY (HRMS)

CDDs/CDFs <sup>1</sup>	CAS registry	Labeled analog	CAS registry
2,3,7,8-TCDD .....	1746-01-6	<sup>13</sup> C <sub>12</sub> -2,3,7,8-TCDD .....	76523-40-5
		<sup>37</sup> Cl <sub>4</sub> -2,3,7,8-TCDD .....	85508-50-5
Total TCDD .....	41903-57-5		
2,3,7,8-TCDF .....	51207-31-9	<sup>13</sup> C <sub>12</sub> -2,3,7,8-TCDF .....	89059-46-1
Total-TCDF .....	55722-27-5		
1,2,3,7,8-PeCDD .....	40321-76-4	<sup>13</sup> C <sub>12</sub> -1,2,3,7,8-PeCDD .....	109719-79-1
Total-PeCDD .....	36088-22-9		
1,2,3,7,8-PeCDF .....	57117-41-6	<sup>13</sup> C <sub>12</sub> -1,2,3,7,8-PeCDF .....	109719-77-9

TABLE 1.—CHLORINATED DIBENZO-P-DIOXINS AND FURANS DETERMINED BY ISOTOPE DILUTION AND INTERNAL STANDARD HIGH RESOLUTION GAS CHROMATOGRAPHY (HRGC)/HIGH RESOLUTION MASS SPECTROMETRY (HRMS)—Continued

CDDs/CDFs <sup>1</sup>	CAS registry	Labeled analog	CAS registry
2,3,4,7,8-PeCDF	57117-31-4	<sup>13</sup> C <sub>12</sub> -2,3,4,7,8-PeCDF	116843-02-8
Total-PeCDF	30402-15-4		
1,2,3,4,7,8-HxCDD	39227-28-6	<sup>13</sup> C <sub>12</sub> -1,2,3,4,7,8-HxCDD	109719-80-4
1,2,3,6,7,8-HxCDD	57653-85-7	<sup>13</sup> C <sub>12</sub> -1,2,3,6,7,8-HxCDD	109719-81-5
1,2,3,7,8,9-HxCDD	19408-74-3	<sup>13</sup> C <sub>12</sub> -1,2,3,7,8,9-HxCDD	109719-82-6
Total-HxCDD	34465-46-8		
1,2,3,4,7,8-HxCDF	70648-26-9	<sup>13</sup> C <sub>12</sub> -1,2,3,4,7,8-HxCDF	114423-98-2
1,2,3,6,7,8-HxCDF	57117-44-9	<sup>13</sup> C <sub>12</sub> -1,2,3,6,7,8-HxCDF	116843-03-9
1,2,3,7,8,9-HxCDF	72918-21-9	<sup>13</sup> C <sub>12</sub> -1,2,3,7,8,9-HxCDF	116843-04-0
2,3,4,6,7,8-HxCDF	60851-34-5	<sup>13</sup> C <sub>12</sub> -2,3,4,6,7,8-HxCDF	116843-05-1
Total-HxCDF	55684-94-1		
1,2,3,4,6,7,8-HpCDD	35822-46-9	<sup>13</sup> C <sub>12</sub> -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	<sup>13</sup> C <sub>12</sub> -1,2,3,4,6,7,8-HpCDF	109719-84-8
1,2,3,4,7,8,9-HpCDF	55673-89-7	<sup>13</sup> C <sub>12</sub> -1,2,3,4,7,8,9-HpCDF	109719-94-0
Total-HpCDF	38998-75-3		
OCDD	3268-87-9	<sup>13</sup> C <sub>12</sub> -OCDD	114423-97-1
OCDF	39001-02-0	Not used.	

<sup>1</sup> Chlorinated dibenzo-p-dioxins and chlorinated dibenzofurans.  
 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.

TABLE 2.—RETENTION TIME REFERENCES, QUANTITATION REFERENCES, RELATIVE RETENTION TIMES, AND MINIMUM LEVELS FOR CDDS AND DCFS

CDD/CDF	Retention time and quantitation reference	Relative retention time	Minimum level <sup>1</sup>		
			Water (pg/L; ppq)	Solid (ng/kg; ppt)	Extract (pg/μL; ppb)
<b>Compounds using <sup>13</sup>C<sub>12</sub>-1,2,3,4-TCDD as the Injection Internal Standard</b>					
2,3,7,8-TCDF	<sup>13</sup> C <sub>12</sub> -2,3,7,8-TCDF	0.999-1.003	10	1	0.5
2,3,7,8-TCDD	<sup>13</sup> C <sub>12</sub> -2,3,7,8-TCDD	0.999-1.002	10	1	0.5
1,2,3,7,8-Pe	<sup>13</sup> C <sub>12</sub> -1,2,3,7,8-PeCDF	0.999-1.002	50	5	2.5
2,3,4,7,8-PeCDF	<sup>13</sup> C <sub>12</sub> -2,3,4,7,8-PeCDF	0.999-1.002	50	5	2.5
1,2,3,7,8-PeCDD	<sup>13</sup> C <sub>12</sub> -1,2,3,7,8-PeCDD	0.999-1.002	50	5	2.5
<sup>13</sup> C <sub>12</sub> -2,3,7,8-TCDF	<sup>13</sup> C <sub>12</sub> -1,2,3,4-TCDD	0.923-1.103			
<sup>13</sup> C <sub>12</sub> -2,3,7,8-TCDD	<sup>13</sup> C <sub>12</sub> -1,2,3,4-TCDD	0.976-1.043			
<sup>13</sup> C <sub>12</sub> -2,3,7,8-TCDD	<sup>13</sup> C <sub>12</sub> -1,2,3,4-TCDD	0.989-1.052			
<sup>13</sup> C <sub>12</sub> -1,2,3,7,8-PeCDF	<sup>13</sup> C <sub>12</sub> -1,2,3,4-TCDD	1.000-1.425			
<sup>13</sup> C <sub>12</sub> -2,3,4,7,8-PeCDF	<sup>13</sup> C <sub>12</sub> -1,2,3,4-TCDD	1.001-1.526			
<sup>13</sup> C <sub>12</sub> -1,2,3,7,8-PeCDF	<sup>13</sup> C <sub>12</sub> -1,2,3,4-TCDD	1.000-1.567			
<b>Compounds using <sup>13</sup>C<sub>12</sub>-1,2,3,7,8,9-HxCDD as the Injection Internal Standard</b>					
1,2,3,4,7,8-HxCDF	<sup>13</sup> C <sub>12</sub> -1,2,3,4,7,8-HxCDF	0.999-1.001	50	5	2.5
1,2,3,6,7,8-HxCDF	<sup>13</sup> C <sub>12</sub> -1,2,3,6,7,8-HxCDF	0.997-1.005	50	5	2.5
1,2,3,7,8,9-HxCDF	<sup>13</sup> C <sub>12</sub> -1,2,3,7,8,9-HxCDF	0.999-1.001	50	5	2.5
2,3,4,6,7,8-HxCDF	<sup>13</sup> C <sub>12</sub> -2,3,4,6,7,8-HxCDF	0.999-1.001	50	5	2.5
1,2,3,4,7,8-HxCDD	<sup>13</sup> C <sub>12</sub> -1,2,3,4,7,8-HxCDD	0.999-1.001	50	5	2.5
1,2,3,6,7,8-HxCDD	<sup>13</sup> C <sub>12</sub> -1,2,3,6,7,8-HxCDD	0.998-1.004	50	5	2.5
1,2,3,7,8,9-HxCDD	( <sup>2</sup> )	1.000-1.019	50	5	2.5
1,2,3,4,6,7,8-HpCDF	<sup>13</sup> C <sub>12</sub> -1,2,3,4,6,7,8-HpCDF	0.999-1.001	50	5	2.5
1,2,3,4,7,8,9-HpCDF	<sup>13</sup> C <sub>12</sub> -1,2,3,4,7,8,9-HpCDF	0.999-1.001	50	5	2.5
1,2,3,4,6,7,8-HpCDD	<sup>13</sup> C <sub>12</sub> -1,2,3,4,6,7,8-HpCDD	0.999-1.001	50	5	2.5
OCDF	<sup>13</sup> C <sub>12</sub> -OCDD	0.999-1.001	100	10	5.0
OCDD	<sup>13</sup> C <sub>12</sub> -OCDD	0.999-1.001	100	10	5.0
1,2,3,4,6,7,8-HxCDF	<sup>13</sup> C <sub>12</sub> -1,2,3,7,8,9-HpCDD	0.949-0.975			
<sup>13</sup> C <sub>12</sub> -1,2,3,7,8,9-HxCDF	<sup>13</sup> C <sub>12</sub> -1,2,3,7,8,9-HpCDD	0.977-1.047			
<sup>13</sup> C <sub>12</sub> -2,3,4,6,7,8-HxCDF	<sup>13</sup> C <sub>12</sub> -1,2,3,7,8,9-HpCDD	0.959-1.021			
<sup>13</sup> C <sub>12</sub> -1,2,3,4,7,8-HxCDF	<sup>13</sup> C <sub>12</sub> -1,2,3,7,8,9-HpCDD	0.977-1.000			

TABLE 2.—RETENTION TIME REFERENCES, QUANTITATION REFERENCES, RELATIVE RETENTION TIMES, AND MINIMUM LEVELS FOR CDDS AND DCFS—Continued

CDD/CDF	Retention time and quantitation reference	Relative retention time	Minimum level <sup>1</sup>		
			Water (pg/L; ppq)	Solid (ng/kg; ppt)	Extract (pg/μL; ppb)
<sup>13</sup> C <sub>12</sub> 1,2,3,6,7,8,-HxCDF	<sup>13</sup> C <sub>12</sub> -1,2,3,7,8,9-HpCDD	0.981–1.003	.....	.....	.....
<sup>13</sup> C <sub>12</sub> 1,2,3,4,6,7,8-HxCDF	<sup>13</sup> C <sub>12</sub> -1,2,3,7,8,9-HpCDD	1.043–1.085	.....	.....	.....
<sup>13</sup> C <sub>12</sub> 1,2,3,4,7,8,9-HxCDF	<sup>13</sup> C <sub>12</sub> -1,2,3,7,8,9-HpCDD	1.057–1.151	.....	.....	.....
<sup>13</sup> C <sub>12</sub> 1,2,3,4,6,7,8-HxCDF	<sup>13</sup> C <sub>12</sub> -1,2,3,7,8,9-HpCDD	1.086–1.110	.....	.....	.....
<sup>13</sup> C <sub>12</sub> OCDD	<sup>13</sup> C <sub>12</sub> -1,2,3,7,8,9-HpCDD	1.032–1.311	.....	.....	.....

<sup>1</sup> The Minimum Level (ML) for each analyte is defined as the level at which the entire analytical system must give a recognizable signal and acceptable calibration point. It is equivalent to the concentration of the lowest calibration standard, assuming that all method-specified sample weights, volumes, and cleanup procedures have been employed.

<sup>2</sup> The retention time reference for 1,2,3,7,8,9-HxCDD is <sup>13</sup>C<sub>12</sub>-1,2,3,6,7,8-HxCDD, and 1,2,3,7,8,9-HxCDD is quantified using the averaged responses for <sup>13</sup>C<sub>12</sub>-1,2,3,4,7,8-HxCDD and <sup>13</sup>C<sub>12</sub>-1,2,3,6,7,8-HxCDD.

TABLE 3.—CONCENTRATION OF STOCK AND SPIKING SOLUTIONS CONTAINING CDDS/CDFS AND LABELED COMPOUNDS

CDD/CDF	Labeled compound stock solution <sup>1</sup> (ng/mL)	Labeled compound spiking solution <sup>2</sup> (ng/mL)	PAR stock solution <sup>3</sup> (ng/mL)	PAR spiking solution <sup>4</sup> (ng/mL)
2,3,7,8-TCDD	.....	.....	40	0.8
2,3,7,8-TCDF	.....	.....	40	0.8
1,2,3,7,8-PeCDD	.....	.....	200	4
1,2,3,7,8-PeCDF	.....	.....	200	4
2,3,4,7,8-PeCDF	.....	.....	200	4
1,2,3,4,7,8-HxCDD	.....	.....	200	4
1,2,3,6,7,8-HxCDD	.....	.....	200	4
1,2,3,7,8,9-HxCDD	.....	.....	200	4
1,2,3,4,7,8-HxCDF	.....	.....	200	4
1,2,3,6,7,8-HxCDF	.....	.....	200	4
1,2,3,7,8,9-HxCDF	.....	.....	200	4
2,3,4,6,7,8-HxCDF	.....	.....	200	4
1,2,3,4,6,7,8-HpCDD	.....	.....	200	4
1,2,3,4,6,7,8-HpCDF	.....	.....	200	4
1,2,3,4,7,8,9-HpCDF	.....	.....	200	4
OCDD	.....	.....	400	8
OCDF	.....	.....	400	8
<sup>13</sup> C <sub>12</sub> -2,3,7,8-TCDD	100	2	.....	.....
<sup>13</sup> C <sub>12</sub> -2,3,7,8-TCDF	100	2	.....	.....
<sup>13</sup> C <sub>12</sub> -1,2,3,7,8-PeCDD	100	2	.....	.....
<sup>13</sup> C <sub>12</sub> -1,2,3,7,8-PeCDF	100	2	.....	.....
<sup>13</sup> C <sub>12</sub> -2,3,4,7,8-PeCDF	100	2	.....	.....
<sup>13</sup> C <sub>12</sub> -1,2,3,4,7,8-HxCDD	100	2	.....	.....
<sup>13</sup> C <sub>12</sub> -1,2,3,6,7,8-HxCDD	100	2	.....	.....
<sup>13</sup> C <sub>12</sub> -1,2,3,4,7,8-HxCDF	100	2	.....	.....
<sup>13</sup> C <sub>12</sub> -1,2,3,6,7,8-HxCDF	100	2	.....	.....
<sup>13</sup> C <sub>12</sub> -1,2,3,7,8,9-HxCDF	100	2	.....	.....
<sup>13</sup> C <sub>12</sub> -2,3,4,6,7,8-HxCDF	100	2	.....	.....
<sup>13</sup> C <sub>12</sub> -1,2,3,4,6,7,8-HpCDD	100	2	.....	.....
<sup>13</sup> C <sub>12</sub> -1,2,3,4,6,7,8-HpCDF	100	2	.....	.....
<sup>13</sup> C <sub>12</sub> -1,2,3,4,7,8,9-HpCDF	100	2	.....	.....
<sup>13</sup> C <sub>12</sub> -OCDD	200	4	.....	.....
Cleanup Standard <sup>5</sup>				
<sup>37</sup> Cl <sub>4</sub> -2,3,7,8-TCDD	0.8	.....	.....	.....
Internal Standards <sup>6</sup>				
<sup>13</sup> C <sub>12</sub> -1,2,3,4-TCDD	200	.....	.....	.....
<sup>13</sup> C <sub>12</sub> -1,2,3,7,8,9-HxCDD	200	.....	.....	.....

<sup>1</sup> Section 7.10—prepared in nonane and diluted to prepare spiking solution.

<sup>2</sup> Section 7.10.3—prepared in acetone from stock solution daily.

<sup>3</sup> Section 7.9—prepared in nonane and diluted to prepare spiking solution.

<sup>4</sup> Section 7.14—prepared in acetone from stock solution daily.

<sup>5</sup> Section 7.11—prepared in nonane and added to extract prior to cleanup.

<sup>6</sup> Section 7.12—prepared in nonane and added to the concentrated extract immediately prior to injection into the GC (Section 14.2).

TABLE 4.—CONCENTRATION OF CDDS/CDFS IN CALIBRATION AND CALIBRATION VERIFICATION SOLUTIONS<sup>1</sup> (SECTION 15.3)

	CDD/CDF	CS2 (ng/mL)	CS3 (ng/mL)	CS4 (ng/mL)	CS5 (ng/mL)
2,3,7,8-TCDD	0.5	2	10	40	200
2,3,7,8-TCDF	0.5	2	10	40	200
1,2,3,7,8-PeCDD	2.5	10	50	200	1000
1,2,3,7,8-PeCDF	2.5	10	50	200	1000
2,3,4,7,8-PeCDF	2.5	10	50	200	1000
1,2,3,4,7,8-HxCDD	2.5	10	50	200	1000
1,2,3,6,7,8-HxCDD	2.5	10	50	200	1000
1,2,3,7,8,9-HxCDD	2.5	10	50	200	1000
1,2,3,4,7,8-HxCDF	2.5	10	50	200	1000
1,2,3,6,7,8-HxCDF	2.5	10	50	200	1000
1,2,3,7,8,9-HxCDF	2.5	10	50	200	1000
2,3,4,6,7,8-HxCDF	2.5	10	50	200	1000
1,2,3,4,6,7,8-HpCDD	2.5	10	50	200	1000
1,2,3,4,6,7,8-HpCDF	2.5	10	50	200	1000
1,2,3,4,7,8,9-HpCDF	2.5	10	50	200	1000
OCDD	5.0	20	100	400	2000
OCDF	5.0	20	100	400	2000
<sup>13</sup> C <sub>12</sub> -2,3,7,8-TCDD	100	100	100	100	100
<sup>13</sup> C <sub>12</sub> -2,3,7,8-TCDF	100	100	100	100	100
<sup>13</sup> C <sub>12</sub> -1,2,3,7,8-PeCDD	100	100	100	100	100
<sup>13</sup> C <sub>12</sub> -PeCDF	100	100	100	100	100
<sup>13</sup> C <sub>12</sub> -2,3,4,7,8-PeCDF	100	100	100	100	100
<sup>13</sup> C <sub>12</sub> -1,2,3,4,7,8-HxCDD	100	100	100	100	100
<sup>13</sup> C <sub>12</sub> -1,2,3,6,7,8-HxCDD	100	100	100	100	100
<sup>13</sup> C <sub>12</sub> -1,2,3,4,7,8-HxCDF	100	100	100	100	100
<sup>13</sup> C <sub>12</sub> -1,2,3,6,7,8-HxCDF	100	100	100	100	100
<sup>13</sup> C <sub>12</sub> -1,2,3,7,8,9-HxCDF	100	100	100	100	100
<sup>13</sup> C <sub>12</sub> -1,2,3,4,6,7,8-HpCDD	100	100	100	100	100
<sup>13</sup> C <sub>12</sub> -1,2,3,4,6,7,8-HpCDF	100	100	100	100	100
<sup>13</sup> C <sub>12</sub> -1,2,3,4,7,8,9-HpCDF	100	100	100	100	100
<sup>13</sup> C <sub>12</sub> -OCDD	200	200	200	200	200
Cleanup Standard: <sup>37</sup> C <sub>14</sub> -2,3,7,8-TCDD	0.5	2	10	40	200
Internal Standards: <sup>13</sup> C <sub>12</sub> -1,2,3,4-TCDD	100	100	100	100	100
<sup>13</sup> C <sub>12</sub> -1,2,3,7,8,9-HxCDD	100	100	100	100	100

TABLE 5.—GC RETENTION TIME WINDOW DEFINING SOLUTION AND ISOMER SPECIFICITY TEST STANDARD (SECTION 7.15)

DB-5 column GC retention-time window defining solution		
CDD/CDF	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-
HxCDF	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 Column TCDD Specificity Test Standard**  
1,2,3,7+1,2,3,8-TCDD  
2,3,7,8-TCDD  
1,2,3,9-TCDD

**DB-225 Column TCDF Isomer Specificity Test Standard**  
2,3,4,7-TCDF  
2,3,7,8-TCDF  
1,2,3,9-TCDF

TABLE 6.—ACCEPTANCE CRITERIA FOR PERFORMANCE TESTS WHEN ALL CDDS/CDFS ARE TESTED<sup>1</sup>

CDD/CDF	Test conc. (ng/mL)	IPR <sup>2,3</sup>		OPR (ng/mL)	VER (ng/mL)
		s (ng/mL)	X (ng/mL)		
2,3,7,8-TCDD	10	2.8	8.3-12.9	6.7-15.8	7.8-12.9

TABLE 6.—ACCEPTANCE CRITERIA FOR PERFORMANCE TESTS WHEN ALL CDDS/CDFS ARE TESTED<sup>1</sup>—Continued

CDD/CDF	Test conc. (ng/mL)	IPR <sup>2,3</sup>		OPR (ng/mL)	VER (ng/mL)
		s (ng/mL)	X (ng/mL)		
2,3,7,8-TCDF	10	2.0	8.7–13.7	7.5–15.8	8.4–12.0
1,2,3,7,8-PeCDD	50	7.5	38–66	35–71	39–65
1,2,3,7,8-PeCDF	50	7.5	43–62	40–67	41–60
2,3,4,7,8-PeCDF	50	8.6	36–75	34–80	41–61
1,2,3,4,7,8-HxCDD	50	9.4	39–76	35–82	39–64
1,2,3,6,7,8-HxCDD	50	7.7	42–62	38–67	39–64
1,2,3,7,8,9-HxCDD	50	11.1	37–71	32–81	41–61
1,2,3,4,7,8-HxCDF	50	8.7	41–59	36–67	45–56
1,2,3,6,7,8-HxCDF	50	6.7	46–60	42–65	44–57
1,2,3,7,8,9-HxCDF	50	6.4	42–61	39–65	45–56
2,3,4,6,7,8-HxCDF	50	7.4	37–74	35–78	44–57
1,2,3,4,6,7,8-HpCDD	50	7.7	38–65	35–70	43–58
1,2,3,4,6,7,8-HpCDF	50	6.3	45–56	41–61	45–55
1,2,3,4,7,8,9-HpCDF	50	8.1	43–63	39–69	43–58
OCDD	100	19	89–127	78–144	79–126
OCDF	100	27	74–146	63–170	63–159
<sup>13</sup> C <sub>12</sub> -2,3,7,8-TCDD	100	37	28–134	20–175	82–121
<sup>13</sup> C <sub>12</sub> -2,3,7,8-TCDF	100	35	31–113	22–152	71–140
<sup>13</sup> C <sub>12</sub> -1,2,3,7,8-PeCDD	100	39	27–184	21–227	62–160
<sup>13</sup> C <sub>12</sub> -1,2,3,7,8-PeCDF	100	34	27–156	21–192	76–130
<sup>13</sup> C <sub>12</sub> -2,3,4,7,8-PeCDF	100	38	16–279	13–328	77–130
<sup>13</sup> C <sub>12</sub> -1,2,3,4,7,8-HxCDD	100	41	29–147	21–193	85–117
<sup>13</sup> C <sub>12</sub> -1,2,3,6,7,8-HxCDD	100	38	34–122	25–163	85–118
<sup>13</sup> C <sub>12</sub> -1,2,3,4,7,8-HxCDF	100	43	27–152	19–202	76–131
<sup>13</sup> C <sub>12</sub> -1,2,3,6,7,8-HxCDF	100	35	30–122	21–159	70–143
<sup>13</sup> C <sub>12</sub> -1,2,3,7,8,9-HxCDF	100	40	24–157	17–205	74–135
<sup>13</sup> C <sub>12</sub> -2,3,4,6,7,8-HxCDF	100	37	29–136	22–176	73–137
<sup>13</sup> C <sub>12</sub> -1,2,3,4,6,7,8-HpCDD	100	35	34–129	26–166	72–138
<sup>13</sup> C <sub>12</sub> -1,2,3,4,6,7,8-HpCDF	100	41	32–110	21–158	78–129
<sup>13</sup> C <sub>12</sub> -1,2,3,4,7,8,9-HpCDF	100	40	28–141	20–186	77–129
<sup>13</sup> C <sub>12</sub> -OCDD	200	95	41–276	26–397	96–415
<sup>37</sup> Cl <sub>4</sub> -2,3,7,8-TCDD	10	3.6	3.9–15.4	3.1–19.1	7.9–12.7

<sup>1</sup> All specifications are given as concentration in the final extract, assuming a 20 µL volume.  
<sup>2</sup> s = standard deviation of the concentration.  
<sup>3</sup> X = average concentration.

TABLE 6A.—ACCEPTANCE CRITERIA FOR PERFORMANCE TESTS WHEN ONLY TETRA COMPOUNDS ARE TESTED<sup>1</sup>

CDD/CDF	Test Conc. (ng/mL)	IPR <sup>2,3</sup>		OPR (ng/mL)	VER (ng/mL)
		s (ng/mL)	X (ng/mL)		
2,3,7,8-TCDD	10	2.7	8.7–12.4	7.314.6	8.2–12.3
2,3,7,8-TCDF	10	2.0	9.1–13.1	8.0–14.7	8.6–11.6
<sup>13</sup> C <sub>12</sub> -2,3,7,8-TCDD	100	35	32–115	25–141	85–117
<sup>13</sup> C <sub>12</sub> -2,3,7,8-TCDF	100	34	35–99	26–126	76–131
<sup>37</sup> Cl <sub>4</sub> -2,3,7,8-TCDD	10	3.4	4.5–13.4	3.7–15.8	8.3–12.1

<sup>1</sup> All specifications are given as concentration in the final extract, assuming a 20 µL volume.  
<sup>2</sup> s = standard deviation of the concentration.  
<sup>3</sup> X = average concentration.

TABLE 7.—LABELED COMPOUNDS RECOVERY IN SAMPLES WHEN ALL CDDS/CDFS ARE TESTED

Compound	Test conc. (ng/mL)	Labeled compound recovery	
		(ng/mL) <sup>1</sup>	(%)
<sup>13</sup> C <sub>12</sub> -2,3,7,8-TCDD	100	25–164	25–164
<sup>13</sup> C <sub>12</sub> -2,3,7,8-TCDF	100	24–169	24–169
<sup>13</sup> C <sub>12</sub> -1,2,3,7,8-PeCDD	100	25–181	25–181
<sup>13</sup> C <sub>12</sub> -1,2,3,7,8-PeCDF	100	24–185	24–185
<sup>13</sup> C <sub>12</sub> -2,3,4,7,8-PeCDF	100	21–178	21–178
<sup>13</sup> C <sub>12</sub> -1,2,3,4,7,8-HxCDD	100	32–141	32–141
<sup>13</sup> C <sub>12</sub> -1,2,3,6,7,8-HxCDD	100	28–130	28–130
<sup>13</sup> C <sub>12</sub> -1,2,3,4,7,8-HxCDF	100	26–152	26–152
<sup>13</sup> C <sub>12</sub> -1,2,3,6,7,8-HxCDF	100	26–123	26–123
<sup>13</sup> C <sub>12</sub> -1,2,3,7,8,9-HxCDF	100	29–147	29–147

TABLE 7.—LABELED COMPOUNDS RECOVERY IN SAMPLES WHEN ALL CDDS/CDFS ARE TESTED—Continued

Compound	Test conc. (ng/mL)	Labeled compound recovery	
		(ng/mL) <sup>1</sup>	(%)
<sup>13</sup> C <sub>12</sub> -2,3,4,6,7,8-HxCDF	100	28–136	28–136
<sup>13</sup> C <sub>12</sub> -1,2,3,4,6,7,8-HpCDD	100	23–140	23–140
<sup>13</sup> C <sub>12</sub> -1,2,3,4,6,7,8-HpCDF	100	28–143	28–143
<sup>13</sup> C <sub>12</sub> -1,2,3,4,7,8,9-HpCDF	100	26–138	26–138
<sup>13</sup> C <sub>12</sub> -OCDD	200	34–313	17–157
<sup>37</sup> Cl <sub>4</sub> -2,3,7,8-TCDD	10	3.5–19.7	35–197

<sup>1</sup> Specification given as concentration in the final extract, assuming a 20-μL volume.

TABLE 7A.—LABELED COMPOUND RECOVERY IN SAMPLES WHEN ONLY TETRA COMPOUNDS ARE TESTED

Compound	Test conc. (ng/mL)	Labeled compound recovery	
		(ng/mL) <sup>1</sup>	(%)
<sup>13</sup> C <sub>12</sub> -2,3,7,8-TCDD	100	31–137	31–137
<sup>13</sup> C <sub>12</sub> -2,3,7,8-TCDF	100	29–140	29–140
<sup>37</sup> Cl <sub>4</sub> -2,3,7,8-TCDD	10	4.2–16.4	42–164

<sup>1</sup> Specification given as concentration in the final extract, assuming a 20 μL volume.

TABLE 8.—DESCRIPTORS, EXACT M/Z'S, M/Z TYPES, AND ELEMENTAL COMPOSITIONS OF THE CDDS AND CDFS

Descriptor	Exact M/Z <sup>1</sup>	M/Z type	Elemental composition	Substance <sup>2</sup>	
1	292.9825	Lock	C <sub>7</sub> F <sub>11</sub>	PFK	
	303.9016	M	C <sub>12</sub> H <sub>4</sub> <sup>35</sup> Cl <sub>4</sub> O	TCDF	
	305.8987	M+2	C <sub>12</sub> H <sub>4</sub> <sup>35</sup> Cl <sub>3</sub> <sup>37</sup> ClO	TCDF	
	315.9419	M	<sup>13</sup> C <sub>12</sub> H <sub>4</sub> <sup>35</sup> Cl <sub>4</sub> O	TCDF <sup>3</sup>	
	317.9389	M+2	<sup>13</sup> C <sub>12</sub> H <sub>4</sub> <sup>35</sup> Cl <sub>3</sub> <sup>37</sup> ClO	TCDF <sup>3</sup>	
	319.8965	M	C <sub>12</sub> H <sub>4</sub> <sup>35</sup> Cl <sub>4</sub> O <sub>2</sub>	TCDD	
	321.8936	M+2	C <sub>12</sub> H <sub>4</sub> <sup>35</sup> Cl <sub>3</sub> <sup>37</sup> ClO <sub>2</sub>	TCDD	
	327.8847	M	C <sub>12</sub> H <sub>4</sub> <sup>37</sup> Cl <sub>4</sub> O <sub>2</sub>	TCDD <sup>4</sup>	
	330.9792	QC	C <sub>7</sub> F <sub>13</sub>	PFK	
	331.9368	M	<sup>13</sup> C <sub>12</sub> H <sub>4</sub> <sup>35</sup> Cl <sub>4</sub> O <sub>2</sub>	TCDD <sup>3</sup>	
	333.9339	M+2	<sup>13</sup> C <sub>12</sub> H <sub>4</sub> <sup>35</sup> Cl <sub>3</sub> <sup>37</sup> ClO <sub>2</sub>	TCDD <sup>3</sup>	
	375.8364	M+2	C <sub>12</sub> H <sub>4</sub> <sup>35</sup> Cl <sub>5</sub> <sup>37</sup> ClO	HxCDFE	
	2	339.8597	M+2	C <sub>12</sub> H <sub>3</sub> <sup>35</sup> Cl <sub>4</sub> <sup>37</sup> ClO	PeCDF
		341.8567	M+4	C <sub>12</sub> H <sub>3</sub> <sup>35</sup> Cl <sub>3</sub> <sup>37</sup> Cl <sub>2</sub> O	PeCDF
		351.9000	M+2	<sup>13</sup> C <sub>12</sub> H <sub>3</sub> <sup>35</sup> Cl <sub>4</sub> <sup>37</sup> ClO	PeCDF
353.8970		M+4	<sup>13</sup> C <sub>12</sub> H <sub>3</sub> <sup>35</sup> Cl <sub>3</sub> <sup>37</sup> Cl <sub>2</sub> O	PeCDF <sup>3</sup>	
354.9792		Lock	C <sub>9</sub> F <sub>13</sub>	PFK	
355.8546		M+2	C <sub>12</sub> H <sub>3</sub> <sup>35</sup> Cl <sub>4</sub> <sup>37</sup> ClO <sub>2</sub>	PeCDD	
357.8516		M+4	C <sub>12</sub> H <sub>3</sub> <sup>35</sup> Cl <sub>3</sub> <sup>37</sup> Cl <sub>2</sub> O <sub>2</sub>	PeCDD	
367.8949		M+2	<sup>13</sup> C <sub>12</sub> H <sub>3</sub> <sup>35</sup> Cl <sub>4</sub> <sup>37</sup> ClO <sub>2</sub>	PeCDD <sup>3</sup>	
369.8919		M+4	<sup>13</sup> C <sub>12</sub> H <sub>3</sub> <sup>35</sup> Cl <sub>3</sub> <sup>37</sup> Cl <sub>2</sub> O <sub>2</sub>	PeCDD <sup>3</sup>	
3		409.7974	M+2	C <sub>12</sub> H <sub>3</sub> <sup>35</sup> Cl <sub>6</sub> <sup>37</sup> ClO	HpCDFE
		373.8208	M+2	C <sub>12</sub> H <sub>2</sub> <sup>35</sup> Cl <sub>5</sub> <sup>37</sup> ClO	HxCDF
			M+4	C <sub>12</sub> H <sub>2</sub> <sup>35</sup> Cl <sub>4</sub> <sup>37</sup> Cl <sub>2</sub> O	HxCDF
		383.8639	M	<sup>13</sup> C <sub>12</sub> H <sub>2</sub> <sup>35</sup> Cl <sub>6</sub> O	HxCDF <sup>3</sup>
		385.8610	M+2	<sup>13</sup> C <sub>12</sub> H <sub>2</sub> <sup>35</sup> Cl <sub>5</sub> <sup>37</sup> ClO	HxCDF <sup>3</sup>
		389.8157	M+2	C <sub>12</sub> H <sub>2</sub> <sup>35</sup> Cl <sub>5</sub> <sup>37</sup> ClO <sub>2</sub>	HxCDD
	391.8127	M+4	C <sub>12</sub> H <sub>2</sub> <sup>35</sup> Cl <sub>4</sub> <sup>37</sup> Cl <sub>2</sub> O <sub>2</sub>	HxCDD	
	392.9760	Lock	C <sub>9</sub> F <sub>15</sub>	PFK	
	401.8559	M+2	<sup>13</sup> C <sub>12</sub> H <sub>2</sub> <sup>35</sup> Cl <sub>5</sub> <sup>37</sup> ClO <sub>2</sub>	HxCDD <sup>3</sup>	
	403.8529	M+4	<sup>13</sup> C <sub>12</sub> H <sub>2</sub> <sup>35</sup> Cl <sub>4</sub> <sup>37</sup> Cl <sub>2</sub> O <sub>2</sub>	HxCDD <sup>3</sup>	
	430.9729	QC	C <sub>9</sub> F <sub>17</sub>	PFK	
	4	445.7555	M+4	C <sub>12</sub> H <sub>2</sub> <sup>35</sup> Cl <sub>6</sub> <sup>37</sup> Cl <sub>2</sub> O	OCDFE
		407.7818	M+2	C <sub>12</sub> H <sup>35</sup> Cl <sub>6</sub> <sup>37</sup> ClO	HpCDF
			M+4	C <sub>12</sub> H <sup>35</sup> Cl <sub>5</sub> <sup>37</sup> Cl <sub>2</sub> O	HpCDF
		417.8253	M	<sup>13</sup> C <sub>12</sub> H <sup>35</sup> Cl <sub>7</sub> O	HpCDF <sup>3</sup>
419.8220		M+2	<sup>13</sup> C <sub>12</sub> H <sup>35</sup> Cl <sub>6</sub> <sup>37</sup> ClO	HpCDF <sup>3</sup>	
423.7766		M+2	C <sub>12</sub> H <sup>35</sup> Cl <sub>6</sub> <sup>37</sup> ClO <sub>2</sub>	HpCDD	
425.7737		M+4	C <sub>12</sub> H <sup>35</sup> Cl <sub>5</sub> <sup>37</sup> Cl <sub>2</sub> O <sub>2</sub>	HpCDD	
430.9729		Lock	C <sub>9</sub> F <sub>17</sub>	PFK	
435.8169		M+2	<sup>13</sup> C <sub>12</sub> H <sup>35</sup> Cl <sub>6</sub> <sup>37</sup> ClO <sub>2</sub>	HpCDD <sup>3</sup>	
437.8140		M+4	<sup>13</sup> C <sub>12</sub> H <sup>35</sup> Cl <sub>5</sub> <sup>37</sup> Cl <sub>2</sub> O <sub>2</sub>	HpCDD <sup>3</sup>	
479.7165		M+4	C <sub>12</sub> H <sup>35</sup> Cl <sub>7</sub> <sup>37</sup> Cl <sub>2</sub> O	NCDPE	

TABLE 8.—DESCRIPTORS, EXACT M/Z'S, M/Z TYPES, AND ELEMENTAL COMPOSITIONS OF THE CDDs AND CDFs—Continued

Descriptor	Exact M/Z <sup>1</sup>	M/Z type	Elemental composition	Substance <sup>2</sup>
5 .....	441.7428	M+2	C <sub>12</sub> <sup>35</sup> Cl <sub>7</sub> <sup>37</sup> ClO .....	OCDF
	442.9728	Lock	C <sub>10</sub> F <sub>17</sub> .....	PFK
	443.7399	M+4	C <sub>12</sub> <sup>35</sup> Cl <sub>6</sub> <sup>37</sup> Cl <sub>2</sub> O .....	OCDF
	457.7377	M+2	C <sub>12</sub> <sup>35</sup> Cl <sub>7</sub> <sup>37</sup> ClO <sub>2</sub> .....	OCDD
	459.7348	M+4	C <sub>12</sub> <sup>35</sup> Cl <sub>6</sub> <sup>37</sup> Cl <sub>2</sub> O <sub>2</sub> .....	OCDD
	469.7779	M+2	<sup>13</sup> C <sub>12</sub> <sup>35</sup> Cl <sub>7</sub> <sup>37</sup> ClO <sub>2</sub> .....	OCDD <sup>3</sup>
	471.7750	M+4	<sup>13</sup> C <sub>12</sub> <sup>35</sup> Cl <sub>6</sub> <sup>37</sup> Cl <sub>2</sub> O <sub>2</sub> .....	OCDD <sup>3</sup>
	513.6775	M+4	C <sub>12</sub> <sup>35</sup> Cl <sub>8</sub> <sup>37</sup> Cl <sub>2</sub> O .....	DCDPE

<sup>1</sup> Nuclidic masses used:

- H = 1.007825.
- O = 15.994915.
- C = 12.00000.
- <sup>35</sup>Cl = 34.968853.
- <sup>13</sup>C = 13.003355.
- <sup>37</sup>Cl = 36.965903.
- F = 18.9984.

<sup>2</sup> TCDD = Tetrachlorodibenzo-p-dioxin.  
 PeCDD = Pentachlorodibenzo-p-dioxin.  
 HxCDD = Hexachlorodibenzo-p-dioxin.  
 HpCDD = Heptachlorodibenzo-p-dioxin.  
 OCDD = Octachlorodibenzo-p-dioxin.  
 HxCDF = Hexachlorodibenzofuran.  
 HpCDF = Heptachlorodibenzofuran.  
 OCDF = Octachlorodibenzofuran.  
 DCDPE = Decachlorodiphenyl ether.  
 TCDF = Tetrachlorodibenzofuran.  
 PeCDF = Pentachlorodibenzofuran.  
 HxCDF = Hexachlorodibenzofuran.  
 HpCDF = Heptachlorodibenzofuran.  
 OCDF = Octachlorodibenzofuran.  
 HpCDPE = Heptachlorodiphenyl ether.  
 NCDPE = Nonachlorodiphenyl ether.  
 PFK = Perfluorokerosene.

<sup>3</sup> Labeled compound.

<sup>4</sup> There is only one m/z for <sup>37</sup>Cl<sub>4</sub>-2,3,7,8,-TCDD (cleanup standard).

TABLE 9.—THEORETICAL ION ABUNDANCE RATIOS AND QC LIMITS

Number of chlorine atoms	M/Z's forming ratio	Theoretical ratio	QC limit <sup>1</sup>	
			Lower	Upper
4 <sup>2</sup> .....	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 <sup>3</sup> .....	M/(M+2) .....	0.51	0.43	0.59
7 .....	(M+2)/(M+4) .....	1.05	0.88	1.20
7 <sup>4</sup> .....	M/(M+2) .....	0.44	0.37	0.51
8 .....	(M+2)/(M+4) .....	0.89	0.76	1.02

<sup>1</sup> QC limits represent ±15% windows around the theoretical ion abundance ratios.

<sup>2</sup> Does not apply to <sup>37</sup>Cl<sub>4</sub>-2,3,7,8-TCDD (cleanup standard).

<sup>3</sup> Used for <sup>13</sup>C<sub>12</sub>-HxCDF only.

<sup>4</sup> Used for <sup>13</sup>C<sub>12</sub>-HpCDF only.

TABLE 10.—SUGGESTED SAMPLE QUANTITIES TO BE EXTRACTED FOR VARIOUS MATRICES <sup>1</sup>

Sample Matrix <sup>2</sup>	Example	Percent solids	Phase	Quantity extracted
Single-phase: Aqueous .....	Drinking water .....	<1	(3) .....	1000 mL.
	Groundwater .....			
	Treated wastewater .....			
Solid .....	Dry soil .....	>20	Solid .....	10 g.
	Compost .....			
	Ash .....			
Organic .....	Waste solvent .....	<1	Organic .....	10 g.
	Waste oil .....			
	Organic polymer .....			
Tissue .....	Fish .....		Organic .....	10 g.
	Human adipose .....			
Multi-phase:				



TABLE 10.—SUGGESTED SAMPLE QUANTITIES TO BE EXTRACTED FOR VARIOUS MATRICES<sup>1</sup>—Continued

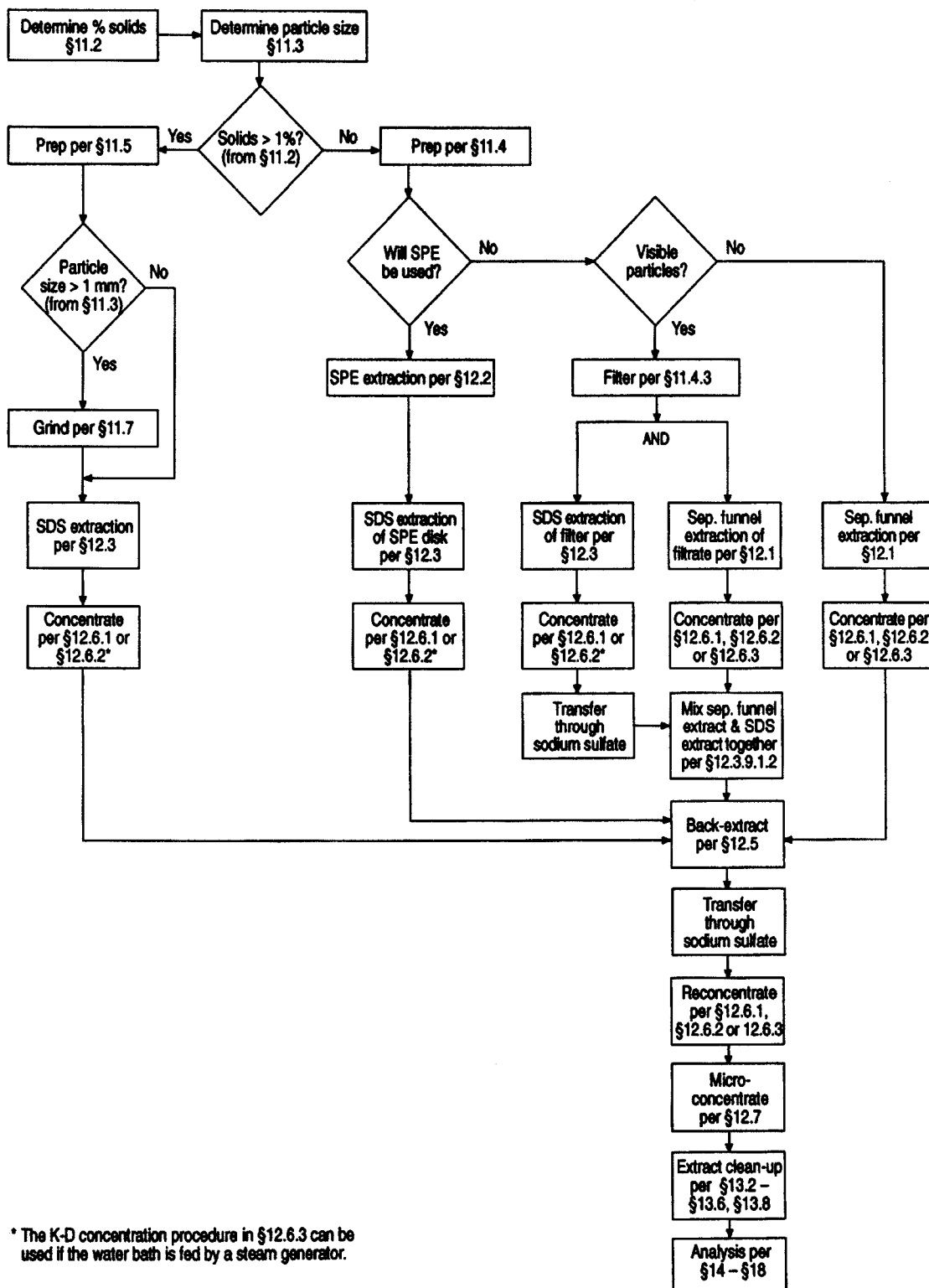
Sample Matrix <sup>2</sup>	Example	Percent solids	Phase	Quantity extracted
Liquid/Solid: Aqueous/Solid .....	Wet soil ..... Untreated effluent. Digested municipal sludge. Filter cake. Paper pulp.	1–30	Solid .....	10 g.
Organic/solid .....	Industrial sludge ..... Oily waste	1–100 .....	Both .....	10 g.
Liquid/Liquid: Aqueous/organic .....	In-process effluent ..... Untreated effluent Drum waste	<1 .....	Organic .....	10 g.
Aqueous/organic/solid .....	Untreated effluent ..... Drum waste	>1 .....	Organic and solid .....	10 g.

<sup>1</sup> The quantity of sample to be extracted is adjusted to provide 10 g of solids (dry weight). One liter of aqueous samples containing 1% solids will contain 10 g of solids. For aqueous samples containing greater than 1% solids, a lesser volume is used so that 10 g of solids (dry weight) will be extracted.

<sup>2</sup> The sample matrix may be amorphous for some samples. In general, when the CDDs/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.

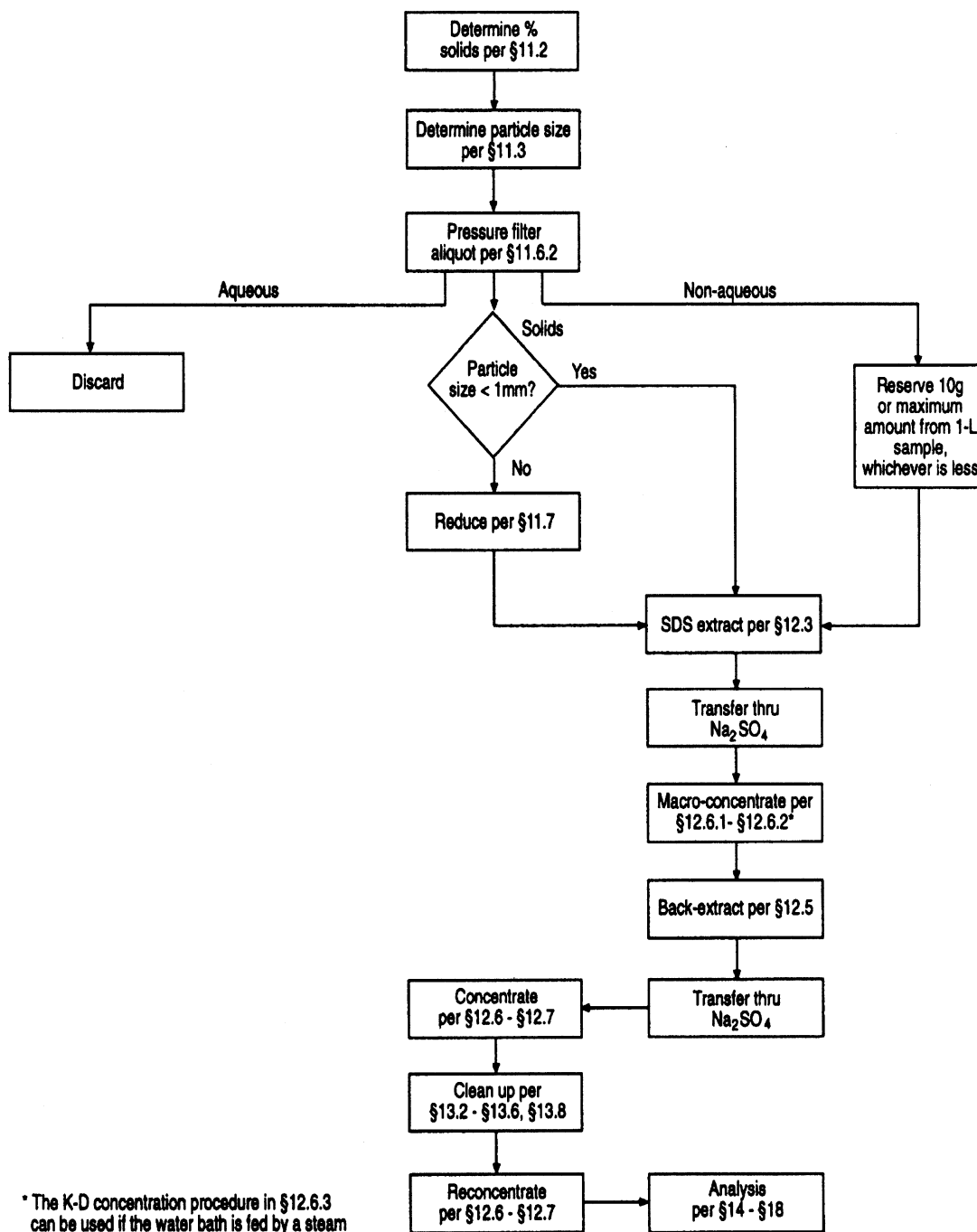
<sup>3</sup> Aqueous samples are filtered after spiking with the labeled compounds. The filtrate and the materials trapped on the filter are extracted separately, and the extracts are combined for cleanup and analysis.

BILLING CODE 6560–50–P



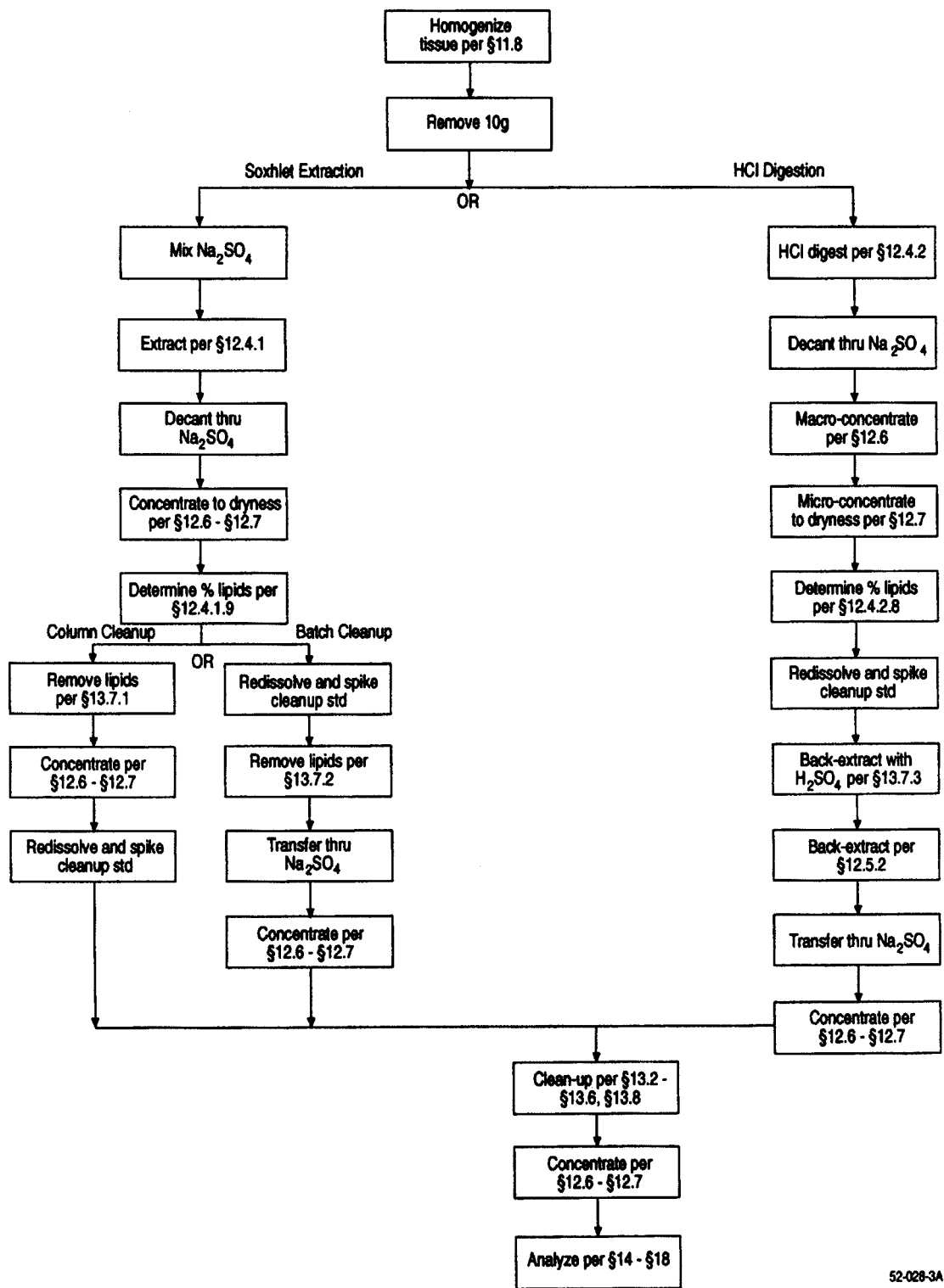
\* The K-D concentration procedure in §12.6.3 can be used if the water bath is fed by a steam generator.

Figure 1. Flow Chart for Analysis of Aqueous and Solid Samples



\* The K-D concentration procedure in §12.6.3 can be used if the water bath is fed by a steam generator.

Figure 2. Flow Chart for Analysis of Multi-Phase Samples



52-028-3A

Figure 3. Flow Chart for Analysis of Tissue Samples

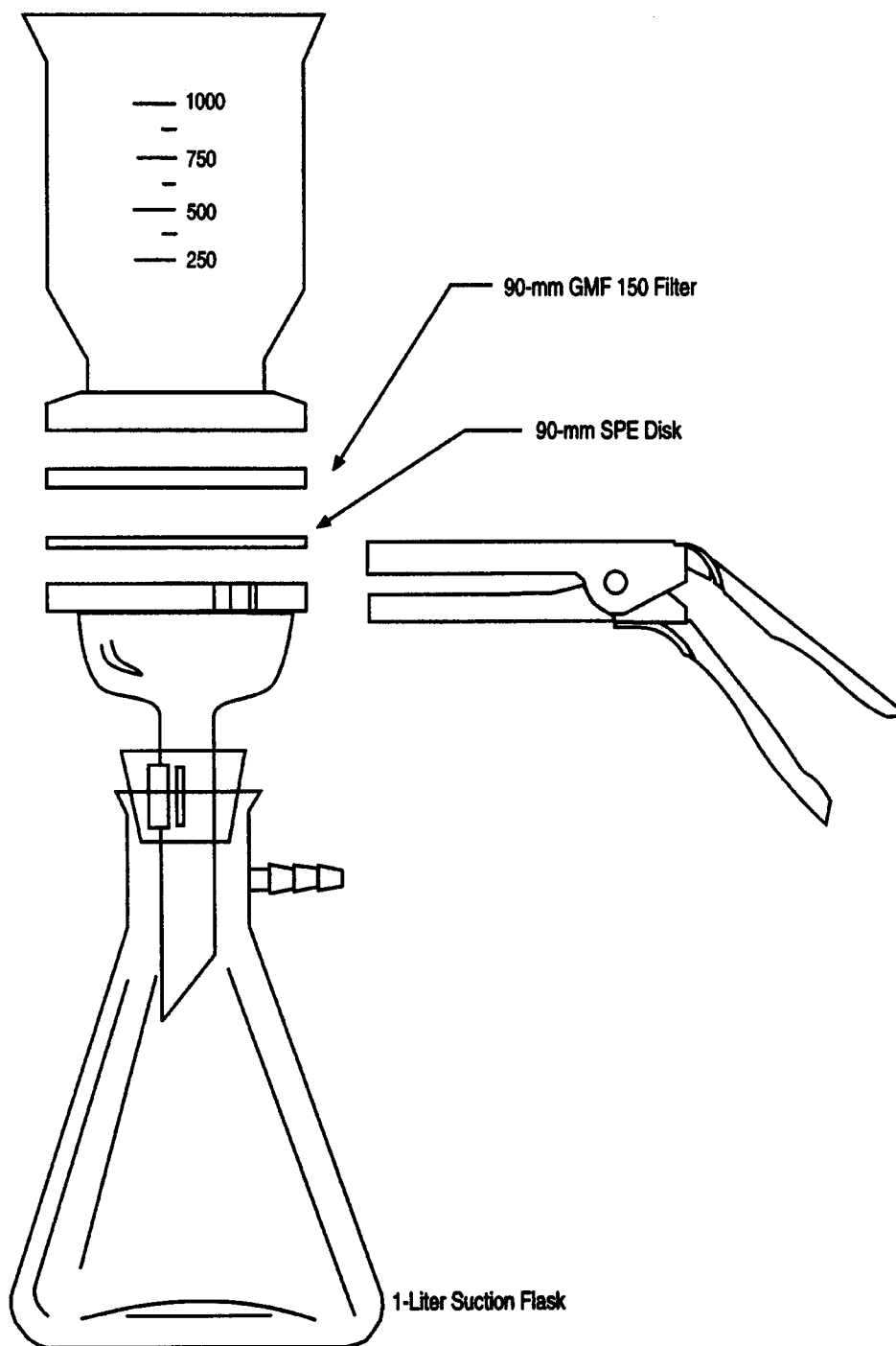
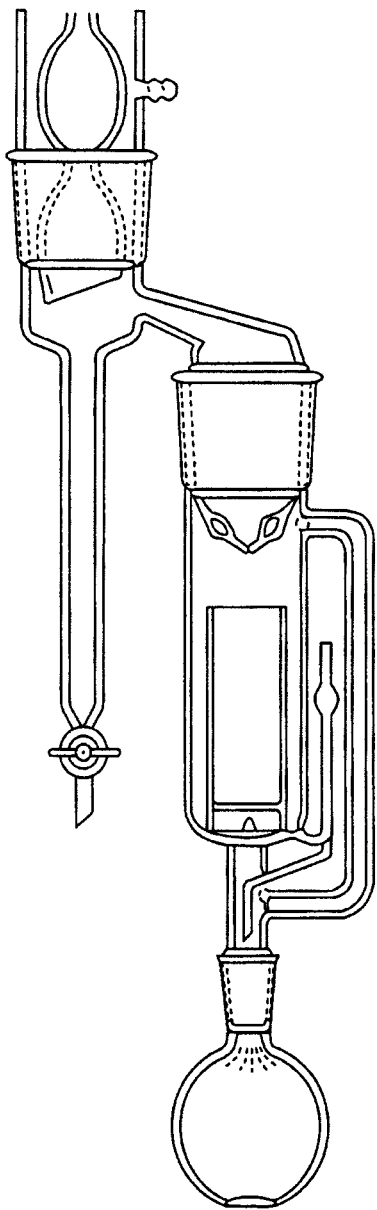


Figure 4. Solid-Phase Extraction Apparatus



52-027-2A

Figure 5. Soxhlet/Dean-Stark Extractor

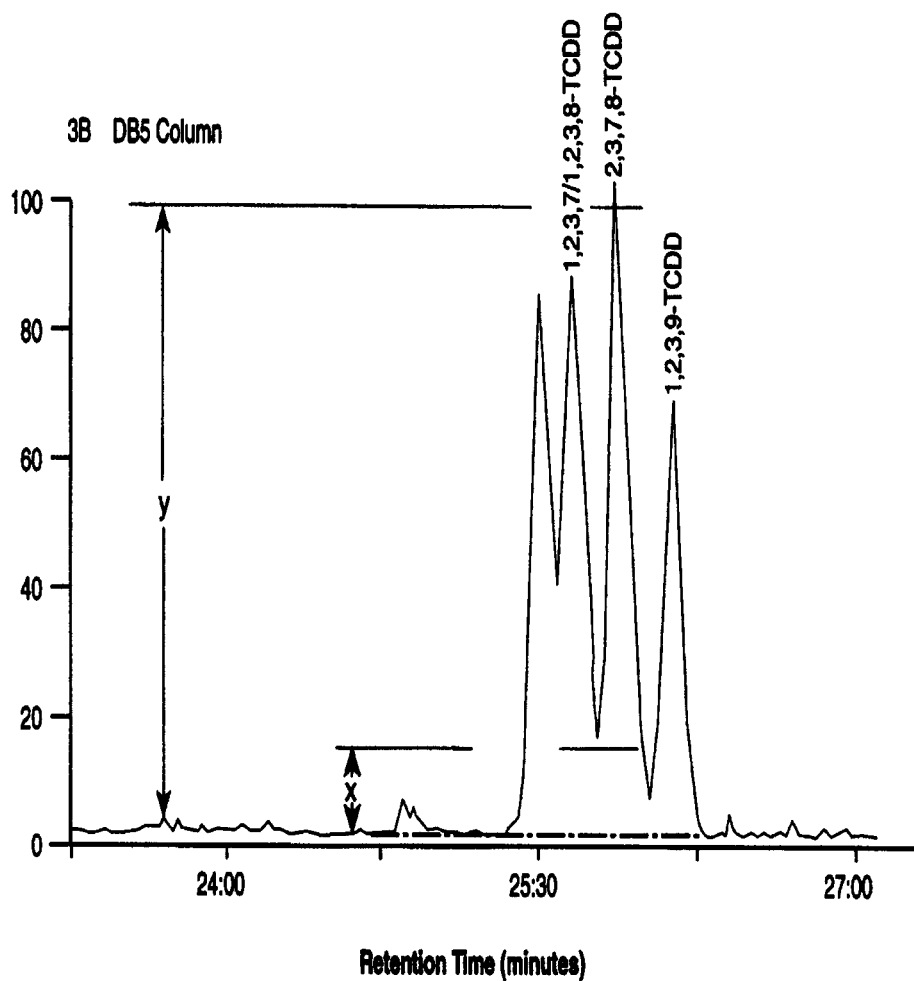


Figure 6. Isomer-Specific Separation of 2,3,7,8-TCDD on DB-5 Column

52-027-03

6-May-88      Sir: Voltage 705      Sys: DB5US  
Sample 1 Injection 1      Group 1      Mass 305.8987  
Text: Column Performance

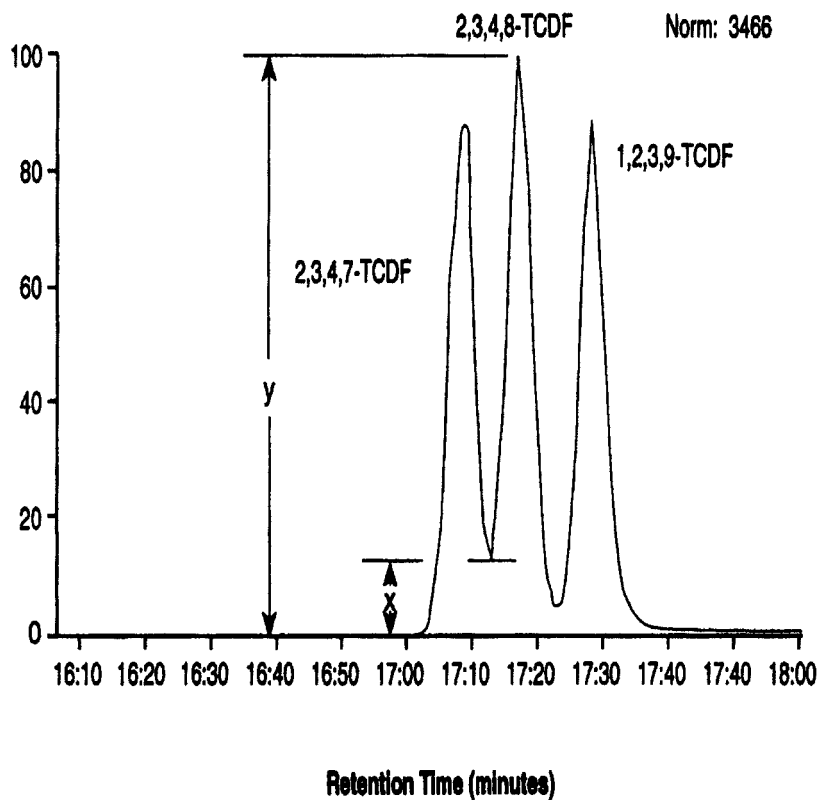


Figure 7. Isomer-Specific Separation of 2,3,7,8-TCDF on DB-5 Column

52-027-4A



## 24.0 Glossary of Definitions and Purposes

These definitions and purposes are specific to this method but have been conformed to common usage as much as possible.

### 24.1 Units of weight and Measure and Their Abbreviations.

#### 24.1.1 Symbols:

°C—degrees Celsius

μL—microliter

μm—micrometer

<—less than

>—greater than

%—percent

#### 24.1.2 Alphabetical abbreviations:

amp—ampere

cm—centimeter

g—gram

h—hour

ID—inside diameter

in.—inch

L—liter

M—Molecular ion

m—meter

mg—milligram

min—minute

mL—milliliter

mm—millimeter

m/z—mass-to-charge ratio

N—normal; gram molecular weight of solute divided by hydrogen equivalent of solute, per liter of solution

OD—outside diameter

pg—picogram

ppb—part-per-billion

ppm—part-per-million

ppq—part-per-quadrillion

ppt—part-per-trillion

psig—pounds-per-square inch gauge

v/v—volume per unit volume

w/v—weight per unit volume

### 24.2 Definitions and Acronyms (in Alphabetical Order).

Analyte—A CDD or CDF tested for by this method. The analytes are listed in Table 1.

Calibration Standard (CAL)—A solution prepared from a secondary standard and/or stock solutions and used to calibrate the response of the instrument with respect to analyte concentration.

Calibration Verification Standard (VER)—The mid-point calibration standard (CS3) that is used in to verify calibration. See Table 4.

CDD—Chlorinated Dibenzo-p-ioxin—The isomers and congeners of tetra-through octa-chlorodibenzo-p-dioxin.

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

CS1, CS2, CS3, CS4, CS5—See Calibration standards and Table 4.

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

GC—Gas chromatograph or gas chromatography.

GPC—Gel permeation chromatograph or gel permeation chromatography.

HPLC—High performance liquid chromatograph or high performance liquid chromatography.

HRGC—High resolution GC.

HRMS—High resolution MS.

IPR—Initial precision and recovery; four aliquots of the diluted PAR standard analyzed to establish the ability to generate acceptable precision and accuracy. An IPR is performed prior to the first time this method is used and any time the method or instrumentation is modified.

K-D—Kuderna-Danish concentrator; a device used to concentrate the analytes in a solvent.

Laboratory Blank—See method blank.

Laboratory Control sample (LCS)—See ongoing precision and recovery standard (OPR).

Laboratory Reagent Blank—See method blank.

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

May Not—This action, activity, or procedural step is prohibited.

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

Minimum Level (ML)—The level at which the entire analytical system must give a recognizable signal and acceptable calibration point for the analyte. It is equivalent to the concentration of the lowest calibration standard, assuming that all method-specified sample weights, volumes, and cleanup procedures have been employed.

MS—Mass spectrometer or mass spectrometry.

Must—This action, activity, or procedural step is required.

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

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

PFK—Perfluorokerosene; the mixture of compounds used to calibrate the exact m/z scale in the HRMS.

Preparation Blank—See method blank.

Primary Dilution Standard—A solution containing the specified analytes that is purchased or prepared from stock solutions and diluted as needed to prepare calibration solutions and other solutions.

Quality Control Check Sample (QCS)—A sample containing all or a subset of the analytes at known concentrations. The QCS is obtained from a source external to the laboratory or is prepared from a source of standards different from the source of calibration standards. It is used to check laboratory performance with test materials prepared external to the normal preparation process.

Reagent Water—Water demonstrated to be free from the analytes of interest and potentially interfering substances at the method detection limit for the analyte.

Relative Standard Deviation (RSD)—The standard deviation times 100 divided by the mean. Also termed "coefficient of variation."

RF—Response factor. See Section 10.6.1.

RR—Relative response. See Section 10.5.2.

RSD—See relative standard deviation.  
SDS—Soxhlet/Dean-Stark extractor; an extraction device applied to the extraction of solid and semi-solid materials (Reference 7).

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

SICP—Selected ion current profile; the line described by the signal at an exact m/z.

SPE—Solid-phase extraction; an extraction technique in which an analyte is extracted from an aqueous sample by passage over or through a material capable of reversibly adsorbing the analyte. Also termed liquid-solid extraction.

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

TCDD—Tetrachlorodibenzo-p-dioxin.

TCDF—Tetrachlorodibenzofuran.

VER—See calibration verification standard.

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