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POLYCHLORINATED DIBENZO DIOXIN/FURANS **USEPA METHODs 8290, 1613, 23, 0023A & TO-9A**			
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This version contains at the end of this document, a summary of the enhanced features or modifications to the reference method and describes any changes or clarifications where the reference method is ambiguous or provides insufficient details. It also embodies information acquired over 29 years of experience in PCDD/Fs analyses.

The procedures described herein are designed with pollution prevention so that they reduce the amount of hazardous substances and lower the hazards to public health. For more details on P2, click here for Pollution Prevention Basics. This revised version allows its presentation (i.e., ensuring hyperlinks are working outside our network environment) on a CD for review by EPA.

Passages considered proprietary are highlighted in red. Should a copy of this SOP be required, the color of the font associated with the text highlighted in red should be changed to red before printing and allowing a copy to leave our facilities. Furthermore, indicate here the date and the purpose for this copy:

Date: **Purpose:**

1. PURPOSE
    1. **This procedure describes the analytical techniques used for extraction and analysis of aqueous, solid, oil, wipe, waste, air and tissue samples for polychlorinated dibenzo dioxins and furans (PCDD/PCDF) by EPA Methods 8290 & 1613.**
2. SCOPE
    1. This SOP is based on the revision of Method 8290. It is consistent with the Final Rule on the Methods Innovation Rule 40 CFR Parts 63, 268, et al.; June 14 th, 2005; pp. 34538-34592.
3. APPARATUS AND MATERIALS
    1. Autosampler
    2. DEC Alpha Station with Opus Quan Data System.
    3. Neslab Water Coolers.
    4. MM Autospec Magnetic Sector High-Resolution Mass Spectrometer.
    5. Pipet, disposable, serological, 10mL.
    6. Amber glass bottles, 1 L (Teflon-lined screw cap).
    7. Two –L separatory funnels.
    8. Teflon boiling chips.
    9. glass chromatographic columns
    10. glass chromatographic columns
        1. Place a glass wool plug at the bottom of the column, pack with 1g of silica gel, 4 g NaOH/silica gel, 1 g of silica gel, 8 g of H 2SO 4/silica gel, 2 g silica gel, 1 g Na 2SO 4.
    11. N-Evaporator.
    12. Conical vials, 2mL.
    13. Glass fiber filters and glass wool plugs.
    14. Funnels, 100 mL.
    15. Dean-Stark Trap, condenser and flask.
    16. Rotary Evaporator.
    17. Round bottom flasks, 500mL.
    18. Top-Loader Balance.
    19. Oven.
4. REAGENTS, STANDARDS AND SOLVENTS

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1. Reagents
    1. Sulfuric acid, concentrated.
    2. Silica gel. Highest purity grade.
    3. Celite 545.
    4. Water, distilled.
    5. Florisil.
    6. Prepurified nitrogen gas.
    7. Anhydrous sodium sulfate.
    8. Sodium Hydroxide. Highest available purity.
2. Solvents
    1. Methylene chloride. Highest available purity.
    2. Hexane. Highest available purity.
    3. Tetradecane. Highest available purity.
    4. Acetone. Highest available purity.
    5. Methanol. Highest available purity.
3. Standards
    1. Analytical standards (Cambridge Isotope Laboratory, Wooburn, MA).

5. System Performance

1. An initial calibration curve is created to demonstrate the linearity of the HRMS system over the calibration range. An initial calibration is repeated once a year, whenever a new set of spiking calibration standards are created or whenever the continuing calibration falls outside the acceptance criteria. To help with the quantitations of high- level samples, a secondary ICAL (concept under evaluation) is available whereby the ES concentrations are 20 times lower compared to the primary ICAL. The secondary ICAL provides RRFs for analytes requiring 20-fold dilutions.

2. ICAL establishes the operating conditions necessary to meet the relative retention time specifications specified in EPA Method 8290/1613. The percent RSD for the mean response factors must be within ± 10 percent for the native standards and within ± 20 percent for extraction standards. Note that these requirements are more stringent than Method 8290 or Method 1613's tolerance windows. In addition, the RRFs must remain within 20 percent of the historical values. All S/N must be at least 10:1. Ion-abundance ratios must be within the 15 percent window of the theoretical ratios. The ICAL used for analytes is summarized in Table 2A.
    1. Inject the reference compound perfluorokerosene (PFK). PFK provides the required lock masses and is used for tuning the mass spectrometer.
    2. The lock-mass ion from PFK is dependent on the masses of the ions monitored within each descriptor. Each descriptor will be monitored in succession as a function of GC retention time to ensure that all PCDD/PCDFs are detected.
    3. An appropriate QC Check Ion will be monitored for each mass descriptor and shall be used to monitor system performance throughout the respective retention time windows. Specific situations where "deflections" are detected are evaluated on a case-by-case basis with emphasis on the impact on the reliability of the analyte data.
3. The instrument is tuned to the minimum required resolving power of 10,000 (10 percent valley or 5 percent crossing over) at an appropriate reference signal close to the masses of interests. Document the resolving power using the mass peak profile display. The latter is obtained at the beginning and the end of each analytical sequence of 12 hours or less.
4. Using the same operating conditions as established for the ICAL, inject 1 mL of the batch control spike (BCS_3). It is a QC sample used for true-stable isotope-dilution GC/MS methodologies to ensure the reliability and accuracy (bias and precision) of the determinations. The BCS_3 is prepared at the same concentration as the CS_3 of the ICAL.
    1. It is a new concept introduced to, not only, enhance the accuracy of the measurements, but to provide a basis for assigning an uncertainty to each measurements (Note: this is limited to the measurement step because it does not directly address the sampling errors), and abridge the level of effort involved in the documentation of the system's performance (i.e., what used to require three separate analyses are now combined into one).

        It is prepared—inside a 4oz. glass jar—in stages at the same time as the batch of field samples; i.e., at each phase involving the addition of the ES, CS or AS, JS to the samples. For air matrices, the BCS_3 is initiated at the same instant as the XAD/PUF cartridges are prepared for sampling. The NS must originate from an independent source.

        One BCS_3 per batch of 20 samples or less -- regardless of the matrix type – is going through the same spiking scheme with the same spiking solutions, same analyst, same delivery system, and at the same time as the field samples. It is the laboratory's responsibility to ensure sufficient BCS_3s are prepared to provide front- and back-end calibration verifications for all the samples as well as re-injections when necessary. For air samples returning with multiple trap preparations for a given project, select one of the BCS_3 (i.e., the one representing the largest number of traps) and use it to verify calibration (PD) and stability (RPD). However, use the ICAL RRFs to process all the samples associated with such project if it can be determined that the data reliability is not adversely affected. The laboratory will make every effort to communicate to the stack-sampling firm the significance of the trap preparation in light of the QA/QC.

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The BCS_3 is then analyzed at the beginning and at the end of a NTE 12-H analytical sequence during which samples are analyzed.

In order to use the front- and back-end BCS_3s averaged RRFs to process the samples, the individual front- and back-end RRFs need to meet a number of requirements (independent verification, RPD, and PD or bias).

The following criteria for the BCS_3 need to be met:

1. The first and last PCDD/F eluters are verified to be within the eight homologue retention time windows.
2. The signal to noise ratio (S/N) exceeds 10:1 for all ions monitored,
3. The ion abundance ratio measurements are within ± 15 percent of the theoretical ratio,
4. The Ax solution should be verified against an independent source. The maximum allowable difference for the "intra-source product area" ratios (*vide infra*) for the unlabeled compound's RRFs is +20 percent (from the laboratory normal source) relative to the ES (from an independent source). This verification should be performed every time a new set of ICAL solutions and new sets of spiking solutions (ES, CS/SS, JS, NS) are prepared from new primary stock standards with a minimum of one verification per year.
5. The ES solution should be verified against an independent source. The maximum allowable difference for the "intra-source product area" ratios (*vide infra*) for the unlabeled compound's RRFs is +20 percent (from an independent source) relative to the ES (from the laboratory normal source). This verification should be performed every time a new set of ICAL solutions and new sets of spiking solutions (ES, CS/SS, JS, Ax) are prepared from new primary stock standards with a minimum of one verification per year.
6. More specifically, it is necessary for the "intra-source product area" ratio below to range from 0.8 to 1.20.

$$\alpha \times \frac{A_X^{ls} \times A_{ES}^{ls}}{A_X^{is} \times A_{ES}^{is}} \quad \begin{matrix} \leftarrow \text{Laboratory Source} \\ \\ \leftarrow \text{Independent Source} \end{matrix}$$

where

A_X^{ls} = the area of the unlabeled analyte from the laboratory source

A_{ES}^{ls} = the area of the labeled extraction standard from the laboratory source

A_X^{is} = the area of the unlabeled analyte from the independent source

A_{ES}^{is} = the area of the labeled extraction standard from the independent source

α is equal to 1 when the concentrations of the respective analytes are the same between the independent and laboratory sources; the appropriate factor should be applied for situations whereby the concentrations of the respective analytes are different.

NOTE: It is highly recommended that the above mixtures be prepared in the same solvent and analyzed under the same GC/MS conditions.

NOTE: Intra-source product area ratios only apply to analytes for which matching standards are available between the laboratory and independent sources.

Similarly, for air samples, it is necessary for the "intra-source product area" ratio below to range from 0.7 to 1.30.

$$\alpha \times \frac{A_{SS}^{ls} \times A_{ES}^{ls}}{A_{SS}^{is} \times A_{ES}^{is}} \quad \begin{matrix} \leftarrow \text{Laboratory Source} \\ \\ \leftarrow \text{Independent Source} \end{matrix}$$

where

A_{SS}^{ls} A = the area of the sampling standard from the laboratory source

A_{ES}^{ls} = the area of the labeled extraction standard from the

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laboratory source

A_{SS}^{is} = the area of the sampling standard from the independent source

A_{ES}^{is} = the area of the labeled extraction standard from the independent source

α is equal to 1 when the concentrations of the respective analytes are the same between the independent and laboratory sources; the appropriate factor should be applied for situations whereby the concentrations of the respective analytes are different.

NOTE: When the SS are used as cleanup standards for non-air matrices, replace SS by CS and ES by JS in the expression above.

The RRFs Percent Differences (PD) relative to the ICAL should remain within

- Twenty percent for the unlabeled compounds
- Thirty percent for the labeled ES compounds
- Twenty percent for air's labeled SS, and
- Thirty percent for non air's labeled CS compounds
- Other requirements are shown in Table Insert 1 and Table Insert 2.

7. The RPDs between the front- and back-end BCS_3s should remain within the limits summarized in the Table Inserts. The RRFs Percent Differences (PD) relative to the ICAL should remain within the limits summarized in the Table Inserts. The addition of both NS and ES should be performed using the same technique and the same volume. That way, any systematic error associated with the ES (within acceptable limits as defined herein) will "ratio out" when the two BCS_3 calibration analyses are used to compute the analyte concentrations in the samples. By using this approach, the accuracy of the measurements is superior to the traditional approaches. It is also a benefit that flows directly from true stable isotope-dilution GC/MS, which until now was regrettably ignored.

   The RPDs between the front- and back-end BCS_3s should remain within:

   - Ten percent for the unlabeled compounds
   - Twenty percent for the labeled compounds

1. For air samples where a split factor is involved, i.e., the sample extract is split and a portion is archived as backup, the BCS_3 is not subjected to an actual physical division. The latter is simulated by the addition of an appropriate volume of the same solvent as for the ICAL and the samples (e.g., if the split factor is 2, then, the BCS_3 needs to be diluted two fold before analysis to allow the analytes to be at the same concentration as for the ICAL CS_3).

   For air samples, the BCS_3 is initiated at the same time as the preparation of the air sampling modules before the sampling session. To that effect, the same amount of the Sampling Standards is added to a vial, which is kept in the laboratory at room temperature and away from light. The corresponding Lab Method Blank prepared with the same batch of sorbent and spiking solution (i.e., 40 g XAD-2 resin, or PUF) is kept refrigerated.

   At the beginning and end of each 12-hour period during which samples are analyzed, an aliquot of the BCS3 is analyzed to demonstrate adequate GC resolution and sensitivity, response factor reproducibility, to establish the PCDD/PCDF retention time windows and isomer-specificities, and to validate the standards and the spiking technique.

   As defined above, the criteria for an acceptable BCS_3 are summarized in the table inserts below. When the BCS3 fails, it is important to discern the following:

   - The fundamental objective of the BCS_3 is to "validate" the standard solutions and spiking technique and the RRFs used to quantitatively characterize the analytes in the samples **at the time** the standards are used to prepare and analyze the samples. They are four types of standards involved in the preparation of the BCS_3 that provide various probes into assessing this "validation" procedure. They are the NS (symbolized as A x in expressions or tables), ES, SS or CS, and JS. The question becomes how can one "extract" the information needed to complete the validation, or how does one "filter" out the irrelevant information to help with the distinction between a critical error and a minor one. A critical error means erroneous data resulting from a seriously flawed spiking technique (e.g., wrong amount of ES added under the OPR/LCS/ICAL system, or the NS under the BCS_3 system) while other minor errors can provide useful information or feedback on the measurement step (e.g., instrumentation variation). The interpretation of the information obtained from the analysis of the BCS_3 is best handled when done contextually. This analytical protocol does not claim that it offers a comprehensive analysis but merely puts forward guidelines to help the analyst in assessing the quality and reliability of the data.

   - A failure on the "**PD**" requirements may be indicative of an instrumentation difficulty or spiking error. The latter can be of Level PD-1 (i.e., at the standard solution level) or Level PD-2 (i.e., at the spiking operation level). A third Level PD-3 is associated with instrumentation. An error at the NS standard solution level (Level PD-1) constitutes, under the BCS_3 system, the most serious failure and requires that a new set of standard solutions be prepared, independently validated (*vide infra*, intra-source ratio study) before repeating the sample extraction and analysis, if called for by the decision flow chart ("*Thinking Method*"). Note that the decision flow charts make a distinction between short- and long-term actions. When appropriate, a new initial calibration may be required before analyzing the BCS_3 and the samples. However, if the error is a Level PD-2 error, a re-

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extraction and analysis is the most suitable action after correcting the flawed spiking technique. As customarily done, a new BCS3 is prepared with a Level PD-2 error. Here again, the decision flow charts should be used since the text herein is only for illustration purposes. Distinction between Levels PD-1 and PD-2 can be accomplished contextually by examination of the initial independent validation study and control charts (showing for instance a trend suggesting a degradation of the ES solution), behavior over time charts, and/or using the matrices shown in Table Inserts 3 or 4. The Level PD-3 error is associated with instrumentation when an out-of-calibration situation is present or a temporary or localized instrumentation variation is operative. Depending on the severity of the Level PD-3 error, a new calibration (either ICAL or rerunning the BCS_3 and all the affected samples) following a new "tuning" of the instrumentation may be required (see Decision Flow Charts for "*Thinking Method*").

- A failure on the "**RPD**" requirements may be indicative of instrumentation instability or inability to sustain the instrumentation's performance over a 12-H period. Again, two levels are possible. Level RPD-1 is strictly associated with instrumentation difficulties that are unrelated to the samples under analysis. A re-analysis (i.e., re-injection) of the BCS_3 **and** of the samples can be considered as a corrective action after correction of the source of the instrumentation's shortfall (see Decision Flow Charts for "*Thinking Method*"). If however, the re-analysis of the BCS_3 fails again, and there are indications that the spiking procedure is questionable (Level PD-1 or PD-2), the associated extraction batch must undergo re-extraction and analysis with the preparation of a new associated BCS3 as discussed above for the BCS_3 PD deviations. A Level RPD-2 BCS_3 failure may be found with the analysis of samples presenting special challenges (i.e., highly complex matrices that do not cleanup well under the various options offered by this protocol). Depending on the severity of the deviation, additional cleanup or other appropriate actions may be required before re-analysis of the samples and associated BCS_3. If such action proves to be ineffective, the data should be qualified accordingly.

- A " **PD**" failure for 2,3,7,8-TCDD and/or 2,3,7,8-TCDF results in the inability to reliably quantify 2,3,7,8-TCDD/F until proper corrective action is implemented (e.g., following GC column maintenance). When the corrective action involves a different liquid phase, the **correct** BCS_3 is used to demonstrate adequate performance. Note that the laboratory is encouraged to adopt a similar stance for 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF (or any other 2,3,7,8-subtituted congeners, which significantly contributes to the TEQ).

- The BCS_3 "PD Requirements" are summarized in the four table inserts below. In addition to the traditional RRFs, another set of "pseudo-RRFs" is computed from the BCS3 data to help with the validation of the ES and RRFs used to report the sample analytes. The pseudo-RRFs are used to further differentiate the various "A" to "C" types PD Requirements. Use Table Insert 3 (non air) or Table Insert 4 (air) for departing-from-the-norm groups of analytes (e.g., all 17 A x or the five SS show a deviation similar in "sign" and "amplitude"). It is also recommended to examine the data contextually (e.g., using QC charts)

Table Insert 1:

Batch CS_3 PD Requirements

Based on Traditional RRFs

("A" to "C"Types)

5.

Type	Analytes	Requirement	Failure Possible Cause[a,b]	Failure Level	Suggested Corrective Action
A	**A_X** vs **ES**	+ 20%	1. Calibration out 2. Spiking error	▪ PD-3 ▪ PD-1/PD-2	▪ New Calibration ▪ New Standards/New Extraction
B	**ES** vs **JS**	+ 30% non air + 30% for air [c]	1. Calibration out 2. Spiking error	▪ PD-3 ▪ PD-1/PD-2	▪ New Calibration ▪ New Standards/New Extraction
C_1	**CS** vs **JS**	+ 30%	1. Calibration out 2. Spiking error	▪ PD-3 ▪ PD-1/PD-2	▪ New Calibration ▪ Affects other Types
C_2	**SS** vs **ES** (air)	+ 20%	1. Calibration out 2. Spiking error	▪ PD-3 ▪ PD-1/PD-2	▪ New Calibration ▪ New Standards/New Extraction/New Sampling[d]

a. Calibration out = usually when one (localized) or several/all analytes are affected; instrumental source.
b. Spiking error = when all analytes are affected with the same "sign" and "amplitude"; must be considered contextually; i.e., using historical data or other information on the set of standards such as the "pseudo-RRFs". Situations when selected analytes degrade are rare but should not be excluded from consideration.
c. By design for air matrices, the amounts of ES and JS added during the preparation of the Batch CS 3 are different. Thus, an additional error is introduced, which can deceive the analyst's interpretation. In this case, the QC emphasis is shifted towards the "C2" type PD requirement.
d. Because of the nature of an "air" sample, there is no additional sample volume

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available to repeat the extraction. The laboratory is required to qualify the data by estimating and documenting accordingly the "error" associated with the reported measurements. If such documentation is not possible, and/or the information points toward a seriously flawed ES addition (as opposed to a spiking error associated with the SS), the data can be rejected and re-sampling efforts may be necessary. See the "Air Spiking Related Error Matrix" tables for an alternative approach whereby the A_x vs SS RRFs are used to determine the analyte's concentrations (Table Insert 4).

Table Insert 2 :

Batch CS_3 PD Requirements

Based on Pseudo-RRFs

("D" to "G"Types)

Type	Analytes[a]	Requirement
D	**A_x** vs **CS/SS**	+/- 25%
E_1	**A_x** vs **JS** (non air)	+/- 35%
E_2	**A_x** vs **JS** (air)	+/- 35%
F_1	**ES** vs **CS** (non air)	+/- 20%
G_2	**SS** vs **JS** (air)	+/- 50%

a. Pseudo-RRFs are limited to analytes, for which an analogous/homologous standard is available:

- 2,3,7,8-TCDD (A_x) vs $^{13}C_{12}$-1,2,3,4-TCDD (JS)
- 2,3,4,7,8-PeCDF (A_x) vs $^{13}C_{12}$-1,2,3,4,6-PeCDF (CS)
- 13 C 12-1,2,3,7,8-PeCDD (ES) vs $^{13}C_{12}$-1,2,3,4,7-PeCDD (CS)
- Do not consider pairs such as OCDD (A_x) vs $^{13}C_{12}$-1,2,3,4,6,8,9-HpCDF (SS) or $^{13}C_{12}$-1,2,3,4,7-PeCDD (CS or SS) vs $^{13}C_{12}$-1,2,3,4,6,7-HxCDD (JS)

Table Insert 3:

"Non-Air" Spiking Related PD Errors

(departing-from-the-norm group of analytes)

"PD Requirements Decision Matrix" – Normal Configuration
(use BCS_3 RRFs)

	Ax	ES	CS	JS
Ax	-	Y	Y	Y
ES	-	-	Y	Y
CS	-	-	-	Y

"PD Requirements Decision Matrix" – Defective A_x Spiking = **Fatal**
(If A_x spiking is shown to be in error, and if no additional or replacement sample is available, use ICAL RRFs if CS_3 is acceptable)

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	Ax	ES	CS	JS
Ax	-	N	N	N
ES	-	-	Y	Y
CS	-	-	-	Y

"PD Requirements Decision Matrix" – Defective JS Spiking
(use BCS_3 RRFs)
percent recovery measurements for CS & ES affected, not the analytes

	Ax	ES	CS	JS
Ax	-	Y	Y	N
ES	-	-	Y	N
CS	-	-	-	N

"PD Requirements Decision Matrix" – Defective CS Spiking
(use BCS_3 RRFs)
percent recovery measurements for CS affected, not the analytes

	Ax	ES	CS	JS
Ax	-	Y	N	Y
ES	-	-	N	Y
CS	-	-	-	N

"PD Requirements Decision Matrix" – Defective ES Spiking
(use BSC_3 RRFs; see Levels PD-1 or PD-2)

	Ax	ES	CS	JS
Ax	-	N	Y	Y
ES	-	-	N	N
CS	-	-	-	Y

Table Insert 4:

"Air" Spiking Related PD Errors

(departing-from-the-norm group of analytes)

"PD Requirements Decision Matrix" – Normal Configuration
(use BCS_3 RRFs)

	Ax	ES	SS	JS
Ax	-	Y	Y	Y
ES	-	-	Y	Y
SS	-	-	-	Y

"PD Requirements Decision Matrix" – Defective A_x Spiking = **Fatal**
(If Ax spiking is shown to be in error, and if no additional or replacement)

	Ax	ES	SS	JS
Ax	-	N	N	N
ES	-	-	Y	Y
SS	-	-	-	Y

"PD Requirements Decision Matrix" – Defective JS Spiking
(use BCS_3 RRFs)
percent recovery measurements for ES affected, not the analytes or the SS

	Ax	ES	SS	JS
Ax	-	Y	Y	N
ES	-	-	Y	N
SS	-	-	-	N

"PD Requirements Decision Matrix" – Defective SS Spiking
(use BCS_3 RRFs)
percent recovery measurements for SS affected, not the analytes

	Ax	ES	SS	JS

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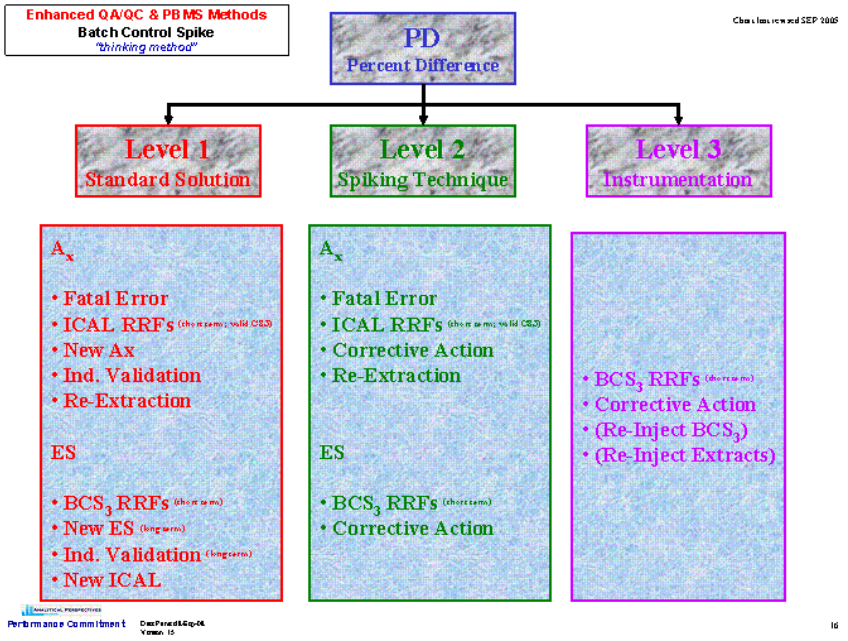
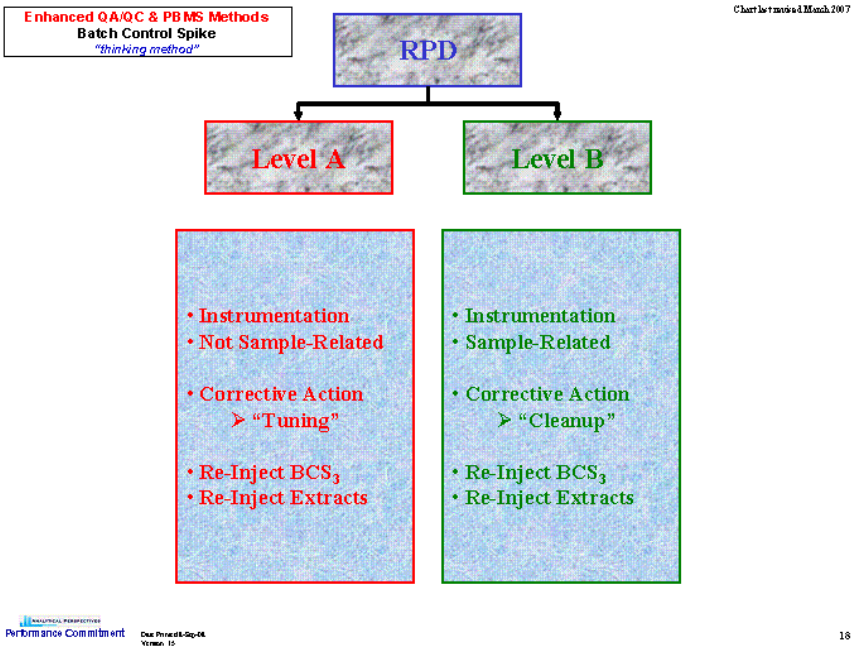
Ax	-	Y	N	Y
ES	-	-	N	Y
SS	-	-	-	N

"PD Requirements Decision Matrix" – Defective ES Spiking
(use BCS_3 RRFs; see Levels PD-1 or PD-2; for air samples only, consider using the A_x vs SS RRFs)

	Ax	**ES**	**SS**	**JS**
Ax	-	N	Y	Y
ES	-	-	N	N
SS	-	-	-	Y

Decision Flow Charts for "*Thinking Method*"





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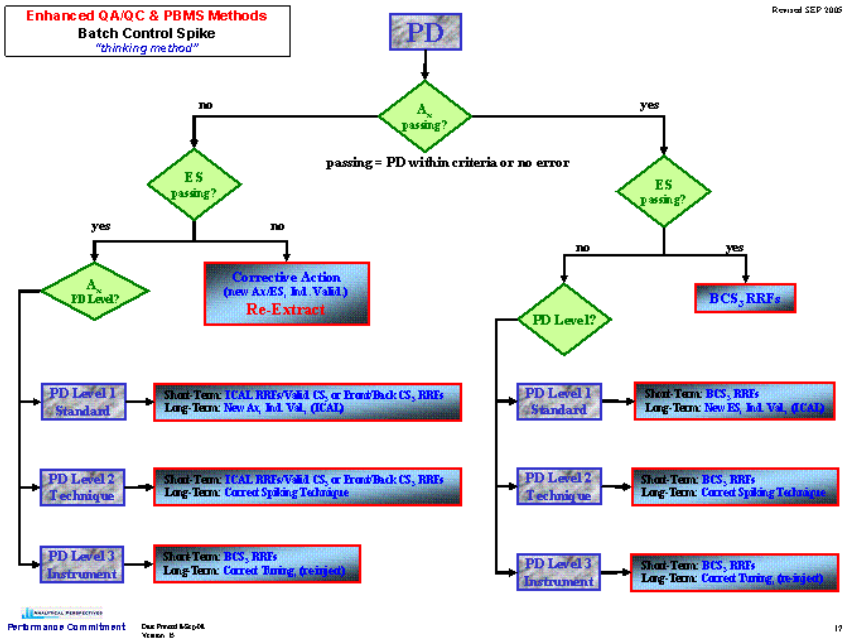

1. Cleanup Calibrator

    1. The purpose of the Cleanup Calibrator QC sample is to verify the ability of recovering all the PCDD/Fs rather than just the seventeen 2,3,7,8-substituted congeners. The Cleanup Calibrator authenticates the fractionation procedure in a similar way GPC separations are calibrated—not only for the seventeen 2,3,7,8-substituted PCDD/F congeners, but also for the first and last eluters of each homologue group as well as the total homologue groups.

    2. Because the fractionation procedure is what really needs to be monitored, this new type of QC sample is used for procedures sharing a common fractionation backbone.

    3. There are a number of viable options that can be followed for the preparation of the Cleanup Calibrator solution:

        1. Acquire from commercial sources a mixture containing the 136 PCDD/F tetra- through octachlorinated congeners

        2. Acquire internally by extracting—without the addition of the ES/CS/JS—an ash sample showing the charateristic "combustion" PCDD/F pattern or fingerprint

        3. Extract and analyze an independent performance evaluation sample containing all the PCDD/F congeners (e.g., M8290B PE samples; M23 audit samples).

        4. If available, acquire from commercial sources the carbon-13 labeled analogues of the first and last eluting PCDD/F congeners, which can be added to every sample undergoing extraction and analysis. While it is recognized that this option only aims at the window defining components, and assumes a similar chromatographic behavior for the analytes on a GC column and a cleanup column, it offers the assurance of recovering "all" the target compounds on a per sample basis

    4. In order to verify that no selective losses or significant distortions of the PCDD/F pattern occur during the fractionation, each inclusive fractionation methodology adopted by the laboratory must be validated at the start (as part of the initial demonstration of performance), once a year (as part of the MDL or the analysis of a suitable PE sample) and whenever a change in the procedure is implemented during which there is a reasonable possibility for selective losses. To that effect, treat the "ash extract" (or the cleanup calibrator solution) as a sample extract by adding the ES and subject the spiked extract to the cleanup steps, followed by the analysis. Note that, when a suitable PE sample is used, the results for all 17 2,3,7,8-substituted congeners as well as each of the eight homologue group totals and the first/last eluting congeners should be rated with the help of this analytical protocol's guidelines.

    5. The "Cleanup Calibrator" concept should be considered and extended to the validation of new extraction techniques particularly when there is a potential for "selective extraction" of PCDD/F congeners (e.g., solid phase extractions when affinities of PCDD/F congeners play a critical role). In other words, the self-referential principle dictates that an alternate extraction technique is validated when it can be demonstrated that no "selective losses" occurred under ther most challenging conditions. The validation of an extraction and/or cleanup procedure that is used "routinely" should not be limited to the seventeen 2,3,7,8-substituted congeners.

    6. Acceptance criteria should be consistent with allowed variations of the RRFs within the initial and continuing calibrations (i.e., 25 percent RPD for the 2,3,7,8-substituted congeners and up to 35 percent RPD for the non specific congeners between the new/alternate and the previously validated approach's results). The comparison is conducted with the data obtained during the initial demonstration of peformance (e.g., PE sample; or using the "traditional" cleanup procedure).

    7. Signs of departures from the norm should be monitored on a daily basis by carefully reviewing the raw data, in particular the SICPs, for distorted patterns (i.e., distribution of the various isomers within an homologue group does not match expectations or experience).

2. Duplicate Analyses (optional)

    Ideally, to be meaningful and of value, a duplicate sample is submitted to the laboratory in a blind fashion. This way, their role is not limited to assessing the performance of the analytical aspects but as a whole, the sampling aspects as well. However, should the scope of the particular work requires the laboratory to select a sample as a

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duplicate, then the results of the laboratory duplicates (concentrations of 2,3,7,8-substituted PCDD/PCDF compounds) should agree within 25 percent relative difference (difference expressed as percentage of the mean) for analytes with a concentration within the calibration curve.

3. Laboratory Method Blank

For a given matrix (i.e., non-biological solid, aqueous, biological tissue, air) a minimum of one Laboratory Method Blank (LMB; mandatory) per batch of 20 samples or less is prepared following the exact same steps as the samples using the same reagents and standards. As part of good laboratory practices, it is recommended that the LMB be extracted on a different extraction position from one batch to the other by tracking and recording the extraction position number of the LMB as well as the associated samples. Similarly when using automated sample fractionation techniques, rotate the position of the LMB from batch to batch, and record that information. For reporting the results, always use the same units and the lowest sample volume or weight from the field samples. Note that, for air samples, the LMB is prepared at the same moment, using the same batch of sorbent materials, spiking solutions and technique, and by the same personnel who prepared the sampling modules before the sampling session.

6. QUALITY CONTROL

1. Method Blank (MB): Method blank is a sand, Hydromatrix TM, Na 2SO 4 or distilled water preparation that is free of native analytes that has been prepared and analyzed using the same procedures followed for the rest of the analytical batch. For air samples, the MB is resin or PUF prepared from the same batch used for the field samples. The MB is kept in house at 4 0 C until the field samples are returned.

    1. A method blank is run with every analytical batch or 20 samples (whichever is less) per matrix type.

    2. For the determination of native 2,3,7,8-substituted congeners, the levels measured in the method blank must be less than the method quantitation limit or ten times lower than the concentration found in any sample within the analytical batch.

    3. All samples within an analytical batch are re-extracted and analyzed if the method blank associated with that batch does not meet the criteria described above.

2. On-Going Precision & Accuracy (OPR = LCS): Applicable only to true 1613 assays. This redundant and useless laboratory control sample is prepared by adding a known quantity of native standards to a matrix and is supposedly used to assess method performance (precision and accuracy). One OPR is analyzed per matrix.

    1. A known amount of native PCDD/Fs is used for spiking.

    2. The OPR of each native isomer should have a relative percent difference as per Method 1613, which defines the tolerance windows that must be met.

    3. Extraction standard recoveries should range between Method 1613's tolerances.

    4. If the Extraction Standard recovery of an isomer in the OPR and the associated sample(s) is also out of the range, the sample and the OPR will be re-extracted and analyzed.

    5. It is AP's policy to develop a database of sample projects where both the OPR and the BCS3 are analyzed for M1613 samples. Once the Laboratory Director has determined that such information has been obtained, then, any project completed under M1613 where an OPR is required will NOT have a BCS3. Only those projects not requiring an OPR will be processed and analyzed under the BCS3 system. Furthermore, when an OPR is required, only a CS3 – spiked with the usual isomeric interferences and the first and last eluters – will be analyzed at the beginning of the sequence. No ending CS3 will be done when OPR is required.

3. Matrix Spike (MS/MSD): Again, this redundant and useless sample is prepared by adding a known quantity of native standards to a sample matrix before the extraction. These are normally done upon request and are considered as billable because we do not believe that there are of any value. Our procedures call for the use of all labeled compounds. Thus, each sample is in fact a matrix spike in itself. MS/MSD samples under isotope-dilution MS do not add any value to the assessment of the system's performance.

    1. A known amount of native PCDD/Fs is used for spiking.

    2. The relative percent difference between MS/MSD samples should be £20 percent provided the matrix used does not contain PCDD/Fs at levels impairing the measurements. Limits are based on the variances that are typically allowed with the methods' relative response factors.

4. Duplicate Samples: Duplicate samples are two separate aliquots taken from the same source. Duplicate samples are analyzed independently to assess laboratory precision.

    1. If the relative percent difference from duplicate sample analyses is greater than 50 percent, then both duplicate samples and the affected batch of samples will be reanalyzed.

7. COLLECTION, PRESERVATION, AND HANDLING

1. Amber glass bottles and jars must be used for collection.

2. Fish tissue is stored at -20 ° C, all other samples are stored at 4 ° C, extracted within 30 days and completely analyzed within 45 days of extraction

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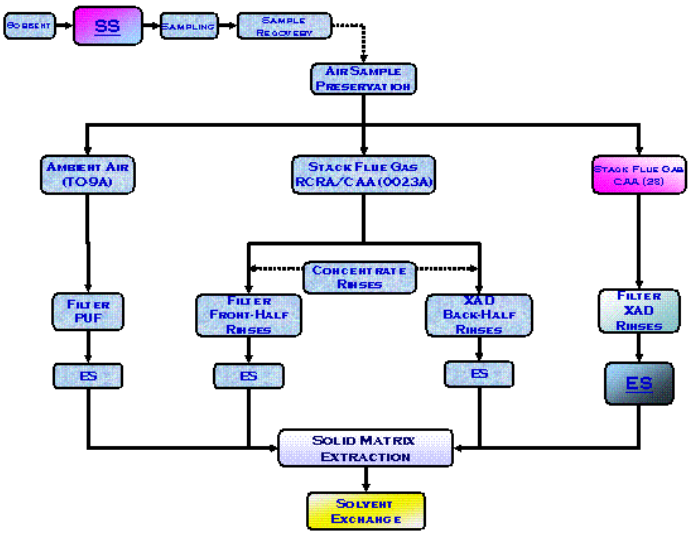
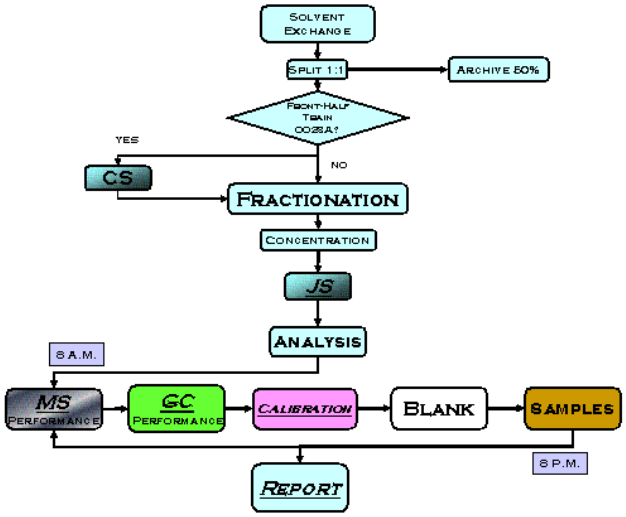


Sample Preparation Flow Chart for Air Matrices



Sample Analysis Flow Chart
(example given for air matrices)

3.

8. EXTRACTION AND CLEANUP PROCEDURES

1. Extraction

1. Determine the percent solids on all samples the day before, except for oil. Weigh out an appropriate weight or volume, dry overnight in 110 °C oven and re-weigh.

2. All samples are extracted on a wet weight basis. For solid samples, adjust the sample amount for extraction to yield a 10-g equivalent dry weight based on the percent solids.

3. In general, if samples are "dirty" or samples contain oil or fuel oil, all cleanup steps may be required.

4. Add the Extraction Standards and native spike (A x) as described in the sample path forms. When appropriate use 1 mL of acetone for spiking.

5. Aqueous or Effluent Samples: If the sample contains >1.0 percent solids, treat the sample as a solid (i.e., isolate a cake). Aliquot an amount of the aqueous sample that will yield a 10-g dry weight equivalent. Filter this aliquot thru a 1.0 mm filter using the filtering setup. Transfer the filter and cake inside the thimble. Spike the appropriate standards using acetone as a carrier. Add 10 g Hydromatrix TM and mix the solids from the filter through the Hydromatrix TM. If solids are still present in the water phase filter the water through 0.7 mm and 0.45 mm filters removing and placing them in the same glass thimble. This insures a complete recovery of the 10-g dry weight equivalent.

   If the solid load is less than 1.0 percent, the sample may be extracted using the TALEX, CLL , or the SPE-C approaches. The details for these procedures are given in the AP-SP-E analytical procedures. The decision to follow one particular approach over the others is dictated by the client's requirements, the type of samples undergoing analysis, and the expertise of the laboratory Director.

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Note: All paper mill effluent samples should be filtered, the isolated solids extracted by SPE and SDS.

6. Solid or Waste Samples: Add 10 g of sample directly into an 8oz jar, spike in acetone, mix sample well, mix with Hydromatrix TM. Soxhlet/Dean Stark extract for 16 hours with toluene (250 mL C 14). Proceed with appropriate cleanup procedures described in section 8.2.

    1. To separate phases of wet (>25 percent water) soil, sediment or paper samples, a Soxhlet/Dean Stark extractor system may be used with toluene as the solvent.

7. Oil Samples: Dilute the sample with 50 mL of hexane. Proceed with appropriate cleanup procedures described in section 8.2.

8. Air Samples: Each sample is usually comprised of a filter, a XAD-2 resin trap, solvent rinses. (Note: aqueous impinger contents and catch for samples are part of CARB Methods 428 & 429.) Samples being prepared by EPA Method 23 are comprised of a filter, a XAD-2 resin trap, solvent rinses and the toluene rinse, which is prepared separately if requested. Method 0023A requires the filter/front half and the XAD-2 resin trap/back half to be extracted separately and analyzed by Method 8290. For ambient air samples, a PUF and filter combination is typical.

    1. Filter and XAD-2 resin trap should be photographed together for documentation purposes prior to combination (Method 23). Line up components next to the corresponding thimbles and take a group photograph, and then take a photograph of each sample's components immediately before combining into the thimble.

    2. Combine resin and filter in a SDS extractor. [redacted]

    3. Concentrate the rinses to less than 5 mL using a rotovap or by other appropriate means. Add concentrated solvent rinses to resin/filter portion in thimble . [redacted]

    4. Into the thimble, spike the appropriate amount, as indicated on the sample path tracking sheets of 23 D/F Extraction Standards. Place extractor atop of a 500-mL round-bottom flask containing Teflon TM boiling chips, and 350 to 400 mL of toluene.

    5. Place a glasswool plug into the thimble on top of the resin. Assemble the entire SDS apparatus to the condenser and place upon heating mantle. Turn on the heating units, cooling water and set temperature for toluene to boil. Allow refluxing for 16 hours at the rate of 3 cycles per hour.

    6. [redacted]

    7. Splitting: Transfer the extract residue with hexane inside a calibrated 15-mL centrifuge tube. Use a total of 12 mL and divide the sample extract in half unless otherwise noted on the process sheet. Store at room temperature the archived portion in the centrifuge tube with a Teflon TM-lined screw top.

9. Tissue Samples: Tissue samples are ground and homogenized. Others can be used as such.

    1. Grind fish using the Hobart meat grinder. Collect the ground tissue directly on cleaned aluminum foil.

    2. Using Tissuemizer blend meat into a creamy paste consistency.

    3. Using a spatula, mix well.

    4. Place two 25-g aliquots of the ground tissue inside two 4-oz jars, label the jars and store in a freezer.

    5. Aliquot another 25 g for analysis or on such requested occasions, aliquot 75 g.

    6. Fortify directly on matrix using acetone as the carrier.

    7. Mix with 20-25 g of Hydromatrix TM , stir until a dry, free-flowing consistency is achieved, adding more Hydromatrix TM as needed, careful not to exceed an amount that can fit into the acid thimble for 25-g sample sizes. Place the mixture on top of a layer of [redacted]

    8. For 75-g samples , [redacted] .The sample is extracted as is with Hydromatrix TM and the oversized extraction setup is used in this case to ensure that enough solvent is used and that there is enough room in the thimble to allow for proper extraction of the sample.

    9. Spike each extraction cell with the appropriate Extraction Standards.

    10. Soxhlet Dean-Stark using hexane for 16 H. For the rare 75-g samples, however, a mixture of 30% Acetone and 70% Toluene should be used rather than Hexane. Again this is to ensure a proper extraction is achieved! Note that when PCBs are extracted as well as PCDD/Fs, the solvent mixture is hexane – acetone (1:1). In case the tissue contains sediment particles, it is recommended to follow the hexane-acetone extraction with a toluene-acetone extraction. Whenever a 75-g sample is extracted, the extraction time is extended from 16 H to 60 H.

    11. Concentrate the extract [redacted] on the Rotovap

    12. Add the CS.

    13. ASECS (sample extract using SOP AP-SP-CU1 current revision).

The section below was, during a recent revision of our SOPs, inserted in the SOP for Homogenization, which should be limited to

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[redacted]

2. Cleanups **(note: refer to specific SOPs for the latest version; this Core Method SOP may not contain the same level of details)**.
    1. If not done, add the Cleanup Standards. Rotoevap to [redacted]. Proceed to first cleanup.
    2. ABP-Acid/base partitioning ("dirty" samples only)
        1. Add 10 mL of hexane to extract in round bottom.
        2. Add 40 mL of concentrated H_2SO_4 into separatory funnel. Add extract. Rinse round bottom with 2 times 10 ml of Hexane and add to separatory funnel. Shake for 2 minutes with periodic venting, allow the layers to separate for a minimum of 5 minutes and discard acid layer. Repeat with 40 mL of fresh acid up to 4 times or when the hexane becomes clear and colorless. Add 40 mL of distilled water into separatory funnel containing sample. Shake and discard. Add 40 mL of 1.0N NaOH into separatory funnel containing sample. Shake and discard. Add 40 mL of distilled water into separatory funnel containing sample. Shake and discard.
        3. Pass hexane extract through Na_2SO_4, then roto-evap to [redacted]. Proceed to the next cleanup procedure.
        4. Acid/Base Silica Column (see SOP AP-SP-CU1 current revision)
        5. [redacted] (see SOP AP-SP-CU1 current revision)

9. GC/MS ANALYSIS
    1. Establish the necessary operating conditions. The following GC operating conditions are for guidance and adjustments may be required.
        1. Injector temperature: 270°

Interface temperature:	200 °C
Initial time:	2 minutes
Temperature program:	200 to 220 ° C, at 5 ° C/min
	220 ° C for 16 minutes
	220 to 235 ° C, at 5 ° C/min
	235 ° C for 7 minutes
	235 to 330 ° C, at 5 ° C/min

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2. The reference compound perfluorokerosene (PFK) provides the required lock masses and is used for tuning the mass spectrometer.
    1. The lock-mass ion from PFK is dependent on the masses of the ions monitored within each descriptor. Each descriptor will be monitored in succession as a function of GC retention time to ensure that all PCDD/PCDFs are detected.
    2. An appropriate lock mass will be monitored for each descriptor and shall not vary by more than ±20 percent throughout the respective retention time window.
    3. The mass resolution check is achieved before any analysis is performed and at the end of each 12-hour shift.
3. Set up the analytical run following this sequential injection pattern: CPSM-CS3, Solvent Blank, Method Blank, Samples, OPR, or BCS_3, Solvent Blank, Samples, BCS_3. (The solvent blank spiked is spiked at a concentration of 1 pg/µL.
4. Continuing Calibration
    1. Follow the BCS_3 requirements. When true 1613 requirements are required, follow the procedures below.
    2. Inject a column performance standard mix (CPSM) to verify retention time windows. This requirement can be accomplished by using the OPR analysis. The latter is prepared with the appropriate congeners. The following criteria must be met:
        - The chromatographic peak separation between 2,3,7,8-TCDD and the closest eluting isomers must be resolved with a valley of £10 percent. Note that the method calls for a less stringent requirement of 25 percent. Analytical Perspectives also requires that the peak shape of the TCDD/F be normal before one can use the DB-5MS column for both TCDD/TCDF isomer-specific determinations.
        - The first and last PCDD/F eluters are verified to be within the eight homologue retention time windows.
    1. Inject a mid-range standard from the initial calibration curve (CS3) at the beginning and end of every 12 hours. The following criteria must be met:
        - The relative response factors for the mid-range standard are within the limits established in Method 1613. That is, the percent RSD for the mean response factors must be within the method's tolerances.
        - The ion ratios are within 15 percent of the theoretical.
        - The signal to noise ratio (S/N) exceeds 10:1 for all ions monitored.
        - The retention times must be within the criteria established in Method 1613.
5. Qualitative Determination
    1. To identify a chromatographic peak as a PCDD or PCDF (either an unlabeled or a labeled compound). It must meet the following criteria:
        - The signals for the two exact m/z being monitored must be present and must maximize within ±2 seconds of one another.
        - The signal-to-noise ratio (S/N) of each of the two exact m/z must be greater than or equal to 2.5:1 for a sample extract, and greater than or equal to 10:1 for a calibration standard.
        - The ion abundance ratios must have a ratio within the limits established for the homologous series.
        - The absolute retention times for non-2,3,7,8-substituted congeners must be within the corresponding windows set by the CPSM.
        - The absolute retention times for 2,3,7,8-substituted congeners must be within 0 to +2 seconds of the isotopically labeled standard; these new limits are derived from over 500 data points obtained from MM1 and MM3 over a 17-week period worth of BCS_3 and CS3/CS0. The 99% confidence intervals suggested 0 to 1 sec.; AP decided to use 2 sec. to cover all analytes (updated on 17 OCT 07). These limits are more representative of our level of performance. Weak peaks will continue to be treated as before with the call made by the chemist.
6. Quantitative Determination
    1. For peaks that meet the criteria listed above, quantitate the PCDD and PCDF peaks from the mean RRF relative to the appropriate Extraction Standard established in the initial calibration.
    2. Recovery of each Extraction Standard versus the Injection Standard should be between 40-135 percent or have a signal to noise ratio >10:1 and a favorable detection limit (Method 8290). See Method 1613 for the acceptable windows.
    3. It is recommended that sample recoveries less than 40 percent or greater than 135 percent be re-extracted and re-analyzed unless the S/N ratio is >10:1 and/or the DL is unacceptable. Similarly, outliers from Method 1613 tolerances should be considered for re-extraction. However, the Lab Director has the option to accept the data provided they meet our extraction requirements. Re-extraction is always required if we can show that the deviations result from an action that can adversely affect the data reliability. See the Decision Flow Charts for the "*Thinking Method*".
    4. Report results in picogram per gram, picogram per liter or picogram per sample.
    5. Any sample in which the 2,3,7,8-TCDF is identified by analysis on a DB-5 GC column and is above the method calibration limit must be confirmed on a DB-225 or equivalent GC column. The use of the DB-5MS for both 2,3,7,8-TCDD and 2,3,7,8-TCDF isomer specificities can also be used. However, the percent valley for 2,3,7,8-TCDD is more stringent (10 percent instead of 25 percent) and the TCDD/TCDF peak shape must be normal (i.e., no tailing) to allow accurate measurement of the 2,3,7,8-TCDF isomer on the DB-5MS column. Analytical Perspectives can provide additional documentation on the validation of the DB-5MS for 2,3,7,8-TCDF determinations. Alternatively, the demonstration of isomer specificity is conducted in conjunction with the BCS_3 analyses (enfolded performance).

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6. For 2,3,7,8-substituted congeners that are not identified, calculate a sample- and analyte-specific estimated detection limit.
7. For a homologous series with no positive identifications, calculate the detection limit.
8. Click here for more information on "Working at the Limits".

10. CALCULATIONS

1. The concentrations for the PCDD or PCDF compounds are calculated by using the formula:

$$C_x = \frac{A_x \times Q_{is}}{A_{is} \times W \times RRF}$$

Where

C_x = Concentration of unlabeled PCDD/PCDF congeners (or group of coeluting isomers within an homologous series) in pg/g.

A_x = Sum of the integrated ion abundances of the quantitation ions (See Table 6 of EPA Method 8290) for unlabeled PCDD/PCDFs.

A_{is} = Sum of the integrated ion abundances of the quantitation ion (See Table 6 of EPA Method 8290) for the labeled Extraction Standards.

Q_{is} = Quantity, in pg, of the Extraction Standard added to the sample before extraction.

W = Weight of the sample (solid or liquid).

RRF = Calculated relative response factor for the analyte.

2. The detection limits for each absent 2,3,7,8-substituted PCDD/PCDF can be calculated using the following formula:

$$DL = \frac{2.5 \times H_N \times Q_{is}}{H_{IS} \times W \times RRF}$$

DL = Estimated detection limit for homologous 2,3,7,8-substituted PCDD/PCDFs.

H_N = Noise height (peak to peak).

H_{IS} = Peak height of the Extraction Standard.

Q_{is} = Quantity, in pg, of the Extraction Standard added to the sample before extraction.

W = Weight of the sample (solid or liquid).

RRF = Calculated relative response factor for the analyte.

11. REFERENCES

USEPA Method 8290, Revision 0, Dated September 1994.

USEPA Method 8290B, Dated July 2002.

USEPA Method 1613, Revision B, Dated October 1994.

- TABLE 1: Theoretical Ion Abundance Ratios and Control Limits for PCDDs and PCDFs

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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	1.02

(a) Used for 13C-HxCDF (IS) only.

(b) Used for 13C-HpCDF (IS) only

TABLE 2A: PRIMARY HIGH-RESOLUTION CONCENTRATION CALIBRATION SOLUTIONS

(Regular Initial Calibration)

Concentrations in pg / µL	CS0	CS1	CS2	CS3	CS4	CS5	CS6
Unlabeled Analytes:							
2,3,7,8-TCDD	0.25	0.5	2	10	40	200	500
2,3,7,8-TCDF	0.25	0.5	2	10	40	200	500
1,2,3,7,8-PeCDD	1.25	2.5	10	50	200	1000	2500
1,2,3,7,8-PeCDF	1.25	2.5	10	50	200	1000	2500
2,3,4,7,8-PeCDF	1.25	2.5	10	50	200	1000	2500
1,2,3,4,7,8-HxCDD	1.25	2.5	10	50	200	1000	2500
1,2,3,6,7,8-HxCDD	1.25	2.5	10	50	200	1000	2500
1,2,3,7,8,9-HxCDD	1.25	2.5	10	50	200	1000	2500
1,2,3,4,7,8-HxCDF	1.25	2.5	10	50	200	1000	2500
1,2,3,6,7,8-HxCDF	1.25	2.5	10	50	200	1000	2500
1,2,3,7,8,9-HxCDF	1.25	2.5	10	50	200	1000	2500
2,3,4,6,7,8-HxCDF	1.25	2.5	10	50	200	1000	2500
1,2,3,4,6,7,8-HpCDD	1.25	2.5	10	50	200	1000	2500
1,2,3,4,6,7,8-HpCDF	1.25	2.5	10	50	200	1000	2500
1,2,3,4,7,8,9-HpCDF	1.25	2.5	10	50	200	1000	2500
OCDD	2.5	5	20	100	400	2000	5000
OCDF	2.5	5	20	100	400	2000	5000
Extraction Standards:							
$^{13}C_{12}$-2,3,7,8-TCDD	100	100	100	100	100	100	100
$^{13}C_{12}$-2,3,7,8-TCDF	100	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8-PeCDF	100	100	100	100	100	100	100
$^{13}C_{12}$-2,3,4,7,8-PeCDF	100	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	100	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	100	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	100	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	100	100	100	100	100	100
$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	100	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	100	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	100	100	100	100	100	100	100
$^{13}C_{12}$-OCDD	200	200	200	200	200	200	200
$^{13}C_{12}$-OCDF	200	200	200	200	200	200	200
Cleanup/Sampling Standards:							
$^{37}Cl_4$-2,3,7,8-TCDD	-	0.5	2	10	40	200	-
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,6,8-HxCDF	100	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,6,9-HxCDF	100	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,6,8,9-HpCDF	100	100	100	100	100	100	100
Injection Standards:							
$^{13}C_{12}$-1,2,3,4-TCDD	100	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4-TCDF	100	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,6,7-HxCDD	100	100	100	100	100	100	100

TABLE 2B: SECONDARY HIGH-RESOLUTION CONCENTRATION CALIBRATION SOLUTIONS (optional)

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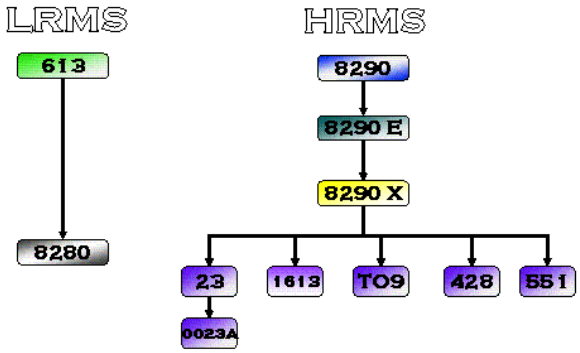
	CS0	CS1	CS2	CS3	CS4	CS5	CS6
Unlabeled Analytes							
2,3,7,8-TCDD	0.25	0.5	2	10	40	200	500
2,3,7,8-TCDF	0.25	0.5	2	10	40	200	500
1,2,3,7,8-PeCDD	1.25	2.5	10	50	200	1000	2500
1,2,3,7,8-PeCDF	1.25	2.5	10	50	200	1000	2500
2,3,4,7,8-PeCDF	1.25	2.5	10	50	200	1000	2500
1,2,3,4,7,8-HxCDD	1.25	2.5	10	50	200	1000	2500
1,2,3,6,7,8-HxCDD	1.25	2.5	10	50	200	1000	2500
1,2,3,7,8,9-HxCDD	1.25	2.5	10	50	200	1000	2500
1,2,3,4,7,8-HxCDF	1.25	2.5	10	50	200	1000	2500
1,2,3,6,7,8-HxCDF	1.25	2.5	10	50	200	1000	2500
1,2,3,7,8,9-HxCDF	1.25	2.5	10	50	200	1000	2500
2,3,4,6,7,8-HxCDF	1.25	2.5	10	50	200	1000	2500
1,2,3,4,6,7,8-HpCDD	1.25	2.5	10	50	200	1000	2500
1,2,3,4,6,7,8-HpCDF	1.25	2.5	10	50	200	1000	2500
1,2,3,4,7,8,9-HpCDF	1.25	2.5	10	50	200	1000	2500
OCDD	2.5	5	20	100	400	2000	5000
OCDF	2.5	5	20	100	400	2000	5000
Extraction Standards							
$^{13}C_{12}$-2,3,7,8-TCDD	5	5	5	5	5	5	5
$^{13}C_{12}$-2,3,7,8-TCDF	5	5	5	5	5	5	5
$^{13}C_{12}$-1,2,3,7,8-PeCDD	5	5	5	5	5	5	5
$^{13}C_{12}$-1,2,3,7,8-PeCDF	5	5	5	5	5	5	5
$^{13}C_{12}$-2,3,4,7,8-PeCDF	5	5	5	5	5	5	5
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	5	5	5	5	5	5	5
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	5	5	5	5	5	5	5
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	5	5	5	5	5	5	5
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	5	5	5	5	5	5	5
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	5	5	5	5	5	5	5
$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	5	5	5	5	5	5	5
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	5	5	5	5	5	5	5
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	5	5	5	5	5	5	5
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	5	5	5	5	5	5	5
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	5	5	5	5	5	5	5
$^{13}C_{12}$-OCDD	10	10	10	10	10	10	10
$^{13}C_{12}$-OCDF	10	10	10	10	10	10	10

(Initial Calibration used for High-Level Samples Requiring 20-Fold Dilution of the Extract)

Methods 8290 / 1613 / 23 / 0023A / TO9

Method 8290—the first high-resolution gas chromatography and high-resolution mass spectrometry method (HRGC-HRMS)—was introduced by the USEPA in 1985. It is capable of measuring low levels of PCDD/Fs in a variety of matrices in support of the RCRA/CERCLA (Superfund) programs. Towards the end of the eighties, the importance and value of HRGC-HRMS were recognized not only in the US but abroad as well.

Method 8290 underwent a number of changes. The changes—designed and implemented by the original author of the method in a commercial environment—led to a series of new analytical protocols in support of different regulatory programs within the Agency. For instance, Method 23—derived from Method 8290X—is the required protocol for the determination of PCDD/Fs in stack emission samples. Similarly, Method TO9-A for PCDD/Fs in ambient air samples is mostly adapted from Method 23. The SDWA and the CWA programs developed Method 1613, which is also derived from Method 8290X.



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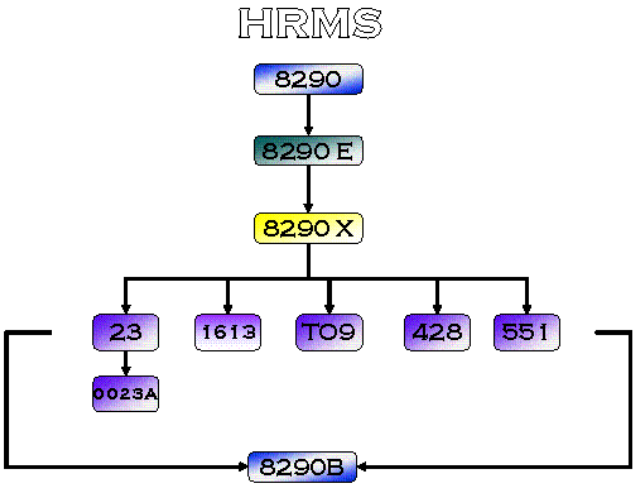

All of the above HRGC-HRMS methods are designed to measure PCDD/Fs. They are based on the same technology; i.e., isotope-dilution HRGC/HRMS. The sample extraction, fractionation and calculation procedures are essentially the same. Overall, the methods' performance (e.g., detection limits, precision, accuracy) is the same. What differentiate them are primarily the QA/QC requirements. For instance, Method 8290 recommends a recovery ranging from 40 to 130 percent regardless of the Extraction standard. On the other hand, Method 1613 specifies a different limit for each Extraction standard (e.g., 25 to 164 percent for 13C 12-2,3,7,8-TCDD; 17 to 157 percent for 13C 12-OCDD). Similarly, the calibration acceptance criteria for Method 8290 are different than those defined in Method 1613 (e.g., for the 13C 12-OCDD's RRF in the continuing calibrations, M1613 specifies a tolerance window ranging from –52 to 215 percent while M8290 requires a +30 percent window).

The published Method 8290 only calls for nine Extraction Standards to be added at the extraction stage. Method 1613 identifies 15 Extraction Standards. However, most laboratories, who recognize the fact that Method 8290 was developed at a time when there was a limited number of Extraction Standards, are in effect using a larger set of Extraction Standards, even surpassing the one specified in Method 1613 (see our comments at the end of this document). Just like in Method 1613, most laboratories will use the 37Cl 4-2,3,7,8-TCDD as a cleanup standard for Method 8290 analyses, even though, such standard is not described in Method 8290.

In essence, and to the benefit of the User, i.e.,

- Improved quality,
- Streamlined operations,
- Cost control, and
- Shorter cycle times

most qualified laboratories have adopted a "universal" methodology whereby the "best" features from each analytical protocol are merged. It was only in the middle of 2008 that we found that our closest competitors finally adopted the use of all 17 13C 12-PCDD/F standards when in fact; AP had been doing this since 2001 along with a number of additional standards (e.g., seven cleanup standards, three injection standards).

This section describes key aspects where modifications and improvements have been implemented by Analytical Perspectives for Methods 8290, 1613 and 23/0023A.

Relevant Modifications & Improvements to the Published Methods 8290/1613/23/0023A/TO9A

(as performed and validated by Analytical Perspectives)

1. Described below are key aspects of the various improvements. For more information and details, refer to Analytical Perspectives Performance Commitment document, which includes QC Acceptance Criteria & Corrective Actions.

2. The reference Method 8290 requires a Soxhlet extraction with toluene for non-biological solid matrices. Our approach calls for the use of Soxhlet Dean-Stark (SDS) extraction with toluene. For biological tissues, hexane SDS extraction is used to prevent precipitation of lipids in the presence of water and methylene chloride. The SDS permits the progressive removal of the entire water contents from the sample matrix and, subsequently, from the extraction solvent system by way of an azeotrope formation between toluene (or when applicable, hexane) and water. During the extraction cycle, the water separates from the organic solvent inside the Dean-Stark arm. Pure organic solvent is now returning to the extraction vessel. The removal of the water from the extraction system ensures the sample matrix is extracted with pure toluene (or when applicable, hexane) rather than a mixture of organic solvent—water as it happens when Soxhlet alone is used. This modification results in a more effective extraction of the organics such as PCDD/Fs, which are more soluble in organic solvents than in water.

3. Our sample extract fractionation procedure is designed to remove a wide variety of interferences. Among the most common interferences, polychlorinated diphenylethers (PCDPEs), when present, can artificially elevate the concentrations of PCDFs. Our cleanup approach removes the bulk of the PCDPEs (or alleged PCDPEs) in air and air-related matrices (e.g., ash) so that their effect is reduced to less than 0.3 to 3 percent positive bias on the total PCDD/Fs. This estimate is based on a worst-case scenario whereby we assume the alleged PCDPE is solely responsible for the signal found in the PCDF channel. Over the years, the application of our cleanup procedures indicate that no PCDPEs are found in our sample extracts (see Fig. below showing an example of M23 data from a competitor lab where numerous PCDPEs constitute a major source of false positives). Our fractionation procedures for fish tissue are designed to eliminate PCDPEs that may interfere with the detection and analysis of PCDFs.

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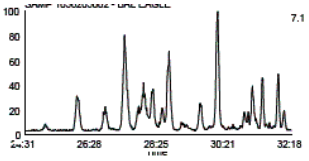
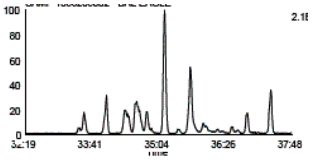


4. HpCDPEs in a M23 Sample as a major source of false positives from a competitor lab



5. OCDPEs in a M23 Sample as a major source of false positives from a competitor lab

6. To eliminate the risk of confusion between methods when referring to the various standards used throughout the procedures, the following nomenclature is applied:

   - Extraction Standard (ES): Extraction standards are the seventeen 13C 12-labeled 2,3,7,8-substituted PCDD/F congeners, which are added to every sample before the extraction. They are used to monitor both the extraction and fractionation efficiencies as well as for quantitative and qualitative purposes. For air matrices, the ES is also used to compute the SS recoveries. Versions of the methods refer to them as “internal standards” or “labeled compounds”.

   - Cleanup Standard (CS) or Sampling Standards (SS): A group of five labeled PCDD/F congeners added to every sample immediately following the extraction and before the fractionation. They are representative of a number of homologue groups and are used to monitor the fractionation efficiencies.

   - Alternate Standards (AS): 13C 12-1,3,6,8-TCDD and 13C 12-1,3,6,8-TCDF are used as additional cleanup standards to monitor for selective losses during the fractionation procedures, which can introduce a bias on totals as well as distort the congener profiles.

   - Injection Standard (JS): Injection standards are a group of three labeled PCDD/F congeners, which are used for the preparation of the final extract for GC/MS analysis. Their function is to measure the recoveries of the ES and CS and to provide a means for insuring that the system’s sensitivity performance remains acceptable. Versions of the methods referred to them as “recovery standards” or “labeled internal standards”.

   - Note that a similar system is adopted for PCBs, PAHs, and USVOAs.

7. The method calls for the use of nine (Method 8290) or 15 carbon-13 labeled Extraction standards (Method 1613) added to the sample before the extraction. Our fortification procedure consists in the addition of 17 carbon-13 labeled extraction standards to the sample before the extraction. One of the additional standards is 13C 12-OCDF, a compound that could not be used in the early eighties when Methods 8290/23/1613 were developed. Indeed, the available technology was unable to achieve chromatographic separation between OCDD and OCDF. From a mass spectrometry point of view, 13C 12-OCDF interferes with the measurement of OCDD and, is interfered by OCDD, especially when OCDD levels are high. With today’s improved gas chromatographic column technology, the separation between the two congeners permits the introduction of such a valuable standard to monitor more specifically the recovery of OCDF and to measure more accurately the amounts of OCDF present in the sample. It is worth mentioning that the author of this document has verified, under certain laboratory lighting conditions, the light-induced thermal degradation of OCDF during the toluene Soxhlet extraction. Up to 80 –90 percent thermally-induced photodegradation of OCDF have been observed while none was found for OCDD under the exact same conditions. In the absence of 13C 12-OCDF, a significant negative bias would result for the measurement of OCDF if the corresponding labeled standard is not used.

8. Each of the methods’ calibration curve is established with **five** individual solutions ranging in concentrations, for instance, from 0.5 pg/ m L to 100 pg/ m L for the TCDD/F. Our **seven-point** initial calibration curve’s dynamic range extends two-fold lower (0.25 pg/ m L) and five-fold higher in concentration (500 pg/ m L). Furthermore, Methods 8290/1613/23/0023A minimum requirements for the acceptance of the initial calibration are RSDs ranging from 20-25 to 35 percent depending on the analyte. Our internal requirement calls for single digit RSDs. Higher precision and accuracy for the PCDD/Fs are the primary benefits of these modifications. Tables 1 and 2 summarize the two sets of ICAL used by Analytical Perspectives. The secondary ICAL—an option offered exclusively by Analytical Perspectives—is used for the analysis of samples containing high-level analytes requiring up to 20-fold dilutions. The secondary ICAL is a product under evaluation, and may not be used systematically until the benefits are fully established.

9. Methods 8290/23/1613/23/0023A do not address the absolute value for the analytes’ RRFs. In order to ensure accurate measurements, Analytical Perspectives verifies that the RRF for specific analytes with an associated carbon-13 labeled extraction standard remains within a reasonable value from historical values (e.g., + 20 percent). The Laboratory Director can reject the ICal and request a new set of standard solutions to be prepared when such deviations are found with a new batch of standards. One critical aspect to successful analysis by isotope-dilution HRGC-HRMS is the reliability of the standards. To that effect, a number of verification procedures with stringent requirements are implemented and enforced (e.g., independent verification of the standards through the use of intra-source area ratios).

10. Methods 23/1613 verification of the initial calibration are conducted **only** at the beginning of every 12-H of operation during which samples are analyzed by HRGC/HRMS. Our procedures based on the BCS3 system require not only a front-end but also a **back-end** continuing calibration verification (concal). Additionally, according to our procedures, both front- and back-end verifications entail a verification of the ability to measure 2,3,7,8-TCDD **and** 2,3,7,8-TCDF in an isomer-specific mode and a verification of the various homologue groups retention time windows. This demonstration and verification is accomplished through the use of a new QA/QC sample concept known internally as the BCS3, which is part of our enfolded performance approach.

    - The BCS3 is a QC sample used for true-stable isotope-dilution GC/MS methodologies to ensure the reliability and accuracy (precision and bias) of the determinations. It is prepared in stages at the same time as the batch of field samples; i.e., at each phase involving the addition of the ES, CS, JS to the samples. For air matrices, the BCS3 is initiated at the preparation stage of the sampling modules (i.e., XAD-2 resin or PUF) during which the SS are introduced inside the sorbent material. The BCS3 is analyzed at the beginning and at the end of a 12-H analytical sequence during which the associated samples are analyzed. In order to use the front- and back-end BCS3 averaged RRFs to process the samples, the individual front- and back-end RRFs need to meet a number of requirements as detailed in section 5.4 of this procedure..

    - It is AP’s policy to develop a database of sample projects where both the OPR and the BCS3 are analyzed for M1613 samples. Once the Laboratory Director has determined that such information has been obtained, then, any project completed under M1613 where an OPR is required will NOT have a BCS3. Only those projects not requiring an OPR will be processed and analyzed under the BCS3 system. Furthermore, when an OPR is required, only a CS3 – spiked with the usual isomeric interferences and the first and last eluters – will be analyzed at the beginning of the sequence. No ending CS3 will be done when OPR is required.

11. Methods 8290/1613/23/0023A injection standards is a mixture containing two carbon-13 labeled PCDD congeners. Our injection standards solution contains three carbon-13

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labeled PCDD/F congeners.

12. The “initial demonstration of performance” is conducted annually while fortifying the sample with the unlabeled target analytes at our reporting limit (i.e., corresponding to the more challenging lowest calibration point of our curve). Precision and accuracy information is available from our Performance Commitment document.

13. For Method 1613 only: Until such a time when the BCS3 can be considered and approved by the Office of Water as a substitute for an on-going precision and accuracy, an OPR quality control sample is prepared with each extraction batch. No OPR is prepared for Method 8290, however. The BCS3 fulfills a more valuable function than the OPR.

14. Our data acquisition and validation procedures require the monitoring and assessment of “quantitative interferences”. When present, specific corrective actions may be necessary when they adversely impact the quality of the data. It is our policy to deliver data of quality; a policy that often results in carrying out additional (and outside the scope of the methods) fractionation procedures for complex samples.

15. Reporting of data is accomplished using a format that is accessible to a wide range of Users. It is designed to minimize the need for our clients to perform additional interpretation of the laboratory data. A three-part set of sample summary results is provided in an electronic format (Excel). Our CLP-like data package comes in both hardcopy and CD versions. The latter is integrated and interactive.

16. A correction for the contribution at m/z 322 from the CS (37Cl 4-2,3,7,8-TCDD) is applied automatically in our calculations.

17. Analytes found at a concentration below 1/10 th our reporting limit are reported as ND with a detection limit equals to 01 xRL.

18. Analytes whose ion-abundance ratio is outside the method’s limits are reported as EMPC – estimated maximum possible concentration; a concept introduced in the early eighties during the development of the first version of USEPA Method 8290. An EMPC is triggered whenever one particular identification criterion for PCDD/Fs is not met. Its value, particularly in risk assessment studies, is found in circumstances when specific analytical factors can lead to possible false negatives. Understanding the analytical factors prompting an EMPC plays an important part in the formulation of effective course of actions or in the development of a strategy for reporting data.We also recognize the different – and sometimes conflicting – realities and needs of the ultimate users; i.e., the regulators, the risk assessors, and the regulated communities. When acquiring data by high-resolution GC/MS, the mass spectrometer is operating in the selected ion-monitoring mode. For each compound of interest, the mass spectrometer records the response of two characteristic ions from the molecular ion cluster. The ion-abundance ratio between the two characteristic ions must fall within accepted ranges for the peak to be identified as a PCDD/F congener. Should the ion-abundance ratio fall outside the permissible range, the corresponding GC peak cannot be identified as a PCDD/F congener. However, if the ratio error is assumed to be due to a contributing interference to one of the ions, then an EMPC may be derived from the unaffected ion and the natural abundance ratio. In essence, one performs a “mathematical” operation removing the “contribution” of the interference, if the latter is the primary reason for triggering the EMPC reporting.

    It is true that a co-eluting chemical interference can cause the ion-abundance ratio to exceed the method’s limits (e.g., the impurity in the commercially available 37Cl 4-2,3,7,8-TCDD). However, for practical purposes, and before rushing into the so-called “confirmation” analyses and/or additional cleanups, which may in some cases amount to unethical practices, it helps to appreciate the fact that over 99 percent of the time, the EMPC is triggered by “ion statistics” and not by a chemical interference.

    Indeed, the uncertainty associated with the measurement of an ion-abundance ratio of two “weak” signals is equal to the sum of the variances associated with the individual signals. The weaker the S/N is, the larger the uncertainty of the measurement is. Ratio ing two numbers with large variances can result in the ion-abundance ratio to easily exceed the +15 percent window. In such case, a “confirmation” analysis is pointless unless the S/N is significantly improved. Our methodology reports EMPCs as such. Whenever a chemical interference can be discerned, we comment in the narrative on the potential impact on the data, or what actions have been taken or attempted to improve the specificity or to remove the interference, or its effect.

    For circumstances whereby an EMPC cannot be reported, we report any out-of-ratio criterion peak eluting at the retention time of a 2,3,7,8-substituted congener as an ND using the peak concentration as the detection limit.

Appendix

Tables 6, 6A, 7, 7A of M1613B
(as requested by SCDHEC)

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TABLE 6. ACCEPTANCE CRITERIA FOR PERFORMANCE TESTS WHEN ALL CDDS/CDFS ARE TESTED [1]

CDD/CDF	Test Conc. (ng/mL)	IPR [2,3] s (ng/mL)	IPR [2,3] X (ng/mL)	OPR (ng/mL)	VER (ng/mL)
2,3,7,8-TCDD	10	2.8	8.3–12.9	6.7–15.8	7.8–12.9
2,3,7,8-TCDF	10	2.0	8.7–13.7	7.5–15.8	8.4–12.0
1,2,3,7,8-PeCDD	50	7.5	38–66	35–71	39–65
1,2,3,7,8-PeCDF	50	7.5	43–62	40–67	41–60
2,3,4,7,8-PeCDF	50	8.6	36–75	34–80	41–61
1,2,3,4,7,8-HxCDD	50	9.4	39–76	35–82	39–64
1,2,3,6,7,8-HxCDD	50	7.7	42–62	38–67	39–64
1,2,3,7,8,9-HxCDD	50	11.1	37–71	32–81	41–61
1,2,3,4,7,8-HxCDF	50	8.7	41–59	36–67	45–56
1,2,3,6,7,8-HxCDF	50	6.7	46–60	42–65	44–57
1,2,3,7,8,9-HxCDF	50	6.4	42–61	39–65	45–56
2,3,4,6,7,8-HxCDF	50	7.4	37–74	35–78	44–57
1,2,3,4,6,7,8-HpCDD	50	7.7	38–65	35–70	43–58
1,2,3,4,6,7,8-HpCDF	50	6.3	45–56	41–61	45–55
1,2,3,4,7,8,9-HpCDF	50	8.1	43–63	39–69	43–58
OCDD	100	19	89–127	78–144	79–126
OCDF	100	27	74–146	63–170	63–159
$^{13}C_{12}$-2,3,7,8-TCDD	100	37	28–134	20–175	82–121
$^{13}C_{12}$-2,3,7,8-TCDF	100	35	31–113	22–152	71–140
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	39	27–184	21–227	62–160
$^{13}C_{12}$-1,2,3,7,8-PeCDF	100	34	27–156	21–192	76–130
$^{13}C_{12}$-2,3,4,7,8-PeCDF	100	38	16–279	13–328	77–130
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	41	29–147	21–193	85–117
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	100	38	34–122	25–163	85–118
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	100	43	27–152	19–202	76–131
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	35	30–122	21–159	70–143
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	100	40	24–157	17–205	74–135
$^{13}C_{12}$-2,3,4,6,7,8,-HxCDF	100	37	29–136	22–176	73–137
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	35	34–129	26–166	72–138
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	41	32–110	21–158	78–129
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	100	40	28–141	20–186	77–129
$^{13}C_{12}$-OCDD	200	95	41–276	26–397	96–415
$^{37}Cl_4$-2,3,7,8-TCDD	10	3.6	3.9–15.4	3.1–19.1	7.9–12.7

[1] All specifications are given as concentration in the final extract, assuming a 20 µL volume.
[2] s = standard deviation of the concentration.
[3] X = average concentration.

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TABLE 6A. ACCEPTANCE CRITERIA FOR PERFORMANCE TESTS WHEN ONLY TETRA COMPOUNDS ARE TESTED [1]

CDD/CDF	Test Conc. (ng/mL)	IPR [2,3] s (ng/mL)	IPR [2,3] X (ng/mL)	OPR (ng/mL)	VER (ng/mL)
2,3,7,8-TCDD	10	2.7	8.7–12.4	7.3–14.6	8.2–12.3
2,3,7,8-TCDF	10	2.0	9.1–13.1	8.0–14.7	8.6–11.6
$^{13}C_{12}$-2,3,7,8-TCDD	100	35	32–115	25–141	85–117
$^{13}C_{12}$-2,3,7,8-TCDF	100	34	35–99	26–126	76–131
$^{37}Cl_4$-2,3,7,8-TCDD	10	3.4	4.5–13.4	3.7–15.8	8.3–12.1

[1] All specifications are given as concentration in the final extract, assuming a 20 µL volume.
[2] s = standard deviation of the concentration.
[3] X = average concentration.

TABLE 7. LABELED COMPOUND RECOVERY IN SAMPLES WHEN ALL CDDS/CDFS ARE TESTED

Compound	Test Conc. (ng/mL)	Labeled Compound Recovery (ng/mL) [1]	Labeled Compound Recovery (%)
$^{13}C_{12}$-2,3,7,8-TCDD	100	25–164	25–164
$^{13}C_{12}$-2,3,7,8-TCDF	100	24–169	24–169
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	25–181	25–181
$^{13}C_{12}$-1,2,3,7,8-PeCDF	100	24–185	24–185
$^{13}C_{12}$-2,3,4,7,8-PeCDF	100	21–178	21–178
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	32–141	32–141
$^{13}C_{12}$-1,2,3,6,7,8,-HxCDD	100	28–130	28–130
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	100	26–152	26–152
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	26–123	26–123
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	100	29–147	29–147
$^{13}C_{12}$-2,3,4,6,7,8,-HxCDF	100	28–136	28–136
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	23–140	23–140
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	28–143	28–143
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	100	26–138	26–138
$^{13}C_{12}$-OCDD	200	34–313	17-157
$^{37}Cl_4$-2,3,7,8-TCDD	10	3.5–19.7	35-197

[1] Specification given as concentration in the final extract, assuming a 20-µL volume.

TABLE 7A. LABELED COMPOUND RECOVERY IN SAMPLES WHEN ONLY TETRA COMPOUNDS ARE TESTED

Compound	Test Conc. (ng/mL)	Labeled compound recovery (ng/mL) [1]	Labeled compound recovery (%)
$^{13}C_{12}$-2,3,7,8-TCDD	100	31-137	31-137
$^{13}C_{12}$-2,3,7,8-TCDF	100	29-140	29-140
$^{37}Cl_4$-2,3,7,8-TCDD	10	4.2–16.4	42–164

[1] Specification given as concentration in the final extract, assuming a 20 µL volume.

List of Abbreviations &Acronyms

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- **AS = alternate standard**
- **A_x = target analyte**
- **BCS_3 = batch control spike**
- **CAA = Clean Air Act**
- **COC = chain of custody**
- **CPSM = column performance standard mixture**
- **CS_3 = calibration solution corresponding to the middle point of the initial calibration curve**
- **CS = cleanup standard**
- **CWA = Clean Water Act**
- ***dfa* Technology = dioxin-furan array (fingerprinting reminiscent of DNA plates)**
- **DOC = documentation of capability**
- **DUP = duplicate**
- **EDL = estimated detection limit (sample- and analyte-specific)**
- **ES = extraction standard (isotopically labeled standard added before the extraction)**
- **GC = gas chromatography**
- **ICAL = initial calibration**
- **ID-HRMS = comprehensive and stable isotope-dilution high-resolution mass spectrometry**
- **Ind. Val. = independent validation**
- **JS = injection standard**
- **LOD = limit of detection**
- **LOQ = limit of quantitation**
- **MB = lab method blank**
- **MDL = method detection limit (not sample-specific)**
- **M/ΔM = mass spectrometer resolving power**
- **MIR = Methods Innovation Rule**
- **ML = minimum level (equivalent to lowest point on the calibration curve)**
- **MS/MSD = matrix spike/matrix spike duplicate**
- **ND = not detected**
- **NELAC = National Environmental Laboratory Accreditation Conference**
- **OPR = on-going precision & recovery (equivalent to LCS or Lab Control Spike)**
- **PAH = polynuclear aromatic hydrocarbon**
- **PBMS = performance-based measurement system**
- **PCB = polychlorinated biphenyl**
- **PCDD/F = polychlorinated dibenzo-*p*-dioxin and dibenzofuran**
- **PD = percent difference**
- **PE = performance evaluation sample**
- **QI = quantitative interference**
- **QuanTIC = selected SVOAs by full-scan GC/MS (ID-HRMS)**
- **RCRA = Resources Recovery Act**
- **RL = reporting limit (usually represents the lowest point on the calibration curve or ML)**
- **RPD = relative percent difference**
- **RRF = relative response factor**
- **RSD = relative standard deviation**
- **RTW = retention time defining window solution**
- **SDWA = Safe Drinking Water Act**
- **SICP = selected ion current profile**
- **S/N = signal-to-noise ratio**
- **SRM = standard reference material**
- **SS = sampling standard**
- **TEQ = toxic equivalency quotient**
- **TSCA = Toxic Substances Control Act**
- **U-SVOA = ultra-semi volatile analyte (selected SVOAs by SIR by ID-HRMS)**
- **VER = continuing calibration verification (equivalent to ConCal)**
- **WHO-2 / WHO-2S = ID-HRMS assay for the 29 World Health Organization target analytes (S = serum)**