

**APPENDIX A TO PART 136
METHODS FOR ORGANIC CHEMICAL ANALYSIS OF MUNICIPAL AND
INDUSTRIAL WASTEWATER**

METHOD 613—2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

1. Scope and Application

1.1 This method covers the determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD). The following parameter may be determined by this method:

Parameter	STORET No.	CAS No.
2,3,7,8-TCDD	34675	1746-01-6

1.2 This is a gas chromatographic/mass spectrometer (GC/MS) method applicable to the determination of 2,3,7,8-TCDD in municipal and industrial discharges as provided under 40 CFR Part 136.1. Method 625 may be used to screen samples for 2,3,7,8-TCDD. When the screening test is positive, the final qualitative confirmation and quantification must be made using Method 613.

1.3 The method detection limit (MDL, defined in Section 14.1)¹ for 2,3,7,8-TCDD is listed in Table 1. The MDL for a specific wastewater may be different from that listed, depending upon the nature of interferences in the sample matrix.

1.4 Because of the extreme toxicity of this compound, the analyst must prevent exposure to himself, of to others, by materials known or believed to contain 2,3,7,8-TCDD. Section 4 of this method contains guidelines and protocols that serve as minimum safe-handling standards in a limited-access laboratory.

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

1.6 This method is restricted to use by or under the supervision of analysts experienced in the use of a gas chromatograph/mass spectrometer and in the interpretation of mass spectra. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 A measured volume of sample, approximately 1 L, is spiked with an internal standard of labeled 2,3,7,8-TCDD and extracted with methylene chloride using a separatory funnel. The methylene chloride extract is exchanged to hexane during concentration to a volume of 1.0 mL or less. The extract is then analyzed by capillary column GC/MS to separate and measure 2,3,7,8-TCDD.2,3

- 2.2 The method provides selected column chromatographic cleanup procedures to aid in the elimination of interferences that may be encountered.

3. Interferences

- 3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated backgrounds at the masses (m/z) monitored. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.1.3.

3.1.1 Glassware must be scrupulously cleaned.⁴ Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. Solvent rinsing should be followed by detergent washing with hot water, and rinses with tap water and distilled water. The glassware should then be drained dry, and heated in a muffle furnace at 400°C for 15-30 minutes. Some thermally stable materials, such as PCBs, may not be eliminated by the treatment. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. Thorough rinsing with such solvents usually eliminates PCB interference. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

- 3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. 2,3,7,8-TCDD is often associated with other interfering chlorinated compounds which are at concentrations several magnitudes higher than that of 2,3,7,8-TCDD. The cleanup procedures in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches^{1,5-7} to eliminate false positives and achieve the MDL listed in Table 1.

- 3.3 The primary column, SP-2330 or equivalent, resolves 2,3,7,8-TCDD from the other 21 TCDD isomers. Positive results using any other gas chromatographic column must be confirmed using the primary column.

4. Safety

- 4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. 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 data handling sheets should also be made available to all personnel involved in the

chemical analysis. Additional references to laboratory safety are available and have been identified⁸⁻¹⁰ for the information of the analyst. Benzene and 2,3,7,8-TCDD have been identified as suspected human or mammalian carcinogens.

4.2 Each laboratory must develop a strict safety program for handling 2,3,7,8-TCDD. The following laboratory practices are recommended:

- 4.2.1 Contamination of the laboratory will be minimized by conducting all manipulations in a hood.
- 4.2.2 The effluents of sample splitters for the gas chromatograph and roughing pumps on the GC/MS should pass through either a column of activated charcoal or be bubbled through a trap containing oil or high-boiling alcohols.
- 4.2.3 Liquid waste should be dissolved in methanol or ethanol and irradiated with ultraviolet light with a wavelength greater than 290 nm for several days. (Use F 40 BL lamps or equivalent). Analyze liquid wastes and dispose of the solutions when 2,3,7,8-TCDD can no longer be detected.

4.3 Dow Chemical U.S.A. has issued the following precautions (revised November 1978) for safe handling of 2,3,7,8-TCDD in the laboratory:

4.3.1 The following statements on safe handling are as complete as possible on the basis of available toxicological information. The precautions for safe handling and use are necessarily general in nature since detailed, specific recommendations can be made only for the particular exposure and circumstances of each individual use. Inquiries about specific operations or uses may be addressed to the Dow Chemical Company. Assistance in evaluating the health hazards of particular plant conditions may be obtained from certain consulting laboratories and from State Departments of Health or of Labor, many of which have an industrial health service. 2,3,7,8-TCDD is extremely toxic to laboratory animals. However, it has been handled for years without injury in analytical and biological laboratories. Techniques used in handling radioactive and infectious materials are applicable to 2,3,7,8-TCDD.

4.3.1.1 Protective equipment—Throw-away plastic gloves, apron or lab coat, safety glasses, and a lab hood adequate for radioactive work.

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

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

4.3.1.4 Confinement—Isolated work area, posted with signs, segregated glassware and tools, plastic-backed absorbent paper on benchtops.

- 4.3.1.5 Waste—Good technique includes minimizing contaminated waste. Plastic bag liners should be used in waste cans. Janitors must be trained in the safe handling of waste.
- 4.3.1.6 Disposal of wastes—2,3,7,8-TCDD decomposes above 800°C. Low-level waste such as absorbent paper, tissues, animal remains, and plastic gloves may be burned in a good incinerator. Gross quantities (milligrams) should be packaged securely and disposed through commercial or governmental channels which are capable of handling high-level radioactive wastes or extremely toxic wastes. Liquids should be allowed to evaporate in a good hood and in a disposable container. Residues may then be handled as above.
- 4.3.1.7 Decontamination—For personal decontamination, use any mild soap with plenty of scrubbing action. For decontamination of glassware, tools, and surfaces, Chlorothene NU Solvent (Trademark of the Dow Chemical Company) is the least toxic solvent shown to be effective. Satisfactory cleaning may be accomplished by rinsing with Chlorothene, then washing with any detergent and water. Dishwater may be disposed to the sewer. It is prudent to minimize solvent wastes because they may require special disposal through commercial sources which are expensive.
- 4.3.1.8 Laundry—Clothing known to be contaminated should be disposed with the precautions described under Section 4.3.1.6. Lab coats or other clothing worn in 2,3,7,8-TCDD work areas may be laundered.

Clothing 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 the problem. The washer should be run through a cycle before being used again for other clothing.

- 4.3.1.9 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 gas chromatography can achieve a limit of sensitivity of 0.1 µg per wipe. Less than 1 µg of 2,3,7,8-TCDD per sample indicates acceptable cleanliness; anything higher warrants further cleaning. More than 10 µg on a wipe sample constitutes an acute hazard and requires prompt cleaning before further use of the equipment or work space. A high (10 µg) 2,3,7,8-TCDD level indicates that unacceptable work practices have been employed in the past.
- 4.3.1.10 Inhalation—Any procedure that may produce airborne contamination must be done with good ventilation. Gross losses to a ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical and animal work presents no inhalation hazards except in the case of an accident.

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

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle—1 L or 1 qt, amber glass, fitted with a screw cap lined with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The bottle and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional)—The sampler must incorporate glass sample containers for the collection of a minimum of 250 mL of sample. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.1.3 Clearly label all samples as “POISON” and ship according to U.S. Department of Transportation regulations.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only)

5.2.1 Separatory funnels—2 L and 125 mL, with Teflon stopcock.

5.2.2 Concentrator tube, Kuderna-Danish—10 mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.3 Evaporative flask, Kuderna-Danish—500 mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.4 Snyder column, Kuderna-Danish—Three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.5 Snyder column, Kuderna-Danish—Two-ball micro (Kontes K-569001-0219 or equivalent).

5.2.6 Vials—10-15 mL, amber glass, with Teflon-lined screw cap.

5.2.7 Chromatographic column—300 mm long x 10 mm ID, with Teflon stopcock and coarse frit filter disc at bottom.

- 5.2.8 Chromatographic column—400 mm long x 11 mm ID, with Teflon stopcock and coarse frit filter disc at bottom.
- 5.3 Boiling chips—Approximately 10/40 mesh. Heat to 400°C for 30 minutes or Soxhlet extract with methylene chloride.
- 5.4 Water bath—Heated, with concentric ring cover, capable of temperature control ($\pm 2^\circ\text{C}$). The bath should be used in a hood.
- 5.5 GC/MS system
- 5.5.1 Gas chromatograph—An analytical system complete with a temperature programmable gas chromatograph and all required accessories including syringes, analytical columns, and gases. The injection port must be designed for capillary columns. Either split, splitless, or on-column injection techniques may be employed, as long as the requirements of Section 7.1.1 are achieved.
- 5.5.2 Column—60 m long x 0.25 mm ID glass or fused silica, coated with SP-2330 (or equivalent) with a film thickness of 0.2 μm . Any equivalent column must resolve 2, 3, 7, 8-TCDD from the other 21 TCDD isomers.¹⁶
- 5.5.3 Mass spectrometer—Either a low resolution mass spectrometer (LRMS) or a high resolution mass spectrometer (HRMS) may be used. The mass spectrometer must be equipped with a 70 V (nominal) ion source and be capable of acquiring m/z abundance data in real time selected ion monitoring (SIM) for groups of four or more masses.
- 5.5.4 GC/MS interface—Any GC to MS interface can be used that achieves the requirements of Section 7.1.1. GC to MS interfaces constructed of all glass or glass-lined materials are recommended. Glass surfaces can be deactivated by silanizing with dichlorodimethylsilane. To achieve maximum sensitivity, the exit end of the capillary column should be placed in the ion source. A short piece of fused silica capillary can be used as the interface to overcome problems associated with straightening the exit end of glass capillary columns.
- 5.5.5 The SIM data acquired during the chromatographic program is defined as the Selected Ion Current Profile (SICP). The SICP can be acquired under computer control or as a real time analog output. If computer control is used, there must be software available to plot the SICP and report peak height or area data for any m/z in the SICP between specified time or scan number limits.
- 5.6 Balance—Analytical, capable of accurately weighing 0.0001 g.
- 6. Reagents**
- 6.1 Reagent water—Reagent water is defined as a water in which an interferent is not observed at the MDL of 2, 3, 7, 8-TCDD.

- 6.2 Sodium hydroxide solution (10 N)—Dissolve 40 g of NaOH (ACS) in reagent water and dilute to 100 mL. Wash the solution with methylene chloride and hexane before use.
- 6.3 Sodium thiosulfate—(ACS) Granular.
- 6.4 Sulfuric acid—Concentrated (ACS, sp. gr. 1.84).
- 6.5 Acetone, methylene chloride, hexane, benzene, ortho-xylene, tetradecane—Pesticide quality or equivalent.
- 6.6 Sodium sulfate—(ACS) Granular, anhydrous. Purify by heating at 400°C for four hours in a shallow tray.
- 6.7 Alumina—Neutral, 80/200 mesh (Fisher Scientific Co., No. A-540 or equivalent). Before use, activate for 24 hours at 130°C in a foil-covered glass container.
- 6.8 Silica gel—High purity grade, 100/120 mesh (Fisher Scientific Co., No. S-679 or equivalent).
- 6.9 Stock standard solutions (1.00 µg/µL)—Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions. Acetone should be used as the solvent for spiking solutions; ortho-xylene is recommended for calibration standards for split injectors; and tetradecane is recommended for splitless or on-column injectors. Analyze stock internal standards to verify the absence of native 2,3,7,8-TCDD.
- 6.9.1 Prepare stock standard solutions of 2,3,7,8-TCDD (mol wt 320) and either 37C14 2,3,7,8-TCDD (mol wt 328) or 13KC112K 2,3,7,8-TCDD (mol wt 332) in an isolated area by accurately weighing about 0.0100 g of pure material. Dissolve the material in pesticide quality solvent and dilute to volume in a 10 mL volumetric flask. When compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.
- 6.9.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store in an isolated refrigerator protected from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards or spiking solutions from them.
- 6.9.3 Stock standard solutions must be replaced after six months, or sooner if comparison with check standards indicates a problem.
- 6.10 Internal standard spiking solution (25 ng/mL)—Using stock standard solution, prepare a spiking solution in acetone of either 13KC112K or 37KC14K 2,3,7,8-TCDD at a concentration of 25 ng/mL. (See Section 10.2)
- 6.11 Quality control check sample concentrate—See Section 8.2.1.

7. Calibration

7.1 Establish gas chromatographic operating conditions equivalent to those given in Table 1 and SIM conditions for the mass spectrometer as described in Section 12.2. The GC/MS system must be calibrated using the internal standard technique.

7.1.1 Using stock standards, prepare calibration standards that will allow measurement of relative response factors of at least three concentration ratios of 2,3,7,8-TCDD to internal standard. Each calibration standard must be prepared to contain the internal standard at a concentration of 25 ng/mL. If any interferences are contributed by the internal standard at m/z 320 and 322, its concentration may be reduced in the calibration standards and in the internal standard spiking solution (Section 6.10). One of the calibration standards should contain 2,3,7,8-TCDD at a concentration near, but above, the MDL and the other 2,3,7,8-TCDD concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the GC/MS system.

7.1.2 Using injections of 2-5 µL, analyze each calibration standard according to Section 12 and tabulate peak height or area response against the concentration of 2,3,7,8-TCDD and internal standard. Calculate response factors (RF) for 2,3,7,8-TCDD using Equation 1.

Equation 1

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

where:

A_s = SIM response for 2,3,7,8-TCDD m/z 320.

A_{is} = SIM response for the internal standard, m/z 332 for ^{13}C 2,3,7,8-TCDD m/z 328 for $^{37}Cl_4$ 2,3,7,8-TCDD.

C_{is} = Concentration of the internal standard (µg/L).

C_s = Concentration of 2,3,7,8-TCDD (µg/L).

If the RF value over the working range is a constant (<10% relative standard deviation, RSD), the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is} , vs. concentration ratios C_s/C_{is} .

7.1.3 The working calibration curve or RF must be verified on each working day by the measurement of one or more 2,3,7,8-TCDD calibration standards. If the response for 2,3,7,8-TCDD varies from the predicted response by more than

*This equation corrects an error made in the original method publication (49 FR 43234, October 26, 1984). This correction will be formalized through a rulemaking in FY97.

±15%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared.

7.2 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. Quality Control

8.1 Each laboratory that uses this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and an ongoing analysis of spiked samples to evaluate and document data quality. The laboratory must maintain records to document the quality of data that is generated. Ongoing data quality checks are compared with established performance criteria to determine if the results of analyses meet the performance characteristics of the method. When results of sample spikes indicate atypical method performance, a quality control check standard must be analyzed to confirm that the measurements were performed in an in-control mode of operation.

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

8.1.2 In recognition of advances that are occurring in chromatography, the analyst is permitted certain options (detailed in Sections 10.5, 11.1, and 12.1) to improve the separations or lower the cost of measurements. Each time such a modification is made to the method, the analyst is required to repeat the procedure in Section 8.2

8.1.3 Before processing any samples, the analyst must analyze a reagent water blank to demonstrate that interferences from the analytical system and glassware are under control. Each time a set of samples is extracted or reagents are changed, a reagent water blank must be processed as a safeguard against laboratory contamination.

8.1.4 The laboratory must, on an ongoing basis, spike and analyze a minimum of 10% of all samples with native 2,3,7,8-TCDD to monitor and evaluate laboratory data quality. This procedure is described in Section 8.3.

8.1.5 The laboratory must, on an ongoing basis, demonstrate through the analyses of quality control check standards that the operation of the measurement system is in control. This procedure is described in Section 8.4. The frequency of the check standard analyses is equivalent to 10% of all samples analyzed but may be reduced if spike recoveries from samples (Section 8.3) meet all specified quality control criteria.

8.1.6 The laboratory must maintain performance records to document the quality of data that is generated. This procedure is described in Section 8.5.

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

8.2.1 A quality control (QC) check sample concentrate is required containing 2,3,7,8-TCDD at a concentration of 0.100 µg/mL in acetone. The QC check sample concentrate must be obtained from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory in Cincinnati, Ohio, if available. If not available from that source, the QC check sample concentrate must be obtained from another external source. If not available from either source above, the QC check sample concentrate must be prepared by the laboratory using stock standards prepared independently from those used for calibration.

8.2.2 Using a pipet, prepare QC check samples at a concentration of 0.100 µg/L (100 ng/L) by adding 1.00 mL of QC check sample concentrate to each of four 1 L aliquots of reagent water.

8.2.3 Analyze the well-mixed QC check samples according to the method beginning in Section 10.

8.2.4 Calculate the average recovery (\bar{X}) in µg/L, and the standard deviation of the recovery (s) in µg/L, for 2,3,7,8-TCDD using the four results.

8.2.5 Compare s and (\bar{X}) with the corresponding acceptance criteria for precision and accuracy, respectively, found in Table 2. If s and \bar{X} meet the acceptance criteria, the system performance is acceptable and analysis of actual samples can begin. If s exceeds the precision limit or \bar{X} falls outside the range for accuracy, the system performance is unacceptable for 2,3,7,8-TCDD. Locate and correct the source of the problem and repeat the test beginning with Section 8.2.2.

8.3 The laboratory must, on an ongoing basis, spike at least 10% of the samples from each sample site being monitored to assess accuracy. For laboratories analyzing one to ten samples per month, at least one spiked sample per month is required.

8.3.1 The concentration of the spike in the sample should be determined as follows:

8.3.1.1 If, as in compliance monitoring, the concentration of 2,3,7,8-TCDD in the sample is being checked against a regulatory concentration limit, the spike should be at that limit or one to five times higher than the background concentration determined in Section 8.3.2, whichever concentration would be larger.

8.3.1.2 If the concentration of 2,3,7,8-TCDD in the sample is not being checked against a limit specific to that parameter, the spike should be at 0.100 µg/L or one to five times higher than the background concentration determined in Section 8.3.2, whichever concentration would be larger.

8.3.1.3 If it is impractical to determine background levels before spiking (e.g., maximum holding times will be exceeded), the spike concentration should be (1) the regulatory concentration limit, if any; or, if none (2) the larger of either five times higher than the expected background concentration or 0.100 µg/L.

8.3.2 Analyze one sample aliquot to determine the background concentration (B) of 2,3,7,8-TCDD. If necessary, prepare a new QC check sample concentrate (Section 8.2.1) appropriate for the background concentration in the sample. Spike a second sample aliquot with 1.0 mL of the QC check sample concentrate and analyze it to determine the concentration after spiking (A) of 2,3,7,8-TCDD. Calculate percent recovery (P) as $100 (A-B)/T$, where T is the known true value of the spike.

8.3.3 Compare the percent recovery (P) for 2,3,7,8-TCDD with the corresponding QC acceptance criteria found in Table 2. These acceptance criteria were calculated to include an allowance for error in measurement of both the background and spike concentrations, assuming a spike to background ratio of 5:1. This error will be accounted for to the extent that the analyst's spike to background ratio approaches 5:1.¹¹ If spiking was performed at a concentration lower than 0.100 µg/L, the analyst must use either the QC acceptance criteria in Table 2, or optional QC acceptance criteria calculated for the specific spike concentration. To calculate optional acceptance criteria for the recovery of 2,3,7,8-TCDD: (1) Calculate accuracy (X') using the equation in Table 3, substituting the spike concentration (T) for C; (2) calculate overall precision (S') using the equation in Table 3, substituting X' for \bar{X} ; (3) calculate the range for recovery at the spike concentration as $(100 X'/T) \pm 2.44(100 S'/T)\%$.¹¹

8.3.4 If the recovery of 2,3,7,8-TCDD falls outside the designated range for recovery, a check standard must be analyzed as described in Section 8.4.

8.4 If the recovery of 2,3,7,8-TCDD fails the acceptance criteria for recovery in Section 8.3, a QC check standard must be prepared and analyzed.

NOTE: The frequency for the required analysis of a QC check standard will depend upon the complexity of the sample matrix and the performance of the laboratory.

8.4.1 Prepare the QC check standard by adding 1.0 mL of QC check sample concentrate (Section 8.2.1 or 8.3.2) to 1 L of reagent water.

8.4.2 Analyze the QC check standard to determine the concentration measured (A) of 2,3,7,8-TCDD. Calculate the percent recovery (P_sK) as $100 (A/T)\%$, where T is the true value of the standard concentration.

8.4.3 Compare the percent recovery (P_sK) with the corresponding QC acceptance criteria found in Table 2. If the recovery of 2,3,7,8-TCDD falls outside the designated range, the laboratory performance is judged to be out of control, and the problem must be immediately identified and corrected. The analytical

result for 2,3,7,8-TCDD in the unspiked sample is suspect and may not be reported for regulatory compliance purposes.

- 8.5 As part of the QC program for the laboratory, method accuracy for wastewater samples must be assessed and records must be maintained. After the analysis of five spiked wastewater samples as in Section 8.3, calculate the average percent recovery (P) and the standard deviation of the percent recovery (s_p). Express the accuracy assessment as a percent recovery interval from $P-2s_p$ to $P+2s_p$. If $P=90\%$ and $s_p=10\%$, for example, the accuracy interval is expressed as 70-110%. Update the accuracy assessment on a regular basis (e.g., after each 5-10 new accuracy measurements).
- 8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to assess the precision of the environmental measurements. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

- 9.1 Grab samples must be collected in glass containers. Conventional sampling practices¹² should be followed, except that the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of Tygon tubing and other potential sources of contamination.
- 9.2 All samples must be iced or refrigerated at 4°C and protected from light from the time of collection until extraction. Fill the sample bottles and, if residual chlorine is present, add 80 mg of sodium thiosulfate per liter of sample and mix well. EPA Methods 330.4 and 330.5 may be used for measurement of residual chlorine.¹³ Field test kits are available for this purpose.
- 9.3 Label all samples and containers "POISON" and ship according to applicable U.S. Department of Transportation regulations.
- 9.4 All samples must be extracted within seven days of collection and completely analyzed within 40 days of extraction.²

10. Sample Extraction

CAUTION: When using this method to analyze for 2,3,7,8-TCDD, all of the following operations must be performed in a limited-access laboratory with the analyst wearing full protective covering for all exposed skin surfaces. See Section 4.2.

- 10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2 L separatory funnel.

- 10.2 Add 1.00 mL of internal standard spiking solution to the sample in the separatory funnel. If the final extract will be concentrated to a fixed volume below 1.00 mL (Section 12.3), only that volume of spiking solution should be added to the sample so that the final extract will contain 25 ng/mL of internal standard at the time of analysis.
- 10.3 Add 60 mL of methylene chloride to the sample bottle, seal, and shake 30 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 to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250 mL Erlenmeyer flask.
- 10.4 Add a second 60 mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.
- 10.5 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10 mL concentrator tube to a 500 mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D concentrator if the requirements of Section 8.2 are met.
- 10.6 Pour the combined extract into the K-D concentrator. Rinse the Erlenmeyer flask with 20-30 mL of methylene chloride to complete the quantitative transfer.
- 10.7 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL of methylene chloride to the top. Place the K-D apparatus on a hot water bath (60-65°C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. 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 with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.
- 10.8 Momentarily remove the Snyder column, add 50 mL of hexane and a new boiling chip, and reattach the Snyder column. Raise the temperature of the water bath to 85-90°C. Concentrate the extract as in Section 10.7, except use hexane to prewet the column. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1-2 mL of hexane. A 5 mL syringe is recommended for this operation. Set aside the K-D glassware for reuse in Section 10.14.
- 10.9 Pour the hexane extract from the concentrator tube into a 125 mL separatory funnel. Rinse the concentrator tube four times with 10 mL aliquots of hexane. Combine all rinses in the 125 mL separatory funnel.

- 10.10 Add 50 mL of sodium hydroxide solution to the funnel and shake for 30-60 seconds. Discard the aqueous phase.
- 10.11 Perform a second wash of the organic layer with 50 mL of reagent water. Discard the aqueous phase.
- 10.12 Wash the hexane layer with a least two 50 mL aliquots of concentrated sulfuric acid. Continue washing the hexane layer with 50 mL aliquots of concentrated sulfuric acid until the acid layer remains colorless. Discard all acid fractions.
- 10.13 Wash the hexane layer with two 50 mL aliquots of reagent water. Discard the aqueous phases.
- 10.14 Transfer the hexane extract into a 125 mL Erlenmeyer flask containing 1-2 g of anhydrous sodium sulfate. Swirl the flask for 30 seconds and decant the hexane extract into the reassembled K-D apparatus. Complete the quantitative transfer with two 10 mL hexane rinses of the Erlenmeyer flask.
- 10.15 Replace the one or two clean boiling chips and concentrate the extract to 6-10 mL as in Section 10.8.
- 10.16 Add a clean boiling chip to the concentrator tube and attach a two-ball micro-Snyder column. Prewet the column by adding about 1 mL of hexane to the top. Place the micro-K-D apparatus on the water bath so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus 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. When the apparent volume of liquid reaches about 0.5 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with 0.2 mL of hexane.

Adjust the extract volume to 1.0 mL with hexane. Stopper the concentrator tube and store refrigerated and protected from light if further processing will not be performed immediately. If the extract will be stored longer than two days, it should be transferred to a Teflon-sealed screw-cap vial. If the sample extract requires no further cleanup, proceed with GC/MS analysis (Section 12). If the sample requires further cleanup, proceed to Section 11.

- 10.17 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000 mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

- 11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. If particular circumstances demand the use of a cleanup procedure, the analyst may use either procedure below or any other appropriate procedure.^{1,5-7} However, the analyst first must demonstrate that the requirements of Section 8.2 can be met using the method as revised to incorporate the cleanup procedure. Two cleanup column

options are offered to the analyst in this section. The alumina column should be used first to overcome interferences. If background problems are still encountered, the silica gel column may be helpful.

11.2 Alumina column cleanup for 2,3,7,8-TCDD

11.2.1 Fill a 300 mm long x 10 mm ID chromatographic column with activated alumina to the 150 mm level. Tap the column gently to settle the alumina and add 10 mm of anhydrous sodium sulfate to the top.

11.2.2 Preelute the column with 50 mL of hexane. Adjust the elution rate to 1 mL/min. Discard the eluate and just prior to exposure of the sodium sulfate layer to the air, quantitatively transfer the 1.0 mL sample extract onto the column using two 2 mL portions of hexane to complete the transfer.

11.2.3 Just prior to exposure of the sodium sulfate layer to the air, add 50 mL of 3% methylene chloride/95% hexane (V/V) and continue the elution of the column. Discard the eluate.

11.2.4 Next, elute the column with 50 mL of 20% methylene chloride/80% hexane (V/V) into a 500 mL K-D flask equipped with a 10 mL concentrator tube. Concentrate the collected fraction to 1.0 mL as in Section 10.16 and analyze by GC/MS (Section 12).

11.3 Silica gel column cleanup for 2,3,7,8-TCDD

11.3.1 Fill a 400 mm long x 11 mm ID chromatographic column with silica gel to the 300 mm level. Tap the column gently to settle the silica gel and add 10 mm of anhydrous sodium sulfate to the top.

11.3.2 Preelute the column with 50 mL of 20% benzene/80% hexane (V/V). Adjust the elution rate to 1 mL/min. Discard the eluate and just prior to exposure of the sodium sulfate layer to the air, quantitatively transfer the 1.0 mL sample extract onto the column using two 2 mL portions of 20% benzene/80% hexane to complete the transfer.

11.3.3 Just prior to exposure of the sodium sulfate layer to the air, add 40 mL of 20% benzene/80% hexane to the column. Collect the eluate in a clean 500 mL K-D flask equipped with a 10 mL concentrator tube. Concentrate the collected fraction to 1.0 mL as in Section 10.16 and analyze by GC/MS.

12. GC/MS Analysis

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are retention times and MDL that can be achieved under these conditions. Other capillary columns or chromatographic conditions may be used if the requirements of Sections 5.5.2 and 8.2 are met.

- 12.2 Analyze standards and samples with the mass spectrometer operating in the selected ion monitoring (SIM) mode using a dwell time to give at least seven points per peak. For LRMS, use masses at m/z 320, 322, and 257 for 2,3,7,8-TCDD and either m/z 328 for $^{37}\text{Cl}_4$ 2,3,7,8-TCDD or m/z 332 for $^{13}\text{C}_{12}$ 2,3,7,8-TCDD. For HRMS, use masses at m/z 319.8965 and 321.8936 for 2,3,7,8-TCDD and either m/z 327.8847 for $^{37}\text{Cl}_4$ 2,3,7,8-TCDD or m/z 331.9367 for $^{13}\text{C}_{12}$ 2,3,7,8-TCDD.
- 12.3 If lower detection limits are required, the extract may be carefully evaporated to dryness under a gentle stream of nitrogen with the concentrator tube in a water bath at about 40°C. Conduct this operation immediately before GC/MS analysis. Redissolve the extract in the desired final volume of ortho-xylene or tetradecane.
- 12.4 Calibrate the system daily as described in Section 7.
- 12.5 Inject 2-5 µL of the sample extract into the gas chromatograph. The volume of calibration standard injected must be measured, or be the same as all sample injection volumes.
- 12.6 The presence of 2,3,7,8-TCDD is qualitatively confirmed if all of the following criteria are achieved:
- 12.6.1 The gas chromatographic column must resolve 2,3,7,8-TCDD from the other 21 TCDD isomers.
- 12.6.2 The masses for native 2,3,7,8-TCDD (LRMS-m/z 320, 322, and 257 and HRMS-m/z 320 and 322) and labeled 2,3,7,8-TCDD (m/z 328 or 332) must exhibit a simultaneous maximum at a retention time that matches that of native 2,3,7,8-TCDD in the calibration standard, with the performance specifications of the analytical system.
- 12.6.3 The chlorine isotope ratio at m/z 320 and m/z 322 must agree to within ±10% of that in the calibration standard.
- 12.6.4 The signal of all peaks must be greater than two and one-half times the noise level.
- 12.7 For quantitation, measure the response of the m/z 320 peak for 2,3,7,8-TCDD and the m/z 332 peak for $^{13}\text{C}_{12}$ 2,3,7,8-TCDD or the m/z 328 peak for $^{37}\text{Cl}_4$ 2,3,7,8-TCDD.
- 12.8 Co-eluting impurities are suspected if all criteria are achieved except those in Section 12.6.3. In this case, another SIM analysis using masses at m/z 257, 259, 320 and either m/a 328 or m/z 322 can be performed. The masses at m/z 257 and m/z 259 are indicative of the loss of one chlorine and one carbonyl group from 2,3,7,8-TCDD. If masses m/z 257 and m/z 259 give a chlorine isotope ratio that agrees to within ±10% of the same cluster in the calibration standards, then the presence of TCDD can be confirmed. Co-eluting DDD, DDE, and PCB residues can be confirmed, but will require another injection using the appropriate SIM masses or full repetitive mass scans. If the response for $^{37}\text{Cl}_4$ 2,3,7,8-TCDD at m/z 328 is too large, PCB contamination is suspected and can be confirmed by examining the response at both m/z 326 and m/z 328. The $^{37}\text{Cl}_4$ 2,3,7,8-TCDD internal standard gives negligible

response at m/z 326. These pesticide residues can be removed using the alumina column cleanup procedure.

- 12.9 If broad background interference restricts the sensitivity of the GC/MS analysis, the analyst should employ additional cleanup procedures and reanalyze by GC/MS.
- 12.10 In those circumstances where these procedures do not yield a definitive conclusion, the use of high resolution mass spectrometry is suggested.⁵

13. Calculations

- 13.1 Calculate the concentration of 2,3,7,8-TCDD in the sample using the response factor (RF) determined in Section 7.1.2 and Equation 2.

*Equation 3***

$$\text{RF} = \frac{(A_s) (I_s)}{(A_{is}) (\text{RF}) (V_o)}$$

where:

A_s = Response for the parameter to be measured.

A_{is} = Response for the internal standard.

I_s = Amount of internal standard added to each extract (μg).

V_o = Volume of water extracted (L).

- 13.2 For each sample, calculate the percent recovery of the internal standard by comparing the area of the m/z peak measured in the sample to the area of the same peak in the calibration standard. If the recovery is below 50%, the analyst should review all aspects of his analytical technique.
- 13.3 Report results in $\mu\text{g/L}$ without correction for recovery data. All QC data obtained should be reported with the sample results.

14. Method Performance

- 14.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.¹ The MDL concentration listed in Table 1 was obtained using reagent water.¹⁴ The MDL actually achieved in a given analysis will vary depending on instrument sensitivity and matrix effects.
- 14.2 This method was tested by 11 laboratories using reagent water, drinking water, surface water, and three industrial wastewaters spiked at six concentrations over the range 0.02-0.20 $\mu\text{g/L}$.¹⁵ Single operator precision, overall precision, and method

**This equation has been amended to reflect the original as published in 40 CFR 136, FRL-2636-6 49 FR 43234, October 26, 1984.

accuracy were found to be directly related to the concentration of the parameter and essentially independent of the sample matrix. Linear equations to describe these relationships are presented in Table 3.

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Table 1—Chromatographic Conditions and Method Detection Limit

Parameter	Retention time (min)	Method detection limit (µg/L)
2,3,7,8-TCDD	13.1	0.002

Column conditions: SP-2330 coated on a 60 m long x 0.25 mm ID glass column with hydrogen carrier gas at 40 cm/sec. linear velocity, splitless injection using tetradecane. Column temperature held isothermal at 200°C for one minute, then programmed at 8°C/min. to 250°C and held. Use of helium carrier gas will approximately double the retention time.

Table 2—QC Acceptance Criteria—Method 613

Parameter	Test Conc. (µg/L)	Limit for s (µg/L)	Range for \bar{X} (µg/L)	Range for P, P _s (%)
2,3,7,8-TCDD	0.100	0.0276	0.0523-0.1226	45-129

s = Standard deviation of four recovery measurements, in µ/L (Section 8.2.4).
 \bar{X} = Average recovery for four recovery measurements, in µ/L (Section 8.2.4).
P, P_s = Percent recovery measured (Section 8.3.2, Section 8.4.2).

NOTE: These criteria are based directly upon the method performance data in Table 3. Where necessary, the limits for recovery have been broadened to assure applicability of the limits to concentrations below those used to develop Table 3.

Table 3—Method Accuracy and Precision as Functions of Concentration—Method 613

Parameter	Accuracy, as recovery, X' ($\mu\text{g/L}$)	Single analyst, precision, s_r ($\mu\text{g/L}$)	Overall precision, S' ($\mu\text{g/L}$)
2-Chloronaphthalene	$0.75C+3.21$	$0.280\bar{X}-1.17$	$0.38\bar{X}-1.39$

X' = Expected recovery for one or more measurements. of a sample containing a concentration of C , in $\mu\text{g/L}$.

s_r' = Expected single analyst standard deviation of measurements at an average concentration found of $X.8$, in $\mu\text{g/L}$.

S' = Expected interlaboratory standard deviation of measurements at an average concentration found of \bar{X} , in $\mu\text{g/L}$.

C = True value for the concentration, in $\mu\text{g/L}$.

\bar{X} = Average recovery found for measurements of samples containing a concentration of C , in $\mu\text{g/L}$.