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PROPOSED REFERENCE METHODS FOR POLYCHLORINATED DIBENZO-p-DIOXINS AND POLYCHLORINATED DIBENZOFURANS

by

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MANAGEMENT PERSPECTIVE

This methodology was developed by the Research and Applications Branch, National Water Research Institute, Environment Canada to provide definitive analysis of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans in pulp and paper related matrices. The limits of detection, precision and accuracy of the method have been estimated in the documentation and these will be further defined during the planned validation study. It is intended that this will serve as a reference method for monitoring and for regulating dioxins and furans in the pulp and paper industry in Canada. Questions concerning the method or its application should be addressed to:

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PERSPECTIVE-GESTION

Cette méthode a été mise au point par la Direction de la recherche et des applications de l'Institut national de recherche sur les eaux, d'Environnement Canada, dans le but d'établir une méthode définitive de dosage des polychlorodibenzo-p-dioxines et des polychlorodibenzofuranes dans des matrices caractéristiques de l'industrie des pâtes et papiers. Les limites de détection ainsi que la précision et l'exactitude de la méthode ont été évaluées dans la documentation et seront définies plus précisément au cours de l'étude de validation qui est prévue. Cette méthode est destinée à servir de méthode de référence pour le contrôle et la réglementation des dioxines et des furanes dans l'industrie des pâtes et papiers au Canada. Veuillez adresser toute question relative à la méthode ou à ses applications à:

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ABSTRACT

Analytical methodology for polychlorinated dioxins and furans (PCDDs & PCDFs) in pulp and paper related matrices is not standardized. Many variations exist for the extraction, cleanup, quantitation and confirmation of these compounds and reported detection limits and recoveries vary over several orders of magnitude. Elements included in this method have been taken from a variety of sources as identified in the reference section.

This manuscript consolidates the work done by many researchers in Canada, U.S.A. and Europe over the past 10-12 years to develop reliable multi-media methodolgy at ultra-trace levels. This method uses matrix-specific extraction and cleanup procedures for pulp and paper effluents and receiving waters. The scope of the method can easily be expanded to cover other pulp and paper related matrices and environmental samples.

Analyte-specific quantitative analysis utilizes high-resolution capillary column gas chromatography - high resolution mass spectrometry (HRGC/HRMS) technqiue for the determination of PCDD and PCDF isomers. The estimated detection limit is 10 Pg/L for 2,3,7,8-TCDD in reagent water.

RÉSUMÉ

Les méthodes d'analyse des polychlorodioxines et des polychlorofuranes (PCDD et PCDF) dans des matrices caractéristiques de l'industrie des pâtes et papiers ne sont pas normalisées. Il existe de nombreuses variantes pour l'extraction, la purification, le dosage et la confirmation de ces composés, et les limites de détection et les taux de récupération signalés varient sur plusieurs ordres de grandeur. Certains éléments de cette méthode ont été tirés de plusieurs sources indiquées à la section Bibliographie.

Nous avons réuni dans le présent manuscrit les résultats des travaux effectués au cours des 10 à 12 dernières années par de nombreux chercheurs du Canada, des États-Unis et d'Europe qui tentaient de mettre au point des méthodes fiables permettant de doser des ultra-traces dans divers milieux. Cette méthode renferme des procédés d'extraction et de purification spécifiques à la matrice qui s'appliquent aux effluents des usines de pâtes et papiers et aux eaux dans lesquelles ils sont rejetés. La méthode peut facilement être appliquée à d'autres matrices caractéristiques de l'industrie des pâtes et papiers et à des échantillons environnementaux.

La méthode d'analyse spécifique à l'analyte fait appel à la chromatographie en phase gazeuse à haute résolution, couplée à la spectrométrie de masse à haute résolution (CGHR/SMHR) pour doser les isomères de PCDD et de PCDF. La limite de détection a été évaluée à 10 pg/L dans le cas de la 2,3,7,8-TCDD dans de l'eau utilisée comme réactif.

PROPOSED REFERENCE METHOD FOR POLYCHLORINATED DIBENZO-p-DIOXINS AND POLYCHLORINATED DIBENZOFURANS

1.0 SCOPE

1.1 This is a qualitative and quantitative high resolution gas chromatography/high resolution mass spectrometric (HRGC/HRMS) method for analysis of tetra-, penta-, hexa-, hepta-, and octachlorinated dibenzo-p-dioxins (PCDD's) and dibenzofurans (PCDFs) in paper and pulp effluents and receiving waters. Proper column selection and access to reference isomer standards, may in certain cases, provide isomer specific data. Special instructions are included which measure 2,3,7,8-substituted congeners.

1.2 The sensitivity of this method will depend upon the level of interferences in a given sample. The estimated limit of detection for this method is 10 pg/L for 2,3,7,8-TCDD. Actual limits of detection and method calibration limits will be provided based on

detailed validation and verification of the performance of this method by the single laboratory and/or multi-laboratory evaluation of this method.

1.3 Sections and subsections included in this method are extracted from many sources identified in the list of references at the end of this method.

1.4 This method is recommended for use only by analysts experience with residue analysis and skilled in various cleanup procedures used in ultra-trace multi-residue analysis and expert knowledge in operation of HRGC/HRMS and interpretation of the resultant data.

1.5 Because of the extreme toxicity of many of these compounds, the analyst must take the necessary precautions to prevent exposure to materials known or believed to contain PCDDs or PCDFs. It is the responsibility of whoever uses this method to consult and establish appropriate safety and health practices and determine the applicability or regulatory limitations prior to use. Specific precautionary statements are given in Section 4.0.

2.0 SUMMARY OF METHOD

2.1 A one litre water sample (larger volumes up to 4L may be used to achieve lower detection limit) is spiked with $^{13}C_{12}$-PCDDs and $^{13}C_{12}$-PCDFs internal standard mixture. It is extracted with methylene chloride using a separatory funnel technique, exchanged to hexane and concentrated. After appropriate cleanup procedures, extracts are brought to 10 uL volume and are analysed by HRGC/HRMS using selected ion monitoring (SIM).

2.2 In some cases, samples may require additional cleanup steps inorder to remove interferences and achieve acceptable detection limits. Depending upon the nature of chemical interferences present, selected cleanup steps, as shown in Figure 1, may be employed.

3.0 INTERFERENCES

3.1 Contamination of solvents, reagents, glassware and other sample processing hardware may yield discrete artifacts and/or elevated background levels which preclude acceptable detection limits and accurate analytical information. All of these materials must be

demonstrated to be free from interferents under the conditions of analysis by running laboratory method blanks.

3.2 High purity reagents, solvents and column materials must be used and should be tested for potential interferences when new lots are introduced.

3.3 Interferents co-extracted from the samples will vary from sample to sample. Retention times of target analytes together with identification criteria for PCDDs and PCDFs must be verified using reference standards.

3.4 High resolution capillary columns are used to resolve as many PCDD and PCDF isomers as possible; however, no single column is known to resolve all of the isomers. Use of several capillary columns will, in fact, be necessary during the determination of the isomers and the toxicity equivalent factors (TEFs).

3.5 Aqueous samples should not be aliquoted from sample containers. The entire sample must be used and the sample container washed/rinsed out with the extracting solvent.

3.6 The analysis of a water sample will include any particulate matter which may be present in the sample. The solubility of 2,3,7,8-TCDD is estimated to be less than 50 ng/L, suggesting that higher detected levels may be due to solids present in the matrix. These particulates may be a contributing factor to positive results and samples should be evaluated accordingly.

4.0 HAZARDS AND SAFETY

4.1 In view of the toxic properties of PCDDs/PCDFs, special precautions must be taken to minimize the risk of human exposure, either through direct contact with contaminated materials, or through inhalation of contaminated air.

4.2 Work with PCDDs and PCDFs, regardless of concentration, should be conducted in an exhaust-ventilated hood with minimal airflow of 150 feet/minute. The hood should be equipped with a HEPA (high efficiency particulate absolute) filter which has been leak-tested (99.5% efficient) and is followed by charcoal filters.

4.3 All work related to PCDDs/PCDFs analysis, including the preparation, handling, and storage of all samples and standards, should be conducted within laboratory specially designed to handle toxic and hazardous chemicals. Such facility should include the following design features:

- restricted access;
- a higher rate of ventilation relative to surrounding areas;
- all exhaust air ducking routed to a common, scrubbed outlet;
- segregation, via doors and air pressure differentials, into low, medium and high hazard areas;
- fully operational capability on auxiliary power in the event of a commerical power failure;
- an independent back-up air supply system designed to come into operation whenever a shut-down of the buildings common air supply system occurs;
- capability of visually monitor ventilation system performance;
- a system of distinctive audio and visual alarms to alert all building personnel to potentially hazardous conditions.

4.4 Samples must be handled from receipt to disposal by qualified personnel only. Analysts must have a working knowledge of safety protocols and be adept at safety procedures. HRGC/HRMS instruments must be equipped with vapour contamination traps on the capillary split and sweep vents and on the rough pump effluent lines prior to use.

4.5 PCDDs and PCDFs contaminated wastes should be minimized as much as possible. Aqueous and solvent waste should be allowed to evaporate in a hood designed as specified in Section 4.2. Contaminated materials and protective clothings worn when working with dioxin and dibenzofuran should be placed in appropriately labelled drums and disposed of in accordance with all applicable local, state and federal regulations.

4.6 Analysts must also follow the additional safety practices as outlined in US EPA Method 613, Section 4 (July 1982 version) with some amendments to cover PCDDs and PCDFs.

5.0 APPARATUS AND EQUIPMENT

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

5.1 Sampling equipment: Grab sample bottle-amber glass, 1-Litre or 1-quart volume. French or Boston Round design is recommended. The container must be acid washed and solvent rinsed before use to minimize interferences.

5.2 Bottle caps: Threaded to screw onto the sample bottles. Caps must be lined with Teflon. Solvent washed foil, used with shiny side towards the sample, may be substituted for Teflon if the sample is not corrosive. Apply Teflon tape around cap to completely seal cap at bottom. Sample containers must be kept refrigerated after sampling.

5.3 Water bath: Heated, with concentric ring cover, capable of temperature control (±2°C). The bath should be used in a hood.

5.4 500-mL Kuderna-Dauish fitted with a 10-mL concentrator tube and three-ball macro Snyder column.

5.5 Separatory funnels, 125-mL to 2000-mL with polytetrafluoroethylene (PTFE) stopcock.

5.6 Funnels, short stem.

5.7 Pipets, disposable, pasture, 150-mm long X 5-mm I.D.

5.8 Pipets, disposable, serological 10-mL for preparation of carbon column specified in paragraph 12.4.

5.9 Reacti-vials 100-uL to 2-mL size, amber glass. These should be salinized prior to use.

5.10 Graduated cylinders, 100-mL to 1000-mL.

5.11 Glass helices, 1/16 inch.

5.12 Erlenmyer flasks, 250-mL and 500-mL size fitted with Teflon stoppers.

5.13 Wrist action shaker.

5.14 Teflon boiling chips. Wash with hexane prior to use.

5.15 15-mL conical concentrator tubes.

5.16 Adaptors for concentrator tubes.

5.17 Volumetric flasks, 100-mL.

5.18 Scintillation vials, 40-mL disposable.

5.19 300-mm X 10.5-mm glass chromatographic column fitted with Teflon stopcock.

5.20 Nitrogen blowdown apparatus (N.Evap (reg. trade mark)) Analytical Evaporator Model III, Organomation Assoc. Inc., Northborough, Mass. or equivalent). Teflon tubing connection to trap and gas regulator is required.

5.21 Balances capable of accurately weighing to 0.01g and 0.00001g.

5.22 Centrifuge.

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

5.24 Glove box.

5.25 Drying oven.

5.26 Glass fibre filters or glass wool plugs are also recommended.

5.27 Solvent reservoir (125-mL) Kontes: (special order) 12.5-cm diameter, compatible with gravity carbon column.

5.28 Desicator.

5.29 Rotary evaporator with a temperature-controlled water bath.

5.30 Glass wool, extracted with methylene chloride, dried and stored in a clean glass jar. Reuse of glassware should be minimized to avoid the risk of contamination. All glassware that is reused must be scrupulously cleaned as soon as possible after use,

applying the following procedure: Rinse glassware with the last solvent used in it, then with high purity acetone and hexane. Wash with hot detergent water. Rinse with copious amounts of tap water and several portions of distilled water. Drain, dry and heat in a muffle furnace at 400°C for 15 to 30 minutes. Volumetric glassware must not be heated in a muffle furnace. After glassware is dry and cool, rinse it with high-purity acetone and hexane and store it inverted or capped with solvent-rinsed alumium foil in a clean environment.

5.31 Carbopack C, 80/100 mesh, product of, and available from, Supelco Inc. Supelco Park, Bellefonte, PA, 16823-0048. Option: Carbon fibré column for HPLC cleanup. The carbon fibre column (4-mm id X 7.2-cm annealed glass fitted with zero dead volume fittings and 2-mm stainless steel frits) contains the packing which is prepared as described below:

Weigh a glass fibre filter paper (0.06g - type GA 200, Toyo Roshi Co. Ltd.) in a 200-mL flask. Add 0.05 g of activated carbon-PX-21 (6.14) along with 100-mL cyclohexane-methylene chloride (50+50, V+V). Mix and shred the mixture with Polytron, so that the fibres are of a size capable of retaining the carbon particles. Pack the slurry of carbon fibres into the column using the aspirator.

5.32 HPLC pump with loop valve (1.0-mL) injector to be used in the optional carbon column cleanup procedure.

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

5.34 Nitrogen Purification Traps: Series of four traps (stainless steel tubes, 1.0-cm OD X 10-cm long) described by T.J. Nestrick and L.L. Lamparski (Analytical chemistry 53, 122, 1981). Trap Number 1 contains a mixture composed of Chromosorb W/AW (60/80 mesh coated with 5% Apiezon L), graphite (100 mesh,

1-M-USP), and activated carbon (50 to 100 mesh), in a 7:1.5:1.5 ratio. Chromosorb W/AW and Apiezon L were obtained from Ultracarbon Corporation, Bay City, Michigan, activated carbon was obtained from Fisher Scientific Co., Cincinnati, Ohio. Trap Number 2 contains Molecular Sieve 13X (60/80 mesh, obtained from Supelco, Inc., Bellefonte, Pennsylvania). Trap Number 3 contains silica gel impregnated with 30% (W/W) sulfuric acid. Trap Number 4 contains Carbosieve S80/100 mesh, (obtained from Supelco, Inc., Bellefonte, Penn.).

5.35 Gas Chromatograph-Mass Spectrometer-Data System (GC-MS-DS). This system must incorporate the following components: (1) A capillary column gas chromatograph with splitless injector. The oven of the gas chromatograph should be temperature programmable; gas chromatograph/mass spectrometer interface components should withstand 350°C. The interface must be designed so that the separation of 2,3,7,8-TCDD from other TCDD isomers achieved in the gas chromatographic column is not appreciably degraded. It is recommended that the

GC column be fitted directly into the mass spectrometer ion source without being exposed to the ionizing electron beam. Graphite ferrules should be avoided in the injection port because they may adsorb the PCDDs and PCDFs. Vespel or equivalent ferrules are recommended.

The static resolving power of the mass spectrometer must be maintained at a minimum 10,000 (10 percent valley). The mass spectrometer must be operated in a selected ion monitoring (SIM) mode with total cycle time (including the voltage reset time) of one second or less. The total cycle time includes the sum of all the dwell times and voltage reset times. At a minimum, the ions listed in Table 1 for each of the five SIM descriptors must be monitored. The selection of the lock-mass ion is left to the performing laboratory.

The recommended mass spectrometer tuning conditions (Section 7.3 and 7.4) are based on the groups of monitored ions shown in Table 1.

A dedicated computer-based data system which is capable of simultaneous control of selected ion monitoring and data acquisition. The data system must be capable of acquiring data at a minimum of 10 ions in a single scan. It is also recommended to have a data system capable of switching to different sets of ions (descriptors) at specified times during an HRGC/HRMS acquisition. The computer system must be able to monitor peak height and peak area and profile ion intensities overtime. The data system should also permit the measurement of noise on the baseline.

5.36 HRGC Columns: In order to have an isomer-specific determination of 2,3,7,8-TCDD and to allow the detection of OCDD/OCDF within a reasonable time interval in one HRGC/HRMS run, the use of 60 meter, 0.25-mm I.D. fused silica capillary column, coated with SP-2331 (or equivalent) at a 0.2 micron film thick-ness is recommended. A 60-m DB 5 fused silica capillary column is also recommended provided minimum acceptable criteria must be demonstrated and documented (Section 7.4).

6.0 REAGENTS, MATERIALS AND STANDARD SOLUTIONS

6.1 Purity of Reagents: Reagent grade chemicals must be used. The quality of these reagents is first ascertained that the reagent is of desired quality to permit its use without lessening the accuracy of the determination.

6.2 Purity of water: Reagent water as defined by Type I of specification D1193. Additionally, the water must be free of interferences, such as are described in Section 6.

6.3 Trisodium Phosphate.$12H_2O$: 0.05M solution in reagent water.

6.4 Sulfuric acid: 10M solution in reagent water.

6.5 Methylene chloride, carbontetrachloride, hexane, benzene, methanol, tridecane, isooctane, toluene, cyclohexane. Distilled in glass or highest available purity.

6.6 Alumina, neutral, Super 1,Woelm® 80/200 mesh: Store in a sealed container at room temperature in a desiccator over self-indicating silica gel.

6.7 Alumina, neutral, 80/100 mesh: Prepared by Soxhlet extraction with methylene chloride for four hours, air dry and activate in an aluminum foil-covered borosilicate glass container for 24 hours at 190°C. Store in amber glass jar in a desiccator. The baked alumina should be used within five days after baking.

6.8 Prepurified nitrogen gas.

6.9 Anhydrous sodium sulfate: Extracted by manual shaking with several portions of hexane and dried at 100°C.

6.10 Sodium chloride: 5 percent (w/v) in reagent water.

6.11 Silica gel (Type 60), EM reagent 70-230 mesh, CMS No. 393-066: Activate silica gel at 130°C for two days and store it in an amber glass bottle in a desiccator.

6.12 Silica gel containing 40% sulfuric acid: mix two parts activated silica gel in a screw-cap bottle. Shake the mixture until it is clump-free (approximately 15 minutes). This mixture should be prepared prior to its use.

6.13 10-um Silica: Sherisorb S10W, Phase Separations, Inc., Norwalk, Conn.

6.14 Carbon: Super Sorb Sample No. N-252, Grade PX21, Amoco Research Co., Chicago, Ill.

6.15 Calibration Solutions

6.15.1 High resolution concentration calibration solutions (Table 1) at known concentrations used to calibrate the instrument.

6.15.2 Store the concentration calibration solutions in 1-mL minivials at room temperature in the dark.

6.16 GC Column Performance Check Solution: This solution contains first and last eluting isomers for each homologous series from tetra- through hepta-chlorinated congeners. The solution also contains a series of other TCDD isomers for the purpose of documenting the chromatographic resolution. The $^{13}C-_{12}$ 2,3,7,8-TCDD is also present. The laboratory is required to use a highly boiling solvent such as

toluene, nonane, decane or tridecane as solvent to adjust the volume so that the final concentration does not exceed 100 pg/L per congener. Table 2 summarized the qualitative composition (minimum requirement) of this performance evaluation solution. The solution is used for defining the homologous GC retention time windows on HRGC column (60-m DB5 and SP-2331).

6.17 Spiking Standard Solution

The isooctane solution contains the nine internal standards at the nominal concentrations that are listed in Table 3. This solution is used to measure the concentrations of the native substances.

7.0 SYSTEM PERFORMANCE CRITERIA

7.1 The laboratory must document that all applicable system performance criteria were met before analysis of any sample is performed. Table 4 provides recommended GC conditions that can be used to satisfy the required criteria.

7.2 GC Column Performance: A GC performance check is required at the beginning of each day or of each 12 hour period during which samples are analysed.

7.2.1 Inject typically 1-, 2- or 3-uL of the column performance check solution (section 6.17) and acquire selected ion monitoring (SIM) data as described in Section 5.35 within a total cycle time of <1 second.

7.2.2 The chromatographic separation between 2,3,7,8-TCDD and the peaks representing any other TCDD isomers, must be resolved with a valley < 25%. It is the responsibility of the laboratory to verify the conditions suitable for the appropriate resolution of 2,3,7,8-TCDD from all other TCDD isomers.

7.2.3 Retention times for the switching of SIM ions characteristic of one homologous series to the next higher homologous series must be indicated in the Selected Ion Current Profile (SCIP). Accurate switching at the appropriate times is absolutely necessary for accurate monitoring of these compounds. Allowable tolerance on the daily verification with the GC performance check solution should be better than 10 seconds for the absolute retention times of all the components of the

mixture. Particular caution should be exercised for the switching time between the last tetrachlorinated congener (i.e., 1,2,8,9-TCDD) and the first pentachlorinated congener (i.e., 1,3,4,6,8-PeCDF), as these two compounds elute within 15 seconds of each other on the 60 m DB-5 column.

7.3 Mass Spectrometer Performance

7.3.1 The mass spectrometer must be operated in the electron impact mode. A static resolving power of at least 10,000 (10 percent valley definition) must be demonstrated at appropriate masses before any analysis is performed. The static resolving power checks must be performed at the beginning and at the end of each 12-hour period of operation.

Chromatography time for PCDDs and PCDFs exceeds the long term mass stability of the mass spectrometer. Because the instrument is operated in the high-resolution mode, mass drifts of few ppm (i.e., 5 ppm in mass) can have serious adverse effects on the

instrumental performances. Therefore, a mass-drift correction is mandatory. To that effect, it is recommended to select a lock-mass ion from the reference compound (PFK is recommended) used for tuning the mass spectrometer. The selection of the lock-mass ion is dependent on the masses of the ions monitored within each descriptor. Table 5 offers some suggestions for the lock-mass ions. The level of reference compound (PFK) metered into the ion chamber during HRGC/HRMS analysis should be adjusted so that the amplitude of the most intense selected lock-mass signal (regardless) of descriptor number) does not exceed 10 percent of the full-scale deflection for a given set of detector parameters*. Under these conditions the sensitivity changes that might occur during the analysis can be more effectively monitored.

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*NOTE: Excessive PFK (or any other reference substance) may cause noise problems and contamination of the ion source resulting in an increase in downtime for source cleaning.

7.3.2 By using a PFK leak, tune the instrument to meet the minimum required resolving power of 10,000 (10 percent valley) at m/z 304.9824 (PFK) or any other reference signal close to m/z 303.9016 (from TCDF). By using the peak matching unit and the aforementioned PFK reference peak, verify that the exact mass of m/z 380.9760 (PFK) is within 5 ppm of the required value. Note that the selection of the low- and high- mass ions must be such that they provide the largest voltage jump performed in any of the five mass descriptors (Table 5).

7.4 Calibration

7.4.1 Two types of calibration procedures are required. One type, initial calibration, is required before any samples are analyzed and is required intermittently throughout sample analysis as dictated by results of routine calibration procedures below. The other type consists of analyzing the column performance check solution and concentration calibration solutions (7.4.2 and 7.4.4). No samples are

to be analyzed until acceptable calibration as described in paragraphs 7.4.1 and 7.4.3 is demonstrated and documented.

7.4.2 Initial Calibration

7.4.2.1 Inject typically 1-, 2- or 3-uL of the concentration calibration standards solutions (Table 1) and acquire SIM data for all the compounds. The total cycle time for data acquisition must be $\leq$ 1 second.

7.4.2.2 The ratio of integrated ion current for the ions appearing in Table 6 must be within the indicated control limits.

7.4.2.3 For each SICP (Selected Ion Current Profile) and each GC signal corresponding to the elution of a target analyte and of its labelled standard, the signal to ratio (S/N) must be better than or equal to 2.5. This measurement must be made for any GC peak that has an apparent S/N of less than 10:1. Attachment 1 describes the procedure to be followed for the measurement of the S/N from conspicously weak signals.

7.4.2.4 Calculate relative response factors (RRFs) for unlabelled target analystes relative to their appropriate internal standard; for the labelled $^{13}C-_{12}$ internal standards relative to recovery standards.

7.4.2.5 Calculate RRFs and their respective precent relative standard deviations for seven calibration solution listed in Table 1. The RRFs are used for the determination of the concentration of isomers in a homologous series.

7.4.3 Criteria for Acceptable Calibration: The following criteria must be met before the analysis is performed.

7.4.3.1 The percent relative standard deviations for the mean response factors from each of the determinands (labelled and unlabelled) must be less than 20 percent.

7.4.3.2 The S/N ratio for the GC signals present in every SICP (including the ones for the labelled standards) must be $\geq$ 2.5.

7.4.3.3 The isotopic ratios (Table 6) must be within the specified control limits.

7.4.4 Routine Calibration

Routine calibration must be performed at the beginning of a day after successful mass resolution and GC resolution performance check.

7.4.4.1 Inject typically 1-, 2- or 3-uL of the concentration calibration solution number 3 containing 5 pg/uL of tetra- and pentachlorinated congeners, 10 pg/L of hexa- and heptachlorinated congeners, and 25 pg/uL of octachlorinated congeners, and respective internal standard and recovery standards. Determine and document an acceptable calibration, using the same HRGC/HRMS conditions as described in Section 7.0.

7.4.5 Criteria for Acceptable Routine Calibration

The following criteria must be met before further analysis is performed. If these criteria are not met, corrective action must be taken.

7.4.5.1 The measured RRFs for unlabelled and labelled standard obtained during routine calibration must be within 20 percent of the mean values established in the initial calibration (7.4.2).

7.4.5.2 The ion-abundance ratios must be within the allowed control limits (Table 6).

7.4.5.3 The chromatographic peak separation between 2,3,7,8-TCDD and 1,2,3,4-TCDD must be resolved with a value of $\leq$ 10 percent. The performance check solution (6.14) must be used to check and document following parameters: (a) the retention windows for each of the homologues; (b) the GC resolution of 2,3,7,8-TCDD and 1,2,3,4-TCDD; and (c) the relative ion abundance criteria listed for PCDDs and PCDFs in Table 6.

7.4.5.4 If one or more of the above criteria is not satisifed, the entire initial calibration process must be repeated.

NOTE: An initial calibration must be carried out whenever the high resolution concentration solution-3, the sample fortification or the recovery standard solution is replaced by a new solution from a different lot.

8.0 QUALITY ASSURANCE/QUALITY CONTROL PROCEDURES

8.1 Before processing any sample, the analyst must demonstrate through the analysis of a method blank that all glassware and reagents are interferent-free at the method detection limit.

8.2 A laboratory "method blank" should be analyzed with each set of samples to ensure that there is no interference from glassware, reagents or laboratory apparatus. The method blank, using one litre of reagent water, should also be spiked with the internal standard.

8.3 The laboratory shall, on an ongoing basis, demonstrate that the analytical system is under control through calibration verification (Table 1), MS resolution verification, control charts for RRFs, the analysis of GC performance evaluation solution (Table 2), analytical precision and sarrogate recoveries. Standard reference materials should also be analyzed to assess the accuracy of the method.

8.4 Field duplicates, field blanks and fortified field blanks/samples should be analyzed periodically to determine the total precision (field and lab).

8.5 Other QA/QC portions of this method have not been completely established at this time. Analysts are advised to refer to Attachment II.

9.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

9.1 The principal factor affecting the precision and accuracy of the PCDDs/PCDFs analysis in water and effluents is the presence of particulates. Many of the determinants are known to strongly bound to particulates in an aquatic environment. Therefore, the sample containing visible particulates or turbidity should be filtered to remove particulates (0.45 um or larger) before sampling. Store the filter with particulate matter at 5°C for analysis at a later date.

9.2 Presampling visit to sampling sites to ensure adequate sampling conditions is recommended. Organically inert sample transfer lines should be used.

9.3 Sample containers must be solvent rinsed and proven clean before use by HRAC/HRMS analysis of bottle rinsings.

9.4 All samples must be collected in triplicate.

9.5 Samples must be collected in glass containers. The bottles must not be prewashed with sample before collection. Sampling equipment must be free from potential sources of contamination.

9.6 Field blanks should be taken on regular basis.

9.7 Samples should be refrigerated at 4°C during shipping, strict time protocol for completion of extractions within five days of receipt at the laboratory should be followed.

9.8 Validation criteria for analytical data must include detection of PCDDs/PCDFs congeners in replicate samples.

9.9 Maximum holding time (MHT)*: The estimated MHT for the samples is 30-45 day. This will be confirmed by a single laboratory at a later date.

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* MHT is that time at which a 10% change in analyte concentration (C_{t10}) occurs and the precision of the method of measurement allows the 10% change to be statistically different from the 0% change (C_{t0}) at the 90% confidence level.

10.0 EXTRACTION OF SAMPLE

10.1 Extraction of Filtered Sample (Soluble PCDDs & PCDFs)

10.1.1 Position a 500-mL Kuderna-Danish (K-D) evaporative flask, fitted with a 10-mL graduated concentrator tube in the hood directly under a 2-L separatory funnel. Add 1/4 inch of glass helices to the K-D apparatus. Plug a glass funnel with glass wool and place it in the top of the evaporative flask. Pour sodium sulfate into the glass funnel to within 1 inch of the top. Pre-wet the entire apparatus with methylene chloride.

10.1.2 Mark the water meniscus on the side of the 1-L sample bottle for later determination of the exact sample volume. Pour the entire sample (approximately 1-L) into a 2-L separatory funnel.

10.1.3 Add 20-uL of the sample spiking solution in Table 3. Rinse the sample bottle with 60-mL of methylene chloride and add to separatory funnel.

10.1.4 Extract the sample with 60-mL of methylene chloride by shaking the funnel for 2 minutes. CAUTION: Vent the separatory funnel frequently during this extraction to relieve pressure inside the funnel. Allow organic and aqueous layers to separate for 10 minutes. If any emulsion forms, phase separation should be attempted using a glass stirring rod, addition of salt or centrifugation.

10.1.5 Collect the methylene chloride extract by allowing it to filter through the sodium sulfate-filled funnel into the K-D apparatus. Extract the sample with two additional portions of methylene chloride (section 10.1.4) and filter the extracts into the K-D apparatus. After the third methylene chloride extract has filtered through the sodium sulfate, pour through an additional 15-20-mL of methylene chloride to quantitatively rinse the apparatus.

10.1.6 Remove the funnel from the top of the K-D apparatus and insert a three-ball macro Snyder column. Prewet the Snyder column with

2-3-mL of methylene chloride and concentrate the extract to approximately 3-mL using a steam bath. Remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

10.1.7 Remove the Snyder column from the K-D assembly and add 50-mL of hexane to the K-D evaporator flask. Reattach the Snyder column and prewet with 2-3-mL of hexane. Add teflon boiling chip to the K-D apparatus before proceeding with second concentration step. Rinse the flask and the lower joint with two 5-mL portions of hexane and combine the rinsate with the extract to givea final volume of about 15-mL.

10.1.8 Determine the original sample volume by filling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5-mL.

10.2 Extraction of Filtered Sample (Soluble PCDDs & PCDFs)

10.2.1 Prepare a clean glass Soxhlet extraction thimble by adding a clean silica to form 3-6-mm layer on the surface of the glass frit at the bottom of the thimble and place a 10-mm layer of glass wool over the layer of silica.

10.2.2 Spike the filtered sample (paragraph 9.1) with 20-uL of the spiking solution (Table 3). Homogenize the filter paper with 10 g of anhydrous sodium sulfate and transfer the extraction thimble. Homogenization may not be required if the diameter of the filter paper is smaller than the diameter of the extraction thimble.

10.2.3 Extract the contents (10.2.2) for 16 hrs. with 100-mL of toluene. Cool and filter the toluene extract through a glass fibre filter paper into a 500-mL round bottom flask. Rinse the filter with 5-mL of toluene. Concentrate the combined toluene solution to near dryness using a rotary evaporator at 50°C.

10.2.4 Add 150-mL of methylene chloride to redissolve the analytes and the coextract-ants. Transfer methylene chloride to the K-D apparatus. Rinse the flask with two additional portions of 20-mL of methylene chloride to quantitatively rinse the round bottom flask (10.2.3). Proceed to step 10.1.6.

11.0 BASIC CLEANUP PROCEDURES: The use of a cleanup may depend upon the extent of interferences in a given sample. The analyst may use any or all of the procedures below or any other appropriate procedure, however, before using a cleanup procedure, the analyst must demonstrate the cleanup procedure remove the interferences without significantly affecting the precision and accuracy of the analytical results.

11.1 <u>Acid/Base Wash (Liquid/Liquid Partition)</u>

11.1.1 In a 125-mL separatory funnel, partition the concentrated extract (10.1.7) against 40-mL of 0.05M solution of trisodium phosphate

Shake for 2 minutes. Remove and discard the aqueous layer (bottom) after allowing phases to separate for 10 minutes. Repeat the base washing until no colour is visible in the bottom layer (perform base washings a maximum of three times).

11.1.2 Partition the extract (11.1.1) against 40-mL of 10M sulfuric acid (6.4). Shake for 2 minutes. Remove and discard the aqueous layer. Repeat the acid washings until no colour is visible in the acid layer (perform acid washings a maximum of three times).

11.1.3 Partition the extract (11.1.2) against 40-mL 0.01M Bismuth (III) nitrate solution in 0.1M hydrochloric acid. Shake for 2 minutes. Remove and discard the aqueous layer. Repeat the step until no precipitate and/or turbidity is visible in bismuth nitrate layer (perform bismuth treatments a maximum of three times).

11.1.4 Partition the organic extract (11.1.3) against 40-mL of 5 percent (WIV) sodium chloride. Shake for 2 minutes. Remove and

discard the aqueous layer. Dry the extract by pouring it through a funnel containing anhydrous sodium sulfate into a 100-mL round bottom flask. Wash the separatory funnel with two 15-mL portion of hexane and combine the extracts. Concentrate the hexane solution to near dryness with rotary evaporator (35° water bath).

11.1.5 Rinse the round bottom flask (11.1.3) with four 0.5-mL portions of hexane, adding each rinse to the 15-mL conical concentrator tube (5.15).

11.1.6 Reduce the extract volume (11.1.4) to 1-mL under stream of purified nitrogen.

11.2 Silica Gel/Alumina Column Chromatography

11.2.1 Prepare two 1 x 50 cm chromatography columns for each sample. Attach the columns to laboratory supports in a hood so that the lower tip of the upper (silica gel) column may be inserted into the top of the lower (alumina) column.

11.2.2 Add 1 gram of silica gel and 4 grams of 40% acid silica sequentially to the top column, tapping the column to settle the contents after each addition. Add 12 grams of activated alumina to the lower column, settling the contents as above, followed by a 1 cm layer of sodium sulfate.

11.2.3 Prewet both columns with enough hexane to remove any trapped air, discard the eluate. Insert the bottom tip of the silica column into the top of the alumina column and adjust flow rate.

11.2.4 Just before the level of hexane reaches the top of the acid silica layer, quantitatively transfer the sample concentrate to the top of the acid silica column, using three 1-mL hexane washes to complete the transfer.

11.2.5 Just prior to exposure of the acid silica layer to air, add a 45-mL portion of hexane to the top column, allowing it to pass through top and bottom column successfully.

Discard the eluate. When the hexane has completely eluted from the silica gel column, remove the column.

11.2.6 Elute the alumina column with a 20-mL of hexane followed by 30-mL of carbon tetrachloride and 30-mL of 3% methylene chloride in hexane. Add each successive solvent to the column when the previous solvent layer is 1/4 inch from the top of the sodium sulfate layer. Discard all eluates.

11.2.7 As the 3% methylene chloride in hexane eluent reaches the top of the sodium sulfate layer, add 40-mL of 35% methylene chloride in hexane to the column and collect this eluate in a 40-mL conical vial.

11.2.8 Concentrate the eluate volume to approximately 0.5-mL and transfer it into a 1.0-mL TFE-lined screw cap vial, using three 0.2-mL hexane washes to complete the transfer. Concentrate the sample just to dryness using a stream of purified nitrogen gas. Add 10-uL of a recovery standard solution (Table 3) and mix throughly. Proceed with HRGC/HRMS.

12.0 ADDITIONAL CLEANUP PROCEDURES

12.1 In most cases, samples will require only basic cleanup, as described in section II, before HRGC/HRMS analysis in order to achieve acceptable detection limits. In some cases, however, sample may contain a variety of interferences which are not removed completely and may require the additional cleanup procedures to eliminate the interferences. If particular circumstances require the use of additional cleanup procedures, the analyst may use any or all of the procedures below or any other appropriate procedure.

12.2 Silica Gel Impregnated With Sulfuric Acid (40% W/W) Column Chromatography

12.2.1 Fabricate a glass chromatography column, using glass disposable pipette, in the following manner: insert glass wool plug into the bottom of the column and pack the column with 3 cm of silica gel impregnated with sulfuric acid (6.12).

12.2.2 Apply the concentrated extract, passed through the basic cleanup as outlined in section 11, to the column. Rinse the

container with additional 10-uL of hexane and apply to the column. Heat the column, in vertical position, in an oven at 130°C for 45 minutes. Cool the column to room temperature.

12.2.3 Elute with 6-7-mL of hexane.

12.2.4 Concentrate the eluent just to dryness. Add 10-uL of recovery standard (Table 3) and analyze for PCDDs and PCDFs using HRGC/HRMS.

12.2.5 If acceptable detection is not attained due to interferences proceed to the following step.

12.3 Micro-Alumina Column Chromatography

12.3.1 Concentrate the extract (12.2.3), passed through cleanup procedures as described in section 11.0 and 12.2, to 1-mL hexane.

12.3.2 Fabricate a glass chromatography column in the following manner: Insert glass wool plug into the bottom of the column. Add a 1-cm layer of sodium sulfate. Pack the column

with 5-cm of alumina and cover the alumina with 2-cm of anhydrous sodium sulfate.

12.3.3 Wash the column with 10-mL of hexane.

12.3.4 Apply the sample extract from section (12.3.1) and a 1-mL hexane rinsate to the alumina column.

12.3.5 Elute the column with 10-mL of toluene into 15 mL conical concentrator tube.

12.3.6 Concentrate the eluate volume just to dryness and redissolve the residue using 10-uL of a recovery standard (Table 3).

12.3.7 Analyze the concentrate by HRGC/HRMS, for PCDDs & PCDFs. If this extract is known to contain high levels of interferences then proceed with the carbon fibre cleanup described below.

12.4 Carbon Column Chromatography: After the above cleanup procedures and HRGC/HRMS analysis, some sample extract may retain organic interferences resulting in

overlapping or high signal-to-notice ratio in the ions of interest. Activated carbon column chromatography will, in most cases, eliminate these remaining interferences.

12.4.1 Preparation of Column Adsorbent: Mix 3.6-g of Carbopack (80/100 mesh) and 16.4-g of Celite 545 in a 40-mL vial. Activate at 130°C for six hours. Cool and store in a descicator. Check each new batch of mixed Carbopac C/ celite to ensure PCDD & PCDF recovery is over 80%.

12.4.2 Preparation of Columns: Insert wad of glass wool into a 150-mL 7mm brosilicate transfer pipet and push securely into the narrow end using a length of narrow plastic tubing. Add the carbopak-Celite mixture to the column through a micro-funnel, using vacuum aspitation at the pointed tip of the pipet to pack a 2-cm column of adsorbent. Support the column using a adjustable clamp.

12.4.3 Pre-elute the column with the following sequence of solvents and solvent mixtures: 2-mL of toluene; 1-mL of methylene

chloride-methanol-benzene (75+20+5, V+V+V); 1-mL of cyclohexane-methylene chloride (50 + 50, V+V); 2-mL of hexane. Discard all eluates.

12.4.4 When the bottle of the hexane miniscus reaches the top the adsorbant, quantitatively transfer the sample extract (12.3.3). Rinse the container with 5 mL of hexane and apply the rinsate to the column.

12.4.5 Elute the column with 1-mL of cyclohexane-methylene chloride (50+50, V+V) followed by 1-mL methylene chloride-methanol-benezene (70+20+5, V+V+V). Discard eluates.

12.4.6 Elute the column with 2-mL of toluene. Collect this fraction in a 40-mL conical vial.

12.5 <u>Alternate Carbon Column HPLC Cleanup</u>

12.5.1 Assemble the HPLC system which consists of the following:

- solvent delivery system capable of constant flow at low back pressures;

- solvent selector valve equipped with 1-mL sample loop;
- 1-mL syringe;
- 4-mm I.D. glasstube 7.2-cm long, previously annealed and fitted with zero dead volume fittings on 2-um stainless steel frits and packed with carbon fibre (5.31-option);
- asssociated tubing and connections.

12.5.2 Dilute the fraction from section 12.3.3 with 1-mL of 1:1 methylene chloride/cyclohexane and quantitatively transfer the solution to the HPLC system.

12.5.3 Elute the carbon fibre HPLC system in succession as follows:

- eluent 1 (44-mL of 1:1 methylene chloride/cyclohexane);
- eluent 2 (30-mL ethyl acetate);
- eluent 3 (40-mL 10% Benzene in ethyl acetate);
- eluent 4 (35-mL 50% Benezene in ethyl acetate);
- eluent 5 (30-mL toluene-elution in the reverse direction).

12.5.4 Concentrate the toluene fraction into 100-mL round bottom flask just to dryness and transfer to 15-mL centrifuge tube with hexane.

12.5.5 Concentrate the hexane to near dryness and 10-uL of the recovery standards solution(Table 3) and analyze the resultant solution by HRGC/HRMS.

13.0 ANALYSIS OF PCDDs & PCDFs IN A SAMPLE

The quantitative determination for each congener can be expressed as:

- dissolved content: the results obtainted by analysing the extracts from paragraph 10.1;

- particulate PCDDs and PCDFs: the results obtained by analysing the sub-sample (paragraphs 9.1 and 10.2);

- and Total PCDDs & PCDFs: the analytical results obtained by adding the dissolved and particulate content for each congener in each homologous series.

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TABLE 1

HIGH-RESOLUTION CONCENTRATION CALIBRATION SOLUTION (HRCC)

CONCENTRATION (pg/uL)

COMPOUND	HRCC 1	2.0	3.0	4.0	5.0	6.0	7.0
UNLABELLED ANALYTES							
2,3,7,8-TCDD	1.0	2.5	5.0	10.0	20.0	40.0	80.0
2,3,7,8-TCDF	1.0	2.5	5.0	10.0	20.0	40.0	80.0
1,2,3,7,8-PeCDD	1.0	2.5	5.0	10.0	20.0	40.0	80.0
1,2,3,7,8-PeCDF	1.0	2.5	5.0	10.0	20.0	40.0	80.0
2,3,4,7,8-PeCDF	1.0	2.5	5.0	10.0	20.0	40.0	80.0
1,2,3,4,7,8-HxCDD	2.5	5.0	10.0	20.0	40.0	80.0	160.0
1,2,3,6,7,8-HxCDD	2.5	5.0	10.0	20.0	40.0	80.0	160.0
1,2,3,7,8,9-HxCDD	2.5	5.0	10.0	20.0	40.0	80.0	160.0
1,2,3,4,7,8-HxCDF	2.5	5.0	10.0	20.0	40.0	80.0	160.0
1,2,3,6,7,8-HxCDF	2.5	5.0	10.0	20.0	40.0	80.0	160.0
1,2,3,7,8,9-HxCDF	2.5	5.0	10.0	20.0	40.0	80.0	160.0
2,3,4,6,7,8-HxCDF	2.5	5.0	10.0	20.0	40.0	80.0	160.0
1,2,3,4,6,7,8-HpCDD	2.5	5.0	10.0	20.0	40.0	80.0	160.0
1,2,3,4,6,7,8-HpCDF	2.5	5.0	10.0	20.0	40.0	80.0	160.0
1,2,3,4,7,8,9-HpCDF	2.5	5.0	10.0	20.0	40.0	80.0	160.0
OCDD	5.0	12.5	25.0	50.0	100.0	200.0	400.0
OCDF	5.0	12.5	25.0	50.0	100.0	200.0	400.0
INTERNAL STANDARDS							
$^{13}C_{12}$-2,3,7,8-TCDD	20.0	20.0	20.0	20.0	20.0	20.0	20.0
$^{13}C_{12}$-2,3,7,8-TCDF	20.0	20.0	20.0	20.0	20.0	20.0	20.0
$^{13}C_{12}$-1,2,3,7,8-PeCDD	20.0	20.0	20.0	20.0	20.0	20.0	20.0
$^{13}C_{12}$-1,2,3,7,8-PeCDF	20.0	20.0	20.0	20.0	20.0	20.0	20.0
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	50.0	50.0	50.0	50.0	50.0	50.0	50.0
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	50.0	50.0	50.0	50.0	50.0	50.0	50.0
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	50.0	50.0	50.0	50.0	50.0	50.0	50.0
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	50.0	50.0	50.0	50.0	50.0	50.0	50.0
$^{13}C_{12}$-OCDD	100.0	100.0	100.0	100.0	100.0	100.0	100.0
RECOVERY STANDARDS							
$^{13}C_{12}$-1,2,3,4-TCDD[a]	20.0	20.0	20.0	20.0	20.0	20.0	20.0
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD[b]	50.0	50.0	50.0	50.0	50.0	50.0	50.0

(a)- Used for recovery determinations of TCDD, TCDF, PeCDD and PeCDF internal standards.

(b)- Used for recovery determinations of HxCDD, HxCDF, HpCDD, HpCDF, and OCDD internal standards.

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

TABLE 2. COMPOSITION OF PCDD AND PCDF GC PERFORMANCE EVALUATION SOLUTION

Column Retention Time Window Defining Standard
(DB-5 and Sp-2330 Columns)

No. of Chlorine Atoms	PCDD-Positional isomer		PCDF-Positional isomer	
	Early Eluter	Late Eluter	Early Eluter	Late Eluter
4	1,3,6,8-	1,2,8,9-	1,3,6,8-	1,2,8,9-
5	1,2,4,7,9-	1,2,3,8,9-	1,3,4,6,8-	1,2,3,8,9-(DB-5) 2,3,4,6,7,8-(Sp2330)
6	1,2,4,6,7,9-	1,2,3,4,6,7-	1,2,3,4,6,8-	1,2,3,4,8,9-(DB-5) 2,3,4,6,7,8-(Sp2330)
7	1,2,3,4,6,7,8-	1,2,3,4,6,7,9-	1,2,3,4,6,7,8-	1,2,3,4,6,7,9-(DB-5) 1,2,3,4,7,8,9-(Sp2330)
8	1,2,3,4,6,7,8,9-		1,2,3,4,6,7,8,9-	

NOTE: The isomers without bracketed information elute identically from both the columns.

TABLE 3

COMPOSITION OF THE SAMPLE SPIKING AND RECOVERY STANDARD SOLUTIONS

Analyte	Sample Spiking Solution Concentration (pg/uL; Solvent: Isooctane)[a]	Recovery Standard Solution Concentration (pg/uL; Solvent: Tridecane)[b]
$^{13}C_{12}$-2,3,7,8-TCDD	10	-
$^{13}C_{12}$-2,3,7,8-TCDF	10	-
$^{13}C_{12}$-1,2,3,4-TCDD	-	20
$^{13}C_{12}$-1,2,3,7,8-PeCDD	10	-
$^{13}C_{12}$-1,2,3,7,8-PeCDF	10	-
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	25	-
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	25	-
$^{13}C_{12}$-1,2,3,4,7,9-HpCDD	-	50
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	25	-
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	25	-
$^{13}C_{12}$-OCDD	50	-

(a) - 20 uL of this solution is added to sample prior to extraction

(b) - 10 uL of this solution is added prior to GC/MS analysis

TABLE 4

CHROMATOGRAPHIC CONDITIONS

Injection Port Temperature	260°C
Separator Temperature	260°C
Initial Temperature	70°C
Initial Time	4 min.
Ramp Rate 1	20°C/min.
Temperature 2	200°C
Hold Time 2	0 min.
Ramp Rate 2	4°C/min.
Temperature 3	260°C
Hold Time 3	5 min.
Split/Sweep	85 sec.

Table 5. Ions Monitored for HRGC/HRMS analysis of PCDD/PCDFs
(S = internal/recovery standard)

Descriptor	Accurate(a) Mass	Ion ID	Elemental Composition	Analyte
1	303.9016	M	$C_{12}H_4{}^{35}Cl_4O$	TCDF
	305.8987	M+2	$C_{12}H_4{}^{35}Cl_3{}^{37}ClO$	TCDF
	315.9419	M	$^{13}C_{12}H_4{}^{35}Cl_4O$	TCDF (S)
	317.9389	M+2	$^{13}C_{12}H_4{}^{35}Cl_3{}^{37}ClO$	TCDF (S)
	319.8965	M	$C_{12}H_4{}^{35}Cl_4O_2$	TCDD
	321.8936	M+2	$C_{12}H_4{}^{35}Cl_3{}^{37}ClO_2$	TCDD
	331.9368	M	$^{13}C_{12}H_4{}^{35}Cl_4O_2$	TCDD (S)
	333.9339	M+2	$^{13}C_{12}H_4{}^{35}Cl_3{}^{37}ClO_2$	TCDD (S)
	375.8364	M+2	$C_{12}H_4{}^{35}Cl_6O$	HxCDPE
	[354.9792]	LOCK	C_9F_{13}	PFK
2	339.8597	M+2	$C_{12}H_3{}^{35}Cl_4{}^{37}ClO$	PeCDF
	341.8567	M+4	$C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O$	PeCDF
	351.9000	M+2	$^{13}C_{12}H_3{}^{35}Cl_4{}^{37}ClO$	PeCDF (S)
	353.8970	M+4	$^{13}C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O$	PeCDF (S)
	355.8546	M+2	$C_{12}H_3{}^{35}Cl_4{}^{37}ClO_2$	PeCDD
	357.8516	M+4	$C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O_2$	PeCDD
	367.8949	M+2	$^{13}C_{12}H_3{}^{35}Cl_4{}^{37}ClO_2$	PeCDD (S)
	369.8919	M+4	$^{13}C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O_2$	PeCDD (S)
	409.7974	M+2	$C_{12}H_3{}^{35}Cl_7O$	HpCDPE
	[354.9792]	LOCK	C_9F_{13}	PFK

(Continued)

Table 5. Continued

Descriptor	Accurate Mass	Ion ID	Elemental Composition	Analyte
3	373.8208	M+2	$C_{12}H_2{}^{35}Cl_5{}^{37}ClO$	HxCDF
	375.8178	M+4	$C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O$	HxCDF
	383.8642	M	$^{13}C_{12}H_2{}^{35}Cl_6O$	HxCDF (S)
	385.8610	M+2	$^{13}C_{12}H_2{}^{35}Cl_5{}^{37}ClO$	HxCDF (S)
	389.8156	M+2	$C_{12}H_2{}^{35}Cl_5{}^{37}ClO_2$	HxCDD
	391.8127	M+4	$C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O_2$	HxCDD
	401.8559	M+2	$^{13}C_{12}H_2{}^{35}Cl_5{}^{37}ClO_2$	HxCDD (S)
	403.8529	M+4	$^{13}C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O_2$	HxCDD (S)
	445.7555	M+4	$C_{12}H_2{}^{35}Cl_6{}^{37}Cl_2O$	OCDPE
	[354.9792]	LOCK	C_9F_{13}	PFK
4	407.7818	M+2	$C_{12}H^{35}Cl_6{}^{37}ClO$	HpCDF
	409.7789	M+4	$C_{12}H^{35}Cl_5{}^{37}Cl_2O$	HpCDF
	417.8253	M	$^{13}C_{12}H^{35}Cl_7O$	HpCDF (S)
	419.8220	M+2	$^{13}C_{12}H^{35}Cl_6{}^{37}ClO$	HpCDF (S)
	423.7766	M+2	$C_{12}H^{35}Cl_6{}^{37}ClO_2$	HpCDD
	425.7737	M+4	$C_{12}H^{35}Cl_5{}^{37}Cl_2O_2$	HpCDD
	435.8169	M+2	$^{13}C_{12}H^{35}Cl_6{}^{37}ClO_2$	HpCDD (S)
	437.8140	M+4	$^{13}C_{12}H^{35}Cl_5{}^{37}Cl_2O_2$	HpCDD (S)
	479.7165	M+4	$C_{12}H^{35}Cl_7{}^{37}Cl_2O$	NCDPE
	[430.9728]	LOCK	C_9F_{17}	PFK

(Continued)

Table 5. Continued

Descriptor	Accurate Mass	Ion ID	Elemental Composition	Analyte
5	441.7428	M+2	$C_{12}{}^{35}Cl_7{}^{37}ClO$	OCDF
	443.7399	M+4	$C_{12}{}^{35}Cl_6{}^{37}Cl_2O$	OCDF
	457.7377	M+2	$C_{12}{}^{35}Cl_7{}^{37}ClO_2$	OCDD
	459.7348	M+4	$C_{12}{}^{35}Cl_6{}^{37}Cl_2O_2$	OCDD
	469.7779	M+2	$^{13}C_{12}{}^{35}Cl_7{}^{37}ClO_2$	OCDD (S)
	471.7750	M+4	$^{13}C_{12}{}^{35}Cl_6{}^{37}Cl_2O_2$	OCDD (S)
	513.6775	M+4	$C_{12}{}^{35}Cl_8{}^{37}Cl_2O$	DCDPE
	[430.9728]	LOCK	C_9F_{17}	PFK

(a)The following nuclidic masses were used:

H = 1.007825	O = 15.994915
C = 12.000000	^{35}Cl = 34.968853
13C = 13.003355	^{37}Cl = 36.965903

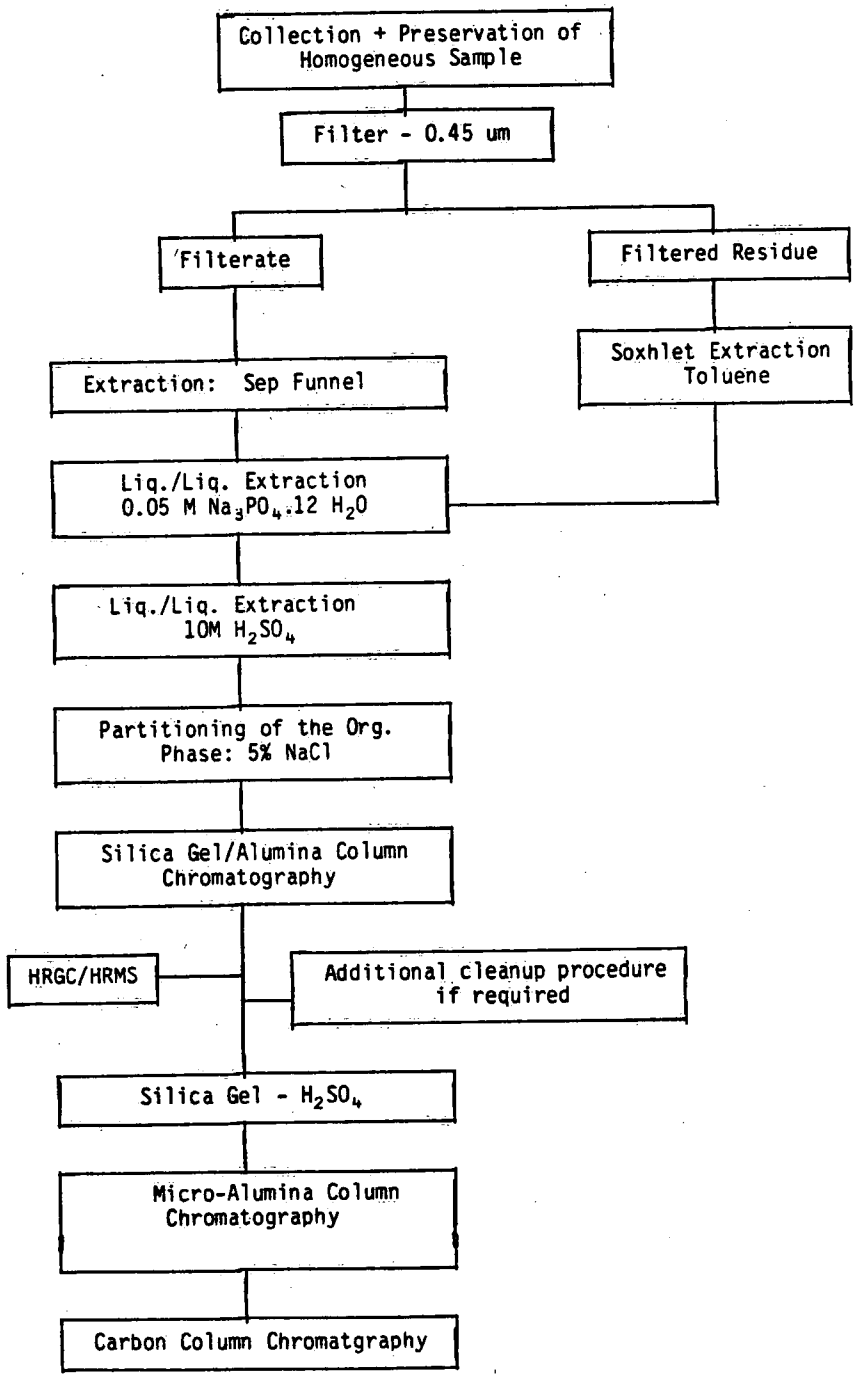
Table 6. Theoretical Ion Abundance Ratios and Their Control Limits for PCDDs and PCDFs

Number of Chlorine Atoms	Ion Type	Theoretical Ratio	Control Limits lower	Control Limits upper
4	$\frac{M}{M+2}$	0.77	0.65	0.89
5	$\frac{M+2}{M+4}$	1.55	1.24	1.86
6	$\frac{M+2}{M+4}$	1.24	1.05	1.43
6(a)	$\frac{M}{M+2}$	0.51	0.43	0.59
7(b)	$\frac{M}{M+2}$	0.44	0.37	0.51
7	$\frac{M+2}{M+4}$	1.04	0.88	1.20
8	$\frac{M+2}{M+4}$	0.89	0.76	0.89

(a)Used only for ^{13}C-HxCDF (IS).
(b)Used only for ^{13}C-HpCDF (IS).

FIGURE 1

METHOD FLOW CHART FOR SAMPLE EXTRACTION, CLEANUP AND HRGC/HRMS ANALYSIS



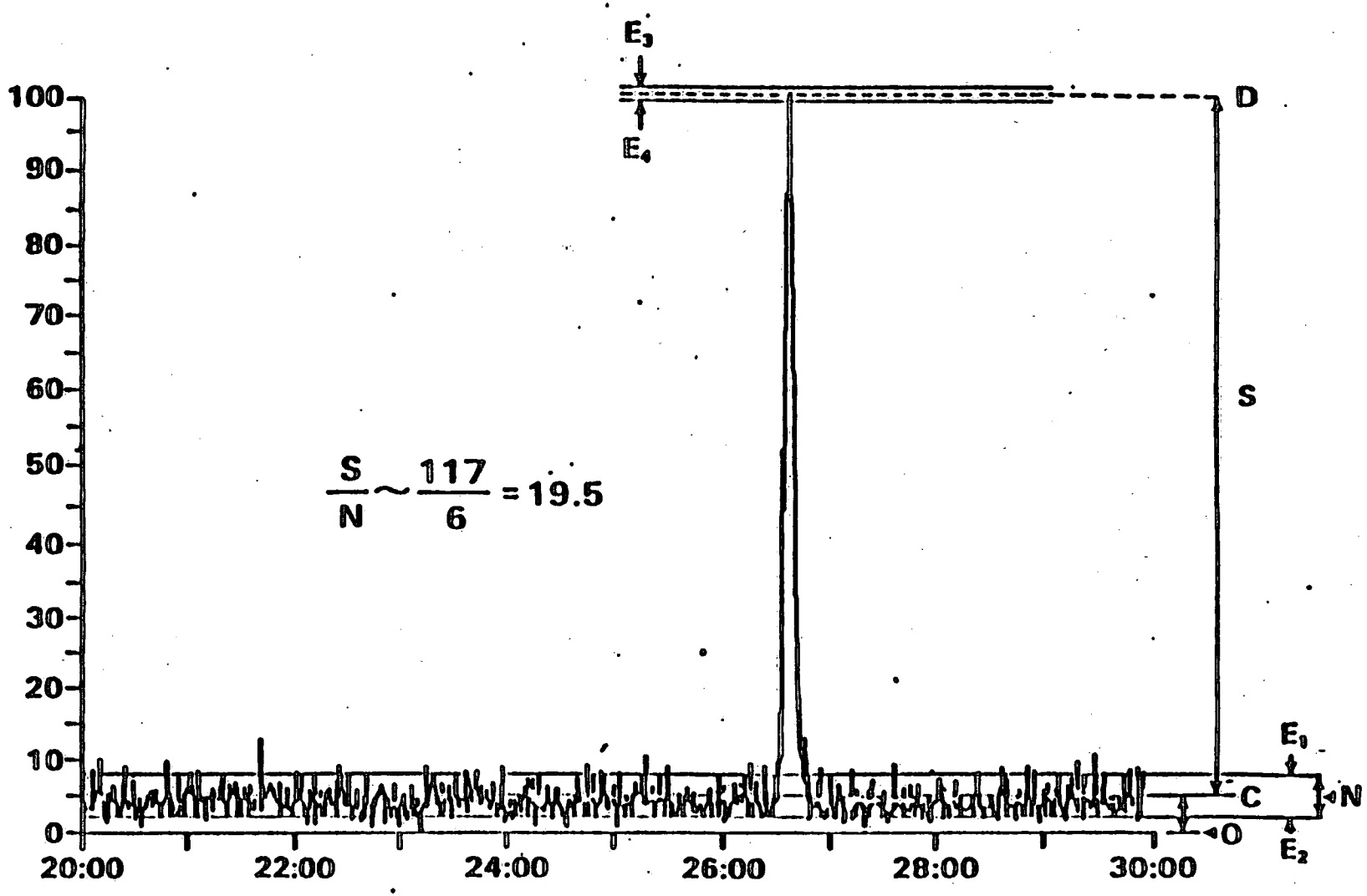

E3
E4
D
S
E1
C
N
O
E2
100
90
80
70
60
50
40
30
20
10
0
20:00
22:00
24:00
26:00
28:00
30:00
S/N ~ 117/6 = 19.5

Figure 2.

ATTACHMENT I

MANUAL DETERMINATION OF S/N

In Figure 2 the peak height is measured between the mean noise (line C and D). These mean signal values are obtained by tracing the line between the baseline average noise extremes, E_1 and E_2, and between the apex average noise extremes, E_3 and E_4, at the apex of the signal. Note, it is imperative that the instrument interface amplifier electronic zero offset be set high enough such that negative-going baseline noise is recorded.

ATTACHMENT II

Final Version January 31, 1989
D.B. Sergeant
DFO, Burlington

QUALITY ASSURANCE PROTOCOL FOR CPPA

NATIONAL DIOXIN CHARACTERIZATION OF CANADIAN BLEACHED

CHEMICAL PULPING OPERATIONS

The Dioxin Quality Assurance Committee (DQAC), which will evaluate and recommend to CPPA either acceptance or rejection (request re-analysis) of results, has developed a set of mandatory quality assurance protocols for the analytical contract laboratories participating in the CPPA survey. The objectives of this intensive QA are:

- to maximize the generation of quality analytical data
- to ensure complete documentation and defensibility of all data generated for CPPA mill studies
- to expedite data evaluation and acceptance with a minimum of inconvenience to DQAC and contract labs
- to clearly define the various QA requirements that must be followed

Since highly accurate and precise results at the parts-per-trillion level are required by the CPPA, the Dioxin Task Force's Analytical Working Group previously evaluated contractor analytical capabilities for the pulp and paper matrices involved by conducting two interlaboratory studies. The protocol for Interlab Study II, the Wright State protocols, U.S. EPA protocols, and several OME quality assurance documents formed the framework from which DQAC developed their QA management plan. The objective of this plan, as stated previously, is to scientifically evaluate the contractor generated data over the time-frame of the CPPA mill study and to identify problems and recommend correction in a timely manner (essential because of variety of matrices and large number of samples to be processed).

The DQAC quality assurance document is divided into 3 sections:

(a) Analytical Procedures Pulp and Paper Matrices

(b) Data Reporting and Evaluation Procedures

(c) Additional QA Requirements

These must be followed explicitly by the contracting laboratory(ies). This does not infer that contracted agency should not follow their own internal QA/QC as well.

A. Analytical Procedures Pulp and Paper Matrices

1. Sample Sets

All samples will be processed in sets. Sample sets forwarded to contractor will consist of 9 samples of the same matrix (sludge, pulp or effluent). These will be processed along with 1 blind replicate (to be designated by CPPA), a method blank[1], and a blind reference material every set (reproducibility check). This reference material may be spiked with known quantities of various isomers. Laboratories will follow their own internal QA/QC protocols as well, however there is no need for duplicates because of the blind replicates and reference samples.

SAMPLE SET	=	9	samples
		1	method blank (internal QC)
		1	blind replicate
		1	blind reference
		12	

2. Sample Size

The following minimum amounts of sample must be extracted for each matrix:

Sludge[2]	5g
Pulp[2]	10g
Effluent	1L

For solid samples (sludge and pulp) oven-dried (O.D.) weight must be determined and used in calculations and for moisture determination. In the case of effluent samples the entire sample is to be extracted. There should be no sample splitting.

1. Method blank is defined as all surrogates, reagents and cleanup steps, that are used on the matrices composing the sample set, with no sample matrix present.
2. Air-dried weight for analysis.

3. Surrogate Spiking

Prior to extraction, each sample must be spiked in its extraction vessel with 100 μL of the surrogate standard mixture provided by CPPA to the contractor. Because of miscibility problems, effluent samples will be spiked by first adding 100 μL of surrogate mixture to 1000 μL acetone and quantitatively transferring this with acetone into the effluent sample. Sample must be thoroughly mixed immediately using a magnetic stirrer and teflon or glass coated stirring bar for 10 minutes to thoroughly disperse the spiking solution.

4. Extraction and Cleanup

All samples will be extracted and cleaned up by the labs' regular validated extraction and cleanup procedures.

5. Performance (Recovery) Standard/Final Extract Volume

The cleaned up extract will be taken just to dryness in a clean, new 100 μL autosampler vial under a gentle stream of nitrogen and 20 μL of 100 pg/μL $^{13}C_{12}$-1,2,3,4-TCDD in toluene (provided by CPPA) will be added. This will be used to assess instrument stability, operator performance and surrogate recoveries.

6. Quantitation

A quantitation standard mixture is provided to all contractors and contains 10 native PCDD/PCDF congeners, the isotopically labelled performance standard and 6 isotopically labelled surrogates. Table 1 details the composition of this analytical standard and the ion masses to be monitored for determining sample results.

TABLE 1 - QUANTITATION STANDARD and SELECTED ION MASSES FOR PCDD/PCDF ANALYSIS

QUANTITATION Standard	CONCENTRATION (pg/μL)	QUANTITATION Ion	CONFIRMATION Ions
DIOXINS			
2,3,7,8-T_4CDD	100	322	320,257
1,2,3,7,8-P_5CDD	100	356	354,293
1,2,3,4,7,8-H_6CDD	100	390	392,327
1,2,3,4,6,7,8-H_7CDD	100	424	426,361
O_8CDD	100	460	458,397
FURANS			
2,3,7,8-T_4CDF	100	306	304,243
1,2,3,7,8-P_5CDF	100	340	338,277
2,3,4,6,7,8-H_6CDF	100	374	376,311
1,2,3,4,6,8-H_7CDF	100	408	410,345
O_8CDF	100	444	442,379
SURROGATES			
$^{13}C_{12}$-2,3,7,8-T_4CDD	100	334	332
$^{13}C_{12}$-1,2,3,7,8-P_5CDD	100	368	366
$^{13}C_{12}$-1,2,3,6,7,8-H_6CDD	200	402	404
$^{13}C_{12}$-1,2,3,4,6,7,8-H_7CDD	200	436	438
$^{13}C_{12}$-O_8CDD	300	472	470
$^{13}C_{12}$-2,3,7,8-T_4CDF	100	318	316
PERFORMANCE			
$^{13}C_{12}$-1,2,3,4-T_4CDD	100	334	332

Laboratories are to use internal standard procedures for quantitation using this mixture. For quantitation by the internal standard method, the isotopically labelled surrogates serve as internal standards to correct for losses during processing of samples and to compensate for errors owing to differences in injected volume and unnoticed variations in instrumental sensitivity.

When using internal standard quantitation, all six isotopically-labelled surrogates must be used as the internal standards. Also, the quantitation mixture must be run daily (see C.15) to determine relative response factors between the labelled and native congeners and to determine surrogate and performance standard recoveries. Results are automatically corrected for surrogate recovery, however, the actual surrogate recovery values must be reported.

Dioxin and furan results for each homologue are corrected for the recovery of the corresponding labelled surrogate dioxin congener, except for tetra-furan which is corrected for the recovery of the labelled surrogate $^{13}C_{12}$-2,3,7,8-TCDF isomer. Contractor will provide examples of all equations used to calculate surrogate recoveries, to determine relative response factors between native and surrogate compounds and to determine PCDD/PCDF concentrations in the sample.

7. Criteria for PCDD/PCDF Identification

Since many compounds can interfere with the determination of PCDDs and PCDFs, it is of utmost importance that positive identifications be made. The criteria for PCDD/PCDF confirmation are listed below:

C1 - Peak reponses of the quantitation ions must be greater than 3 times the background noise level.

C2 - Peak area (or height) ratios or spectral ion intensity ratios (from intensity list of spectrum of analyte peak) of the two monitored molecular ions for each congener group must be within $\pm$20% of the ratio obtained for the corresponding components in the QUANTITATION standard mixture.

C3 - Peak maxima for all three monitored ions must coincide within $\pm$2 scan units for it to be included in total congener summation.

C4 - For isomer specific identification, peaks may be identified as 2,3,7,8-TCDD, 2,3,7,8-TCDF, O_8CDF and O_8CDD if they meet the first two criteria and co-elute with their isotopically-labelled surrogates within $\pm$2 scan units.

C5 - no response must be seen at m/e 374 at the retention time of 2,3,7,8-TCDF. This M+ ion of hexachlorodiphenyl ether gives the same fragment ions as TCDF and yields a false positive TCDF result.

C6 - surrogate recoveries must fall within acceptable windows in Table 3 below.

8. Detection Limits

The detection limit must be reported for all sample results - not just for N.D. values. Details on how detection limits are to be calculated will be provided. The concentration units used to report the detection limit must be the same as that used for the sample. Detection limits must be corrected for surrogate recovery.

Table 2 below gives detection limits which must be met for data to be considered acceptable.

D. L's.

TABLE 2

DETECTION LIMITS (ppt)

	Pulp	Sludge
T_4CDD/F	5	20
P_5CDD/F	10	40
H_6CDD/F	15	60
H_7CDD/F	20	80
O_8CDD/F	25	100

TCDD / F 2
P_5CDD 5
H_6CDD 10
H_7CDD 15
O_8CDD 20

An analyte is detectable when the quantitat greater than 3 times S/N ratio. However, if other qualitative criterea are not met, such as, correct ion ratios (isomer specific determinations only), that measurement is reported as ND(R). An analyte is non-detectable when the quantitation ion response is less than 3 times S/N ratio. This will be reported as ND.

Detection limits corrected for surrogate recovery will be calculated in both cases as follows:

$$\text{D.L. (ppt)} = \frac{(3 \times N) \times \text{Pg/unit height} \times 100}{\text{g sample} \times \text{\% recovery of surrogate}}$$

B. Data Reporting and Evaluation Procedures

Data Reporting

All results must be reported on Form A, (attached) or computer generated form of identical format. For each total congener concentration reported the number of positive individual isomers found must be reported. This uniformity of reporting format is essential for ease of evaluation of data sets originating from more than one lab. Six legible copies of each must be provided.

Accompanying each data set will be original printer hardcopies of all GCMS ion chromatograms, with areas and retention times clearly marked in ink for positive analyte peaks not printed by printer, spectra and spectral ion intensity lists used for ratio calculations for isomer specific congeners of all analytical standards, sample and blank extracts and check solutions with all peaks identified as PCDD or PCDF congeners - clearly indicated directly on the printouts in ink. This is a mandatory requirement. The original and 5 legible copies must be provided. All materials submitted to DQAC must be clear and legible otherwise no review or payments will be made.

It is mandatory that all information requested on Form A be supplied. The ratio ranges must be determined by the contractor on their GCMS by analysis of the standard provided and determination of individual isomer's ion ratio from the areas or spectral (ion) intensities.

Data Evaluation

The DQAC will use a Deliverables Checklist to ensure that all mandatory requirements of sample analysis and data reporting are available for evaluation. If any element is missing the DQAC will request it be provided before any evaluation is attempted.

The DQAC will examine all material requested and either accept or reject the data based upon the identification criteria, surrogate recoveries, comparision of blind duplicates and reference materials, performance on solutions of standards, etc. Laboratory performance may be further evaluated by inter-laboratory study with spiked or unspiked matrices. Blind duplicate relative percent differences must agree to within $\leq$50% for TCDD, TCDF, OCDD and OCDF.

Surrogate recoveries outlined in Table 3 below must be achieved for all matrices. In cases where recoveries of surrogates are less than the minimum recovery (in the 20 - 40% range with all other criterea being met) and there is a significant positive isomer value at greater than twice the detection limit the data will be closely examined by the DQAC and may be accepted. However, if results are N.D. repeat analysis will be requested.

TABLE 3

SURROGATE STANDARD RECOVERY CRITERRA

Surrogate Standard	Amount Spiked (ng)	Acceptable Recovery (%)
$^{13}C_{12}$-2,3,7,8-T_4CDD	2	40-120
$^{13}C_{12}$-1,2,3,7,8-P_5CDD	2	35-120
$^{13}C_{12}$-1,2,3,6,7,8-H_6CDD	4	30-120
$^{13}C_{12}$-1,2,3,4,6,7,8-H_7CDD	4	25-130
$^{13}C_{12}$-O_8CDD	6	20-120
$^{13}C_{12}$-2,3,7,8-T_4CDF	2	40-120

C. Additional QA Requirements

Most of the following QA requirements are probably routine procedure in contract laboratories. However, they form an integral part of a QA protocol and must be followed:

(1) Once the study begins no changes to methods or procedures are to take place unless written permission is received from the CPPA after review by the DQAC.

(2) Good laboratory practice - all equipment, sample concentrators, glassware, benches, etc. shall be kept clean during processing of these samples. No high level samples such as flyash shall be processed simultaneously with the CPPA samples. This could lead to cross-contamination problems.

(3) Sample storage during workups: - all sample extracts shall be refrigerated at 4°C in the dark when not needed for various cleanup steps.

(4) Record keeping: - a set of laboratory notebooks will be dedicated to these analyses and all records of sample treatment shall be recorded in these logs, which will be checked, signed and dated daily by a senior chemist. Such notebooks shall be available for audit upon request by DQAC.

(5) Laboratory audits: - the DQAC may wish to audit individual contract laboratories and their operating procedures during this study. The analyst-in-charge may be required to review some of the data with the DQAC.

(6) The contractor is responsible for the disposal of unused samples. Such disposal shall not take place without the approval of DQAC. Samples will be suitably stored until then by contractor to ensure their integrity.

(7) All final sample extracts will be retained in new, unused, precleaned 100 µl autosampler vials,. When contractor completes his analyses these extracts (gently evaporated to dryness under nitrogen) will be delivered to CPPA. Tightly sealed with teflon-lined caps, under chain-of-custody protocols. Vial containers will not be replaced by DQAC or CPPA.

(8) Raw GCMS data shall be backed up onto tape or diskette daily and in duplicate. These tapes shall be stored in two different secure locations and may not be erased without express written permission of DQAC and CPPA.

(9) The common set of analytical standards and spiking surrogate mixtures (provided by CPPA) will be used for all quantitative determinations by all contractors.

(10) DQAC will critically examine all positive and negative 2,3,7,8-TCDF values and may audit these results by submitting a portion of the samples for confirmatory analysis such as high resolution GC - high resolution MS or isomer specific GCMS.

(11) Contractor(s) shall be responsible for GC column performance checking and shall provide details of such checks with sample reports:

(i) check for isomer specificity 2,3,7,8-TCDD and -TCDF

(ii) use commercial window defining mixtures to set up GCMS windows for the various congener groups.

(iii) verify performance of column by running analytical standards and maintaining a ±20% response on all ions monitored across the congener groups.

(iv) periodically a column blank shall be run to assess carryover.

(v) verify and demonstrate that 10 pg 2,3,7,8-TCDD can be accurately seen at greater than 3 times S/N prior to each set of analyses.

(12) Additional performance audits may involve blind analysis of spiked matrix samples, split audit samples sent to government labs, etc.

(13) Instrument mass range shall be calibrated daily. Linearity of system shall be determined at the start of the project by a minimum of 5 point calibration covering a concentration range of 0.02 to 2ng/µl PCDD/PCDF. Relative response factors (RRF's) (native std./labelled std.) are established using these 5 mixtures. The contractor shall be responsible for obtaining these mixtures but will provide all details to DQAC. Linearity shall be rechecked periodically and in particular, after each column change.

(14) The RRF's established in (13) above will be updated to those of the single quantitation standard provided by the CPPA prior to any analyses. RRF's must be checked daily (at beginning of the day and every 8 hrs. thereafter, including after the last sample analysis) by single point calibration. The daily RRF's must be $\pm$15% of the originally determined values. If RRF's outside this range are obtained RRF's must be completely re-established by repeating the original RRF determination procedure.

(15) Analytical standard and surrogate mixtures will be replaced periodically throughout the study.

(16) COCl loss must be monitored.

NOTE: As in AWG's Interlaboratory Studies I and II the wording isomer specific, applied to a 30m DB-5 column or its equivalent, represents a worse case scenario. The possibility of other co-eluting PCDD and PCDF isomers exists. For unequivocal 2,3,7,8-TCDD and 2,3,7,8-TCDF isomer specific analysis longer columns with other liquid phases are required.

FORM A
TABLE: 1 REPORT DATE: _____

SAMPLE TYPE: _____

FINAL VOLUME: _____ µL

SAMPLE SIZE: _____ g (air dry) SAMPLE CODE: _____

_____ L (liquid) CONTRACT LAB CODE: _____

ISOMER	pg/O.D.g or pg/L	DL	NI	RATIO	ACCEPTABLE RATIO RANGE	ANALYTE REJECTION[3]
2,3,7,8-T_4CDD						
T_4CDD (total)						
P_5CDD						
H_6CDD						
H_7CDD						
O_8CDD						
2,3,7,8-T_4CDF						
T_4CDF (total)						
P_5CDF						
H_6CDF						
H_7CDF						
O_8CDF						
Sur.Rec. (%)						
$^{13}C_{12}$-TCDF						
$^{13}C_{12}$-TCDD						
$^{13}C_{12}$-P_5CDD						
$^{13}C_{12}$-H_6CDD						
$^{13}C_{12}$-H_7CDD						
$^{13}C_{12}$-O_8CDD						
Perf.Std.Rec.(%)						

1. Results are corrected for surrogate recovery.
2. DL/NI = Detection Limit/Number of Isomers (or Peaks Resolved).
3. Coded criteria designation from A7.
4. O.D.g = oven-dried weight in grams

APPROVED _____ DATE _____