

TOXAPHENE AND TOXAPHENE CONGENERS BY GAS CHROMATOGRAPHY/NEGATIVE ION
CHEMICAL IONIZATION MASS SPECTROMETRY (GC-NICI/MS)

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 formally trained in the basic principles of chemical analysis and in the use of the subject technology.

In addition, SW-846 methods, with the exception of required 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. Performance data included in this method are for guidance purposes only, and must not be used as absolute quality control (QC) acceptance criteria for the purposes of laboratory QC or accreditation.

1.0 SCOPE AND APPLICATION

Please see Appendix A at the back of this document for a summary of revisions to Method 8276 (From March 2010).

1.1 This method is used to determine the concentrations of various toxaphene congeners (see table below) and technical toxaphene (along with the possible addition of other toxaphene congeners and compounds from Method 8081) in extracts from solid and liquid matrices, using fused-silica, open-tubular capillary columns with negative ion chemical ionization mass spectrometry (NICI/MS). This approach emphasizes the analytical conditions recommended for technical toxaphene and for toxaphene congeners as compared to weathered toxaphene. Technical toxaphene can be definitively quantitated by NICI/MS while weathered toxaphene may only appear to be present based on the detection of ions found in toxaphene or the presence of known degradation products of toxaphene (e.g., Hx-Sed and Hp-Sed). For this reason, the quantitation of weathered toxaphene may be considered subjective and qualitative with the success highly dependent on matching the calibration standards to the weathered peak pattern. For clarification, any notation of toxaphene in the method hereafter will refer to technical toxaphene.

| Compound | CAS Registry No. ^a |
|---|-------------------------------|
| Toxaphene | 8001-35-2 |
| Toxaphene Congeners: | |
| 2-exo,3-endo,6-exo,8,9,10-Hexachlorobornane (Hx-Sed) | 57981-29-0 |
| 2-endo,3-exo,5-endo,6-exo,8,9,10-Heptachlorobornane (Hp-Sed) | 70649-42-2 |
| 2-endo,3-exo,5-endo,6-exo,8,8,10,10-Octachlorobornane (P26) | 142534-71-2 |
| 2-endo,3-exo,5-endo,6-exo,8,9,10,10-Octachlorobornane (P40) | 166021-27-8 |
| 2-exo,3-endo,5-exo,8,9,9,10,10-Octachlorobornane (P41) | 165820-16-6 |
| 2-exo,5,5,8,9,9,10,10-Octachlorobornane (P44) | 165820-17-7 |
| 2-endo,3-exo,5-endo,6-exo,8,8,9,10,10-Nonachlorobornane (P50) | 6680-80-8 |
| 2,2,5,5,8,9,9,10,10-Nonachlorobornane (P62) | 154159-06-5 |

^a Chemical Abstract Service Registry Number

1.2 The analyst should select gas chromatography (GC) columns, detectors, and calibration procedures most appropriate for the specific analytes of interest in a particular project application. Matrix-specific performance data should be generated, and the stability of the analytical system and instrument calibration must be established for each analytical matrix (e.g., hexane solutions from various sample matrix extractions). Example chromatograms and GC-NICI/MS conditions are provided as guidance.

1.3 Although performance data are presented only for toxaphene and toxaphene congeners, in future method revisions additional target analytes (e.g., from Method 8081) may be added if acceptable performance can be demonstrated. When more analytes are included, it may become likely that not all of them can be determined in a single analysis. The chemical and chromatographic behaviors of these additional chemicals may result in coelution of some target analytes. Several cleanup/fractionation schemes are provided in this method. For additional details refer to Method 3600.

1.4 Multi-component mixtures (e.g., chlordane and toxaphene) present additional difficulties that include the need to separate congeners (Secs. 3.1 and 3.3) of the mixture. When samples contain more than one multi-component analyte, a higher level of expertise is necessary to attain acceptable levels of qualitative and quantitative analysis. The same is true of multi-component analytes that have been subjected to degradation by the environment or treatment technologies. These processes result in “weathered” multi-component mixtures that may have significant differences in peak patterns compared to those of the standards.

1.5 Unless present in relatively high concentrations, 4,4'-DDD, 4,4'-DDE, 4,4'-DDT and related compounds will not pose significant interferences to toxaphene analysis by NICI/MS because they do not respond with similar sensitivity.

1.6 This method has primarily been validated for the analysis of target analytes listed in Sec. 1.1. Extracts suitable for analysis by this method may also be analyzed for other organochlorine and organophosphorus pesticides (Methods 8081 and 8141) provided acceptable performance data can be generated. Additionally, some extracts may also be suitable for triazine herbicide analysis. However, low recoveries of triazine herbicides may result from lack of sample preservation. In addition, should users generate acceptable performance data for organochlorine pesticide compounds as noted in Method 8081, this method may be considered as an appropriate alternative to Method 8081.

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, and 8000) for additional information on QC procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts also should consult the disclaimer statement at the front of the SW-846 manual and the information in Chapter Two for 1) guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies; and 2) the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the target analytes in the matrix of interest, and at the level(s) 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 the Environmental Protection Agency (EPA) as guidance to be used by the analyst and the regulatory community in making judgments necessary to generate results that meet the data quality objectives (DQOs) for the intended application.

1.8 Application of this method is restricted to use by, or under the supervision of, personnel appropriately experienced and trained in the use of GC-NICI/MS and skilled in the interpretation of applicable chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method.

1.9 This method depends on resonance electron capture (electron attachment) or dissociative electron capture in the ion source when the instrument is operated under methane chemical ionization (CI) conditions. Thus, the technique produces a transient ionic species that can be detected as a molecular anion of a compound or as a dissociated ion of that species produced by dissociative electron capture. Other moderating gases besides methane may be used provided acceptable project-specific performance data can be generated. Within this description, the technique does not strictly result from a CI event (i.e., the result of an ion-molecule reaction) and refers to the production of negative ions by any of these processes as NICI/MS. See Ref. 1 for a more detailed discussion of NICI/MS theory.

2.0 SUMMARY OF METHOD

2.1 The samples are prepared for analysis by GC-NICI/MS using the appropriate sample preparation (refer to Method 3500) and, if necessary, sample cleanup procedures (refer to Method 3600).

2.2 The extract is analyzed by injection of a sample aliquot into a gas chromatograph with a narrow-bore fused-silica capillary column interfaced to a mass spectrometer capable of performing NIMS under CI conditions with methane as the introduced gas.

2.3 Analysis of toxaphene involves monitoring a series of ions representing various congener groups found in the mixture and integrating all of these signals for a total toxaphene response. In the case of the toxaphene congeners, individual compounds are quantitated separately and reported separately.

2.4 This method does not address all the congeners that comprise toxaphene. Toxaphene congeners can be added to the method by identifying retention windows with the additional congeners, assuming their responses are resolved from other congeners. Any added analytes must meet performance-based QC acceptance criteria.

3.0 DEFINITIONS

3.1 Toxaphene - A complex mixture of polychlorinated monoterpenes (primarily bornanes and camphenes) produced commercially from 1947 to 1982 and purported to contain 600-plus separate congeners. Also known as camphechlor, Melipax, chlorocamphene, polychlorocamphene, and chlorinated camphene.

3.2 GC-NICI/MS - All techniques producing negatively charged ions used for confirmation and quantitation of analytes. See Sec. 1.9 for additional details.

3.3 Congener - One of many variants or configurations of a common starting material. Most often the term is used to describe a chlorinated or brominated species of a given starting material that gives rise to multiple levels of halogenation to fully halogenated compounds.

3.4 Parlar number - Individual toxaphene congeners based on GC elution order are also known as "Parlars". For ease of reference, specific congeners have been given a Parlar number (e.g., P26, P50). More information on Parlar nomenclature can be found in reference 28.

3.5 Weathered toxaphene - In the environment, technical toxaphene degrades over time via both biotic and abiotic processes. The major processes of this degradation seem to be dechlorination and dehydrochlorination that lead to a pronounced shift toward lower chlorinated homologs. Weathered toxaphene may have a different chromatographic profile and NICI/MS detector response when compared to "virgin" toxaphene; this is a result of the composition changes which occurs from environmental degradation.

3.6 Total Ion Current (TIC) - The sum of the separate ion currents carried by the different ions contributing to the spectrum (sometimes called the reconstructed ion current).

3.7 Refer to Chapter One, "Quality Control", for guidance on quality assurance (QA) and quality control (QC) protocols, and the instrument manufacturer's instructions for additional 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 during sample analysis. All of these materials must be demonstrated to be free from interferences under conditions of analysis by method blanks. Specific selection of reagents may be necessary. Refer to each method used for specific guidance on QC procedures and to Chapter Four for general guidance on glassware cleaning. Also refer to Methods 3500, 3600, and 8000 for a discussion of interferences.

4.2 Interferences co-extracted from the samples will vary considerably from matrix to matrix. While general cleanup techniques are referenced or provided as part of this method, unique samples may require additional cleanup approaches to achieve desired degrees of discrimination and quantitation. Sources of interference in this method can be grouped into three broad categories as follows.

4.2.1 Contaminated solvents, reagents, or sample processing hardware

4.2.2 Contaminated GC carrier gas, parts, column surfaces, or detector surfaces

4.2.3 Compounds extracted from the sample matrix to which the detector will respond

4.3 Interferences by phthalate esters introduced during sample preparation can pose a major problem in some pesticide determinations but not for toxaphene analysis provided the scan window or ion monitoring signal is greater than m/z 300. However, extremely high levels of phthalate contamination could adversely affect quantitative responses of coeluting target components by reducing the availability of thermal electrons or reagent ions needed to ionize target analytes. Interferences from phthalate esters can best be minimized by avoiding contact

with any plastic materials and checking all solvents and reagents for phthalate contamination.

4.3.1 Common flexible plastics contain varying amounts of phthalate esters that are easily extracted or leached from such materials during laboratory operations.

4.3.2 Exhaustive cleanup of solvents, reagents, and glassware may be necessary to eliminate background phthalate ester contamination.

4.3.3 Phthalate esters may be removed prior to analysis using Method 3640 (gel-permeation cleanup, GPC) or Method 3630 (silica gel cleanup).

4.3.3 Cross-contamination of clean glassware routinely occurs when plastics are handled during extraction steps, especially when solvent-wetted surfaces are manipulated.

4.4 The presence of sulfur will result in broad peaks that interfere with the detection of early-eluting compounds only if the monitored ions occur near m/z 256, 224, 192, 160, 128, and 96 (as well as the M+2 isotope due to ^{34}S and the corresponding M+1 peaks from ^{33}S). Therefore, sulfur should not interfere with monitored ions from toxaphene but should nevertheless be removed because of deleterious effects on chromatography and on the response of the NICI/MS ion source during coelution. Sulfur contamination should be expected with sediment samples.

Method 3660 is suggested for removal of sulfur. Because the recovery of endrin aldehyde is drastically reduced when using the tetrabutylammonium (TBA) procedure in Method 3660, it must be determined prior to sulfur cleanup if it is an analyte of interest. Endrin aldehyde is not affected by copper powder, so it can be determined after the removal of sulfur by using the copper powder technique in Method 3660. However, as indicated in Method 3660, copper powder may adversely affect the recoveries of other potential analytes of interest, including some organochlorine compounds and many organophosphorus compounds.

4.5 Waxes, lipids, and other high molecular weight materials can be removed by Method 3640 (GPC) but are generally transparent to NICI/MS. Naturally occurring compounds such as flavonoids (e.g., coumarins) that contain conjugated carbonyl groups may respond sensitively under GC-NICI/MS conditions as will other kinds of compounds containing functional groups that facilitate ionization under these conditions.

4.6 Other halogenated pesticides or industrial chemicals may interfere with the analysis of pesticides. Certain coeluting organophosphorus pesticides may be eliminated using Method 3640 (pesticide option). Coeluting chlorophenols may be removed with Methods 3630 (silica gel), Method 3620 (Florisil®), or Method 3610 (alumina).

4.7 PCB Interference

4.7.1 Polychlorinated biphenyls (PCBs) also may interfere with the analysis of organochlorine pesticides. The problem may be most severe for the analysis of multicomponent analytes such as chlordane, toxaphene, and Strobane. If PCBs are known or expected to occur in samples, the analyst should consult Methods 3620 and 3630 for techniques that may be used to separate the pesticides from the PCBs. Under NICI/MS conditions in the absence of oxygen (oxygen reacts with PCBs), PCBs should not constitute an interference concern for toxaphene ions. The presence of the oxygen

reaction does not preclude analysis, but must lead to reproducible results and would require removal of PCBs from extracts prior to toxaphene analysis. Overall, the success of analysis is strongly dependent on adequate cleanup, separations of congeners/analytes, and quantitative recoveries.

4.7.2 Based on limited studies to date, the oxygen reaction observed with PCBs that gives rise to ions potentially interfering with toxaphene determination (i.e., ions at the same nominal mass but not the same elemental composition) is completely eliminated in modern instruments under appropriate conditions. The use of a PCB congener (e.g., PCB #204) serves to monitor this situation by the absence of $(M - Cl + O)^-$ ions (e.g., m/z 411) at its retention time (less than 0.5% possible attributable response). Thus the success of this method depends heavily on the proper extraction, cleanup, concentration, separations, and mass spectrometric conditions to achieve the quantitation and confirm the target analytes.

4.8 Coelution among the target analytes in this method can cause interference problems. Toxaphene congener coelution may be a problem for all possible GC columns regardless of the proposed analysis scheme.

4.9 Chlordane interference

4.9.1 Under GC-NICI/MS conditions, the determination of toxaphene and individual congeners of toxaphene may be affected by the presence of chlordane. In addition, chlordane congeners may coelute with certain toxaphene congeners and the ^{13}C isotope of the chlordane ion may add intensity to the corresponding isobaric ion of toxaphene.

4.9.2 As shown in Table 3, ions for monitoring the presence of chlordane indicate that their ^{13}C isotopes can contribute signals to monitored toxaphene ions. Therefore, use Table 3 to confirm whether chlordane is present at levels which significantly affect toxaphene quantitation.

4.10 Table 4 gives ions that can be used to confirm the presence of organochlorine pesticides in the sample. None of these compounds should result in interference with toxaphene determination.

5.0 SAFETY

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 specified in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

6.0 EQUIPMENT AND SUPPLIES

The mention of trade names or commercial products in this manual is for illustrative purposes only, and does not constitute an EPA endorsement or exclusive recommendation for use. The products and instrument settings cited in SW-846 methods represent those used during method development or subsequently evaluated by the Agency. Glassware, reagents, supplies, equipment, and settings other than those listed in this manual may be employed provided that method performance appropriate for the intended application has been demonstrated and documented.

6.1 Gas chromatograph (GC) - An analytical system equipped with a temperature programmable oven suitable for any applicable injection technique and all required accessories including syringes, analytical columns, and gases. The capillary column should elute directly into the ion source of the mass spectrometer.

6.2 GC columns - The columns listed in this section were used in developing the method and are not intended to exclude others that are available or may be developed. Laboratories may use these columns or other columns provided that they document method performance data (e.g., chromatographic resolution, analyte breakdown, and sensitivity) appropriate for the intended application.

Given the performance data available to date, the DB-XLB stationary phase columns may provide the maximum practical resolving power for congener separations.

6.2.1 30-m x 0.25-mm ID fused-silica capillary column DB-XLB MSD (J&W Scientific), 0.25- μ m film thickness

6.2.2 30-m x 0.25-mm ID fused silica capillary column ZB-MultiResidue-1 (Phenomenex), 0.25- μ m film thickness. This column appears similar to DB-XLB MSD.

6.2.3 40-m x 0.18-mm ID fused silica capillary column DB-5MS (J&W Scientific), 0.18- μ m film thickness

NOTE: DB-5 columns have been problematic, however, in separating some of the Parlars, which thus results in summed values.

6.3 Column rinsing kit - Bonded-phase column rinse kit (J&W Scientific, Catalog No. 430-3000 or equivalent).

6.4 Mass spectrometer

6.4.1 The mass spectrometer must be capable of producing a mass spectrum in the negative ion mode under chemical ionization conditions. Before acquiring spectra in this mode the instrument must be tuned under NCI conditions according to the guidance outlined in Sec. 11.4.1 and using a customized NCI tune table.

6.4.2 An ion trap mass spectrometer may be used if capable of axial modulation to reduce ion-molecule reactions and the production of NCI spectra. The mass spectrometer must be capable of producing a spectrum using a NCI-customized tuning file according to the guidance outlined in Sec. 11.4.

6.4.3 GC/MS interface - Any GC-to-MS interface may be used that gives acceptable calibration points for each compound of interest and achieves acceptable tuning performance criteria. For a narrow-bore capillary column, the interface is usually capillary-direct into the mass spectrometer source.

6.5 Data system - A computer system should be interfaced to the mass spectrometer. The system must allow the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program. The computer should have software that can search any GC/MS data file for ions of a specific mass and can plot such ion abundances versus time or scan number. This type of plot is defined as an extracted ion current profile (EICP). Software should also be available that allows integrating the abundances in any EICP between specified time or scan-number limits.

6.6 Analytical balance, capable of weighing to 0.0001 g

7.0 REAGENTS AND STANDARDS

7.1 Reagent-grade or pesticide-grade chemicals, at a minimum, should be used in all tests. Unless otherwise indicated, all reagents should conform to specifications (where available) of the Committee on Analytical Reagents of the American Chemical Society. Other grades may be used provided 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 leaching of contaminants from plastic containers.

NOTE: Store all standard solutions (stock, composite, calibration, internal, and surrogate) at $\leq 6^{\circ}\text{C}$ in polytetrafluoroethylene (PTFE) sealed glass containers in the dark. When a standard lot is prepared, aliquots should be stored in individual small vials. All stock standards must be replaced after one year or sooner if routine QC (Sec. 9.0) indicates a problem. All other standard solutions must be replaced after six months or sooner if routine QC (Sec. 9.0) deteriorates.

7.2 Solvents used in the extraction and cleanup procedures (see appropriate 3500 and 3600 series methods) may include *n*-hexane, diethyl ether, methylene chloride (dichloromethane), acetone, ethyl acetate, and isooctane (2,2,4-trimethylpentane). All solvents should be pesticide grade quality or equivalent, and each lot of solvent should be demonstrated to have negligible contamination of both target and non-target compounds (e.g., phthalates) at a minimum below the typical laboratory reporting limit.

7.3 Organic-free reagent water - Reagent water must be interference free. All references to water in this method refer to organic-free reagent water unless otherwise specified.

7.4 Stock standard solutions (1000 mg/L) - May be prepared from pure standard materials or can be purchased as certified solutions. Other concentrations may be used as appropriate for the intended application. If sufficient neat material exists, prepare stock standard solutions by accurately weighing 0.0100 g of pure compound. Dissolve the compound in isooctane or hexane and dilute to volume in a 10-mL volumetric flask. If compound purity is $\geq 96\%$, the weight may be used without correction to calculate the concentration of the stock standard solution. Commercially prepared stock standard solutions may be used at any concentration if they are certified by the manufacturer or by an independent source.

7.5 Composite stock standard - May be prepared from individual stock solutions.

7.5.1 As an example, for composite stock standards containing <25 components, add solvent to 1 mL of each individual stock solution at a concentration of 1000 mg/L (or other concentrations as appropriate) and mix the solutions in a 25-mL volumetric flask. The resulting concentration of each component in the mixture, after the volume is adjusted to 25 mL, will be 1 mg/25 mL or 40 mg/L. This composite solution can be further diluted to obtain the desired concentrations.

7.5.2 For composite stock standards containing > 25 components, use volumetric flasks of the appropriate volume (e.g., 50-mL, 100-mL), and follow the procedure described above.

7.6 Calibration standards - Should be prepared at a minimum of five different concentrations by dilution of the composite stock standard with isooctane or hexane and should match the sample extract solvent. Recommended standard concentrations for establishing a calibration curve for toxaphene are 50 - 400 pg/ μ L and 1.0 - 500 pg/ μ L for all toxaphene congeners, except Parlar 62. The recommended standard concentrations for establishing a calibration curve for Parlar 62 are 5 - 500 pg/ μ L. Ranges may be extended provided that the linear response can be adequately verified through satisfaction of all calibration criteria and QC requirements. The low standard must be equivalent to or below the lowest result to be reported. All reported results must be within the calibration range. Concentrations should correspond to the expected amounts found in real samples and should bracket the linear range of the detector. See Method 8000 for additional information on the preparation of calibration standards.

Analysts should evaluate the specific toxaphene standard carefully. Some toxaphene components, particularly the more heavily chlorinated, are subject to dechlorination reactions. Consequently, standards from different vendors may exhibit marked differences which could lead to possible false negatives or to large differences in quantitative results.

7.7 Internal standard - PCB congener #204 at a concentration range of 50 - 100 pg/ μ L in the final extract is suggested for use as an internal standard for the quantitation of toxaphene and toxaphene congeners. For example, spike 2 - 4 μ L of a 25-ng/ μ L solution into each 1 mL of sample extract for a final concentration of 50 - 100 ng/mL (pg/ μ L). Other concentrations and volumes are acceptable.

7.8 Surrogate standards - The performance of the method should be monitored using surrogate compounds. Surrogate standards are added to all samples, method blanks, matrix spikes, and calibration standards. The following compounds are recommended as possible surrogates. Other surrogates may be used provided that the analyst can demonstrate and document performance appropriate for the data quality needs of the particular application. Method 3500 describes the procedures for preparing these surrogates.

7.8.1 ϵ -hexachlorocyclohexane (ϵ -HCH) and decachlorobiphenyl (PCB congener #209) have been found to be a useful pair of surrogates. Decachlorobiphenyl should have a minimum retention time of 45 minutes to ensure adequate resolution of target compounds.

7.8.2 Alternatively, if an adequate response can be achieved for the particular application, tetrachloro-*m*-xylene or possibly isotopically labeled toxaphene congeners may be used.

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 See Chapter Four, "Organic Analytes" for sample collection and preservation instructions.

8.2 Store the sample extracts at ≤ 6 °C, protected from light, in sealed vials (e.g., screw-cap or crimp-capped vials) equipped with intact PTFE-lined septa. Extracts should be analyzed within 40 days of extraction.

9.0 QUALITY CONTROL

9.1 Refer to Chapter One, "Quality Control", for guidance on QA and QC protocols. When inconsistencies exist among QC guidelines, method-specific criteria take precedence over both technique-specific and Chapter One criteria. Technique-specific QC criteria take precedence over Chapter One criteria. 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), Standard Operating Procedure (SOP) or a Sampling and Analysis Plan (SAP); these documents translate project objectives and specifications into directions for implementing the project and assessing the results.

Each laboratory should maintain a formal QC program and maintain records to document the quality of the data generated. Development of in-house QC limits for each method is encouraged. Use of instrument specific QC limits is encouraged provided such limits will generate data appropriate for use in the intended application. All data sheets and QC data should be saved for reference or inspection.

9.2 Refer to Method 8000 for specific determinative method QC procedures and to Method 3500 and 5000 for QC procedures to ensure the proper operation of the various sample preparation techniques. If an extract cleanup procedure is performed, refer to Method 3600 for the appropriate QC procedures. Any more specific QC procedures provided in this method will supersede those noted in Methods 8000, 5000, 3500, or 3600.

9.3 QC procedures necessary to evaluate the GC system operation are found in Method 8000 and include evaluation of retention time windows, calibration verification, and chromatographic analysis of samples. In addition, discussions regarding the instrument QC requirements listed below can be found in the referenced sections of this method.

9.3.1 The GC/MS is tuned and calibrated using a NICI-customized tune table. The analytical system should be tuned prior to the initial calibration and for each occurrence when a new initial calibration may be necessary. See Sec. 11.4.1 for further details.

9.3.2 There must be an initial calibration of the GC/MS system and subsequent second source verification as described in Sec.11.4.

9.3.3 The GC/MS system must meet the calibration verification acceptance criteria in Sec. 11.5 every 12 hours.

9.3.4 The absolute or relative retention time (RRT) of each sample component must fall within the specified window of its corresponding standard component provided in Sec. 11.4.4.

9.4 Initial demonstration of proficiency - The initial demonstration of method proficiency (IDP) must be performed by the laboratory prior to independently running an analytical method, and should be repeated if other changes occur (e.g., instrument repair, significant change in procedure, and change in analyst). Refer to Method 8000 Sec. 9.0 for additional information regarding instrument, procedure, and analyst IDPs. An IDP must consist of replicate reference samples from each sample preparation and determinative method combination it utilizes by generating data of acceptable accuracy and precision for target analytes in a clean reference matrix taken through the entire preparation and analysis. If an autosampler is used to perform sample dilutions, prior to use, the laboratory should satisfy itself that those dilutions are of equivalent or better accuracy than is achieved by an experienced analyst performing manual dilutions.

9.5 Method blank - Before processing any samples, the analyst should demonstrate that all parts of the equipment in contact with the sample and reagents are interference-free through analysis of a method blank. The blank should be carried through all stages of sample preparation and analysis. As a continuing check, each time samples are extracted, cleaned up, and analyzed, a method blank must be prepared and analyzed for the compounds of interest as a safeguard against laboratory contamination. If a peak is observed within the retention time window of an analyte that would prevent its determination, the source must be found and eliminated, if possible, before samples are processed. Method blank re-extraction may be necessary if the source of contamination cannot be determined.

When new reagents or chemicals are received, the lab should monitor the preparation and/or analysis blanks associated with samples for any signs of contamination. It is not necessary to test every new batch of reagents or chemicals prior to sample preparation if the source shows no prior problems. However, if reagents are changed during a preparation batch, separate blanks need to be prepared for each set of reagents.

9.6 Sample QC for preparation and analysis - The laboratory must also have procedures for documenting matrix effect on method performance (precision, accuracy, method sensitivity). Minimally, this should include analysis of QC samples including a method blank, a matrix spike/matrix spike duplicate (MS/MSD) or a sample duplicate (if a matrix spike duplicate cannot be prepared and sufficient sample exists), and a laboratory control sample (LCS) in each analytical batch. Addition of surrogates to each field sample and QC sample is also recommended. Method blanks, matrix spike samples, and replicate

samples should be subjected to the same analytical procedures (Sec. 11.0) as those used on actual samples.

9.6.1 Documenting the effect of the matrix should include analysis of at least one matrix spike and one duplicate unspiked sample or one MS/MSD pair. The decision on whether to prepare and analyze duplicate samples or a MS/MSD must be based on knowledge of the samples in the sample batch. If samples are expected to contain target analytes, laboratories may use a matrix spike and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes, then laboratories should use a MS and MSD.

9.6.2 An LCS should be included with each analytical batch and consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS is spiked in identical manner as the matrix spike, when appropriate. When results of the matrix spike analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix.

9.6.3 Also see Method 8000 for the details on carrying out sample QC procedures for preparation and analysis. In-house method performance criteria for evaluating method performance should be developed using the guidance found in Method 8000.

9.7 Lower limit of quantitation (LLOQ) check standard

The laboratory shall establish the LLOQ as the lowest point of quantitation, which in most cases, is the lowest concentration in the calibration curve. The LLOQ verification is recommended for each project application to validate quantitation capability at low analyte concentration levels. This verification may be accomplished with either clean control material (e.g., reagent water, solvent blank, Ottawa sand, diatomaceous earth, etc.) or a representative sample matrix, free of target compounds. Optimally, the LLOQ should be less than the desired regulatory action levels based on the stated DQOs.

9.7.1 Determination of LLOQs using spiked clean control material represents a best-case scenario, and does not evaluate potential matrix effects of real-world samples. For the application of LLOQs on a project-specific basis with established DQOs, a representative matrix-specific LLOQ verification may provide a more reliable estimate of the lower quantitation limit capabilities.

9.7.1.1 A LLOQ check standard (not part of an initial calibration) is prepared by spiking a clean control material with the analyte(s) of interest at the predicted LLOQ concentration level(s). Alternatively, a representative sample matrix may be spiked with the analytes of interest at the predicted LLOQ concentration levels. The LLOQ check is carried through the same preparation procedures as the environmental samples and other QC samples.

9.7.1.2 Recovery of target analytes in the LLOQ check standard should be within established in-house limits, or other such project-specific acceptance limits, to demonstrate acceptable method performance at the LLOQ. Until the laboratory has sufficient data to determine acceptance limits, the LCS criteria $\pm 20\%$ may be used for the LLOQ acceptance criteria. This acknowledges

the poorer overall response at the low end of the calibration curve. Historically-based LLOQ acceptance criteria should be determined as soon as practical once sufficient data points have been acquired.

9.7.2 In-house acceptance criteria for recovery of the LLOQ check standard for a sample matrix can be calculated when sufficient data points exist. The laboratory should have a documented procedure for establishing in-house acceptance ranges.

9.8 Surrogate recoveries - The laboratory should evaluate recovery data from individual samples against historically developed surrogate control limits. See Method 8000 for information on evaluating surrogate data and developing and updating surrogate limits. Procedures for evaluating recoveries of multiple surrogates and associated corrective actions when they are deemed unacceptable should be defined in a QAPP or SOP.

9.9 The experience of the analyst performing GC-NICI/MS is invaluable to the success of the method. Each day that analysis is performed, the calibration verification standard should be evaluated to determine if the chromatographic system is operating properly. Do the peaks look normal? Is the response obtained comparable to the response from previous calibrations?

Careful examination of the standard chromatogram can indicate if the column is still performing acceptably, the injector is leaking, the injector septum needs replacing, etc. When any changes are made to the system (e.g., the column or septum is changed), see guidance in Method 8000 regarding whether recalibration of the system must take place.

9.10 It is recommended that the laboratory adopt additional QA practices for use with this method. Specific practices that are most productive depend upon the needs of the laboratory, the nature of the samples, and project-specific requirements. Field duplicates may be analyzed to assess precision of the environmental measurements. When doubt exists over identification of a peak on the chromatogram, confirmatory techniques such as GC with a dissimilar column, element-specific detector, or mass spectrometer (selected ion monitoring or full scan) must be used. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

10.0 CALIBRATION AND STANDARDIZATION

See Sec. 11.4 and 11.5 for information on calibration and standardization.

11.0 PROCEDURE

11.1 Sample preparation

Samples are normally prepared by one of the following methods prior to GC-NICI/MS analysis.

| <u>Matrix</u> | <u>Methods</u> |
|---------------|------------------------------------|
| Water | 3510, 3520 |
| Soil/sediment | 3540, 3541, 3545, 3546, 3550, 3562 |
| Tissue | 3540, 3541, 3545, 3550, 3562 |

11.2 Extract cleanup - Cleanup procedures may not be necessary for a relatively clean sample matrix, but most extracts from environmental and waste samples will require additional preparation before analysis. The specific cleanup procedure used will depend on the nature of the sample to be analyzed, expected interferences coextracted from the sample, and the DQOs. General guidance on the selection of cleanup methods is provided in Sec. 11.2 and in Method 3600. Refer to Table 13 for a data comparison of real-world samples extracted with and without cleanup procedures.

11.2.1 Method 3610 (alumina) may be used to remove phthalate esters.

11.2.2 Method 3620 (Florisil®) may be used to separate organochlorine pesticides from aliphatic compounds, aromatics, and nitrogen-containing compounds.

11.2.3 Method 3630 (silica gel) may be used to separate single- and multi- component organochlorine compounds from interferences.

11.2.4 Method 3640 (GPC - pesticide option) is a size exclusion procedure that may be used to remove high molecular weight materials.

11.2.5 Method 3660 (sulfur cleanup) should be used to remove possible sulfur interfering compounds which may be present in certain sediments and industrial wastes. (These compounds should not interfere with monitored ions from toxaphene but should nevertheless be removed because of deleterious effects on chromatography and on the response of the NICI/MS ion source during coelution.)

11.2.6 Method 3665 (sulfuric acid/permanganate) may be used prior to toxaphene analysis to remove more fragile organic contaminants.

11.3 GC conditions - This method uses a single column in conjunction with an electron capture NICI/MS detector. If an autosampler is not used, the use of an internal standard is required. It is highly recommended to use an internal standard in all samples even if an autosampler is used. The list in Sec. 6.0 indicates the columns used to develop the method performance data in the tables at the end of this method and is not intended to exclude other columns that are available or may be developed. Laboratories may use these or other capillary columns (including columns of other dimensions), provided that they document method performance data (e.g., chromatographic resolution, analyte breakdown, and sensitivity) appropriate for the intended application.

11.4 Tuning, initial calibration, and second-source calibration accuracy check - Establish the GC-NICI/MS operating conditions according to the recommendations in Table 1. Alternative operating conditions may be acceptable provided that appropriate compound sensitivity and selectivity can be achieved.

11.4.1 Prior to analysis of samples or calibration standards, the instrument must be tuned for NCI conditions. If possible, use the CI autotune procedure provided by the instrument manufacturer. Without specific manufacturer guidance, the following generic recommendations should be followed.

11.4.1.1 The source is operated in the CI mode using methane as the reagent gas.

11.4.1.2 When the source pressure has stabilized at a value known to produce satisfactory results, the instrument is manually tuned and calibrated using a NCI-customized tuning file. Pressure stabilization usually takes about 45 minutes.

NOTE: The internal standard noted in Sec. 7.7 should permit most of the components of interest in a chromatogram to have retention times of 0.80-1.20 relative to it. Use the base peak ion from the internal standard as the primary ion for quantitation (Table 2).

11.4.2 Analyze 1 µL of each calibration standard (containing the quantitation compounds, as well as, the appropriate surrogates and internal standard) and calculate the response factor (RF) as shown in Sec. 11.4.3. A set of at least five calibration standards is necessary (Sec. 7.6 and Method 8000). Alternate injection volumes may be used if applicable QC requirements are met, but must be the same for all standards and sample extracts. Figure 1 is a representative chromatogram of select congeners.

NOTE: Toxaphene will require a separate calibration curve from the congeners due to possible congener interferences present in the toxaphene calibration standard chromatograms.

11.4.3 Initial calibration calculations

11.4.3.1 Calculate a RF for each target analyte relative to one of the internal standards as follows:

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

where:

A_s = Peak response of the analyte or surrogate.

A_{is} = Peak response of the internal standard.

C_s = Concentration or mass of the analyte or surrogate in the sample aliquot.

C_{is} = Concentration or mass of the internal standard in the sample aliquot.

11.4.3.2 Calculate the average RF and the percent relative standard deviation (%RSD) of the RFs for each target analyte using the equations below. The %RSD should be $\leq 20\%$ for each target analyte. It is also recommended that a minimum RF for the most common target analytes be demonstrated for each individual calibration level to ensure that these compounds are behaving as expected. In addition, meeting the minimum RF criteria for the lowest calibration standard is critical in establishing and demonstrating the desired sensitivity.

Due to the large number of compounds that may potentially be analyzed by this method, some compounds will fail to meet these criteria. For these occasions, it is acknowledged that the failing compounds may not be critical to the specific project and therefore they may be used as qualified data or estimated values for screening purposes. The analyst should strive to place more emphasis on meeting the calibration criteria for compounds that are critical to the project.

$$\text{Average RF} = \overline{RF} = \frac{\sum_{i=1}^n RF_i}{n} \qquad SD = \sqrt{\frac{\sum_{i=1}^n (RF_i - \overline{RF})^2}{n-1}}$$

$$RSD = \frac{SD}{\overline{RF}} \times 100$$

where:

RF_i = RF for each of the calibration standards.

\overline{RF} = Average RF for each compound from the initial calibration.

n = Number of calibration standards (e.g., 5).

11.4.3.3 If more than 10% of the compounds included in the initial calibration exceed the 20% RSD limit or do not meet the minimum coefficient of determination ($r^2 = 0.99$) for alternate curve fits, the chromatographic system may be considered too reactive for analysis to begin. Clean or replace the injector liner, capillary column, and/or ion source, and then repeat the calibration procedure beginning with Sec. 11.4.

11.4.4 Either of the two procedures described in Sec. 11.4.4.1 or 11.4.4.2 may be used to determine calibration function acceptability. These include refitting the calibration data back to the model or the determination of the relative standard error (RSE) for the curve when comparing the actual response with the predicted response.

11.4.4.1 Refitting the calibration data back to the model or calculating the % Error is determined by using the following equation:

$$\% \text{ Error} = \frac{x_i - x'_i}{x_i} \times 100$$

where:

x'_i = Measured amount of analyte at calibration level i , in mass or concentration units.

x_i = True amount of analyte at calibration level i , in mass or concentration units.

Percent error between the calculated and expected amounts of an analyte should be $\leq 30\%$ for all standards. For some data uses, $\leq 50\%$ may be acceptable for the lowest calibration point.

11.4.4.2 Relative Standard Error is calculated by using the following equation:

$$RSE = 100 \times \sqrt{\frac{\sum_{i=1}^n \left[\frac{x'_i - x_i}{x_i} \right]^2}{(n - p)}}$$

where:

x_i = True amount of analyte in calibration level i , in mass or concentration units.

x'_i = Measured amount of analyte in calibration level i , in mass or concentration units.

p = Number of terms in the fitting equation.
(average = 1, linear = 2, quadratic = 3, cubic = 4)

n = Number of calibration points.

RSE acceptance limit criterion for the calibration model is $\leq 20\%$.

11.4.5 Evaluation of retention times - Either the absolute or relative retention time (RRT) used in conjunction with spectral matching is allowed for use by the laboratory to establish peak identity. The absolute or RRT of each target analyte in each calibration standard should agree within established limits set by the laboratory in their SOP. These limits should be appropriate for reliable identification of the target analyte. If this criterion is not met and there are no other indicators of an analyte's identification (such as a very unique and high probability mass spectral match), that analyte may not be considered as identified using either RRT or absolute retention times. The RRT equation follows units.

$$RRT = \frac{\text{Retention time of the Analyte}}{\text{Retention time of the Internal Standard}}$$

11.4.6 Linearity of target analytes - If the RSD of any target analyte is $\leq 20\%$, then the relative response is assumed to be constant over the calibration range, and the average RF may be used for quantitation (Sec. 11.8.2).

11.4.6.1 If the RSD of any target analyte is $>20\%$, refer to Method 8000 for additional calibration options. One of the options must be applied to the GC/MS calibration in this situation, or a new initial calibration must be performed. The average RF should not be used for compounds that have an RSD $>20\%$ unless the concentration is reported as estimated.

11.4.6.2 When the RSD is $>20\%$, plotting and visual inspection of a calibration curve can be a useful diagnostic tool. The inspection may indicate analytical problems, including errors in standard preparation, presence of active sites in the chromatographic system, analytes that exhibit poor chromatographic behavior, etc.

11.4.6.3 Due to the large number of compounds that may be analyzed by this method, some compounds may fail to meet either the 20% RSD, minimum coefficient of determination ($r^2 = 0.99$), or the acceptance criteria for alternative calibration procedures in Method 8000. Any calibration method described in Method 8000 may be used for a compound, but it should be used consistently until the next calibration is performed. If compounds fail to meet these criteria, the associated concentrations may still be determined but they must be reported as estimated. In order to report non-detects, it must be demonstrated that there is adequate sensitivity to detect the failed compounds at the applicable lower quantitation limit.

11.4.6.4 The method of linear regression analysis has the potential for a significant bias at the lower portion of a calibration curve, while the average response factor and quadratic methods of calibration do not typically have this problem. Also, when a least squares linear regression calibration model is used, inverse weighting of the concentrations used in the initial calibration has been shown to emphasize precision at the low end of the calibration range (see Method 8000 for all calibration model options).

When evaluating the calibration curves with the linear regression model, the analyst should perform a minimum quantitation check on the viability of the lowest calibration point by refitting the response from the low concentration calibration standard back into the curve (see Method 8000 for additional details). It is not necessary to reanalyze a low concentration standard; rather the data system can recalculate this low concentration as if it were an unknown sample. The recalculated concentration of the low calibration point should be $\pm 50\%$ (or in-house historically based acceptance criteria) of the standard's true concentration.

Other recovery criteria may be applicable depending on the project's DQOs. For those situations, the minimum quantitation check criteria should be outlined in a laboratory SOP, or a project-specific QAPP. Analytes which do not meet the minimum quantitation calibration refitting criteria should be considered

"out-of-control." Corrective actions may be appropriate such as redefining the LLOQ and/or reporting "out-of-control" target analytes as estimated when the concentration is at or near the lowest calibration point.

11.4.7 The initial calibration function for each target analyte should be checked by injecting the second-source standard immediately after the standards used for initial calibration. The second-source standard should be prepared at a concentration near the middle of the calibration range with a standard from a source different (if available) from that used for the initial calibration. The measured values of the parameters in the second-source check standard should fall within 70 - 130% of the expected value(s). Due to the variability of technical mixtures (e.g., technical toxaphene), this criterion for the second source check standard should be applied with caution. An alternative recovery limit may be appropriate based on the desired project-specific DQOs. Quantitative sample analyses should not proceed for those analytes that fail the second-source standard initial calibration verification. However, analyses may continue for those analytes that fail the criteria with an understanding these results could be used for screening purposes and would be considered estimated values.

11.5 Calibration verification consists of several steps that are performed at the beginning of each 12-hour analytical shift.

11.5.1 Calibration verification - Initial calibration (Sec. 11.4) for each compound of interest should be verified once every 12 hours prior to sample analysis, with the introduction technique and conditions used for samples. This is accomplished by analyzing a calibration verification standard (containing all the compounds for quantitation) at a concentration either near the midpoint concentration for the calibration range of the GC-NICI/MS or near the action level for the project. The results must be compared against the most recent initial calibration curve and should meet the verification acceptance criteria provided in Sec. 11.5.1.1 through 11.5.1.5.

11.5.1.1 Each of the target analytes in the calibration verification standard should meet the appropriate minimum RFs. These criteria are particularly important when the target analytes are also critical project-required compounds. This is the same check that is applied during the initial calibration.

11.5.1.2 If minimum RFs are not met, the system should be evaluated, and corrective action taken before sample analysis begins. Possible problems include standard mixture degradation; contamination of the injection port inlet, source and/or front end of the column; active sites in the column or chromatographic system; etc.

11.5.1.3 All target compounds of interest must be evaluated using a 20% criterion: percent difference when performing the average RF model calibration and percent drift when calibrating with a regression fit model. Refer to Method 8000 for guidance on calculating percent difference and drift.

11.5.1.4 If the percent difference or percent drift for a compound is $\leq 20\%$, the initial calibration for that compound is assumed to be valid. Due to large numbers of compounds that may potentially be analyzed by this method, it is expected that some compounds will fail to meet the criterion. If the criterion is

not met for more than 20% of the compounds included in the initial calibration, then corrective action must be taken prior to the analysis of samples. When compounds fail, they may still be reported as non-detects if it can be demonstrated that there was adequate sensitivity to detect the compound at the applicable quantitation limit. For situations when the failed compound is present, the concentrations in the affected samples must be reported as estimated.

11.5.1.5 Problems similar to those listed under initial calibration could affect the ability to pass the calibration verification standard analysis. If the problem cannot be corrected by other measures, a new initial calibration must be generated. Calibration verification criteria must be met before sample analysis may begin.

11.5.2 Method blank - A method blank should be analyzed prior to sample analysis to demonstrate that the total system (introduction device, transfer lines and GC-NICI/MS system) is free of contaminants. If the method blank indicates contamination, it may be appropriate to analyze a solvent blank to demonstrate that the contamination is not a result of carryover from standards or samples. See Method 8000 for information regarding method blank performance criteria.

11.5.3 Internal standard retention time - Retention times of the internal standard in the calibration verification standard must be evaluated immediately during or after data acquisition. If the retention time for any internal standard changes by >30 seconds from that in the midpoint standard level of the most recent initial calibration sequence, the chromatographic system must be inspected for malfunctions and corrections must be made, as required. When corrections are made, reanalysis of samples analyzed while the system was malfunctioning is required.

11.5.4 Internal standard response - If the EICP area for the internal standard in the calibration verification standard changes by greater than a factor of two (-50% to +100%) from that in the midpoint standard level of the most recent initial calibration sequence, the mass spectrometer must be inspected for malfunctions and corrections must be made, as appropriate. When corrections are made, reanalysis of samples analyzed while the system was malfunctioning is required.

NOTE: A decrease in the EICP area of the internal standard may be due to the use of a hydrocarbon reagent gas (e.g., methane) in the negative ion chemical ionization mode and a decreased source temperature (160 °C, versus 250 °C for EI). These conditions make the ion source susceptible to a buildup of a hydrocarbon film on its surface. This in turn may lead to a lower signal. If this decrease of signal is gradual, it should not signify anything worse than this hydrocarbon buildup. If the signal drops drastically over one or two runs, something else is happening and measures should be taken immediately to remedy this.

11.6 GC-NICI/MS analysis of samples

11.6.1 It is highly recommended that sample extracts be screened on a GC with an electron capture detector (ECD) using the same type of capillary column employed in the GC-NICI/MS system. Pre-screening can detect the possible presence of interferences (e.g., sulfur, PCBs) to determine if extract cleanups are needed.

Screening will minimize contamination of the GC-NICI/MS system from unexpectedly high concentrations of organic compounds. The QAPP often contains historical contaminant information of the sampling site which may indicate if screening is necessary.

11.6.2 Allow the sample extract to warm to room temperature. Just prior to analysis, add an aliquot of the internal standard solution to the concentrated sample extract. The internal standard concentration in the injected sample extract must be the same as that in the calibration standards.

11.6.3 Inject an aliquot of the sample extract using the same operating conditions employed for the calibration (Sec. 11.4). The volume to be injected should include an appropriate concentration that is within the calibration range of the standards as noted in Sec. 7.7. The injection volume must be the same volume used for the calibration standards.

11.6.4 If any quantitation ion response exceeds the GC-NICI/MS system initial calibration range, the sample extract must be diluted and reanalyzed. Additional internal standard solution must be added to the diluted extract to maintain the same concentration as in the calibration standards (usually 50 - 100 pg/ μ L or other concentrations as appropriate).

NOTE: It may be a useful diagnostic tool to monitor internal standard retention times in all samples, spikes, blanks, and standards to check drifting, method performance, poor injection execution, and the need for system inspection and/or maintenance. Internal standard responses (area counts) must be monitored in all samples, spikes, blanks for similar reasons. If the EICP area for the internal standard in samples, spikes and blanks changes by a factor of two (-50% to +100%) from the areas determined in the calibration verification standard analyzed that day, corrective action must be taken. The samples, spikes, or blanks should be reanalyzed or the data qualified.

11.6.4.1 When ions from a compound in the sample saturate the detector, this analysis should be followed by an instrument blank consisting of clean solvent. If the blank analysis is not free of interferences, then the system must be decontaminated. Sample analysis may not resume until the blank analysis is demonstrated to be free of interferences. Contamination from one sample to the next on the instrument usually takes place in the syringe. If adequate syringe washes are employed, carryover from high concentration samples can usually be avoided.

11.6.4.2 All dilutions should keep the response of the major constituents (previously saturated peaks) within the linear range of the curve.

11.6.5 The use of selected ion monitoring (SIM) is preferred. Using the primary ion for quantitation and the secondary ions for confirmation sets up the collection groups based on their retention times. Most compounds have a small mass defect in their spectra, usually <0.2 amu. These mass defects should be accounted for

during data acquisition. The total scan time should be <1 second and produce at least 5 -10 scans per chromatographic peak. The start and stop times for the SIM groups are determined from a full scan analysis.

11.7 Analyte identification

11.7.1 The qualitative identification of compounds determined by this method is based on retention time and on comparison of the sample mass spectrum, after background correction, with characteristic ions in a reference mass spectrum. The reference mass spectrum must be generated by the laboratory using the conditions of this method. The characteristic ions from the reference mass spectrum are defined as the three ions of greatest relative intensity, or any ions over 30% relative intensity, if fewer than three such ions occur in the reference spectrum. Compounds are identified when the following criteria are met.

11.7.1.1 The intensities of the characteristic ions of a compound must maximize in the same scan or within one scan of each other. Selection of a peak by a data system target compound search routine where the search is based on the presence of a target chromatographic peak containing ions specific for the target compound at a compound-specific retention time will be accepted as meeting this criterion.

11.7.1.2 Either absolute or RRT, used in conjunction with spectral matching is allowed for use by the laboratory to establish peak identity. The absolute or RRT of each target analyte, when compared against the daily calibration verification standard, should agree within established limits set by the laboratory in their SOP. These limits should be appropriate for reliable identification of the target analyte. If this criterion is not met and there are no other indicators of an analyte's identification (such as a very unique and high probability mass spectral match), that analyte may not be considered as identified using either RRT or absolute retention times.

11.7.1.3 The relative intensities of the characteristic ions agree within 30% of the relative intensities of these ions in the reference spectrum. (e.g., for an ion with an abundance of 50% in the reference spectrum, the corresponding abundance in a sample spectrum should be 20 - 80%.) Use professional judgment when interferences are observed.

11.7.1.4 Structural isomers that produce very similar mass spectra should be identified as individual isomers if they have sufficiently different GC retention times. Sufficient GC resolution is achieved if the height of the valley between two isomer peaks is less than 50% of the average of the two peak heights. Otherwise, structural isomers are identified as isomeric pairs. The resolution should be verified on the midpoint concentration of the initial calibration as well as the laboratory-designated calibration verification standard level if closely eluting isomers are to be reported.

CAUTION: Because of the complexity of toxaphene, decreasing the run time will likely cause additional isomers to be reported as the target congeners, thus inflating concentrations.

11.7.1.5 Identification is hampered when sample components are not resolved chromatographically and produce mass spectra containing ions contributed by more than one analyte. When GC peaks obviously represent more than one sample component (i.e., a broadened peak with shoulder(s) or a valley between two or more maxima), appropriate selection of analyte spectra and background spectra is important.

11.7.1.6 Examination of EICPs of appropriate ions can aid in the selection of spectra and in qualitative identification of compounds. When analytes coelute (i.e., only one chromatographic peak is apparent), the identification criteria may be met, but each analyte spectrum will contain extraneous ions contributed by the coeluting compound.

11.7.2 The identification of mixtures such as toxaphene is not based on a single peak, but rather on the characteristic peaks that comprise the "fingerprint" of the mixture, using both the retention times and shapes of the indicator peaks. See Method 8000 for information on confirmation of tentative identifications.

11.8 Quantitation

11.8.1 Multi-component analytes such as toxaphene - Complex analytes present problems in measurement. Quantitation is based on the areas of the characteristic peaks as compared to the areas of the corresponding peaks at the same retention times in the calibration standard, using either internal or external calibration procedures. Suggestions are offered in the following sections for handling toxaphene.

11.8.1.1 Calibrate the instrument for toxaphene using the guidance noted in Sec. 11.4

11.8.1.2 Quantitate toxaphene by summing the area of all peaks originating from this compound using the total area of the toxaphene pattern or using the 4 to 6 major peaks that closely match the corresponding peaks in the most recent calibration verification standard. Whichever approach is employed should be documented and available to the data user, if necessary.

11.8.1.3 While toxaphene contains a large number of compounds that will produce well-resolved peaks in a GC-NICI/MS chromatogram, it also contains many other components that are not chromatographically resolved. This unresolved complex mixture results in the "hump" in the chromatogram that is characteristic of this compound. Although the resolved peaks are important for identification, the area of the unresolved complex mixture contributes a significant portion of the area of the total response.

11.8.1.4 To measure total area, construct the baseline of toxaphene in the sample chromatogram between the retention times of the first and last eluting toxaphene components in the standard. In order to use the total area approach, the pattern in the sample chromatogram must be compared to that of the standard to ensure that all of the major components in the standard are present in the sample. Otherwise, the sample concentration may be significantly underestimated. As an example, either manually or by using the

data system's control settings, integrate the entire area under the total ion chromatogram for the toxaphene response and subtract out the internal standard response (m/z 429.8) and ions associated with surrogate or other compounds if they are present.

11.8.1.5 When toxaphene is determined using the 4 to 6 peaks approach, the analyst must take care to evaluate the relative areas of the peaks chosen in the sample and standard chromatograms. It is highly unlikely that the peaks will match exactly, but the analyst should not employ peaks from the sample chromatogram whose relative sizes or areas appear to be disproportionately larger or smaller in the sample compared to the standard.

11.8.1.6 The heights or areas of the selected 4 - 6 peaks should be summed together and used to determine the toxaphene concentration. Alternatively, utilize each peak in the standard to calculate a calibration factor for that peak, using the total mass of toxaphene in the standard. These calibration factors are then used to calculate the concentration of each corresponding peak in the sample chromatogram and the 4 - 6 resulting concentrations are averaged to provide the final result for the sample.

11.8.2 Once a target compound has been identified, the quantitation of that compound will be based on the integrated abundance of the primary characteristic ion from the EICP.

11.8.2.1 Use the integration produced by the software if the integration is correct because the software should produce more consistent integrations. However, manual integrations may be necessary when the software does not produce proper integrations because baseline selection is improper, the correct peak is missed, a coelution is integrated, the peak is partially integrated, etc. The analyst is responsible for ensuring that the integration is correct whether performed by the software or done manually.

11.8.2.2 Manual integrations should not be substituted for proper maintenance of the instrument or setup of the method (e.g., retention time updates, integration parameter files, etc). The analyst should seek to minimize manual integration by properly maintaining the instrument, updating retention times, and configuring peak integration parameters.

11.8.3 If the RSD of a compound's response factor is $\leq 20\%$, the concentration in the extract may be determined using the average RF from initial calibration data (Sec. 11.4.4). See Method 8000 for the equations describing internal standard calibration and either linear or non-linear calibrations.

11.8.4 Table 5 lists example retention times for the target analytes. The retention times listed in this table are provided for illustrative purposes only. Each laboratory must determine retention times and retention time windows for their specific application of the method.

12.0 DATA ANALYSIS AND CALCULATIONS

See Sections 11.4 through 11.8 and Method 8000 for information on data analysis and calculations.

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. Performance data must not be used as absolute QC acceptance criteria for the purposes of laboratory QC or accreditation. All performance data included resulted from both single and multi-laboratory analyses.

13.2 The chromatographic separations in this method were tested in multiple laboratories by using clean hexane and liquid/solid extracts spiked with the test compounds at various concentrations. Single-operator and multi-laboratory precision and method accuracy were found to be related to the concentration of the compound and the type of matrix.

13.3 The levels of accuracy and precision that can be achieved with this method depend on the sample matrix, sample preparation, optional cleanup techniques, and calibration procedures used.

13.4 Tables 2 through 4 represent SIM for method analytes using GC-NICI/MS.

13.5 Table 5 contains representative retention times.

NOTE: Decachlorobiphenyl should have a minimum RT of 45 minutes to ensure adequate resolution of target compounds.

13.6 Table 6 contains single-laboratory performance data on spiked soil for toxaphene and chlordane. Data are provided for guidance purposes only.

13.7 Table 7 contains single-laboratory performance data on spiked soil for toxaphene congeners. Data are provided for guidance purposes only.

13.8 Table 8 contains single-laboratory performance data on spiked soil for organochlorine pesticides. Data are provided for guidance purposes only.

13.9 Table 9 contains multi-laboratory phase I validation data using unknown standard concentrations.

13.10 Table 10 contains multi-laboratory phase II validation data using spiked extracts of uncontaminated real-world sample matrices.

13.11 Table 11 contains multi-laboratory phase II validation data using real-world samples from Terry Creek in Brunswick, Georgia.

13.12 Table 12 contains multi-laboratory phase II validation data using real world

samples from Terry Creek in Brunswick, Georgia. Extracts from this study were further subjected to copper, acid, and silica gel cleanup methods.

13.13 Table 13 includes a Terry Creek real-world sample data comparison between extraction only to those extracts subjected to copper, acid, and silica gel cleanup methods.

13.14 Table 14 contains multiple-laboratory validation data using multiple technical toxaphene spiking protocols of uncontaminated real world sample matrices.

13.15 Table 15 contains multi-laboratory phase III validation data using spiked extracts of uncontaminated real world sample matrices.

13.16 Table 16 contains multi-laboratory phase III validation data using real world samples from Terry Creek in Brunswick, Georgia.

13.17 Figure 1 is an example chromatogram of a mixed toxaphene congeners standard.

13.18 Figure 2 is an example chromatogram of a fish tissue extract containing weathered toxaphene and spiked with decachlorobiphenyl.

13.19 Figure 3 is an example chromatogram of a toxaphene congener validation standard.

13.20 Figure 4 is an example chromatogram of an EPA Region 4 sediment extract spiked with decachlorobiphenyl.

NOTE: Not all components shown in the figures may be target analytes.

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 EPA requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. Laboratories are urged to protect 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 Waste Management Manual for Laboratory Personnel* available from the American Chemical Society at the address listed in Sec. 14.2.

16.0 REFERENCES

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17.0 TABLES, VALIDATION DATA, FIGURES

The following pages contain the tables and figures referenced by this method.

Table 1

RECOMMENDED GC-NICI/MS OPERATING CONDITIONS FOR TOXAPHENE AND
TOXAPHENE CONGENERS USING A NARROW-BORE CAPILLARY COLUMN¹

Column - 30-m x 0.25-mm ID fused silica capillary column J&W DB-XLB, 0.25- μ m film thickness.

| | |
|----------------------------|--|
| Carrier gas | Helium |
| Carrier gas pressure | 10.4 psi |
| Carrier gas flow | 1.2 mL/min |
| EPC | Constant flow |
| Injector temperature | 205°C |
| Purge flow | 60 mL/min |
| Purge time | 1 min |
| Injector mode | Splitless |
| Injector liner | 4-mm I.D. splitless |
| Transfer line temperature | 280°C |
| Initial oven temperature | 60°C, hold 1 min |
| Temperature program | 60 °C to 150 °C at 10 °C/min, followed by 150 °C to 260 °C at 3 °C/min, followed by 260 °C to 320 °C at 20 °C/min, hold 0.33 min followed by 320 °C to 330 °C at 50 °C/min, hold 3 min |
| Final oven temperature | 330 °C |
| MS mode | Negative CI |
| CI gas | Methane |
| CI gas flow | 40% |
| Source pressure | N/A |
| MS quad temperature | 150 °C |
| MS Source temperature | 160 °C |
| Solvent delay | 15 min |
| Acquisition mode | SIM |
| SIM parameters | |
| Resolution | Low |
| Dwell time | 35 |
| Group 1 | Ions |
| Time, initial | 306.9, 308.9, 310.9, 340.9, 342.9, 344.9, 376.9, 378.9, 380.9, 410.9, 412.9, 414.9, 444.8, 446.8, 448.8 |
| | 429.8 (IS), 410.8 (IS O ₂ rxn) 254.9 (Surr), 497.7 (Surr) |
| Scan parameters (optional) | 300-500, 0.5 sec/scan |

¹Data provided courtesy of Scott Sivertsen from US EPA Region 4 Laboratory.

Table 2

SELECTED ION MONITORING FOR ANALYTES BY GC-NICI/MS

| Compound | Quantitation Ion | Other Ions | Comments |
|---------------|------------------|---|-------------------------|
| Toxaphene | TIC ¹ | 306.9, 308.9, 310.9, 340.9, 342.9, 344.9, 376.9, 378.9, 380.9, 410.8, 412.8, 414.8, 444.8, 446.8, 448.8 | Manual Integration |
| Parlar 26 | 378.9 | 376.9, 380.9 | |
| Parlar 40, 41 | 378.9 | 376.9, 380.9 | |
| Parlar 50, 62 | 412.8 | 410.8, 414.8 | |
| Hx-Sed | 308.9 | 306.9, 310.9 | |
| Hp-Sed | 342.9 | 340.9, 344.9 | |
| IS, PCB 204 | 429.8 | 410.8 | Monitor oxygen reaction |
| Surr, PCB 209 | 497.7 | | |
| Surr, ε-HCH | 254.9 | | |

¹Total Ion Current

Table 3

SELECTED ION MONITORING FOR CHLORDANE BY GC-NICI/MS

| Compound | Quantitation Ion | Other Ions | Comments |
|------------------------|------------------|--|-----------------------|
| Chlordane ¹ | TIC ² | 303.9, 305.9, 337.9, 339.9, 341.9, 371.8, 373.8, 375.8, 407.8, 409.8, 411.8, 441.8, 443.8, 445.8 | Manual integration |
| IS, PCB#204 | 429.8 | | QC check for response |

¹Ions are responses observed for technical chlordane; they may be used to confirm the presence of chlordane in the sample. ¹³C-isotope contributions from the higher members of the chlordane congeners contribute to monitored ions for technical toxaphene and some toxaphene congener ions.

²Total Ion Current

Table 4

SELECTED ION MONITORING FOR ANALYTES BY GC-NICI/MS

| Compound | Quantitation Ion ¹ | Other Ions | Comments |
|--|-------------------------------|--------------|-------------------|
| BHC | 254.9 | 252.9, 256.9 | M-Cl |
| Heptachlor ² | 299.9 | 297.9, 301.9 | M-Cl ₂ |
| Aldrin ² | 329.9 | 327.9, 331.9 | M-Cl+H |
| Dieldrin ² , endrin ² , endrin aldehyde ² | 379.8 | 377.9, 381.9 | M |
| Heptachlor epoxide ² | 387.8 | 385.9, 389.9 | M |
| Endosulfan I, II | 403.7 | 405.7, 407.7 | M |
| Endosulfan sulfate | 421.7 | 419.7, 423.7 | M |
| IS, PCB 204 | 429.8 | | QC |

¹Ions are responses observed for organochlorine pesticides; they may be used to confirm the presence of pesticides in the sample. These compounds do not pose major interferences to toxaphene determination under NICI/MS conditions.

²These compounds may exhibit fragmentation resulting in a response at m/z 234.9, 236.9, and 238.9 that aids in confirmation.

Table 5

EXAMPLE GAS CHROMATOGRAPHIC RETENTION TIMES FOR TOXAPHENE
CONGENERS USING A NARROW-BORE CAPILLARY COLUMN (DB-XLB MSD)

| Compound | Retention Time (min) |
|--------------------------|-----------------------------|
| Hx-Sed | 31.31 |
| Hp-Sed | 31.95 |
| Parlar 26 | 32.38 |
| Parlar 41 | 36.69 |
| Parlar 40 | 37.03 |
| Parlar 44 | 37.35 |
| Parlar 50 | 37.89 |
| Parlar 62 | 41.91 |
| Decachlorobiphenyl (DCB) | 48.30 |

Table 6

QUANTITATIONS OF SPIKED SOIL FOR TOXAPHENE (25 ppb, 5-g sample) AND CHLORDANE (50 ppb, 5-g sample)¹

| Replicate No. | Toxaphene (ppb) | Chlordane (ppb) |
|----------------------|------------------------|------------------------|
| 1 | 34.2 | 31.4 |
| 2 | 46.4 | 35.2 |
| 3 | 42.7 | 29.4 |
| 4 | 35.0 | 41.6 |
| 5 | 27.0 | 27.0 |
| 6 | 42.0 | 27.8 |
| 7 | 43.9 | 28.0 |
| Avg | 38.7 | 31.5 |
| % RSD | 17.8 | 16.7 |

¹Data provided courtesy of Dr. Bill Brumley from US EPA ORD, 2008. Results were obtained using a 40-m Agilent J&W 0.18-mm ID, 0.18- μ m film thickness column.

Table 7

QUANTITATIONS OF SPIKED SOIL FOR TOXAPHENE CONGENERS (500 ppt, 5-g sample)¹

| Replicate No. | Hx-Sed | Hp-Sed | Parlar 26 |
|----------------------|---------------|---------------|------------------|
| 1 | 468 | 532 | 552 |
| 2 | 690 | 722 | 788 |
| 3 | 768 | 746 | 778 |
| 4 | 688 | 724 | 808 |
| 5 | 780 | 752 | 804 |
| 6 | 778 | 786 | 822 |
| 7 | 672 | 784 | 706 |
| Avg | 692 | 720 | 752 |
| % RSD | 15.8 | 12.1 | 12.7 |

| Replicate No. | Parlar 38, 40, 41 | Parlar 44 | Parlar 50, 62 |
|----------------------|--------------------------|------------------|----------------------|
| 1 | 508 | 696 | 544 |
| 2 | 792 | 930 | 812 |
| 3 | 808 | 876 | 702 |
| 4 | 856 | 1,016 | 844 |
| 5 | 860 | 986 | 790 |
| 6 | 826 | 942 | 750 |
| 7 | 866 | 1,270 | 772 |
| Avg | 788 | 958 | 744 |
| % RSD | 16.1 | 17.8 | 13.4 |

¹Data provided courtesy of Dr. Bill Brumley from US EPA ORD, 2008. Results were obtained using a 40-m Agilent J&W 0.18-mm ID, 0.18- μ m film thickness column.

Table 8

QUANTITATIONS OF SPIKED SOIL FOR
ORGANOCHLORINE PESTICIDES (50 ppb, 5-g sample)¹

| Replicate No. | α -BHC | β -BHC | δ -BHC | Heptachlor | Aldrin | Heptachlor Epoxide |
|---------------|---------------|--------------|---------------|------------|--------|--------------------|
| 1 | 28.0 | 52.0 | 39.8 | 34.8 | 40.8 | 51.2 |
| 2 | 40.6 | 57.6 | 50.0 | 44.6 | 53.4 | 53.0 |
| 3 | 33.0 | 46.4 | 37.8 | 35.4 | 42.0 | 46.2 |
| 4 | 27.0 | 71.6 | 40.4 | 28.2 | 34.4 | 51.8 |
| 5 | 42.4 | 71.4 | 53.4 | 49.2 | 56.6 | 59.6 |
| 6 | 43.2 | 64.4 | 51.2 | 53.0 | 59.8 | 62.4 |
| 7 | 37.6 | 43.2 | 32.2 | 49.0 | 53.0 | 54.2 |
| Avg | 36.0 | 58.0 | 43.6 | 42.0 | 48.6 | 54.0 |
| % RSD | 18.7 | 19.9 | 18.3 | 22.0 | 19.5 | 10.0 |

| Replicate No. | Endosulfan I | Dieldrin | Endrin | Endosulfan II | Endrin Aldehyde | Endosulfan Sulfate |
|---------------|--------------|----------|--------|---------------|-----------------|--------------------|
| 1 | 16.8 | 73.8 | 73.6 | 17.7 | 26.0 | 36.6 |
| 2 | 21.4 | 70.4 | 72.6 | 29.4 | 44.6 | 47.4 |
| 3 | 37.8 | 61.2 | 63.6 | 38.4 | 19.7 | 37.6 |
| 4 | 51.0 | 69.6 | 68.6 | 43.0 | 25.6 | 42.8 |
| 5 | 25.2 | 74.4 | 72.8 | 37.8 | 44.2 | 51.0 |
| 6 | 20.4 | 74.2 | 70.6 | 29.8 | 42.8 | 52.4 |
| 7 | 13.0 | 62.8 | 66.6 | 11.1 | 37.8 | 46.2 |
| Avg | 26.6 | 69.4 | 69.8 | 29.6 | 34.4 | 44.8 |
| % RSD | 50.1 | 7.9 | 5.3 | 39.2 | 30.1 | 13.8 |

¹Data provided courtesy of Dr. Bill Brumley from US EPA ORD, 2008. Results were obtained using a 40-m Agilent J&W 0.18-mm ID, 0.18- μ m film thickness column and are included for informational purposes only to indicate that quantitation and acceptable recoveries of other organochlorine pesticide compounds using GC-NICI/MS are possible.

Table 9

PHASE I MULTI-LABORATORY VALIDATION DATA FOR UNKNOWN SPIKED STANDARDS¹**Technical Toxaphene**

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility (RSD) |
|-----------|-------------|--------------------------|--------------------------|----------------|-----------------------|
| Unknown 1 | 9 | 10.0 | 13.0 | 5.7 | 44% |
| Unknown 2 | 10 | 125 | 130 | 17.4 | 13% |
| QC | 9 | 150 | 148 | 10.3 | 7% |

Hx-SED

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility (RSD) |
|-------------|-------------|--------------------------|--------------------------|----------------|-----------------------|
| P-Unknown 1 | 10 | 10.0 | 9.3 | 2.1 | 23% |
| P-Unknown 2 | 10 | 125 | 126 | 10.9 | 9% |

Hp-SED

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility (RSD) |
|-------------|-------------|--------------------------|--------------------------|----------------|-----------------------|
| P-Unknown 1 | 10 | 10.0 | 9.1 | 2.1 | 23% |
| P-Unknown 2 | 10 | 125 | 124 | 9.3 | 7% |

P26

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility (RSD) |
|-------------|-------------|--------------------------|--------------------------|----------------|-----------------------|
| P-Unknown 1 | 10 | 10.0 | 9.7 | 2.7 | 28% |
| P-Unknown 2 | 10 | 125 | 123 | 7.8 | 6% |
| P-QC | 9 | 200 | 200 | 22.5 | 11% |

P40

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility (RSD) |
|-------------|-------------|--------------------------|--------------------------|----------------|-----------------------|
| P-Unknown 1 | 9 | 10.0 | 9.4 | 2.6 | 28% |
| P-Unknown 2 | 9 | 125 | 121 | 9.0 | 7% |
| P-QC | 8 | 200 | 182 | 22.5 | 12% |

Table 9 (cont.)

PHASE I MULTI-LABORATORY VALIDATION DATA FOR UNKNOWN SPIKED STANDARDS

P41

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility (RSD) |
|-------------|-------------|--------------------------|--------------------------|----------------|-----------------------|
| P-Unknown 1 | 9 | 10.0 | 9.6 | 3.8 | 40% |
| P-Unknown 2 | 9 | 125 | 121 | 9.8 | 8% |
| P-QC | 8 | 200 | 176 | 22.0 | 13% |

P44

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility (RSD) |
|-------------|-------------|--------------------------|--------------------------|----------------|-----------------------|
| P-Unknown 1 | 10 | 10.0 | 9.2 | 3.0 | 33% |
| P-Unknown 2 | 10 | 125 | 121 | 15.0 | 12% |
| P-QC | 9 | 200 | 269 | 41.9 | 16% |

P50

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility (RSD) |
|-------------|-------------|--------------------------|--------------------------|----------------|-----------------------|
| P-Unknown 1 | 10 | 10.0 | 9.1 | 2.9 | 32% |
| P-Unknown 2 | 10 | 125 | 122 | 9.2 | 8% |
| P-QC | 9 | 200 | 182 | 22.0 | 12% |

P62

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility (RSD) |
|-------------|-------------|--------------------------|--------------------------|----------------|-----------------------|
| P-Unknown 1 | 10 | 10.0 | 7.1 | 2.7 | 38% |
| P-Unknown 2 | 10 | 125 | 127 | 19.4 | 15% |
| P-QC | 9 | 200 | 320 | 62.0 | 19% |

¹The values of the quality control standards were unknown to the analysts.

Table 10

PHASE II MULTI-LABORATORY VALIDATION DATA USING SPIKED EXTRACTS OF
UNCONTAMINATED REAL WORLD SAMPLE MATRICES¹

Technical Toxaphene

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility (RSD) |
|--------|-------------|-----------------------------|-----------------------------|-------------------|--------------------------|
| GW 2 | 9 | 600 | 694 | 58.6 | 8.4% |
| Sed 2 | 9 | 700 | 881 | 159 | 18.0% |
| Soil 2 | 9 | 400 | 458 | 71.0 | 15.5% |

Hx-SED

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility (RSD) |
|--------|-------------|-----------------------------|-----------------------------|-------------------|--------------------------|
| GW 1 | 9 | 1.0 | 0.98 | 0.11 | 11.2% |
| GW 2 | 9 | 150 | 146 | 9.2 | 6.3% |
| Sed 1 | 9 | 4.0 | 3.57 | 0.36 | 10.1% |
| Sed 2 | 9 | 200 | 198 | 22.0 | 11.1% |
| Soil 1 | 9 | 2.0 | 1.92 | 0.26 | 13.5% |
| Soil 2 | 9 | 250 | 253 | 25.7 | 10.2% |

Hp-SED

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility (RSD) |
|--------|-------------|-----------------------------|-----------------------------|-------------------|--------------------------|
| GW 1 | 9 | 1.0 | 1.00 | 0.11 | 11.0% |
| GW 2 | 9 | 150 | 148 | 7.9 | 5.3% |
| Sed 1 | 9 | 4.0 | 3.61 | 0.44 | 12.2% |
| Sed 2 | 9 | 200 | 198 | 18.9 | 9.5% |
| Soil 1 | 9 | 2.0 | 1.99 | 0.26 | 13.1% |
| Soil 2 | 9 | 250 | 255 | 24.1 | 9.5% |

¹Liquid samples were solvent extracted with methylene chloride according to Method 3510 using a sample volume of one L and concentrated to a final extract volume of 5 mL in isooctane. Solid samples were solvent extracted using methylene chloride according to Method 3540 with a sample mass of 30-g and concentrated to a final extract volume of 5 mL in isooctane.

Table 10 (cont.)

PHASE II MULTI-LABORATORY VALIDATION DATA USING SPIKED EXTRACTS OF
UNCONTAMINATED REAL WORLD SAMPLE MATRICES¹

P26

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility (RSD) |
|--------|-------------|-----------------------------|-----------------------------|-------------------|--------------------------|
| GW 1 | 9 | 1.0 | 0.96 | 0.12 | 12.5% |
| GW 2 | 9 | 150 | 145 | 6.8 | 4.7% |
| Sed 1 | 9 | 4.0 | 3.56 | 0.34 | 9.6% |
| Sed 2 | 9 | 200 | 196 | 17.9 | 9.1% |
| Soil 1 | 9 | 2.0 | 1.95 | 0.27 | 13.8% |
| Soil 2 | 9 | 250 | 257 | 23.0 | 8.9% |

P40

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility (RSD) |
|--------|-------------|-----------------------------|-----------------------------|-------------------|--------------------------|
| GW 1 | 9 | 1.0 | 1.03 | 0.15 | 14.6% |
| GW 2 | 9 | 150 | 149 | 7.1 | 4.8% |
| Sed 1 | 9 | 4.0 | 3.72 | 0.47 | 12.6% |
| Sed 2 | 9 | 200 | 203 | 20.7 | 10.2% |
| Soil 1 | 9 | 2.0 | 2.11 | 0.25 | 11.8% |
| Soil 2 | 9 | 250 | 267 | 26.6 | 10.0% |

P41

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility (RSD) |
|--------|-------------|-----------------------------|-----------------------------|-------------------|--------------------------|
| GW 1 | 9 | 1.0 | 0.97 | 0.12 | 12.4% |
| GW 2 | 9 | 150 | 146 | 9.0 | 6.2% |
| Sed 1 | 9 | 4.0 | 3.68 | 0.46 | 12.5% |
| Sed 2 | 9 | 200 | 196 | 23.8 | 12.1% |
| Soil 1 | 9 | 2.0 | 2.04 | 0.28 | 13.7% |
| Soil 2 | 9 | 250 | 263 | 27.3 | 10.4% |

¹ Liquid samples were solvent extracted with methylene chloride according to Method 3510 using a sample volume of one L and concentrated to a final extract volume of 5 mL in isoctane. Solid samples were solvent extracted using methylene chloride according to Method 3540 with a sample mass of 30-g and concentrated to a final extract volume of 5 mL in isoctane.

Table 10 (cont.)

PHASE II MULTI-LABORATORY VALIDATION DATA USING SPIKED EXTRACTS OF UNCONTAMINATED REAL WORLD SAMPLE MATRICES¹**P44**

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility (RSD) |
|--------|-------------|--------------------------|--------------------------|----------------|-----------------------|
| GW 1 | 9 | 1.0 | 0.93 | 0.18 | 19.4% |
| GW 2 | 9 | 150 | 149 | 13.1 | 8.8% |
| Sed 1 | 9 | 4.0 | 3.91 | 1.05 | 26.9% |
| Sed 2 | 9 | 200 | 208 | 34.4 | 16.5% |
| Soil 1 | 9 | 2.0 | 2.01 | 0.30 | 14.9% |
| Soil 2 | 9 | 250 | 282 | 32.1 | 11.4% |

P50

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility (RSD) |
|--------|-------------|--------------------------|--------------------------|----------------|-----------------------|
| GW 1 | 9 | 1.0 | 0.97 | 0.18 | 18.6% |
| GW 2 | 9 | 150 | 148 | 8.0 | 5.4% |
| Sed 1 | 9 | 4.0 | 3.68 | 0.44 | 12.0% |
| Sed 2 | 9 | 200 | 199 | 21.2 | 10.7% |
| Soil 1 | 9 | 2.0 | 2.00 | 0.27 | 13.5% |
| Soil 2 | 9 | 250 | 265 | 22.7 | 8.6% |

P62

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility (RSD) |
|--------|-------------|--------------------------|--------------------------|----------------|-----------------------|
| GW 1 | 9 | 1.0 | 0.96 | 0.33 | 34.4% |
| GW 2 | 9 | 150 | 143 | 18.6 | 13.0% |
| Sed 1 | 9 | 4.0 | 4.09 | 1.08 | 26.4% |
| Sed 2 | 9 | 200 | 198 | 44.5 | 22.5% |
| Soil 1 | 9 | 2.0 | 2.02 | 0.29 | 14.4% |
| Soil 2 | 9 | 250 | 273 | 57.9 | 21.2% |

¹ Liquid samples were solvent extracted with methylene chloride according to Method 3510 using a sample volume of one L and concentrated to a final extract volume of 5 mL in isoctane. Solid samples were solvent extracted using methylene chloride according to Method 3540 with a sample mass of 30-g and concentrated to a final extract volume of 5 mL in isoctane.

Table 11

PHASE II MULTI-LABORATORY VALIDATION DATA USING REAL WORLD SAMPLES FROM
TERRY CREEK IN BRUNSWICK, GEORGIA¹

SEDIMENT

| ID | No. of Labs | Grand Mean (pg/ μ L) | Grand Mean (mg/kg) | Std. Deviation (pg/ μ L) | Reproducibility (RSD) |
|-----------|-------------|-----------------------------|-----------------------|---------------------------------|--------------------------|
| Toxaphene | 9 | 173,000 | 36,300 | 91,000 | 53% |
| Hx-Sed | 9 | 3,600 | 754 | 366 | 10% |
| Hp-Sed | 9 | 3,700 | 776 | 526 | 14% |
| P26 | 8 | 1,250 | 261 | 163 | 13% |
| P40 | 9 | 2,660 | 557 | 951 | 36% |
| P41 | 9 | 1,310 | 273 | 1530 | 117% |
| P44 | 8 | 902 | 189 | 172 | 19% |
| P50 | 9 | 1,780 | 372 | 231 | 13% |
| P62 | 8 | 3,840 | 803 | 1,410 | 37% |

SOIL

| ID | No. of Labs | Grand Mean (pg/ μ L) | Grand Mean (mg/kg) | Std. Deviation (pg/ μ L) | Reproducibility (RSD) |
|-----------|-------------|-----------------------------|-----------------------|---------------------------------|--------------------------|
| Toxaphene | 9 | 2,980 | 624 | 1,730 | 58% |
| Hx-Sed | 9 | 153 | 32 | 37 | 24% |
| Hp-Sed | 9 | 129 | 27 | 30 | 23% |
| P26 | 8 | 6.74 | 1.4 | 2.08 | 31% |
| P40 | 9 | 15.0 | 3.1 | 7.4 | 49% |
| P41 | 9 | 6.0 | 1.3 | 8.5 | 142% |
| P44 | 8 | 2.53 | 0.5 | 1.30 | 51% |
| P50 | 9 | 4.0 | 0.8 | 1.2 | 30% |
| P62 | 8 | 20.7 | 4.3 | 45.8 | 221% |

¹Samples were solvent extracted using methylene chloride according to Method 3540 with a sample mass of 30 g and concentrated to a final extract volume of 5 mL in isoctane.

Table 12

PHASE II MULTI-LABORATORY VALIDATION DATA USING REAL WORLD SAMPLES FROM
TERRY CREEK IN BRUNSWICK, GEORGIA WITH EXTRACTS SUBJECTED TO CLEANUP¹

Technical Toxaphene

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Grand Mean (mg/kg) | % Rec. | Std. Dev. (pg/ μ L) | Reproducibility (RSD) |
|---------------------------|-------------|-----------------------------|-----------------------------|-----------------------|--------|----------------------------|--------------------------|
| TC Sed | 4 | --- | 305,000 | 63,900 | --- | 47,200 | 15% |
| TC Soil | 4 | --- | 15,600 | 3,260 | --- | 4,570 | 29% |
| PCB 209 Sed Surr | 4 | 100 | 106 | --- | 106% | 4.0 | 4% |
| ϵ -HCH Sed Surr | 4 | 250 | 231 | --- | 92% | 21.0 | 9% |
| PCB 209 Soil Surr | 4 | 100 | 106 | --- | 106% | 1.0 | 1% |
| ϵ -HCH Soil Surr | 4 | 250 | 240 | --- | 96% | 11.8 | 5% |
| LCS | 4 | 200 | 228 | --- | 114% | 27.5 | 12 |

Hx-SED

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Grand Mean (mg/kg) | % Rec. | Std. Dev. (pg/ μ L) | Reproducibility (RSD) |
|---------------------------|-------------|-----------------------------|-----------------------------|-----------------------|--------|----------------------------|--------------------------|
| TC Sed | 4 | --- | 3,680 | 772 | --- | 183 | 5% |
| TC Soil | 4 | --- | 1,380 | 290 | --- | 91 | 7% |
| PCB 209 Sed Surr | 4 | 100 | 106 | --- | 106% | 4.5 | 4% |
| ϵ -HCH Sed Surr | 4 | 250 | 234 | --- | 94% | 11.9 | 5% |
| PCB 209 Soil Surr | 4 | 100 | 121 | --- | 121% | 9.7 | 8% |
| ϵ -HCH Soil Surr | 4 | 250 | 246 | --- | 98% | 4.8 | 2% |
| LCS | 4 | 200 | 211 | --- | 105% | 12.8 | 6% |

Hp-SED

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Grand Mean (mg/kg) | % Rec. | Std. Dev. (pg/ μ L) | Reproducibility (RSD) |
|---------------------------|-------------|-----------------------------|-----------------------------|-----------------------|--------|----------------------------|--------------------------|
| TC Sed | 4 | --- | 4,220 | 883 | --- | 993 | 24% |
| TC Soil | 4 | --- | 1,100 | 231 | --- | 29 | 3% |
| PCB 209 Sed Surr | 4 | 100 | 106 | --- | 106% | 4.5 | 4% |
| ϵ -HCH Sed Surr | 4 | 250 | 234 | --- | 94% | 11.9 | 5% |
| PCB 209 Soil Surr | 4 | 100 | 121 | --- | 121% | 9.7 | 8% |
| ϵ -HCH Soil Surr | 4 | 250 | 246 | --- | 98% | 4.8 | 2% |
| LCS | 4 | 200 | 212 | --- | 106% | 14.2 | 7% |

¹Samples were solvent extracted using methylene chloride according to Method 3540 with a sample mass of 30 g and concentrated to a volume of 10 mL. These extracts were further subjected to clean-up using a copper, acid, and silica gel approach according to Methods 3660, 3665, and 3630, respectively, to a final extract volume of 5 mL in isoctane.

Table 12 (cont.)

PHASE II MULTI-LABORATORY VALIDATION DATA USING REAL WORLD SAMPLES FROM
TERRY CREEK IN BRUNSWICK, GEORGIA WITH EXTRACTS SUBJECTED TO CLEANUP¹

P26

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Grand Mean (mg/kg) | % Rec. | Std. Dev. (pg/ μ L) | Reproducibility (RSD) |
|---------------------------|-------------|-----------------------------|-----------------------------|-----------------------|--------|----------------------------|--------------------------|
| TC Sed | 4 | --- | 1,870 | 392 | --- | 156 | 8% |
| TC Soil | 4 | --- | 60 | 13 | --- | 6 | 11% |
| PCB 209 Sed Surr | 4 | 100 | 106 | --- | 106% | 4.5 | 4% |
| ϵ -HCH Sed Surr | 4 | 250 | 234 | --- | 94% | 11.9 | 5% |
| PCB 209 Soil Surr | 4 | 100 | 121 | --- | 121% | 9.7 | 8% |
| ϵ -HCH Soil Surr | 4 | 250 | 246 | --- | 98% | 4.8 | 2% |
| LCS | 4 | 200 | 216 | --- | 108% | 22.8 | 11% |

P40

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Grand Mean (mg/kg) | % Rec. | Std. Dev. (pg/ μ L) | Reproducibility (RSD) |
|---------------------------|-------------|-----------------------------|-----------------------------|-----------------------|--------|----------------------------|--------------------------|
| TC Sed | 4 | --- | 4,200 | 880 | --- | 1,890 | 45% |
| TC Soil | 4 | --- | 143 | 30 | --- | 60 | 42% |
| PCB 209 Sed Surr | 4 | 100 | 106 | --- | 106% | 4.5 | 4% |
| ϵ -HCH Sed Surr | 4 | 250 | 234 | --- | 94% | 11.9 | 5% |
| PCB 209 Soil Surr | 4 | 100 | 121 | --- | 121% | 9.7 | 8% |
| ϵ -HCH Soil Surr | 4 | 250 | 246 | --- | 98% | 4.8 | 2% |
| LCS | 4 | 200 | 219 | --- | 110% | 24.3 | 11% |

P41

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Grand Mean (mg/kg) | % Rec. | Std. Dev. (pg/ μ L) | Reproducibility (RSD) |
|---------------------------|-------------|-----------------------------|-----------------------------|-----------------------|--------|----------------------------|--------------------------|
| TC Sed | 4 | --- | 1,230 | 258 | --- | 175 | 14% |
| TC Soil | 4 | --- | 33 | 7 | --- | 17 | 51% |
| PCB 209 Sed Surr | 4 | 100 | 106 | --- | 106% | 4.5 | 4% |
| ϵ -HCH Sed Surr | 4 | 250 | 234 | --- | 94% | 11.9 | 5% |
| PCB 209 Soil Surr | 4 | 100 | 121 | --- | 121% | 9.7 | 8% |
| ϵ -HCH Soil Surr | 4 | 250 | 246 | --- | 98% | 4.8 | 2% |
| LCS | 4 | 200 | 220 | --- | 110% | 28.1 | 13% |

¹Samples were solvent extracted using methylene chloride according to Method 3540 with a sample mass of 30 g and concentrated to a volume of 10 mL. These extracts were further subjected to clean-up using a copper, acid, and silica gel approach according to Methods 3660, 3665, and 3630, respectively, to a final extract volume of 5 mL in isoctane.

Table 12 (cont.)

PHASE II MULTI-LABORATORY VALIDATION DATA USING REAL WORLD SAMPLES FROM
TERRY CREEK IN BRUNSWICK, GEORGIA WITH EXTRACTS SUBJECTED TO CLEANUP¹

P44

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Grand Mean (mg/kg) | % Rec. | Std. Dev. (pg/ μ L) | Reproducibility (RSD) |
|---------------------------|-------------|-----------------------------|-----------------------------|-----------------------|--------|----------------------------|--------------------------|
| TC Sed | 3 | --- | 1,390 | 292 | --- | 194 | 14% |
| TC Soil | 3 | --- | 30 | 6 | --- | 5 | 18% |
| PCB 209 Sed Surr | 4 | 100 | 106 | --- | 106% | 4.5 | 4% |
| ϵ -HCH Sed Surr | 4 | 250 | 234 | --- | 94% | 11.9 | 5% |
| PCB 209 Soil Surr | 4 | 100 | 121 | --- | 121% | 9.7 | 8% |
| ϵ -HCH Soil Surr | 4 | 250 | 246 | --- | 98% | 4.8 | 2% |
| LCS | 4 | 200 | 209 | --- | 104% | 29.8 | 14% |

P50

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Grand Mean (mg/kg) | % Rec. | Std. Dev. (pg/ μ L) | Reproducibility (RSD) |
|---------------------------|-------------|-----------------------------|-----------------------------|-----------------------|--------|----------------------------|--------------------------|
| TC Sed | 4 | --- | 2,920 | 612 | --- | 342 | 12% |
| TC Soil | 4 | --- | 69 | 14 | --- | 7 | 11% |
| PCB 209 Sed Surr | 4 | 100 | 106 | --- | 106% | 4.5 | 4% |
| ϵ -HCH Sed Surr | 4 | 250 | 234 | --- | 94% | 11.9 | 5% |
| PCB 209 Soil Surr | 4 | 100 | 121 | --- | 121% | 9.7 | 8% |
| ϵ -HCH Soil Surr | 4 | 250 | 246 | --- | 98% | 4.8 | 2% |
| LCS | 4 | 200 | 221 | --- | 110% | 23.8 | 11% |

P62

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | Grand Mean (mg/kg) | % Rec. | Std. Dev. (pg/ μ L) | Reproducibility (RSD) |
|---------------------------|-------------|-----------------------------|-----------------------------|-----------------------|--------|----------------------------|--------------------------|
| TC Sed | 3 | --- | 8,840 | 1,850 | --- | 1,550 | 18% |
| TC Soil | 3 | --- | 219 | 46 | --- | 54 | 24% |
| PCB 209 Sed Surr | 4 | 100 | 106 | --- | 106% | 4.5 | 4% |
| ϵ -HCH Sed Surr | 4 | 250 | 234 | --- | 94% | 11.9 | 5% |
| PCB 209 Soil Surr | 4 | 100 | 121 | --- | 121% | 9.7 | 8% |
| ϵ -HCH Soil Surr | 4 | 250 | 246 | --- | 98% | 4.8 | 2% |
| LCS | 4 | 200 | 226 | --- | 113% | 27.6 | 12% |

¹ Samples were solvent extracted using methylene chloride according to Method 3540 with a sample mass of 30 g and concentrated to a volume of 10 mL. These extracts were further subjected to clean-up using a copper, acid, and silica gel approach according to Methods 3660, 3665, and 3630, respectively, to a final extract volume of 5 mL in isoctane.

Table 13

PHASE II MULTI-LABORATORY VALIDATION DATA COMPARISON USING REAL WORLD
 SAMPLES FROM TERRY CREEK IN BRUNSWICK, GEORGIA (EXTRACTION ONLY COMPARED
 TO EXTRACTS SUBJECTED TO CLEANUP)

SEDIMENT

| ID | No. of Labs ¹ | | Grand Mean (pg/ μ L) ¹ | | Grand Mean (mg/kg) ¹ | |
|-----------|--------------------------|---------|---------------------------------------|---------|---------------------------------|---------|
| | Extraction only | Cleanup | Extraction only | Cleanup | Extraction only | Cleanup |
| Toxaphene | 9 | 4 | 173,000 | 305,000 | 36,300 | 63,900 |
| Hx-Sed | 9 | 4 | 3,600 | 3,680 | 754 | 772 |
| Hp-Sed | 9 | 4 | 3,700 | 4,220 | 776 | 883 |
| P26 | 8 | 4 | 1,250 | 1,870 | 261 | 392 |
| P40 | 9 | 4 | 2,660 | 4,200 | 557 | 880 |
| P41 | 9 | 4 | 1,310 | 1,230 | 273 | 258 |
| P44 | 8 | 3 | 902 | 1,390 | 189 | 292 |
| P50 | 9 | 4 | 1,780 | 2,920 | 372 | 612 |
| P62 | 8 | 3 | 3,840 | 8,840 | 803 | 1,850 |

SOIL

| ID | No. of Labs ¹ | | Grand Mean (pg/ μ L) ¹ | | Grand Mean (mg/kg) ¹ | |
|-----------|--------------------------|---------|---------------------------------------|---------|---------------------------------|---------|
| | Extraction only | Cleanup | Extraction only | Cleanup | Extraction only | Cleanup |
| Toxaphene | 9 | 4 | 2,980 | 15,600 | 624 | 3,260 |
| Hx-Sed | 9 | 4 | 153 | 1,380 | 32 | 290 |
| Hp-Sed | 9 | 4 | 129 | 1,100 | 27 | 231 |
| P26 | 8 | 4 | 6.74 | 60 | 1.4 | 13 |
| P40 | 9 | 4 | 15.0 | 143 | 3.1 | 30 |
| P41 | 9 | 4 | 6.0 | 33 | 1.3 | 7 |
| P44 | 8 | 3 | 2.53 | 30 | 0.5 | 6 |
| P50 | 9 | 4 | 4.0 | 69 | 0.8 | 14 |
| P62 | 8 | 3 | 20.7 | 219 | 4.3 | 46 |

¹ Samples were solvent extracted using methylene chloride according to Method 3540 with a sample mass of 30 g and concentrated to a final extract volume of 5 mL in isoctane.

² Samples were solvent extracted using methylene chloride according to Method 3540 with a sample mass of 30 g and concentrated to a volume of 10 mL. These extracts were further subjected to clean-up using a copper, acid, and silica gel approach according to Methods 3660, 3665, and 3630, respectively, to a final extract volume of 5 mL in isoctane.

Table 14

PHASE II MULTI-LABORATORY VALIDATION DATA USING MULTIPLE TECHNICAL
TOXAPHENE SPIKING PROTOCOLS FOR UNCONTAMINATED REAL WORLD SAMPLE
MATRICES¹

Technical Toxaphene
Spiked Prior to Extraction and Cleanup

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | % Recovery | Std. Deviation (pg/ μ L) | Reproducibility RSD |
|----------------------|-------------|-----------------------------|-----------------------------|------------|------------------------------------|------------------------|
| Sed 1L | 4 | 75 | 78 | 105% | 6.9 | 9% |
| PCB 209 Surr | 4 | 100 | 109 | 109% | 2 | 2% |
| ϵ -HCH Surr | 4 | 250 | 275 | 110% | 10 | 4% |
| Sed 1M | 4 | 150 | 148 | 99% | 16 | 11% |
| PCB 209 Surr | 4 | 100 | 97 | 97% | 5 | 5% |
| ϵ -HCH Surr | 4 | 250 | 238 | 95% | 17 | 7% |
| LCS L | 4 | 75 | 71 | 95% | 9 | 12% |
| LCS M | 4 | 200 | 185 | 93% | 27 | 15% |
| Low-level Check | 4 | 40 | 49 | 121% | 7 | 14% |

Technical Toxaphene
Spiked After Extraction and Prior to Clean-up

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | % Recovery | Std. Deviation (pg/ μ L) | Reproducibility RSD |
|----------------------|-------------|-----------------------------|-----------------------------|------------|------------------------------------|------------------------|
| Sed 2L | 4 | 75 | 73 | 97% | 9.1 | 12% |
| PCB 209 Surr | 4 | 100 | 100 | 100% | 4 | 4% |
| ϵ -HCH Surr | 4 | 250 | 258 | 103% | 14 | 5% |
| Sed 2M | 4 | 150 | 153 | 102% | 18 | 12% |
| PCB 209 Surr | 4 | 100 | 99 | 99% | 6 | 6% |
| ϵ -HCH Surr | 4 | 250 | 255 | 102% | 19 | 7% |
| LCS L | 4 | 75 | 71 | 95% | 9 | 12% |
| LCS M | 4 | 200 | 185 | 93% | 27 | 15% |
| Low-level Check | 4 | 40 | 49 | 121% | 7 | 14% |

¹ Samples were solvent extracted using methylene chloride according to Method 3540 with a sample mass of 30 g and concentrated to a volume of 10 mL. These extracts were further subjected to clean-up using a copper, acid, and silica gel approach according to Methods 3660, 3665, and 3630, respectively, to a final extract volume of 5 mL in isooctane.

Table 14
(cont.)

PHASE II MULTI-LABORATORY VALIDATION DATA USING MULTIPLE SPIKING
PROTOCOLS FOR UNCONTAMINATED REAL WORLD SAMPLE MATRICES¹

Technical Toxaphene
Spiked in the Final Extract

| ID | No. of Labs | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | % Recovery | Std. Deviator (pg/ μ L) | Reproducibility RSD |
|----------------------|-------------|-----------------------------|-----------------------------|------------|--------------------------------|------------------------|
| Sed 3L | 4 | 75 | 86 | 114% | 10 | 12% |
| PCB 209 Surr | 4 | 100 | 105 | 105% | 5 | 5% |
| ϵ -HCH Surr | 4 | 250 | 276 | 111% | 21 | 7% |
| Sed 3M | 4 | 150 | 157 | 105% | 21 | 13% |
| PCB 209 Surr | 4 | 100 | 105 | 105% | 5 | 5% |
| ϵ -HCH Surr | 4 | 250 | 273 | 109% | 18 | 7% |
| LCS L | 4 | 75 | 71 | 95% | 9 | 12% |
| LCS M | 4 | 200 | 185 | 93% | 27 | 15% |
| Low-level Check | 4 | 40 | 49 | 121% | 7 | 14% |

¹ Samples were solvent extracted using methylene chloride according to Method 3540 with a sample mass of 30 g and concentrated to a volume of 10 mL. These extracts were further subjected to clean-up using a copper, acid, and silica gel approach according to Methods 3660, 3665, and 3630, respectively, to a final extract volume of 5 mL in isoctane.

Table 15

PHASE III MULTI-LABORATORY VALIDATION DATA USING SPIKED LABORATORY QUALITY CONTROL SAMPLES CARRIED THROUGH EXTRACTION AND CLEANUP¹

Technical Toxaphene

| ID | No. of Participants | True Value (pg/μL) | Grand Mean (pg/μL) | % Recovery | Std. Deviation | Reproducibility (% RSD) |
|------------------------|---------------------|--------------------|--------------------|------------|----------------|-------------------------|
| Second Source Standard | 8 | 150 | 159 | 106 | 9.85 | 6.20 |
| LCS 1 | 8 | 50 | 57.3 | 115 | 9.74 | 16.9 |
| LCS 2 | 8 | 200 | 207 | 104 | 13.5 | 6.50 |
| PCB 209 LCS1 | 8 | 100 | 95.0 | 95.0 | 1.93 | 2.03 |
| ε-HCH LCS 1 | 8 | 250 | 231 | 92.2 | 21.0 | 9.10 |
| PCB 209 LCS2 | 8 | 100 | 90.2 | 90.2 | 5.36 | 5.95 |
| ε-HCH LCS2 | 8 | 250 | 240 | 95.8 | 15.4 | 6.46 |

Hx-Sed

| ID | No. of Participants | True Value (pg/μL) | Grand Mean (pg/μL) | % Recovery | Std. Deviation | Reproducibility (% RSD) |
|------|---------------------|--------------------|--------------------|------------|----------------|-------------------------|
| LCS1 | 8 | 100 | 98.4 | 98.4 | 5.84 | 5.94 |
| LCS2 | 8 | 2 | 1.95 | 97.4 | 0.16 | 8.00 |

Hp-Sed

| ID | No. of Participants | True Value (pg/μL) | Grand Mean (pg/μL) | % Recovery | Std. Deviation | Reproducibility (% RSD) |
|------|---------------------|--------------------|--------------------|------------|----------------|-------------------------|
| LCS1 | 8 | 100 | 98.9 | 98.9 | 5.77 | 5.84 |
| LCS2 | 8 | 2 | 1.99 | 99.3 | 0.13 | 6.30 |

P26

| ID | No. of Participants | True Value (pg/μL) | Grand Mean (pg/μL) | % Recovery | Std. Deviation | Reproducibility (% RSD) |
|------|---------------------|--------------------|--------------------|------------|----------------|-------------------------|
| LCS1 | 8 | 100 | 97.9 | 97.9 | 5.27 | 5.39 |
| LCS2 | 8 | 2 | 1.83 | 91.4 | 0.36 | 19.7 |

Table 15 (cont.)

P40

| ID | No. of Participants | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | % Recovery | Std. Deviation | Reproducibility (% RSD) |
|------|---------------------|--------------------------|--------------------------|------------|----------------|-------------------------|
| LCS1 | 8 | 100 | 98.1 | 98.1 | 6.33 | 6.45 |
| LCS2 | 8 | 2 | 1.94 | 97.2 | 0.15 | 7.46 |

P41

| ID | No. of Participants | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | % Recovery | Std. Deviation | Reproducibility (% RSD) |
|------|---------------------|--------------------------|--------------------------|------------|----------------|-------------------------|
| LCS1 | 8 | 100 | 98.6 | 98.6 | 6.31 | 6.40 |
| LCS2 | 8 | 2 | 2.03 | 102 | 0.17 | 8.32 |

P44

| ID | No. of Participants | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | % Recovery | Std. Deviation | Reproducibility (RSD) |
|------|---------------------|--------------------------|--------------------------|------------|----------------|-----------------------|
| LCS1 | 8 | 100 | 99.4 | 99.4 | 8.85 | 8.91 |
| LCS2 | 8 | 2 | 1.99 | 99.7 | 0.16 | 8.09 |

P50

| ID | No. of Participants | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | % Recovery | Std. Deviation | Reproducibility (% RSD) |
|------|---------------------|--------------------------|--------------------------|------------|----------------|-------------------------|
| LCS1 | 8 | 100 | 96.9 | 96.9 | 7.47 | 7.71 |
| LCS2 | 8 | 2 | 2.01 | 101 | 0.07 | 3.55 |

P62

| ID | No. of Participants | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | % Recovery | Std. Deviation | Reproducibility (% RSD) |
|------|---------------------|--------------------------|--------------------------|------------|----------------|-------------------------|
| LCS1 | 8 | 226 | 237.0 | 105 | 26.0 | 11.0 |
| LCS2 | 8 | 22.6 | 21.0 | 92.9 | 4.51 | 21.5 |

Table 15 (cont.)

Congeners - PCB 209

| ID | No. of Participants | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | % Recovery | Std. Deviation | Reproducibility (% RSD) |
|------|---------------------|--------------------------|--------------------------|------------|----------------|-------------------------|
| LCS1 | 8 | 100 | 95.1 | 95.1 | 5.13 | 5.39 |
| LCS2 | 8 | 100 | 94.9 | 94.9 | 5.55 | 5.85 |

Congeners – ϵ -HCH

| ID | No. of Participants | True Value (pg/ μ L) | Grand Mean (pg/ μ L) | % Recovery | Std. Deviation | Reproducibility (% RSD) |
|------|---------------------|--------------------------|--------------------------|------------|----------------|-------------------------|
| LCS1 | 8 | 250 | 248.0 | 99.0 | 11.7 | 4.72 |
| LCS2 | 8 | 250 | 247.0 | 98.9 | 7.84 | 3.17 |

¹Samples were extracted in hexane in an Accelerated Solvent Extractor (ASE) using a modified version of Method 3545 with a sample mass of 10 g of solid material (Ottawa sand). In-cell cleanup was done in the ASE extraction cell using activated, acidified (30% H₂SO₄) silica gel per Method 3665 and Method 3630. These extracts were further subjected to cleanup using a Florisil® cartridge (1% deactivated with DI water) approach according to Method 3620. The sample was in hexane throughout the process and was concentrated to a final extract volume of 1 mL.

Table 16

PHASE III MULTI-LABORATORY VALIDATION DATA USING FISH SAMPLES COLLECTED FROM TERRY CREEK IN BRUNSWICK, GEORGIA WITH EXTRACTS SUBJECTED TO CLEANUP¹

Technical Toxaphene

| ID | No. of Participants | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility % RSD |
|------|---------------------|--------------------------|----------------|-----------------------|
| TC 1 | 8 | 351 | 71.9 | 20.5 |
| TC 2 | 8 | 317 | 79.3 | 25.0 |

Hx-Sed

| ID | No. of Participants | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility % RSD |
|-----|---------------------|--------------------------|----------------|-----------------------|
| TC1 | 8 | 80.3 | 5.59 | 6.97 |
| TC2 | 8 | 54.2 | 4.49 | 8.27 |

Hp-Sed

| ID | No. of Participants | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility % RSD |
|-----|---------------------|--------------------------|----------------|-----------------------|
| TC1 | 8 | 91.8 | 5.66 | 6.17 |
| TC2 | 8 | 75.6 | 5.80 | 7.67 |

P26

| ID | No. of Participants | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility % RSD |
|-----|---------------------|--------------------------|----------------|-----------------------|
| TC1 | 8 | 8.05 | 0.40 | 4.93 |
| TC2 | 8 | 11.6 | 0.52 | 4.46 |

P40

| ID | No. of Participants | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility % RSD |
|-----|---------------------|--------------------------|----------------|-----------------------|
| TC1 | 8 | 11.1 | 1.02 | 9.19 |
| TC2 | 8 | 12.6 | 0.71 | 5.66 |

Table 16 (cont.)

P41

| ID | No. of Participants | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility % RSD |
|-----|---------------------|--------------------------|----------------|-----------------------|
| TC1 | 8 | 4.49 | 0.19 | 4.31 |
| TC2 | 8 | 5.29 | 0.21 | 3.90 |

P44

| ID | No. of Participants | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility % RSD |
|-----|---------------------|--------------------------|----------------|-----------------------|
| TC1 | 8 | 4.76 | 0.33 | 6.86 |
| TC2 | 8 | 6.60 | 0.55 | 8.33 |

P50

| ID | No. of Participants | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility % RSD |
|-----|---------------------|--------------------------|----------------|-----------------------|
| TC1 | 8 | 8.34 | 0.73 | 8.76 |
| TC2 | 8 | 12.9 | 1.06 | 8.18 |

P62

| ID | No. of Participants | Grand Mean (pg/ μ L) | Std. Deviation | Reproducibility % RSD |
|-----|---------------------|--------------------------|----------------|-----------------------|
| TC1 | 8 | 10.5 | 2.81 | 26.9 |
| TC2 | 8 | 14.8 | 4.13 | 27.8 |

¹Samples were extracted in hexane in an Accelerated Solvent Extractor (ASE) using a modified version of Method 3545 with a sample mass of 10 g of solid material (Ottawa sand). In-cell cleanup was done in the ASE extraction cell using activated, acidified (30% H₂SO₄) silica gel per Method 3665 and Method 3630. These extracts were further subjected to cleanup using a Florisil® cartridge (1% deactivated with DI water) approach according to Method 3620. The sample was in hexane throughout the process and was concentrated to a final extract volume of 1 mL.

FIGURE 1

EXAMPLE GAS CHROMATOGRAM OF A MIXED CONGENER STANDARD

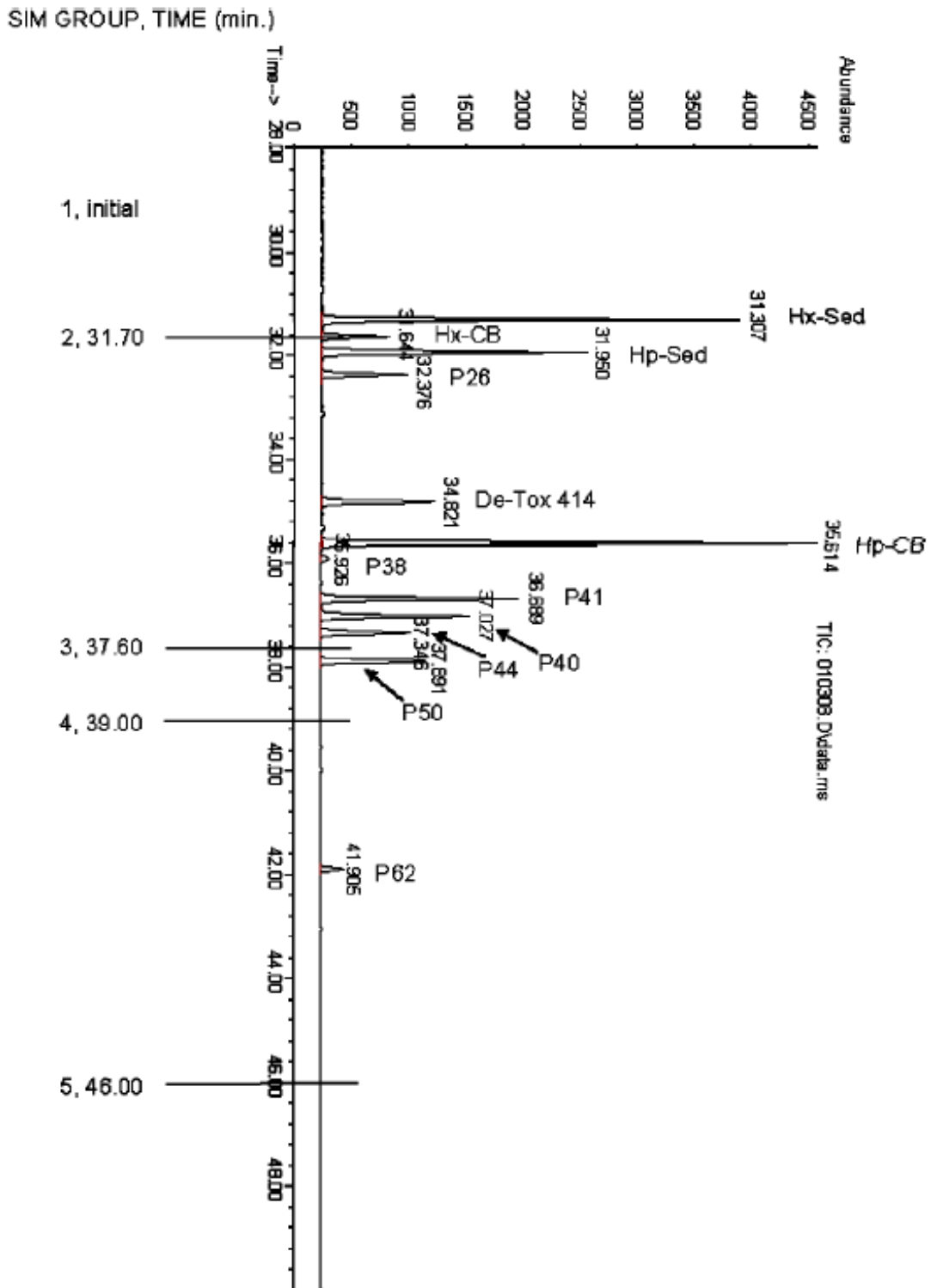


FIGURE 2

EXAMPLE GAS CHROMATOGRAM OF A FISH TISSUE EXTRACT

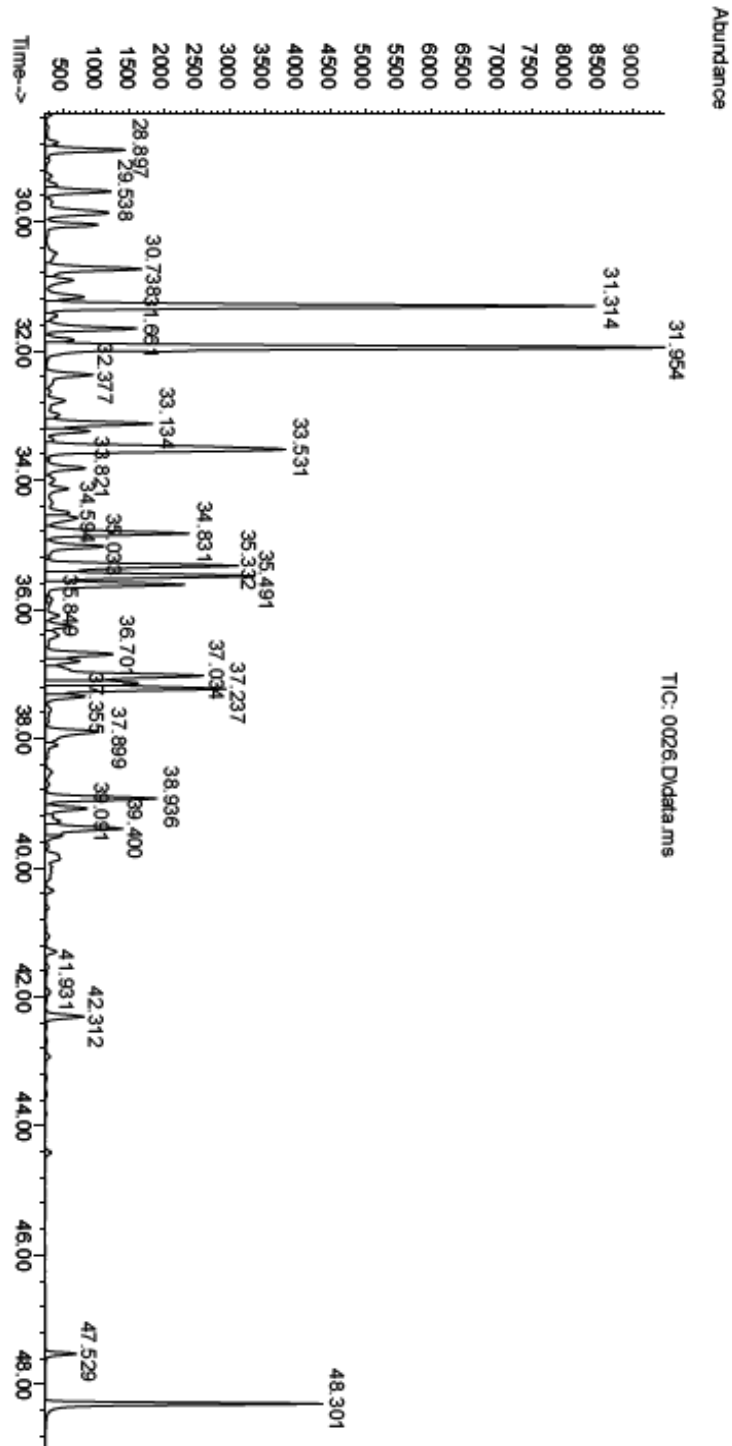


FIGURE 3

EXAMPLE GAS CHROMATOGRAM OF A 100 pg/ μ L CONGENER CALIBRATION VERIFICATION STANDARD

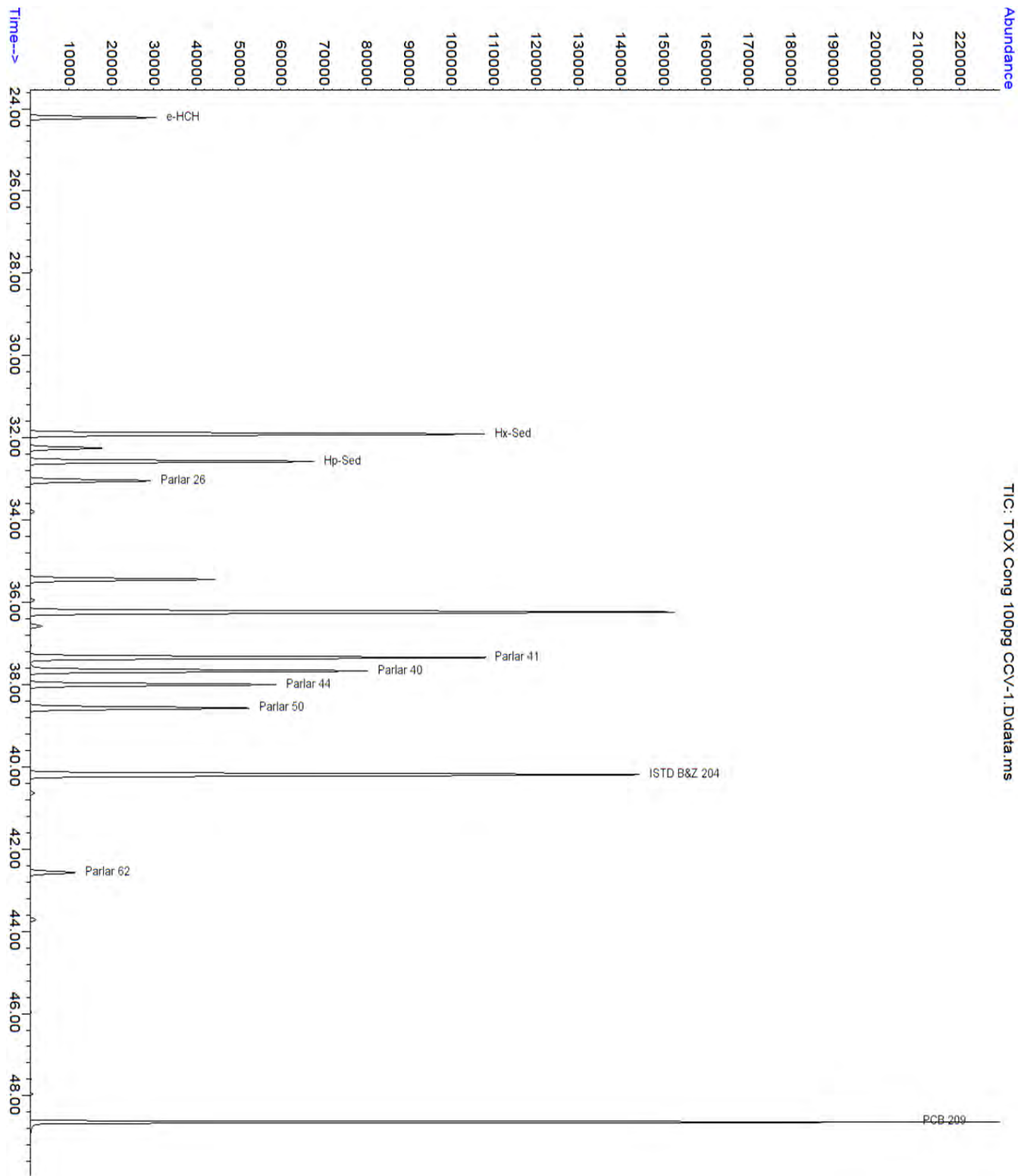
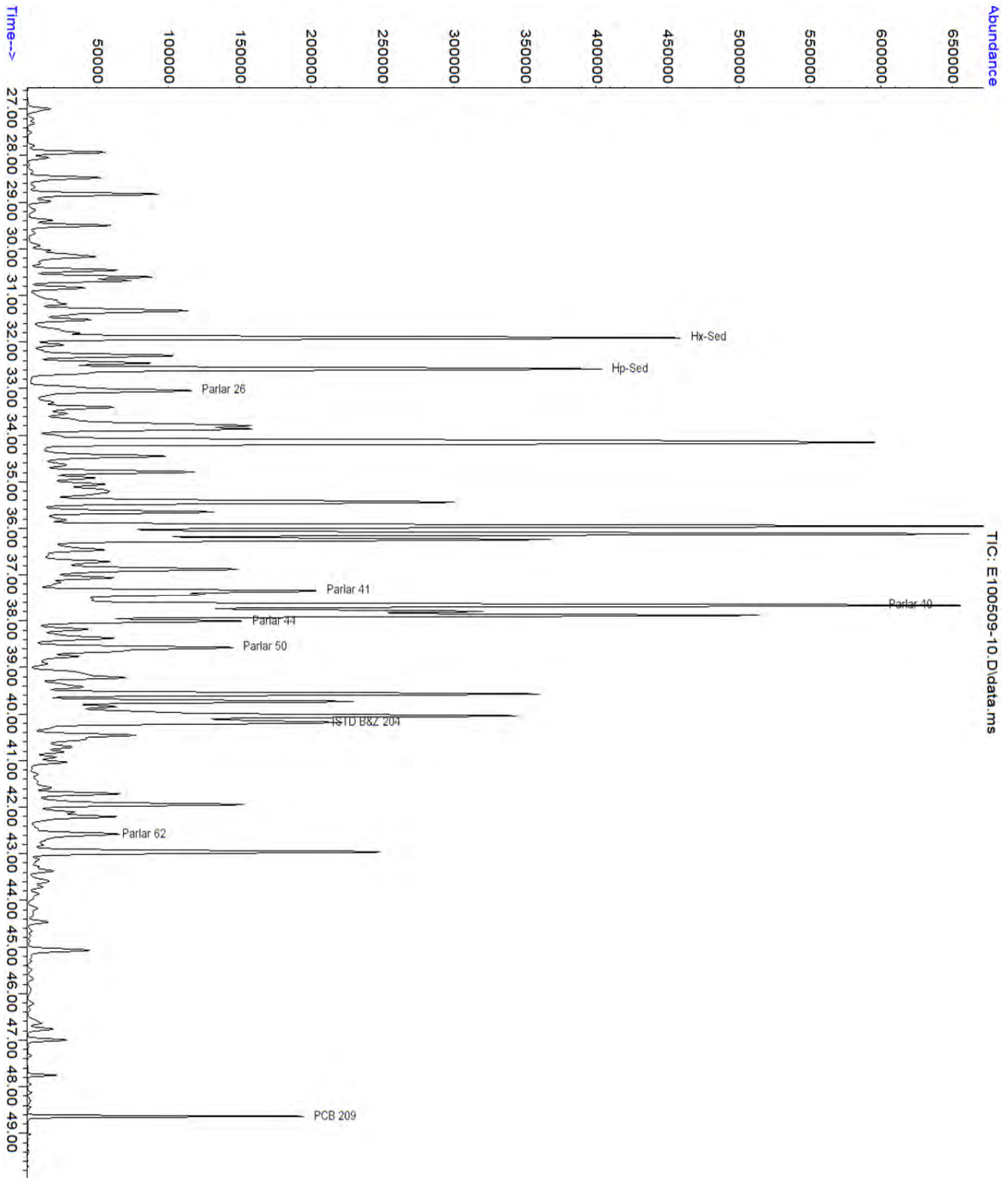


FIGURE 4

EXAMPLE GAS CHROMATOGRAM OF AN EPA REGION 4 SEDIMENT EXTRACT SPIKED USING DECACHLOROBIPHENYL



Appendix A: Changes in Revision 1 from the Revision 0, 2010 Version.

1. The acronym NIMS (negative ion mass spectroscopy) has been replaced with NICI/MS (negative ion chemical ionization/mass spectrometry).
2. Section 2.0 now points to Method 3500 for sample preparation guidance, rather than listing all methods specifically.
3. References to the method quantitation limit (MQL) have been replaced with the lower limit of quantitation (LLOQ).
4. Section 6.4.1 no longer specifies exact mass spectrometer conditions, instead it states that the detector must be tuned per Section 11.4.1 and Table 1 conditions.
5. Sections 9.4 (IDP) and 9.7 (LLOQ) were expanded to reflect current guidance.
6. Section 11.1 was re-organized into a table to increase clarity and ease of preparatory method selection.
7. Improved overall method formatting for consistency with new SW-846 methods style guidance. The format was updated to Microsoft Word 2007.docx. Many minor editorial and technical revisions were made throughout to improve method clarity.
8. The revision number was changed to 1 and the date published was changed to September 2012.
9. This appendix was added showing changes from the previous revision.