SCREENING FOR POLYCHLORINATED DIBENZODIOXINS AND POLYCHLORINATED DIBENZOFURANS (PCDD/PCDFs) BY IMMUNOASSAY

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 procedure for the analysis of polychlorinated dibenzodioxins and polychlorinated dibenzofurans (PCDDs/PCDFs) in soil at 500 ppt (pg/g) using a simple one-step liquid phase oxidative cleanup or, at mid to low ppt levels, using a two step coupled column cleanup (oxidation and activated carbon binding) adapted from Method 8290. The analysis method uses a commercially-available enzyme immunoassay (EIA) test kit containing a polyclonal antibody specific for PCDDs/PCDFs. The EIA kit is designed for the screening of samples according to their toxic equivalent concentration (TEQ) by responding to the toxic PCDD/PCDF congeners in approximate correlation with their toxic equivalency factors (TEFs). The test is capable of multiple congener recognition and preferentially targets congeners with high TEF values; i.e., those with the highest toxicity relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD). The final measured EIA response is the sum of the individual congener responses. This response correlates with TEQ because the immunoassay cross-reaction profile for PCDDs/PCDFs correlates with TEF values. See Table 2 for a detailed cross-reaction profile.

1.2 The testing product evaluated for this method employs a competitive enzyme immunoassay. It is important to note that this method differs from other 4000 series immunoassay methods due to the specific chemistry of the analyte. The chemistry of dioxin analysis is more difficult, as shown by the cost and turnaround time differences between dioxin/furan analysis (e.g., Methods 8280/8290) and PCB analysis (e.g., Method 8082). Dioxins are 3 to 4 orders of magnitude less water soluble than most other 4000 series target analytes. Additionally, sensitivity targets can be orders of magnitude lower than for PCBs and other analytes, so the matrix concentration factors can be extreme and extracts generally require partial cleanup.

1.3 The PCDD/PCDF congener composition of samples can be highly variable. Because PCDDs/PCDFs are formed unintentionally by a variety of chemical and combustion processes, samples usually contain a mixture of many different congeners. Samples from different sources often have very different mixtures of congeners which are consistent within the source. In most samples, the majority of the PCDD/PCDF mass present does not contribute significantly to the total sample TEQ. Also, in most samples, only a few PCDD/PCDF congeners are responsible for the majority of the TEQ.

1.4 Detection for the test kit from which this method was developed is based upon structure. Strong recognition by the antibody used in this immunoassay kit generally requires both the dioxin/furan core structure and the 2,3,7,8- chlorination pattern. For example, test response to non-toxic PCDDs/PCDFs is greatly reduced because of their deviation from this chlorination pattern. Recognition of the PCBs that are most similar to 2,3,7,8-TCDD (PCBs 77 and 126) is very limited because the core structure to which the chlorines are attached is not a dioxin or furan. When both core structure and chlorination pattern are changed, as for PCB 153, there is no detectable recognition. See Table 2 for more detailed information on cross-reactivity.

1.5 The sensitivity submitted by the manufacturer of this testing product is approx. 4 pg of 2,3,7,8-TCDD, based on a standard curve from 3.2 to 100 pg per EIA tube. The ultimate limit of detection in soil and sediment samples is determined by the amount of sample extracted, multiplied by the fraction of that prepared sample extract which is added to the EIA tube. The EIA sensitivity is sufficient for analysis at 10 ppt (pg/g) TEQ using a 5 g sample (or 5 ppt using a 10 g sample), prepared using the two step column cleanup (Refs. 7, 8). Analysis based on the single step oxidative cleanup is designed to provide screening at 500 ppt. Data supporting this sensitivity are given in Table 1.

1.6 This immunoassay is designed to measure sample TEQ by responding to the toxic PCDD/PCDF congeners in correlation with their TEFs. Variation in accuracy among samples may occur solely because of the variability of congener composition noted above. To maximize accuracy, the variability of congener composition in the target sample population should be known. Best method performance is achieved when all samples are from a single group that shares as many properties as possible (common source of contamination, similar congener composition, similar sample matrix, etc.). Data supporting this approach are given in Ref. 7 and 8. However, without such information it is still possible to use this method for blind screening at mid to low ppt levels. Data supporting this approach are given in Ref. 9.

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 intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies, and 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 required 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 supervision of, personnel appropriately experienced and trained in the performance and interpretation of immunoassay methods. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 Sample preparation and analysis procedures are described in the manufacturer's literature. In addition, EIA test kits and sample preparation kits are commercially available for this method and the kit instructions used to develop this method can be downloaded from the following Web address: www.cape-tech.com. Using the EIA test kit from which this method was developed, the following steps are followed for the two different extraction and cleanup methods (using sample preparation kits from the same manufacturer); Section 2.1.1 for the one step oxidative cleanup and Section 2.1.2 for the two step column cleanup.

2.1.1 One Step Oxidative Cleanup

2.1.1.1 For the less sensitive analysis using the one step oxidative cleanup, sodium sulfate is added to a soil sample and mixed. Dimethylformamide (DMF) is added to the soil sample and the soil is extracted by shaking for two hours. The supernatant DMF extract is removed. DMF extracts are stable for weeks to months at room temperature.

2.1.1.2 Interferences are removed by chemical oxidation. Hexane is added to an aliquot of the DMF extract, then treated with 15% SO_3 in concentrated H_2SO_4 (fuming sulfuric acid). The supernatant hexane is removed and exchanged to a water-miscible organic solvent solution. This hexane-based fuming sulfuric acid cleanup is sufficient for most samples, but in certain circumstances an additional cleanup step may be required. This is the case for samples that contain large amounts of non-volatile aliphatic oils. When the DMF extracts of such soils are cleaned using fuming sulfuric acid, the oil is not oxidized, and it remains after evaporation of the hexane, causing a biphasic system when introduced to the EIA first incubation. Such EIA samples appear opalescent or milky and their results will be invalid because the biphasic system prevents capture of analyte by the antibody. For these samples, a new aliquot of DMF extract is cleaned by carbon adsorption or a new soil sample is extracted and cleaned using the more sensitive method (Sec. 2.1.2). In either case, the final solvent in the cleanup procedure is toluene rather than hexane.

2.1.2 Two Step Column Cleanup

2.1.2.1 For the more sensitive analysis using the two step coupled column cleanup, sodium sulfate is added to a soil sample and mixed. A mixture of 1:1 hexane:acetone (HA) is added to the soil sample and the soil is extracted by shaking for 4 to 6 hours. The supernatant HA extract is removed for immediate processing.

2.1.2.2 Polar interferences are removed by oxidation and non-polar interferences are removed by non-binding during the adsorption of the PCDDs/PCDFs on activated carbon. The two step cleanup is as follows: The HA extract is evaporated to a tetradecane keeper to remove the acetone, which is reactive with concentrated sulfuric acid (H_2SO_4), then the tetradecane solution is diluted with hexane. Polar interferences are removed by chemical oxidation (Ref. 11) by first pretreating with loose bulk acid silica (conc. sulfuric acid adsorbed to chromatographic silica) until the liquid phase is clear. The pretreated sample is then added to an acid silica column coupled to an activated carbon column. Pressure is applied until the entire sample enters the

acid silica column. Hexane is then added to the acid silica column and the system is pressurized again to force the sample and additional hexane through the carbon column. The sample and hexane washes are discarded after passing through the columns. Non-polar interferences, which are not reactive with the acid silica, are removed by passing the extract through an activated carbon column, which captures the PCDDs/PCDFs while allowing non-aromatic non-polar compounds to flow through without binding. The carbon column, which contains the adsorbed PCDDs/PCDFs, is then removed and washed with 1:1 hexane:toluene in the forward direction, removing more interferences, including the lower chlorinated PCDDs/PCDFs and dioxin-like PCBs (Refs.10, 11). The carbon column is then eluted with toluene in the reverse direction. The captured toluene eluate is then exchanged to a water-miscible organic solvent solution. This cleanup is sufficient, in terms of both recovery and interference removal, for analysis of most samples by HRGC-HRMS as well as by EIA (Ref.12).

2.2 The cleaned sample in hexane or toluene is exchanged to a water-miscible organic solvent solution for EIA analysis. PCDDs/PCDFs have very low volatility and are retained during this solvent exchange in a small volume of keeper (Triton X-100 detergent in polyethylene glycol [PEG]) after evaporation of the original solvent. Methanol is added to dilute this solution and the methanol-PEG-Triton mixture added directly to the EIA tubes. It should be noted that the literature value for solubility of 2,3,7,8-TCDD in methanol is 10 ppm, which is 5000 times higher than the concentration of the highest standard recommended for the kit used to develop this method. Additionally, the solubility of PCDDs/PCDFs in methanol is augmented significantly by the addition of PEG and Triton X-100.

2.3 An accurately measured volume of negative control, standard or prepared sample is mixed with an aqueous sample diluent in test tubes with anti-dioxin antibody immobilized on the surface. The mixture is incubated at the temperature, and for the time, described in the manufacturer's instructions.

2.4 After incubation, antibody tubes are washed and 0.5 mL of Horseradish Peroxidase (HRP) Competitor Conjugate is added to each tube using a repeater pipettor. Bound PCDDs/PCDFs occupy the dioxin binding sites of the antibodies in proportion to the PCDD/PCDF content of the sample and prevent binding of the competitor-HRP conjugate. After a short incubation, unbound conjugate is removed and the test tubes are washed thoroughly.

2.5 A solution of chromogenic HRP substrate and hydrogen peroxide is added to the test tubes. Color development is directly proportional to enzyme concentration and inversely related to the PCDD/PCDF concentration in the original sample. Stop solution is added to each tube using a repeater pipettor in order to fix the amount of color development.

2.6 The test tubes are analyzed using a tube reader or spectrophotometer to measure the optical density (OD) at 450 nm. The test is interpreted by measuring the signal produced by a sample and determining the concentration from a dose-response curve constructed from standards tested at the same time.

3.0 DEFINITIONS

See Method 4000 for a glossary of basic immunoassay terms and see the glossary at the end of this method for procedure-specific terms. Also refer to the manufacturer's instructions, Chapter One and Methods 8280 and 8290 for other definitions that may be relevant.

4.0 INTERFERENCES

4.1 The anti-dioxin antibody noted in the kit used to develop this method, binds to different PCDD/PCDF congeners with different affinities. The specificity of the test is predominantly for PCDDs/PCDFs which contain 3 to 6 chlorines, with a strong preference for the 2,3,7,8 chlorinated congeners. Cross reactivity data showing test recognition relative to 2,3,7,8-TCDD are given in Table 2. These data demonstrate a very restricted recognition profile, indicating very limited potential for positive interference due to specific binding. Negative interference is possible through indirect action on analyte solubilization by substances that do not bind to the analyte-binding sites of the antibody. Sample preparation and cleanup procedures required for this method reduce the potential for positive and negative interference by removal of all readily oxidizable organic compounds. Substances stable to fuming sulfuric acid, that are also extracted by the extraction solvent, may also cause interference. These can be removed by an additional cleanup step as noted in 2.1.1.2 and summarized in 2.1.2.2. The more rigorous column cleanup described in 2.1.2.2 removes both positive and negative interferences well enough that these samples can be analyzed by Method 8290 (Ref. 10).

4.2 The antibody used in this immunoassay recognizes PCDD/PCDF congeners based on structure, not mass. Therefore, conventional stable isotope labeled internal standards are detected as native material. Typical levels of conventional stable isotope labeled internal standards cannot be used with this EIA. Consult the manufacturer's literature for specific recommendations regarding mass labeled internal standards.

4.3 Non-specific interferences such as sample pH, temperature, osmolarity, solvents, surfactants, and the presence of metal ions can affect immunoassay performance. Samples should be tested at the pH and temperature range specified by the testing product manufacturer. Review the product literature with regard to other potential interferences.

4.4 Storage temperatures may alter the useful life of the testing product reagents and supplies. Follow the manufacturer's directions for storage and use of all reagents and supplies.

5.0 SAFETY

5.1 This method does not address all safety issues associated with its use. The user 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 (MSDSs) should be available to all personnel involved in these analyses.

5.2 The commercially-available test kits should only be used by properly trained personnel in an appropriate laboratory environment.

5.3 Treat PCDDs/PCDFs, solutions that contain PCDDs/PCDFs, and potentially contaminated samples as hazardous materials.

5.4 Use gloves, proper protective clothing, and means to contain and handle hazardous material where appropriate.

5.5 Obtain (if appropriate) permits pertaining to the handling, analysis and transport of dioxin-containing materials.

5.6 Stop solution is 1N hydrochloric acid. Handle carefully.

5.7 Fuming sulfuric acid, concentrated sulfuric acid, and acid silica are corrosive and hygroscopic; carefully follow handling and storage instructions.

6.0 EQUIPMENT AND SUPPLIES

6.1 Each test product will specify the apparatus and materials provided, as well as any additional apparatus and materials necessary for performance of the test. The immunoassay testing products of SW-846 immunoassay methods were submitted to EPA, evaluated by the Agency, and found to meet the performance specification necessary for inclusion in SW-846. As additional testing products are evaluated by EPA and found to provide equivalent performance, information will be made available by the Office of Solid Waste regarding those testing products that are capable of meeting the performance specifications in the methods (see http://www.epa.gov/epaoswer/hazwaste/test/pdfs/kits.pdf). However, the methods will not be revised solely to include information on additional testing products. Descriptions and materials lists for products relevant to the methods should be given in the manufacturer's literature.

6.2 Most testing products provide the supplies specific to the immunoassay, including the tubes or plates containing the immobilized antibody, and the immunochemical reagents. Do not mix the equipment, supplies, and reagents from the testing products for different analytes or from the testing products from different manufacturers. Testing products contain immunochemical reagents that are evaluated by the manufacturer on a lot-specific basis. Do not mix the reagents from one lot with those from another lot unless expressly allowed by the manufacturer. Other equipment that may be required, but is not supplied with the testing product, includes common laboratory items such as precision pipetting devices, vortex mixers, etc.

6.3 Additional information regarding equipment requirements is located in the relevant manufacturer's literature or can be downloaded from their website or obtained by email.

6.4 The immunoassay testing product listed below has been submitted to EPA, evaluated by the Agency, and found to meet the performance specifications necessary for inclusion in SW-846. As additional testing products are evaluated by EPA and found to provide equivalent performance, information will be made available by the Office of Solid Waste regarding those testing products that are capable of meeting the performance specifications in this method (see http://www.epa.gov/epaoswer/hazwaste/test/pdfs/kits.pdf). However, this procedure will not be revised solely to include information on additional testing products. Descriptions and materials lists for products relevant to this method are given in the manufacturer's literature.

DF1 High Performance Dioxin/Furan Immunoassay Kit (CAPE Technologies), www.cape-tech.com.

7.0 REAGENTS AND STANDARDS

7.1 Reagent-grade chemicals must be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on 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 As with the equipment and supplies, each commercially available testing product will supply or specify the reagents necessary for successful completion of the test. This includes the calibrators (standards) employed in the immunoassay. Detailed information on reagent requirements is given in the manufacturer's literature. As noted in Sec. 6.2, do not mix the equipment, supplies, and reagents from the testing products for different analytes or from the testing products from different manufacturers. Store all reagents and standards according to the manufacturer's instructions, and, where applicable, discard any that are past the expiration date assigned by the manufacturer.

8.0 SAMPLE COLLECTION AND STORAGE

Sample collection, preservation, and storage requirements may vary by EPA program and may be specified in the 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 Sample collection

The immunoassay testing products employ very small sample volumes. Therefore, sample collection procedures must focus on the amounts and procedures necessary to ensure that the sample is representative of the source.

The distribution of PCDDs/PCDFs can be extremely heterogeneous. The analyst is responsible for ensuring that soil samples are homogeneous and representative of the area from which they are taken. Subsamples selected for immunoassay analysis should be representative of the samples taken from the site. Water content should be low enough to avoid the presence of standing water in sample containers. If this is not possible, then standing water must be removed before homogenizing for sub-sampling. Additionally, visibly wet samples must be pre-dried before extraction (such as by placing on clean paper towels) to guarantee that sodium sulfate remains in excess during the extraction procedure. Samples which are insufficiently dried will not be extracted effectively by either DMF or hexane:acetone, potentially giving false negative results. QA samples for verification of adequate sample drying are essential.

8.2 Extract holding time

The sample extracts in DMF are stable for up to three months when stored in a tightly sealed vial at a temperature range of 2 ºC - 6 ºC (36 ºF - 43 ºF). Extracts are less stable when stored at room temperature. Extracts in hexane:acetone should not be stored because of their volatility, but can be exchanged to tetradecane or a similar oil such as paraffin oil for storage. Holding times beyond three months may be acceptable as long as it can be substantiated with relevant performance data.

8.3 Storage and use of kit

8.3.1 Do not freeze test kit components or expose them to temperatures above 37 ºC (99 ºF).

8.3.2 If desiccant in tube bag is not blue, do not use kit; contact manufacturer.

8.3.3 Do not expose substrate to direct sunlight.

8.3.4 If substrate is blue before adding to EIA tubes, do not use; contact manufacturer.

8.3.5 Store all test kit components at 2 ºC - 6 ºC (36 ºF - 43 ºF) when not in use.

8.3.6 Storage at ambient temperature (20 ºC - 27 ºC or 68 ºF - 81 ºF) on the day of use or overnight before the day of use is acceptable. Do not store at ambient temperature for extended periods.

8.3.7 Allow all reagents to reach ambient temperature (20 ºC - 27 ºC or 68 ºF - 81 ºF) before beginning the test. This typically requires at least 60 min at ambient temperature to warm from recommended storage conditions. Warming occurs faster if bottles and tube bags are removed from the kit box.

8.3.8 Do not use test kit after the expiration date.

8.3.9 Do not use components from one test kit with components from a different test kit.

9.0 QUALITY CONTROL

9.1 Follow the manufacturer's instructions for the quality control procedures specific to use of the testing product. Also refer to Chapter One for guidance on quality assurance (QA) and quality control (QC) protocols that may be applicable. 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.

9.2 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. 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. Because of the chemistry of PCDDs/PCDFs, the potential for poor extraction and adsorptive loss of analyte during sample preparation procedures is much greater than with other 4000 series methods. Therefore, demonstration of analyte recovery is critical to the success of this method.

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, Section 9.3 for information on how to accomplish a demonstration of proficiency.

9.3 Any method blanks, blank or 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.

9.4 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.

9.5 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 antibody in this immunoassay 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.

9.6 Routine quality control procedures associated with this method include the analyses of standards, matrix blank and spike samples, laboratory control samples, method blanks, and duplicate or replicate analyses (as specified by the manufacturer). All of these analyses must be conducted simultaneously, e.g., as part of the same batch of samples. A batch of samples consists of up to 20 field samples prepared and analyzed at the same time, or the maximum number of samples that can be analyzed along with the standards, controls, and other analyses specified by the manufacturer using a single testing product, whichever is fewer. The batch must include any duplicate or replicate analyses specified by the manufacturer as well as all additional quality control tests specified in this procedure.

9.7 Other quality control considerations:

9.7.1 Do not use testing products past their expiration date.

9.7.2 Do not mix the equipment, supplies, and reagents from the testing products for different analytes or from the testing products from different manufacturers.

9.7.3 Use the testing products within the storage temperature and operating temperature limits specified by the manufacturer.

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.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 As with the specific formats of the testing products and the reagents and supplies, the specifics of the required calculations may vary by manufacturer. Some testing products may provide measuring devices such as optical density readers or spectrophotometers and may include software for performing all the necessary calculations. Other testing products may require the analyst to plot results manually, using graph paper that may or may not be provided with the testing product, and determine sample results by interpolation from a standard curve. Whichever approach is used, the laboratory records (bench notes, etc.) should clearly indicate how the results were obtained. Records specific to each determination, whether in hard copy or in electronic form, should be retained by the laboratory to substantiate the results.

12.2 Follow the manufacturer's instructions regarding calculation of all testing product results. For each batch of samples, use the calibration curve generated concurrently with that EIA run.

13.0 METHOD PERFORMANCE

13.1 Performance data and related information should be provided by the manufacturer in the package insert.

13.2 In the case of this method (which may be used in either the field or the laboratory), any test kits used must be able to meet the performance specifications for the intended application. However, required performance criteria for a particular testing product may be included in the manufacturer's instructions.

13.3 Table 1 provides data of accumulated responses for 2,3,7,8-TCDD standards over ten months, including QA acceptance ranges.

13.4 Table 2 summarizes the cross reactivity of various PCDD/PCDF and PCB congeners relative to 2,3,7,8-TCDD.

13.5 Tables 3 and 4 present correlation data between GC/MS and immunoassay for soil samples from two contaminated sites.

13.6 Table 5 presents data from screening analysis of negative soil extracts spiked at levels near the 500 pg/g target level.

13.7 Table 6 summarizes the accuracy and precision data from the screening analysis noted in Sec. 13.4.

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 operations. 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. DF1 High Performance Dioxin/Furan Immunoassay Kit (CAPE Technologies), www.cape-tech.com.

2. CAPE Technologies Application Notes and related product literature, www.cape-tech.com.

3. Carlson, Robert, E., Harrison, Robert, O., Measurement of PCDD/F TEQ by Immunoassay: Concept Development and Validation. Organohalogen Compounds, vol. 5, Stockholm, Sweden (1998).

4. Harrison, Robert, O., Carlson, Robert, E., Measurement of PCDD/F TEQ by Immunoassay: Demonstration Using Real World Samples, Organohalogen Compounds, vol. 5, Stockholm, Sweden (1998).

5. Harrison, Robert, O., Role of Quality Assurance in Immunoassay Methods Used for Field Screening. Immunochemical Technology for Environmental Applications, American Chemical Society, Washington D.C. (1997).

6. Van den Berg, et. al., “The 2005 World Health Organization Re-evaluation of Human and Mammalian Toxic Equivalency Factors (TEFs) of Dioxins and Dioxin-like Compounds,” Toxicological Sciences, 93(2): 223-241, July 7, 2006.

7. "Correlation between EIA Results and GC-MS TEQ," revised Figure 2 from document listed in Ref. 8. (www.cape-tech.com, Tech References).

8. "Performance of the CAPE Technologies DF1 Dioxin/Furan Immunoassay Kit for Soil and Sediment Samples," EPA 540/R-08/002, February 2008. (www.cape-tech.com, Tech References).

9. "Technologies for Monitoring and Measurement of Dioxin and Dioxin-like Compounds in Soil and Sediment (CAPE Technologies LLC, DF1; Dioxin/Furan Immunoassay Kit; PCB TEQ Immunoassay Kit)," EPA 540/R-05/004, March 2005. (www.cape-tech.com).

10. Technical Note TN-005, CAPE Technologies (www.cape-tech.com, Technical Notes)

11. Application Note AN-008, CAPE Technologies (www.cape-tech.com, Application Notes)

12. "An Efficient and Green Cleanup System for Analysis of Dioxins/Furans, Dioxin-like PCBs and PBDES," (www.cape-tech.com)

13. Immunoassay Sample Prep Kit SP3-60, CAPE Technologies (www.cape-tech.com, Material Lists).

14. Sample Prep Starter Kit SP3-ST, CAPE Technologies (www.cape-tech.com, Material Lists).

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

The pages to follow contain the tables referenced by this method.

TABLE 1

SENSITIVITY AND REPRODUCIBILITY OF THE EIA STANDARD CURVE [a]

Standard Number	1	2	3	4
ng/mL 2378-TCDD in standard (50 mL per EIA tube)	0.064	0.2	0.64	2
pg 2378-TCDD per EIA tube	3.2	10	32	100
mean percent of negative control (%NC)	87	66	41	29
standard deviation (SD)	6	7	7	6
range of mean±2SD	74-99	51-80	27-55	17-40

[a] Data are accumulated responses for 2,3,7,8-TCDD standards over ten months. No sample matrix was present. A total of 41 tests were run in four different labs. The detection limit, which is approximated by the I85 or the concentration giving 85% of the negative control OD, was 3.9±1.4 pg/tube (mean±SD). The midpoint of the curve, defined as the I50 or the concentration giving 50% of the negative control OD, was 21.9±7.4 pg/tube (mean±SD).

TABLE 2

SPECIFICITY OF THE EIA [a]

Compound	Percent Crossreactivity
Toxic Dioxin Congeners	
2,3,7,8-TCDD	100
1,2,3,7,8-PeCDD	105
1,2,3,4,7,8-HxCDD	1.6
1,2,3,6,7,8-HxCDD	7.9
1,2,3,7,8,9-HxCDD	39
1,2,3,4,6,7,8-HpCDD	0.7
OCDD	<0.001
Toxic Furan Congeners	
2,3,7,8-TCDF	20
1,2,3,7,8-PeCDF	4.6
2,3,4,7,8-PeCDF	17
1,2,3,4,7,8-HxCDF	0.4
1,2,3,6,7,8-HxCDF	1.0
1,2,3,7,8,9-HxCDF	3.3
2,3,4,6,7,8-HxCDF	4.9
1,2,3,4,6,7,8-HpCDF	0.02
1,2,3,4,7,8,9-HpCDF	0.9
OCDF	<0.001
Other PCDD/PCDF Congeners	
2,3-dichlorodibenzo-p-dioxin	0.13
2,7-dichlorodibenzo-p-dioxin	0.003
2,3-dichlorodibenzofuran	0.02
2,7-dichlorodibenzofuran	<0.002
2,3,7-trichlorodibenzo-p-dioxin	24
2,3,8-trichlorodibenzofuran	0.26
1,2,3,4-TCDD	<0.001
1,2,3,4-TCDF	<0.001
1,3,6,8-TCDD	0.05
1,3,6,8-TCDF	0.007
PolyChlorinated Biphenyls	
3,3',4,4' (PCB 77)	0.4
3,3',4,4',5 (PCB 126)	0.5
2,2',4,4',5 (PCB 153)	<0.1
3,3',4,4',5,5' (PCB 169)	<0.1
Aroclor 1254	<0.1

TABLE 2
(Continued)

Compound	Percent Crossreactivity
Other PCDD/PCDF Congeners	
2,3-dichlorodibenzo-p-dioxin	0.13
2,7-dichlorodibenzo-p-dioxin	0.003
2,3-dichlorodibenzofuran	0.02
2,7-dichlorodibenzofuran	<0.002
2,3,7-trichlorodibenzo-p-dioxin	24
2,3,8-trichlorodibenzofuran	0.26
1,2,3,4-TCDD	<0.001
1,2,3,4-TCDF	<0.001
1,3,6,8-TCDD	0.05
1,3,6,8-TCDF	0.007
PolyChlorinated Biphenyls	
3,3',4,4' (PCB 77)	0.4
3,3',4,4',5 (PCB 126)	0.5
2,2',4,4',5 (PCB 153)	<0.1
3,3',4,4',5,5' (PCB 169)	<0.1
Aroclor 1254	<0.1

[a] Response curves were prepared for each congener as noted. The percent crossreactivity = (((2,3,7,8-TCDD I50)) (congener I50)) x 100). Values are typically based on 2 to 4 independent curves, each containing at least 4 concentrations.

TABLE 3

CORRELATION BETWEEN IMMUNOASSAY SCREENING ANALYSIS AND TEQ AS DETERMINED BY HIGH RESOLUTION GAS CHROMATOGRAPHY-HIGH RESOLUTION MASS SPECTROMETRY (HRGC-HRMS) [a]

Sample ID	ppt TEQ	EIA Result
1	94338	CP
2	1528119	CP
3	234492	CP
4	822885	CP
5	73750	CP
6	4733	CP
7	39	CN
8*	6278	CP
9	276	FP
10	2390	CP
11	2101	CP
12	30	CN
13	14	CN
14	210	CN
15	5860	CP
16	2191	CP
17	343	FP
18	18	CN
19	25	CN
20	70	CN
21	599	CP
22	217	CN
23	27	CN
24	43	CN
25	18	CN
26	18	CN
27	14	CN
28	26	CN
29	13	CN
30	14	CN
31	11	CN
32	579	CP
33	220	CN
34	13	CN
35	1501	CP

TABLE 3
(Continued)

Sample ID	ppt TEQ	EIA Result
36	3702	CP
37	248	CN
38	216	FP
39	1088	CP
40	1210	CP
41	13	CN
42	451	CN
43	16	FP
44	105	CN
45	16	CN
46	10	CN
47	211	FP
48	1725	CP
49	41	CN
50	551	CP
51	622	CP
52*	26856	CP
53	2122	CP
54	24	CN
55	31	CN
56	31	CN

[a] Fifty six soil samples from Site 1 were prepared and analyzed following the manufacturer's instructions for the DF1 High Performance Dioxin/Furan Immunoassay Kit (CAPE Technologies). An empirically determined calibration factor of 1.07 was applied to all EIA results before semiquantitative scoring. Results by category are 29 correct negative (CN), 0 false negative (FN), 22 correct positive (CP), and 5 false positive (FP) (91% correct, 9% FP). Two immunoassay samples which appeared heterogeneous or contained visible precipitate during the first EIA incubation were interpreted as giving invalid results. These samples were put through an additional carbon column cleanup for oil removal, were analyzed again by EIA, and the latter results reported (*).

TABLE 4

CORRELATION BETWEEN IMMUNOASSAY SCREENING ANALYSIS AND TEQ AS DETERMINED BY HIGH RESOLUTION GAS CHROMATOGRAPHY-HIGH RESOLUTION MASS SPECTROMETRY (HRGC-HRMS) [a]

ppt by GC-MS	EIA run 1	EIA run 2
2730	CP	FN**
470	FP	FP
65	CN	CN
1400	CP	CP
70		CN
4900	CP	CP
245		FP
7300	CP	CP
365		CN
360	CN	CN
140000	CP	CP
7000		CP
33	CN	CN
990	CP	CP
4100	CP	CP
205		CN
360	CN	CN
65	CN	CN

**actual value with no calibration adjustment was 497 ppt (run 1 was 630 ppt)

8 CN, 7 CP, 2 FP, 1 FN (**) from 18 samples

[a] Eighteen soil samples from Site 2 were prepared and analyzed following the manufacturer's instructions for the DF1 High Performance Dioxin/Furan Immunoassay Kit (CAPE Technologies). No calibration adjustment factor was applied to EIA results before semiquantitative scoring. None of these samples required additional cleanup before EIA analysis. Several days later, aliquots of 13 of the 18 DMF extracts were oxidized and analyzed in a second EIA and the concentrations were calculated based on a new standard curve. The mean coefficient of variation for the 13 pairs of duplicate ppt values was 35%.

TABLE 5

EXAMPLE FALSE POSITIVE/FALSE NEGATIVE RATES [a]

		Spike Level			
		250 ppt spike		1000 ppt spike	
		Oxidation Replicate		Oxidation Replicate	
Sample #	EIA Replicate	1	2	1	2
13	1	CN	CN	CP	CP
	2	CN	CN	CP	CP
18	1	CN	CN	CP	CP
	2	CN	CN	CP	CP
19	1	CN	CN	CP	CP
	2	CN	CN	CP	CP
25	1	CN	CN	CP	CP
	2	CN	CN	CP	CP
27	1	CN	CN	CP	CP
	2	CN	CN	CP	CP
28	1	CN	CN	CP	CP
	2	CN	CN	CP	CP
29	1	CN	CN	CP	CP
	2	CN	CN	CP	CP
30	1	CN	CN	CP	CP
	2	CN	CN	CP	CP
31	1	CN	CN	CP	CP
	2	CN	CN	CP	CP
34	1	CN	CN	CP	CP
	2	CN	CN	CP	CP
41A	1	CN	CN	CP	CP
	2	CN	CN	CP	CP
41B	1	CN	CN	CP	CP
	2	CN	CN	CP	CP
45	1	CN	CN	CP	CP
	2	CN	CN	CP	CP

TABLE 5
(Continued)

		Spike Level			
		250 ppt spike		1000 ppt spike	
		Oxidation Replicate		Oxidation Replicate	
Sample #	EIA Replicate	1	2	1	2
46*	1	CN*	CN*	*FN**	*FN**
	2	CN*	CN*	*FN**	***FN***
46 (oxid. x 2)	1			CP	
	2			CP	

Summary of FN/FP results (based on repeat oxidation data for sample 46):

Correct Negatives: 52 Correct Positives: 54

False Positives: 0 False Negatives: 0

[a] False positive/false negative data for negative soil extracts spiked near the 500 ppt decision level. Fourteen site 1 soil extracts ranging from 10 to 26 ppt by GC-MS were spiked with 2,3,7,8-TCDD at levels equivalent to 250 and 1000 ppt in the original sample. Extracts were oxidized and analyzed by EIA following the manufacturer's instructions for the DF1 High Performance Dioxin/Furan Immunoassay Kit (CAPE Technologies). Results for each run were calculated according to the manufacturer=s instructions. Controls consisting of DMF spiked at both levels were processed with each run and used to correct for spike recovery. Individual EIA replicate ppt values were scored as positive if equal to or greater than 500 ppt and were scored as negative if less than 500 ppt. Each semi-quantitative score below corresponds to one EIA tube. Sample 41A and 41B were field duplicates that appeared different based on gross color and particle size. Extract 46 was nearly black in color and totally opaque. Initial data for sample 46 (*) indicated false negatives at the high spike level (individual EIA replicates were 401, 366, 366, and 332 ppt). The unusually dark appearance of the acid phase after the first oxidation indicated that a second oxidation would be required to reduce the interferences to the same level as the other samples. The analysis of sample 46 was repeated using a second oxidation of the first hexane supernatant (plus a fresh aliquot of DMF). The appearance of the second phase matched the other samples. The individual EIA replicates of 1872 and 1318 ppt gave a correct positive interpretation (the corresponding twice oxidized DMF controls were 1068 and 846 ppt).

TABLE 6

EXAMPLE ACCURACY AND PRECISION DATA [a]

A. Summary of quantitative ppt data from DMF controls		
spike level	2	1000 ppt spike
number of runs (2 or 4 EIA replicates within each run)	4	5
mean±SD of within run means (ppt)	271±74	957±174
coefficient of variation of ppt	27%	18%

B. Summary of quantitative ppt data from spiked extracts (sample 46 data includes only 2x oxidation		
spike level	250 ppt spike	1000 ppt spike
number of individual EIA replicates	54	56
overall mean±SD (ppt)	266±61	984±356
coefficient of variation of ppt	23%	36%

C. Coefficients of variation for ppt within runs (all EIA replicates at each spike level for each run)		
	spiked extracts	DMF controls
number of runs	9	9
mean of within run %cv values	18%	15%

D. Summary of EIA replicate precision (both spike levels combined)		
	spiked extracts	DMF controls
number of pairs of EIA replicates	57	11
mean of all EIA replicate %cv values for	10%	13%

[a] Summary of quantitative data on accuracy and precision from the false positive/false negative experiment of Table 5. Data are based on four parameter curves and calculated ppt values for each of 5 runs on 5 separate days. The accuracy and precision shown in parts A and B support the semi-quantitative screening method described here. These data also support quantitative use of the test in certain situations with sufficient quality assurance samples. Note that the data of section B include variation among the 14 different soils, as well as the intrinsic method variability. The precision data shown in parts C and D support screening analysis based on un-replicated sample oxidation and un-replicated EIA tubes for both standards and samples.

Congeners	Compounds containing different numbers and positions of chlorination (or other single atom substituents) on the same base structure. For example, dioxin congeners all contain the same dibenzo-p-dioxin nucleus, but are chlorinated to different levels and in different ring positions.
Keeper	A high boiling material used to keep analyte in solution during sample evaporation. In GC methods, typically tetradecane or similar hydrocarbon. In this method, a combination of detergent and high-boiling glycol used for exchanging sample from a non-polar solvent to an aqueous system for EIA analysis.
TEF	Toxicity equivalency factor - Toxicity values given to several of the halogenated aryl hydrocarbons relative to the most toxic congener, 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8 TCDD).
TEQ	Toxicity equivalent concentration - A toxicity weighted concentration which accounts for both the concentration of individual congeners and their different TEF values. Calculated as TEQ = $\sum$ (congener concentration x congener TEF) for all congeners having assigned TEF values.

Appendix A:

Summary of Revisions to Method 4025 (as compared to previous Revision 0, October 2002)

1. Improved overall method formatting for consistency with new SW-846 methods style guidance. The format was updated to Microsoft Word .docx.
2. The revision number was changed to 1 and the date published was changed to July 2014.
3. Additional information provided concerning method sensitivity (Sec. 1.5) and cleanup procedures (Sec. 2.0).
4. The Reference section (16) has been updated to include more current documentation for the method.
5. Minor editorial and technical revisions were made throughout to improve method clarity.
6. This appendix was added showing changes from the previous revision.
7. Section 9.2.1 was added to discuss the need for initial demonstration of proficiency (IDP).