METHOD 4430

SCREENING FOR POLYCHLORINATED DIBENZO-*p*-DIOXINS (PCDDs) AND POLYCHLORINATED DIBENZOFURANS (PCDFs) IN SOILS AND SEDIMENTS USING AN ARYL HYDROCARBON RECEPTOR-BASED PCR ASSAY

SW-846 is not intended to be an analytical training manual. Therefore, method procedures are written based on the assumption that they will be performed by analysts who are formally trained in at least the basic principles of chemical analysis and in the use of the subject technology.

In addition, SW-846 methods, with the exception of required method use for the analysis of method-defined parameters, are intended to be guidance methods which contain general information on how to perform an analytical procedure or technique which a laboratory can use as a basic starting point for generating its own detailed standard operating procedure (SOP) either for its own general use or for a specific project application. The performance data included in this method are for guidance purposes only, and are not intended to be and must not be used as absolute QC acceptance criteria for the purposes of laboratory accreditation.

1.0 SCOPE AND APPLICATION

For a summary of changes in this version, please see Appendix A at the end of this document.

1.1 This method is a screening procedure for polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in soils and sediments. This method uses a commercially-available aryl hydrocarbon receptor (AhR)-based polymerase chain reaction (PCR) assay. The AhR-PCR assay screens samples by their toxicity equivalent quotient (TEQ) by responding to individual PCDD/PCDF congeners in approximate correlation to their toxicity equivalent factors (TEFs). The TEQ measured by the AhR-PCR assay is the sum of the response from the individual congeners. See Table 2 for a detailed list of response factors for individual PCDD/PCDF congeners. More information about the AhR-PCR assay can be found at www.eichrom.com/dioxin/products.

1.2 The AhR-PCR assay employs AhR proteins extracted from mammalian cells to bind PCDD/PCDFs. Upon binding PCDD/PCDFs, the AhR forms an activated receptor complex with an aryl hydrocarbon nuclear translocator (ARNT) and a specific DNA response element (DRE). This activated receptor complex is captured onto a microwell in a 96-well plate and isolated from unreacted AhR, ARNT and DRE by washing on a 96-well plate washer. The level of PCDD/PCDF contamination is then measured indirectly by amplifying and measuring the DRE using real time PCR. Since the interaction of the AhR with individual PCDD/PCDF congeners is proportional to the TEF values of the individual PCDD/PCDF congeners, the response measured by the AhR-PCR assay correlates very well with the TEQ of the sample.

1.3 The AhR-PCR assay and the associated sample preparation method outlined in this document provide a technique for the estimation of the total PCDD/PCDF TEQ. The method will not provide concentrations of individual PCDD/PCDF congeners, as the individual congeners are not separated during the sample preparation. During the sample preparation, the PCDD/PCDFs are isolated as a group from potentially interfering compounds such as polychlorinated biphenyls (PCB) and polynuclear aromatic hydrocarbons (PAH). A complete list of compounds which have been identified to have significant response on the AhR-PCR assay is provided in Table 2.

1.4 The AhR-PCR assay responds to PCDD/PCDF congeners in proportion to their concentration and TEF value. However, the response factors for individual PCDD/PCDF congeners on the AhR-PCR assay are not identical to the TEF values assigned by the World Health Organization (WHO). Therefore, variation in the accuracy among samples may occur solely because of variability in congener composition. A comparison of AhR-PCR response factors and WHO TEF values is provided in Table 2.

1.5 This method details a single extraction technique, pressurized fluid extraction (PFE), and sample preparation method which has been shown to effectively extract the PCDD/PCDFs from soil and sediment samples and isolate the PCDD/PCDFs from most interfering classes of compounds. Other extraction methods, solvent systems or sample clean-up methods may be used, provided adequate performance of these methods is demonstrated for the analytes of interest from the matrix of interest.

1.6 The limit of detection submitted by the manufacturer of this testing product is 0.4 pg of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD). This detection limit is provided as guidance only, and the limit achievable by a user for a given sample will be dependent on several factors including the type of sample matrix.

1.7 Prior to employing this method, analysts are advised to consult the base method for each type of procedure that may be employed in the overall analysis (e.g., Methods 3500, 3600, 4000, 5000, and 8000) and the manufacturer's instructions for additional information on quality control procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts also should consult the disclaimer statement at the front of the manual and the information in Chapter Two for guidance on the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrix of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly specified in a regulation, the use of SW-846 methods is *not* mandatory in response to Federal testing requirements. The information contained in this method is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to generate results that meet the data quality objectives for the intended application.

1.8 This method is restricted to use by, or under the supervision of, personnel appropriately experienced and trained in the use of general laboratory techniques including sample extraction, column chromatography and polymerase chain reaction (PCR). Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 Detailed sample preparation and analysis procedures for this method are available in the manufacturer's literature at www.eichrom.com/dioxin/products. A brief description of the key steps is provided below.

2.2 Diatomaceous earth is added to the dry sample and mixed. The sample is then extracted using pressurized fluid extraction (PFE).

2.3 Following PFE, the solvent is exchanged to hexane, and the PCDD/PCDFs are isolated from interfering compounds by column chromatography using commercially available silica gels and Florisil®. The PCDD/PCDFs are recovered in 50% methylene chloride:hexane. The sample is concentrated and redissolved in heptane.

2.4 An accurately measured volume of blank, sample, standard or recovery standard in heptane is added to a glass vial containing a mixture of AhR, ARNT and DRE and mixed for one hour.

2.5 An accurately measured volume of the AhR, ARNT, DRE that has been equilibrated with blank, sample, standard or recovery standard for one hour is then added to a plastic microwell and mixed for 30 min. PCDD/PCDF-AhR-ARNT-DRE complexes bind to the walls of the microwell, and excess AhR, ARNT and DRE are removed by rinsing on a 96-well plate washer.

2.6 PCR reagents are added to the microwell and the amount of DRE, which is proportional to the TEQ of the sample, is measured using PCR. The response from the PCR is related to TEQ by a dose-response curve generated from 2,3,7,8-TCDD standards. The PCR can analyze up to 38 samples in duplicate, with blanks, standards and recovery standards simultaneously in 90 min.

3.0 DEFINITIONS

See the glossary at the end of this method for procedure-specific terms. Also, refer to Chapter One, Methods 8280 and 8290, and the manufacturer's instructions for definitions that may be relevant to this procedure.

4.0 INTERFERENCES

4.1 Solvents, reagents, glassware and other sample processing hardware may yield artifacts and/or interferences to sample analysis. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing method blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be necessary. Refer to each method to be used for specific guidance on quality control procedures and to Chapter Four and Method 8290 for general guidance on the cleaning of glassware.

4.2 The AhR used in this method binds to the seventeen PCDD/PCDF congeners with established TEF values, and other aryl hydrocarbon compounds, such as PCBs, PAHs, brominated and mixed brominated/chlorinated dibenzo-*p*-dioxins and furans, PCDD/PCDF congeners without established TEF values and other compounds similar in structure to 2,3,7,8-TCDD. The highest response is observed for 2,3,7,8-TCDD and compounds of similar structure and degree of chlorination. A complete list of compounds for which the response of the AhR-PCR assay has been observed is given in Table 2.

4.3 The AhR used in this method binds to PCDD/PCDF and similar molecules based on structure, not mass. Therefore, ^{13}Carbon- or other stable isotope-labeled standards are detected to the same degree as native compounds. These labeled standards cannot be used as internal standards with this method.

4.4 High levels (10^6 x that of the desired analyte) of selected PAH compounds and high molecular weight aliphatic hydrocarbons have been shown to inhibit the response of the AhR-PCR assay. However, it is unlikely that high levels of these compounds will remain with the PCDD/PCDF fraction if the sample preparation instructions provided by the manufacturer are followed.

5.0 SAFETY

5.1 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals listed in this method. A reference file of material safety data sheets (MSDS) should be available to all personnel involved in these analyses.

5.2 The AhR-PCR assay should only be used by properly trained personnel in an appropriate laboratory environment. Personnel should wear appropriate personal protective equipment, including safety glasses, lab coat and gloves.

5.3 WARNING: PCDD/PCDF standards, solutions containing PCDD/PCDFs and potentially contaminated samples should be treated as hazardous materials.

5.4 Handle all organic solvents (toluene, acetone, hexane, heptane and methylene chloride) in a fume hood.

5.5 Silica gels and Florisil should be treated as respiratory hazards and treated with the appropriate care.

5.6 Silica gels impregnated with sulfuric acid, potassium hydroxide and silver nitrate should be treated with care as they can cause skin irritation, discoloration and/or burns.

5.7 Some test components are stored in freezers (-20 ºC and -80 ºC). Care should be taken to avoid direct contact of frozen components with skin.

6.0 EQUIPMENT AND SUPPLIES

6.1 This section does not list all common laboratory glassware (e.g., beakers and flasks). A complete list of the equipment and supplies can be found in the manufacturer's literature (www.eichrom.com/dioxin/products).

6.2 The AhR-PCR assay requires a real-time PCR instrument with FAM and ROX detection capabilities. Please see the manufacturer's literature for a complete list of compatible models.

6.3 Other significant equipment required for the AhR-PCR assay includes a 96-well platewasher (Biotek Elx50 or equivalent), a 96-well plateshaker (Heidolph Titramax 1000 or equivalent), a refrigerator/freezer (≤6 ºC/-20 ºC) and a -80 ºC freezer (or liquid nitrogen vial storage Dewar) for storage of the test kit components.

6.4 The manufacturer will supply or specify equipment, apparatus and materials necessary for the successful completion of the test. Do not mix the equipment, supplies or reagents from other test kits. The AhR-PCR assay contains reagents that are evaluated by the manufacturer on a lot to lot basis. Do not mix the reagents from multiple lots, unless expressly allowed by the manufacturer.

6.5 Incorporation of apparatus for the capture of evaporated solvents during concentration procedures may be required by Federal, State or local municipality regulations that govern air emissions of volatile organics. EPA recommends the incorporation of this type of

reclamation system as a method to implement an emissions reduction program. Solvent recovery is a means to conform with waste minimization and pollution prevention initiatives.

7.0 REAGENTS AND STANDARDS

7.1 Reagent-grade or pesticide-grade chemicals must be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the committee of Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. Reagents should be stored in glass to prevent the leaching of contaminants from plastic containers.

7.2 Extraction solvents -- This method has been validated using a 3:7 mixture of acetone and toluene for the extraction solvent and a pressurized fluid extraction system. Other solvent systems or another extraction apparatus may also be used provided that they have been shown to provide reproducibly high recovery of the analytes of interest from the matrix of interest. The choice of extraction solvent and apparatus will depend on the analytes of interest and the matrix of interest, and no single solvent or apparatus is universally applicable to all analytes or matrices. Whatever solvent system and apparatus is employed, including those specifically listed in this method, the analyst must demonstrate adequate performance for the analytes of interest, at the levels of interest, from the matrix of interest. See Sec. 9.2.1 of this method for more information about how to demonstrate adequate performance. .

7.3 Detailed information on reagent requirements is given in the manufacturer's literature (www.eichrom.com/dioxin/products). Do not mix the equipment, supplies or reagents from different testing products or different lots of the AhR-PCR assay. Store all reagents and standards according to the manufacturer's instructions, and discard any reagents that are past the expiration date assigned by the manufacturer.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

Sample collection, preservation and storage requirements may vary by EPA program and may be specified in a regulation or project planning document that requires compliance monitoring for a given contaminant. Where such requirements are specified in the regulation, follow those requirements. In the absence of specific regulatory requirements, use the following information as guidance in determining the sample collection, preservation and storage requirements.

8.1 The AhR-PCR assay requires small sample masses (typically 5-10 g). Sample collection procedures should ensure that the sample is representative of the source. The distribution of PCDD/PCDFs in the sample can be heterogeneous. Samples should be homogenized to ensure that any subsamples are representative of the whole sample. Water content should be low enough to avoid standing water in the sample containers and ideally should be removed before homogenizing and subsampling.

8.2 Sample extracts are stable for up to three months when refrigerated (≤6 ºC) and stored in tightly sealed vials. Longer storage times may be possible if validated with performance data.

8.3 Store all test kit components as outlined in the manufacturer's literature.

8.4 Some components must be thawed before use. Unless otherwise instructed by the manufacturer, thaw components immediately prior to use to near room temperature. Do not use hot water to thaw components as this can lead to decreased performance of the test.

8.5 Do not use test kits or components after the expiration date.

8.6 Do not use test kit components with components from a different lot of the test kit or with components from test kits from different manufacturers.

9.0 QUALITY CONTROL

9.1 Refer to Chapter One for guidance on quality assurance (QA) and quality control (QC) protocols. When inconsistencies exist between QC guidelines, method-specific QC criteria take precedence over both technique-specific criteria and those criteria given in Chapter One, and technique-specific QC criteria take precedence over the criteria in Chapter One. Any effort involving the collection of analytical data should include development of a structured and systematic planning document, such as a Quality Assurance Project Plan (QAPP) or a Sampling and Analysis Plan (SAP), which translates project objectives and specifications into directions for those that will implement the project and assess the results. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated. All data sheets and quality control data should be maintained for reference or inspection.

9.2 Refer to Methods 3500, 3600, 3620 and 3630 for QC procedures to ensure the proper operation of the various extraction and sample preparation techniques. Any more specific QC procedures provided in this method will supersede those noted in Methods 3500, 3600, 3620 or 3630.

9.2.1 Initial demonstration of proficiency

Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes by generating data of acceptable accuracy and precision for target analytes in a clean matrix. At a minimum, such a demonstration will encompass the initial demonstration of proficiency described in method 3500, using a clean reference matrix. The laboratory must also repeat the demonstration of proficiency whenever new staff members are trained or significant changes in instrumentation are made. See Method 8000D, Sec. 9.3 for information on how to accomplish a demonstration of proficiency.

9.3 Initially, before processing any samples, the analyst should demonstrate that all parts of the equipment in contact with the sample and reagents are interference-free. This is accomplished through the analysis of a method blank. As a continuing check, each time samples are extracted, cleaned up, and analyzed, and when there is a change in reagents, a method blank should be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination.

9.4 Any method blanks, matrix spike samples, or replicate samples should be subjected to the same analytical procedures (Sec. 11.0) as those used on actual samples. For sample extracts that are cleaned up using this method, the associated quality control samples must also be processed through this cleanup method.

9.5 The commercially available testing product used to develop this method represents a performance-based analytical technique. Therefore, it is imperative that the manufacturer's

instructions and specifications be followed closely. Follow the manufacturer's instructions for the testing product being used for the quality control procedures specific to that testing product. The following discussion of quality control requirements relies heavily on the analyst's knowledge and understanding of the manufacturer's instructions.

9.6 Stringent quality assurance protocols should be maintained throughout each stage of the testing procedure; i.e., sample extraction, sample preparation, and immunoassay analysis. Various QA actions check for failure at each of these points in the process. Duplicate, check samples, standard reference materials, and other QA samples and methods can and should be used with this kit, with the exception of conventional isotope labelled internal standards. The AhR-PCR assay recognizes PCDD/PCDF congeners based on structure and not on mass. Therefore, conventional isotope-labelled internal standards are detected as native material and cannot be used with this method.

9.7 As outlined above, routine quality control procedures associated with this method include the measurement of standards, assay blanks, method blanks and recovery standards. It is recommended that all measurements be performed in duplicate and that all measurements be performed as part of the same batch of samples. A batch of samples includes up to 38 field samples prepared and analyzed at the same time, along with standards, assay blanks, method blanks and recovery standards.

9.8 Additional quality control considerations

9.8.1 Do not use test kit components past their expiration dates.

9.8.2 Do not mix the equipment, supplies or reagents from test kits from different manufacturers or from different lots of the test kit.

9.8.3 Use the test components within the storage temperature and operating temperature limits specified by the manufacturer.

9.8.4 The AhR-PCR assay identifies and measures PCDD/PCDF congeners based on structure, not mass. Therefore isotope-labeled internal standards are detected as native material and cannot be used with this method.

10.0 CALIBRATION AND STANDARDIZATION

See the manufacturer’s instructions for information on calibration and standardization.

11.0 PROCEDURE

Follow the manufacturer’s instructions for the test kit being used. Instructions can be found on the test kit package insert or at www.eichrom.com/dioxin/products.

12.0 DATA ANALYSIS AND CALCULATIONS

Follow the instructions provided by the manufacturer for the calculation of all testing product results. For each batch of samples, use the calibration curve generated concurrently with that AhR-PCR assay run.

13.0 METHOD PERFORMANCE

13.1 Performance data and related information are provided in SW-846 methods only as examples and guidance. The data do not represent required performance criteria for users of the methods. Instead, performance criteria should be developed on a project-specific basis, and the laboratory should establish in-house QC performance criteria for the application of this method. These performance data are not intended to be and must not be used as absolute QC acceptance criteria for purposes of laboratory accreditation.

In the case of this method, any test kit used must be able to meet the performance specifications for the intended application. Also, follow the manufacturer's instructions for quality control procedures specific to the test kit used.

13.2 Table 1 depicts response on the AhR-PCR assay for 2,3,7,8-TCDD over 1 year for four different laboratories along with QA acceptance ranges. These data are provided for guidance purposes only.

13.3 Table 2 depicts cross-reactivity on the AhR-PCR assay relative to 2,3,7,8-TCDD. These data are provided for guidance purposes only.

13.4 Table 3 summarizes correlation data for AhR-PCR and GC-HRMS for spiked sand and soil samples. These data are provided for guidance purposes only.

13.5 Table 4 summarizes correlation data for AhR-PCR and GC-HRMS for real-world soil and sediment samples from US EPA Superfund Sites. These data are provided for guidance purposes only.

13.6 Table 5 summarizes typical false positive and false negative rates for AhR-PCR near the action level of 50 pg/g TEQ. These data are provided for guidance purposes only.

13.7 Table 6 summarizes intra-lot variation on the AhR-PCR assay. These data are provided for guidance purposes only.

13.8 Table 7 summarizes inter-lot variation on the AhR-PCR assay. These data are provided for guidance purposes only.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, a free publication available from the American Chemical Society (ACS), Committee on Chemical Safety, http://portal.acs.org/portal/fileFetch/C/WPCP_012290/pdf/WPCP_012290.pdf.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult the ACS publication listed in Sec. 14.2.

16.0 REFERENCES

1. Dioxins and Furans in Soil and Sediment, Eichrom Method DFS01, www.eichrom.com/dioxin/products.

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11. D. R. McAlister, M. J. Fern, R. L. Allen; "Rapid Aryl Hydrocarbon Receptor-based Polymerase Chain Reaction Screening Assay for Polychlorinated Dibenzo-*p*-Dioxins and Furans in Soil and Sediment," *Talanta*, **2007**, in press, doi.org/10.1016/j.talanta.2007.08.005.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

The following pages contain the tables referenced by this method. A glossary is provided after the tables.

Table 1. Standard Curve Metrics (Ct vs pg/mL 2,3,7,8-TCDD)*

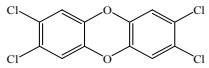
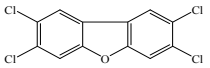
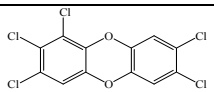
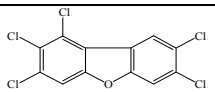
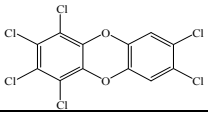
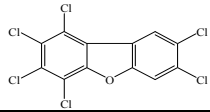
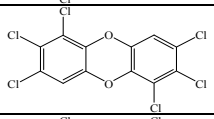
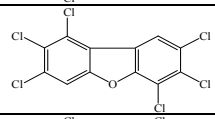
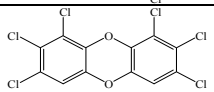
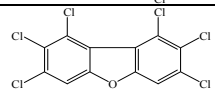
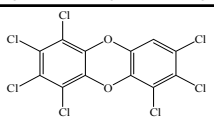
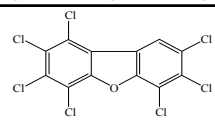
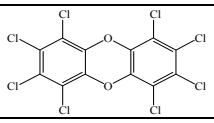
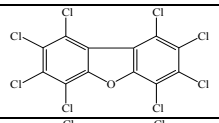
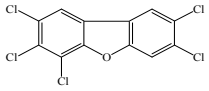
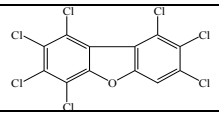
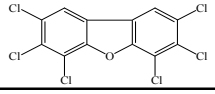
	Eichrom Historical	Eichrom Multi-lab	Multi-lab Site 2	Multi-lab Site 3	Multi-lab Site 4	Acceptable Range
Ct range 0-5000	5.5	5.8	5.7	5.0	4.9	>4.5
R^2 (78-5000)	0.980	0.984	0.992	0.984	0.994	>0.97
average SD of replicates	0.20	0.20	0.20	0.25	0.13	<0.30
number of curves generated	25	6	5	4	3	

* Data are from 43 determinations of 2,3,7,8-TCDD Response over more than one year for 7 point standard curve + blank in heptane (5000, 2500, 1250, 625, 313, 156, 78 and 0 pg 2,3,7,8-TCDD/mL heptane) generated in four different laboratories. The assay detection limit, approximated from the lowest standard concentration, 78 pg/mL, which lies >3σ from the assay blank, is 0.4 pg 2,3,7,8-TCDD per well. Actual achievable method lower limits of quantitation will depend on several factors including sample size, extract volume and recoveries of analytes through extraction and clean-up steps.

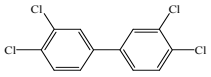
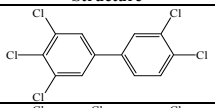
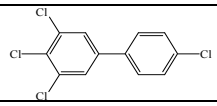
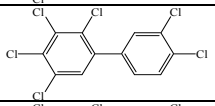
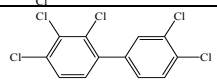
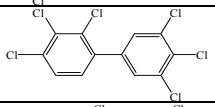
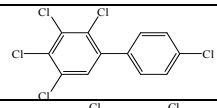
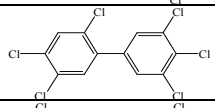
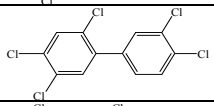
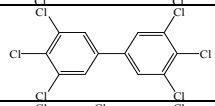
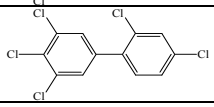
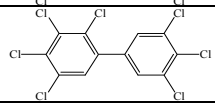
These data are provided for guidance purposes only.

Table 2. Cross-reactivity on the AhR-PCR Assay

Response for Chlorinated Dioxins and Furans on AhR-PCR Assay

Structure	compound	AhR-PCR Response	compound	AhR-PCR Response	Structure
	2,3,7,8 TCDD	1 TEF(1)	2,3,7,8 TCDF	0.06 TEF (0.1)	
	1,2,3,7,8 PCDD	0.6 TEF (1)	1,2,3,7,8 PCDF	0.1 TEF (0.03)	
	1,2,3,4,7,8 HxCDD	0.4 TEF (0.1)	1,2,3,4,7,8 HxCDF	0.4 TEF (0.1)	
	1,2,3,6,7,8 HxCDD	0.1 TEF (0.1)	1,2,3,6,7,8 HxCDF	0.2 TEF (0.1)	
	1,2,3,7,8,9 HxCDD	0.5 TEF (0.1)	1,2,3,7,8,9 HxCDF	0.3 TEF (0.1)	
	1,2,3,4,6,7,8 HpCDD	0.01 TEF (0.01)	1,2,3,4,6,7,8 HpCDF	0.05 TEF (0.01)	
	1,2,3,4,6,7,8,9 OCDD	0.000003 TEF (0.0003)	1,2,3,4,6,7,8,9 OCDF	0.0005 TEF (0.0003)	
	2,3,4,7,8 PCDF	0.3 TEF (0.3)	1,2,3,4,7,8,9 HpCDF	0.02 TEF (0.01)	
	2,3,4,6,7,8 HxCDF	0.1 TEF (0.1)			

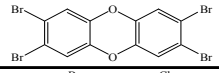
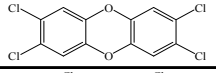
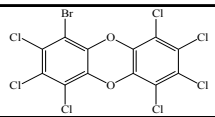
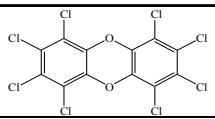
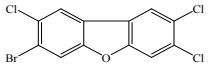
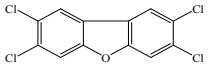
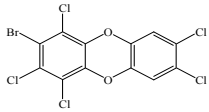
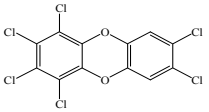
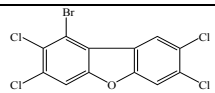
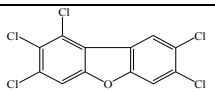
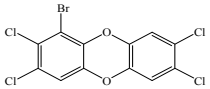
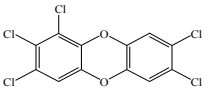
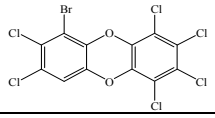
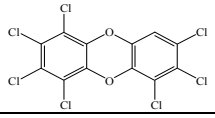
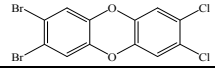
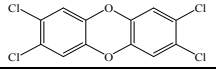
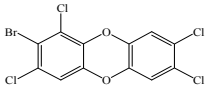
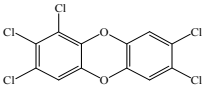
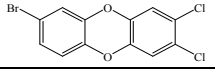
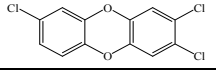
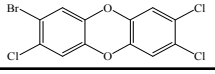
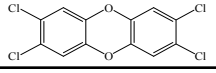
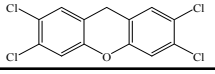
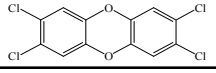
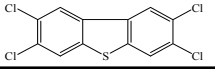
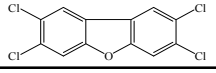
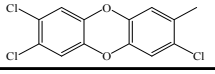
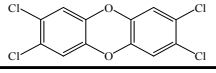
Response for Polychlorinated Biphenyls on AhR-PCR Assay

Structure	compound	AhR-PCR Response	compound	AhR-PCR Response	Structure
	PCB-77 (3,3',4,4')	0.00003 TEF (0.0001)	PCB-126 (3,3',4,4',5)	0.01 TEF (0.1)	
	PCB-81 (3,4,4',5)	0.00005 TEF (0.0003)	PCB-156 (2,3,3',4,4',5)	0.00003 TEF (0.00003)	
	PCB-105 (2,3,3',4,4')	<3 x 10-7 TEF (0.00003)	PCB-157 (2,3,3',4,4',5')	0.00004 TEF (0.00003)	
	PCB-114 (2,3,4,4',5)	0.00001 TEF (0.00003)	PCB-167 (2,3',4,4',5,5')	0.000001 TEF (0.00003)	
	PCB-118 (2,3',4,4',5)	<3 x 10-7 TEF (0.00003)	PCB-169 (3,3',4,4',5,5')	0.001 TEF (0.03)	
	PCB-123 (2',3,4,4',5)	0.000009 TEF (0.00003)	PCB-189 (2,3,3',4,4',5,5')	<3 x 10-7 TEF (0.00003)	

These data are provided for guidance purposes only.

Table 2. Cross-reactivity on the AhR-PCR Assy (continued)

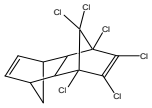
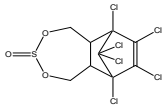
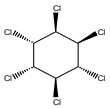
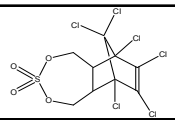
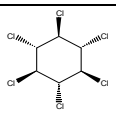
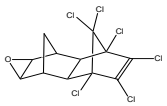
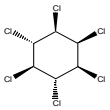
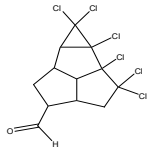
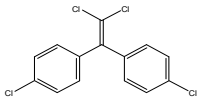
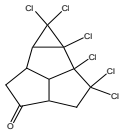
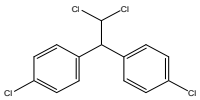
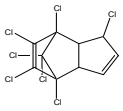
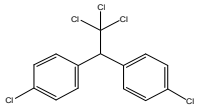
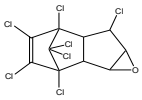
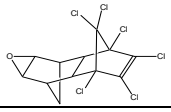
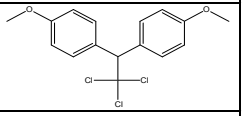
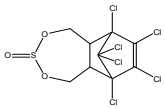
Response for Bromo/chloro Dioxins and Furans on AhR-PCR Assay

Structure	compound	AhR-PCR Response	analogous chloro-compound	AhR-PCR Response	Structure
	2,3,7,8-TBrDD	0.3	2,3,7,8-TCDD	1.0	
	1-Br-2,3,4,6,7,8,9-HpCDD	0.00004	1,2,3,4,6,7,8,9-OCDD	0.000003	
	3-Br-2,7,8-TriCDF	0.4	2,3,7,8-TCDF	0.1	
	2-Br-3,6,7,8,9-PCDD	0.05	1,2,3,4,7,8-HxDD	0.4	
	1-Br-2,3,7,8-TCDF	0.3	1,2,3,7,8-PCDF	0.1	
	1-Br-2,3,7,8-TCDD	0.4	1,2,3,7,8-PCDD	0.6	
	1-Br-2,3,6,7,8,9-HxCDD	0.06	1,2,3,4,6,7,8-HpCDD	0.01	
	2,3-Br-7,8-DiCDD	0.4	2,3,7,8-TCDD	1.0	
	2-Br-1,3,7,8-TCDD	0.6	1,2,3,7,8-PCDD	0.6	
	2-Br-7,8-DiCDD	$<10^{-6}$	2,3,7-TriCDD	N/A	
	2-Br-3,7,8-TriCDD	0.5	2,3,7,8-TCDD	1.0	
	2,3,6,7-TCl-xanthene	0.7	2,3,7,8-TCDD	1.0	
	2,3,7,8-TCl-thiophene	0.2	2,3,7,8-TCDF	0.1	
	2,3,7-Cl-8-methyl-dibenzo-p-dioxin	0.3	2,3,7,8-TCDD	1.0	

These data are provided for guidance purposes only.

Table 2. Cross-reactivity on the AhR-PCR Assy (continued)

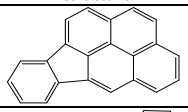
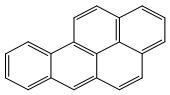
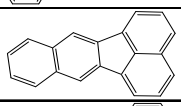
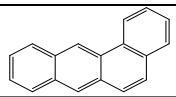
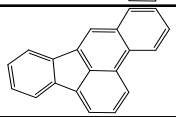
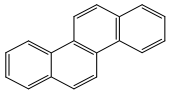
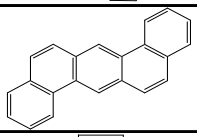
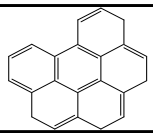
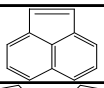
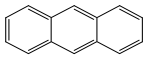
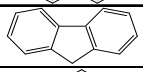
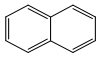
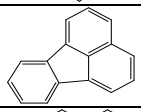
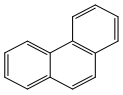
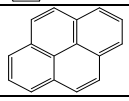
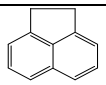
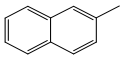
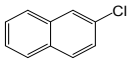
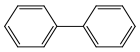
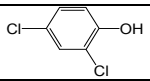
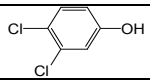
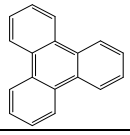
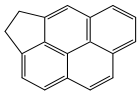
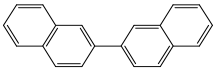
Response of Pesticide Compounds on AhR-PCR Assay

Structure	Compound	AhR-PCR Response	Compound	AhR-PCR Response	Structure
	aldrin	no measurable response from 2000 ppt to 2000 ppm	Endosulfan II	no measurable response from 2000 ppt to 2000 ppm	
	alpha-BHC	no measurable response from 2000 ppt to 2000 ppm	Endosulfan sulfate	no measurable response from 2000 ppt to 2000 ppm	
	beta-BHC	no measurable response from 2000 ppt to 2000 ppm	Endrin	no measurable response from 2000 ppt to 2000 ppm	
	delta-DHC	no measurable response from 2000 ppt to 2000 ppm	Endrin Aldehyde	no measurable response from 2000 ppt to 2000 ppm	
	p,p'-DDE	no measurable response from 2000 ppt to 2000 ppm	Endrin Ketone	no measurable response from 2000 ppt to 2000 ppm	
	p,p'-DDD	no measurable response from 2000 ppt to 2000 ppm	Heptachlor	no measurable response from 2000 ppt to 2000 ppm	
	p,p'-DDT	no measurable response from 2000 ppt to 2000 ppm	Heptachlor epoxide	no measurable response from 2000 ppt to 2000 ppm	
	Dieldrin	no measurable response from 2000 ppt to 2000 ppm	Methoxychlor	no measurable response from 2000 ppt to 2000 ppm	
	Endosulfan I	no measurable response from 2000 ppt to 2000 ppm			

These data are provided for guidance purposes only.

Table 2. Cross-reactivity on the AhR-PCR Assy (continued)

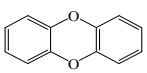
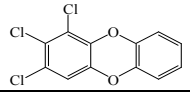
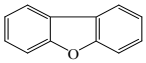
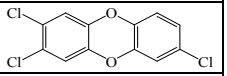
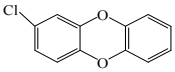
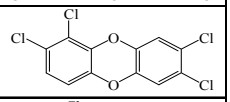
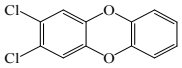
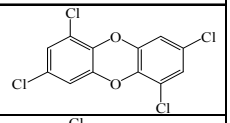
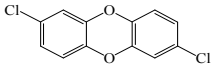
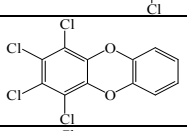
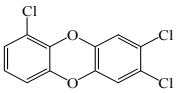
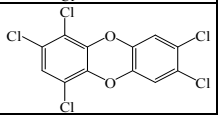
Response of PAH Compounds on AhR-PCR Assay

structure	compound	AhR-PCR Response	compound	AhR-PCR Response	Structure
	Indeno(1,2,3-cd)pyrene	0.8	Benzo(a)pyrene	0.1	
	Benzo(k)fluoranthene	0.5	Benzo(a)anthracene	0.05	
	Benzo(b)fluoranthene	0.6	Chrysene	0.04	
	Dibenzo(ah)anthracene	0.3	Benzo(ghi)perylene	0.004	
	acenaphthylene	No response at 200 ppt to 10 ppm	anthracene	No response at 200 ppt to 10 ppm	
	fluorene	No response at 200 ppt to 10 ppm	naphthalene	No response at 200 ppt to 10 ppm	
	fluoranthene	No response at 200 ppt to 10 ppm	phenanthrene	No response at 200 ppt to 10 ppm	
	pyrene	No response at 200 ppt to 10 ppm	acenaphthene	No response at 200 ppt to 10 ppm	
	2-methylnaphthalene	No response at 200 ppt to 10 ppm	2-chloronaphthalene	No response at 200 ppt to 10 ppm	
	biphenyl	No response at 200 ppt to 10 ppm	2,4-dichlorophenol	No response at 200 ppt to 10 ppm	
	3,4-dichlorophenol	No response at 200 ppt to 10 ppm	toluene	No response at 200 ppt to 10 ppm	
	triphenylene	0.001	cyclopenta[c,d]pyrene	0.0002	
	2,2'-binaphthyl	0.05			

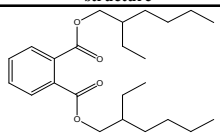
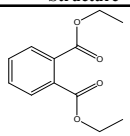
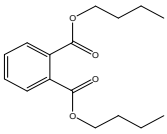
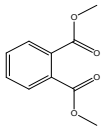
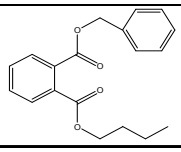
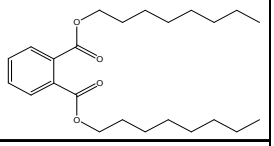
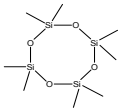
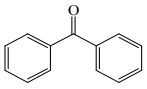
These data are provided for guidance purposes only.

Table 2. Cross-reactivity on the AhR-PCR Assy (continued)

Response of non-2,3,7,8 Dioxins/Furans on AhR-PCR Assay

structure	compound	AhR-PCR Response	compound	AhR-PCR Response	Structure
	dibenzo-p-dioxin	$< 10^{-6}$	1,2,3-TriCDD	0.006	
	dibenzo-p-furan	$< 10^{-6}$	2,3,7-TriCDD	0.08	
	2-MCDD	$< 10^{-6}$	1,2,7,8-TCDD	0.9	
	2,3-DiCDD	$< 10^{-6}$	1,3,6,8-TCDD	0.001	
	2,7-DiCDD	$< 10^{-6}$	1,2,3,4-TCDD	0.007	
	1,7,8-TriCDD	0.004	1,2,4,7,8-PCDD	0.3	

Response of Miscellaneous Compounds on AhR-PCR Assay

structure	compound	AhR-PCR Response	compound	AhR-PCR Response	Structure
	bis-(2-ethylhexyl)phthalate	$< 7 \times 10^{-7}$	diethylphthalate	$< 7 \times 10^{-7}$	
	di-n-butylphthalate	$< 7 \times 10^{-7}$	dimethylphthalate	$< 7 \times 10^{-7}$	
	butylbenzylphthalate	$< 7 \times 10^{-7}$	di-n-octylphthalate	$< 7 \times 10^{-7}$	
$C_{10}H_{22}$	decane	$< 9 \times 10^{-7}$	eicosane	$< 9 \times 10^{-7}$	$C_{20}H_{42}$
$C_{12}H_{26}$	dodecane	$< 9 \times 10^{-7}$	docosane	$< 9 \times 10^{-7}$	$C_{22}H_{246}$
$C_{14}H_{30}$	tetradecane	$< 9 \times 10^{-7}$	tetracosane	$< 9 \times 10^{-7}$	$C_{24}H_{50}$
$C_{16}H_{34}$	hexadecane	$< 9 \times 10^{-7}$	hexacosane	$< 9 \times 10^{-7}$	$C_{26}H_{54}$
$C_{18}H_{38}$	octadecane	$< 9 \times 10^{-7}$	octacosane	$< 9 \times 10^{-7}$	$C_{28}H_{58}$
	octamethylcyclotetrasiloxane	no measurable response from 2000 ppt to 2000 ppm	benzophenone	$< 1 \times 10^{-6}$	

These data are provided for guidance purposes only.

Table 3. Correlation of AhR-PCR with GC-HRMS for Spiked sand and loamy soil

sand			loamy soil		
GC-MS TEQ	AhR-PCR TEQ	50pg/g screen	GC-MS TEQ	AhR-PCR TEQ	50pg/g screen
0.03	0.6	CN	0.03	0.4	CN
0.03	0.4	CN	0.03	0.3	CN
0.03	0.8	CN	0.03	0.5	CN
0.03	0.4	CN	0.03	0.5	CN
0.03	0.6	CN	0.03	0.4	CN
0.03	0.6	CN	0.03	0.3	CN
5	6.9	CN	5	4.8	CN
5	5.9	CN	5	3.6	CN
5	4.6	CN	5	6.0	CN
5	4.9	CN	5	3.7	CN
5	4.7	CN	5	3.3	CN
5	4.5	CN	5	3.1	CN
25	22	CN	25	30	CN
25	29	CN	25	22	CN
25	29	CN	25	26	CN
25	28	CN	25	21	CN
25	35	CN	25	32	CN
25	22	CN	25	28	CN
50	45	FN	50	54	CP
50	50	CP	50	48	FN
50	52	CP	50	66	CP
50	31	FN	50	39	FN
50	62	CP	50	37	FN
50	76	CP	50	29	FN
75	80	CP	75	74	CP
75	90	CP	75	59	CP
75	103	CP	75	54	CP
75	82	CP	75	75	CP
75	83	CP	75	84	CP
75	52	CP	75	65	CP
500	610	CP	500	534	CP
500	652	CP	500	305	CP
500	627	CP	500	409	CP
500	372	CP	500	467	CP
500	646	CP	500	483	CP
500	497	CP	500	306	CP
1000	1275	CP	1000	939	CP
1000	1006	CP	1000	898	CP
1000	1078	CP	1000	771	CP
1000	710	CP	1000	1007	CP
1000	720	CP	1000	937	CP
1000	1040	CP	1000	930	CP
1500	1126	CP	1500	1214	CP
1500	1224	CP	1500	1187	CP
1500	1570	CP	1500	1168	CP
1500	876	CP	1500	961	CP
1500	1584	CP	1500	1234	CP
1500	1538	CP	1500	1293	CP

CP = Correct Positive, CN = Correct Negative, FP = False Positive, FN = False Negative

Table 4. Correlation of GC-HRMS and AhR-PCR Assay for Real World Soils

Raritan Bay Sediment

GC-HRMS TEQ (pg/g)	AhR-PCR TEQ (pg/g)	50pg/g screen
0.03	0.6	CN
14	14	CN
14	11	CN
14	6.8	CN
14	14	CN
12	8.8	CN
12	3.9	CN
12	11	CN
12	10	CN
15	15	CN
15	10	CN
15	10	CN
15	10	CN
14	15	CN
14	8.4	CN
14	15	CN
14	9.3	CN
13	14	CN
13	10	CN
13	10	CN
13	10	CN

Newark Bay Sediment

GC-HRMS TEQ (pg/g)	AhR-PCR TEQ (pg/g)	50pg/g screen
0.03	2.1	CN
16	22	CN
16	18	CN
16	17	CN
16	19	CN
32	32	CN
32	37	CN
32	35	CN
32	25	CN
38	29	CN
38	36	CN
38	43	CN
38	26	CN
45	53	FP
45	40	CN
45	44	CN
45	36	CN
62	61	CP
62	55	CP
62	45	FN
62	37	FN

Tittabawasse River Soil

GC-HRMS TEQ (pg/g)	AhR-PCR TEQ (pg/g)	50pg/g screen
0.03	0.7	CN
0.03	1	CN
0.03	1	CN
0.03	1	CN
42	62	FP
42	21	CN
42	26	CN
42	21	CN
435	435	CP
435	637	CP
435	406	CP
435	255	CP
808	597	CP
808	2383	CP
808	423	CP
808	1276	CP
1048	1246	CP
1048	1136	CP
1048	1237	CP
1048	877	CP
3127	1548	CP
3127	995	CP
3127	5578	CP
3127	3083	CP

Solutia Soils

GC-HRMS TEQ (pg/g)	AhR-PCR TEQ (pg/g)	50pg/g screen
0.03	6.2	CN
48	359	FP
48	465	FP
48	479	FP
48	371	FP
846	721	CP
846	667	CP
846	541	CP
846	466	CP
1279	859	CP
1279	1201	CP
1279	599	CP
1279	1123	CP
1833	1833	CP
1833	1938	CP
1833	1516	CP
1833	1243	CP
3257	4541	CP
3257	3083	CP
3257	2944	CP
3257	2692	CP
3951	4631	CP
3951	3935	CP
3951	3214	CP
3951	3352	CP

Winona Post Soils

GC-HRMS TEQ (pg/g)	AhR-PCR TEQ (pg/g)	50pg/g screen
0.03	5.2	CN
8648	16393	CP
8648	6684	CP
8648	9972	CP
8648	12430	CP
8831	9889	CP
8831	10716	CP
8831	9183	CP
8831	19528	CP
11071	11018	CP
11071	17503	CP
11071	20696	CP
11071	28013	CP
11259	33839	CP
11259	8139	CP
11259	7410	CP
11259	26151	CP
11410	15053	CP
11410	15297	CP
11410	7100	CP
11410	12760	CP

Table 5. Example False Positive and Negative Rates Near Action Level (50pg/g)

Spiked Sand			Spiked Soil		
GC-MS TEQ (pg/g)	Procept TEQ (pg/g)	Screen at 50 pg/g Result	GC-MS TEQ (pg/g)	Procept TEQ (pg/g)	Screen at 50 pg/g Result
25	22	True Negative	25	30	True Negative
25	29	True Negative	25	22	True Negative
25	29	True Negative	25	26	True Negative
25	28	True Negative	25	21	True Negative
25	35	True Negative	25	32	True Negative
25	22	True Negative	25	28	True Negative
75	80	True Positive	75	74	True Positive
75	90	True Positive	75	59	True Positive
75	103	True Positive	75	54	True Positive
75	82	True Positive	75	75	True Positive
75	83	True Positive	75	84	True Positive
75	52	True Positive	75	65	True Positive

Table 6. Same lot variation of Measurement

Spiked Sample[1] (pg/g TEQ)	Mean Procept Response (ppt)[2]	Standard Deviation[2]	% RSD[2,3]	% Recovery[4]
Blank	0.8	0.1	17	N/A
5.0	6.3	0.7	12	126
25	26	5.0	20	104
50	46	10	22	91
75	70	15	21	93
500	720	130	18	143
1000	1400	260	19	138
1500	1900	460	24	127

[1]Sand matrix spiked with 7 Dibenzo-*p*-Dioxin/Furan Standards (tetra-hexachloro)

[2]Eight measurements at each concentration on single lot of test kit on same day

[3]Relative standard deviation (standard deviation*100%/mean value)

[4](Mean AhR-PCR Response)*100%/Spike Concentration (pg/g TEQ)

Table 7. Variation of Measurement Between 3 Lots

Spiked Sample[1] (pg/g TEQ)	mean lot 101806[2]	mean lot 01106[2]	mean lot 033006[2]	Inter-lot Mean AhR-PCR Response (pg/g TEQ)	Inter-lot % RSD[3]
Blank	0.8	0.3	0.4	0.5	61
5.0	6.3	8.4	8.2	7.6	15
25	26	32	36	31	16
50	46	51	43	46	9
75	70	62	83	72	15
500	720	560	620	630	13
1000	1400	1200	1400	1300	9
1500	1900	1400	1500	1600	17

[1]Sand matrix spiked with 7 Dibenzo-*p*-Dioxin/Furan Standards (tetra-hexachloro)

[2]Mean of Four to Eight measurements on each lot

[3]Relative standard deviation (standard deviation*100%/mean value)

GLOSSARY

2,3,7,8-TCDD - 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

AhR (aryl hydrocarbon receptor) -- A chemical-responsive DNA-binding protein that is responsible for producing the toxic and biological effects of dioxin and related chemicals

ARNT - Aryl hydrocarbon receptor nuclear translocator protein

Congener - Compounds containing different numbers and position of chlorine atoms (or other substituent) on the same base structure.

Ct (threshold cycle) - This is the output of the real-time PCR instrument. Threshold cycle corresponds to the number of PCR temperature cycles at which the measured fluorescence of a sample well exceeds a threshold value. The threshold value is set by the user, or software, to optimize the linearity of the standard curve. Comparing the Ct of an unknown sample to an appropriate standard curve allows the analyst to relate Ct to concentration or TEQ.

DRE - DNA response element

PAHs - Polycyclic aromatic hydrocarbons

PCBs - Polychlorinated biphenyls

PCDD/PCDFs - Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans.

PCR (polymerase chain reaction) - A technique employed to replicate and measure the concentration of DNA fragments.

TEF (toxicity equivalent factor) - Toxicity values established by the World Health Organization (WHO) for chlorinated dibenzo-*p*-dioxins and furans and polychlorinated biphenyls relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

TEQ (toxicity equivalent quotient) - The sum of the concentration of dioxin congeners multiplied by their TEF value. TEQ = $\sum$(congener concentration) x (TEF).

Appendix A:

Summary of Revisions to Method 4430 (as compared to previous Revision 0, December 2007)

1. Improved overall method formatting for consistency with new SW-846 methods style guidance. The format was updated to Microsoft Word .docx.
2. Minor editorial and technical revisions were made throughout to improve method clarity.
3. The revision number was changed to 1 and the date published was changed to July 2014.
4. This appendix was added showing changes from the previous revision.
5. Sec. 9.2.1 was added to discuss the need for initial demonstration of proficiency (IDP).