TOXAPHENE AND TOXAPHENE CONGENERS BY GAS CHROMATOGRAPHY / NEGATIVE ION MASS SPECTROMETRY (GC / NIMS)

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

1.0 SCOPE AND APPLICATION

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 mass spectrometry (NIMS). The 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 NIMS 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. 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/NIMS 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 all of them cannot 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. 2.5 and 3.1) 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 NIMS 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 guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies, and on the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the 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 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/NIMS 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 NIMS. See Ref. 1 for a more detailed discussion of NIMS theory.

2.0 SUMMARY OF METHOD

2.1 A measured volume or weight of liquid or solid sample is extracted using the appropriate matrix-specific sample extraction technique.

2.1.1 Aqueous samples may be extracted at neutral pH with methylene chloride using either Method 3510 (separatory funnel), Method 3520 (continuous liquid-liquid extraction), Method 3535 (solid-phase extraction), or other appropriate technique.

2.1.2 Solid samples may be extracted with hexane-acetone (1:1) or methylene chloride-acetone (1:1) using Method 3540 (Soxhlet), Method 3541 (automated Soxhlet), Method 3545 (pressurized fluid extraction), Method 3546 (microwave extraction), Method 3550 (ultrasonic extraction), Method 3562 (supercritical fluid extraction), or other appropriate technique or solvents.

2.2 A variety of cleanup steps may be applied to the extract, depending on the nature of the matrix interferences and the target analytes. Suggested cleanups include Method 3610 (alumina), Method 3620 (Florisil®), Method 3630 (silica gel), Method 3640 (gel permeation chromatography, GPC), Method 3660 (sulfur), and Method 3665 (sulfuric acid/permanganate).

2.3 After cleanup, the extract is analyzed by injecting an aliquot of sample into a gas chromatograph (GC) with a narrow-bore fused-silica capillary column interfaced to a mass spectrometer capable of performing NIMS under CI conditions using methane as the introduced gas.

2.4 Analysis of toxaphene (a mixture of polychlorinated monoterpenes) 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.

2.5 This method does not address all the estimated 600-plus 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, Strobane, Melipax, chlorocamphene, polychlorocamphene, and chlorinated camphene.

3.2 GC/NIMS - 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 ranging up to fully halogenated compounds.

3.4 Parlar number - Individual toxaphene congeners based on GC elution order are also known as "Parlars" and specific congeners have been given a Parlar number to easily refer to them (e.g., P26, P50).

3.5 Weathered toxaphene - Once in the environment, technical toxaphene is degraded by both biotic and abiotic processes. The major processes of this degradation seem to be dechlorination and dehydrochlorination which leads to a pronounced shift toward lower chlorinated homologs. Weathered toxaphene may have a different chromatographic profile and NIMS detector response when compared to “virgin” toxaphene, a result of the composition change 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 (this is sometimes called the reconstructed ion current).

3.7 Refer to Chapter One 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 to 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 and purification of solvents by distillation in all-glass systems may be necessary. Refer to each method used for specific guidance on QC procedures and to the chapter text 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 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 which 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 (GPC) or Method 3630 (silica gel cleanup).

4.4 Cross-contamination of clean glassware routinely occurs when plastics are handled during extraction steps, especially when solvent-wetted surfaces are manipulated. Glassware must be scrupulously cleaned.

Clean all glassware as soon as possible after use by rinsing with the last solvent employed. This should be followed by detergent washing with hot water, and rinsing with tap water and organic-free reagent water. Drain the glassware and dry it in an oven at 130°C for several hours, or rinse with methanol and drain. Store dry glassware in a clean environment. (Other appropriate glassware cleaning procedures may be employed.)

4.5 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 NIMS 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 when 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.

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

4.7 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).

4.8 PCB Interference

4.8.1 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 NIMS 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.

4.8.2 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., #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.

4.9 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.

4.10 Chlordane interference

4.10.1 Under GC/NIMS 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.

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

4.11 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 listed in this method. A reference file containing each material safety data sheet (MSDS) 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.

This section does not list common laboratory glassware (e.g., beakers and flasks).

6.1 Gas chromatograph - 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 those 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 40-m x 0.18-mm ID and DB-XLB stationary phase columns may provide the maximum practical resolving power for congener separations. The DB-5 columns have been problematic, however, in the ability to adequately separate Parlars 40 and 41, resulting in a single summed value for the concentration of these two compounds.

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

6.2.2 30-m x 0.25-mm ID fused silica capillary column ZB-MultiResidue-1 (Phenomenex), 0.25-µm 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-µm film thickness.

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 Capable of scanning from 35 to 500 amu every 1 sec or less, using 150 volts (nominal) electron energy in the negative chemical ionization (NCI) mode. The mass spectrometer must be capable of producing a mass spectrum using a NCI-customized

tuning file according to the guidance outlined in Sec. 11.4.

6.4.2 An ion trap mass spectrometer may be used if it is capable of axial modulation to reduce ion-molecule reactions and can produce NCI spectra. The mass spectrometer must be capable of producing a mass 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 must be used in all tests. Unless otherwise indicated, all reagents should conform to specifications of the Committee on Analytical Reagents of the American Chemical Society, where available. 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 the leaching of contaminants from plastic containers.

NOTE: Store all standard solutions (stock, composite, calibration, internal, and surrogate) at $\leq 6^{\circ}C$ 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) indicates a problem.

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 in 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 The following solvents may be necessary for the preparation of standards.

7.3.1 Acetone, $(CH_3)_2CO$

7.3.2 Toluene, $C_6H_5CH_3$

7.3.3 n-Hexane, C_6H_{14}

7.3.4 Isooctane, C_8H_{18}

7.3.5 Methylene chloride, CH_2Cl_2

7.4 Organic-free reagent water - All references to water in this method refer to organic-free reagent water as defined in Chapter One of SW-846.

7.5 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 can 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.

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

7.6.1 As an example, for composite stock standards containing fewer than 25 components, take 1 mL of each individual stock solution at a concentration of 1000 mg/L (or other concentrations as appropriate), add solvent, and mix the solutions in a 25-mL volumetric flask. For a composite containing 20 individual standards, 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.

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

7.7 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 - 750 pg/µL and 0.5 - 500 pg/µL for toxaphene congeners. These 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. The concentrations should correspond to the expected range of concentrations 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.

7.8 Internal standard - PCB congener #204 at a concentration range of 50 to 100 pg/µL 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 µL of a 25-ng/µL solution into each 1 mL of sample extract for a final concentration 50 - 100 ng/mL (pg/µL). Other concentrations and volumes are acceptable.

7.9 Surrogate standards - The performance of the method should be monitored using surrogates. 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.

7.9.1 Decachlorobiphenyl (PCB congener #209) and ε-hexachlorocyclohexane (ε -HCH) 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.

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

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 See the introductory material to SW-846 Chapter Four, "Organic Analytes."

8.2 Store the sample extracts at $\leq 6^{\circ}C$, protected from light, in sealed vials (e.g., screw-cap or crimp-capped vials) equipped with un-pierced PTFE-lined septa. Extracts should be analyzed within 40 days of extraction.

9.0 QUALITY CONTROL

9.1 Refer to SW-846 Chapter One for guidance on quality assurance (QA) and QC protocols. When inconsistencies exist between QC guidelines, method-specific criteria take precedence over both technique-specific and Chapter One criteria. Technique-specific QC criteria take precedence over the criteria in Chapter One. Any effort involving the collection of analytical data should include development of a structured and systematic planning document, such as a Quality Assurance Project Plan (QAPP), Standard Operating Procedure (SOP) or a Sampling and Analysis Plan, which translates project objectives and specifications into directions for those implementing the project and assessing the results. Each laboratory should maintain a formal QA program. The laboratory should also maintain records to document the quality of the data generated. All data sheets and QC data should be maintained for reference or inspection.

9.2 Refer to Method 8000 for determinative method QC procedures. Refer to Method 3500 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 specific QC procedures provided in this method will supersede those noted in Methods 8000, 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.

- The GC/MS is tuned and calibrated using a NCI-customized tuning file. 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 for further details.

- There must be an initial calibration of the GC/MS system as described in Sec. 11.4. In addition, the initial calibration curve should be verified immediately after performing the standard analyses, preferably with a certified standard from a source independent of the primary standard. Alternatively, if a standard from an independent supplier is not available, a second lot number from the manufacturer of the primary source is sufficient. The suggested acceptance limits for this initial calibration verification analysis are 70 - 130%. Alternative acceptance limits may be appropriate based on the desired project-specific DQOs. Quantitative sample analyses should not proceed for those compounds that fail the second-source standard initial calibration verification. However, analyses may continue for those compounds that fail the criteria with an understanding that these results could be used for screening purposes and would be considered estimated values.

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

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

9.4 Initial demonstration of proficiency - Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes by generating data of acceptable accuracy and precision for target analytes in a clean matrix. The laboratory must also repeat the these operations whenever new staff members are trained or significant changes in instrumentation are made. See Method 8000 for information on how to accomplish a demonstration of proficiency.

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. This demonstration is accomplished through the analysis of a method blank. 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 any analyte that would prevent the determination of that analyte, the source must be determined and eliminated, if possible, before samples are processed. Method blank re-extraction may be necessary if the source of contamination cannot be determined. The blank should be carried through all stages of sample preparation and analysis. 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 the effect of the matrix on method performance (precision, accuracy, method sensitivity). At a minimum, this should include the analysis of QC samples including a method blank, matrix spike/matrix spike duplicate, sample duplicate (if a matrix spike duplicate cannot be prepared for whatever reason and sufficient sample exists), and laboratory control sample (LCS) in each analytical batch. The addition of surrogates to each field sample and QC sample when surrogates are used is also recommended. Any 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.

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

the guidance found in Method 8000.

9.6.1 Documenting the effect of the matrix should include the analysis of one matrix spike/matrix spike duplicate pair or at least one matrix spike and one duplicate unspiked sample. The decision to prepare and analyze duplicate samples or a matrix spike/matrix spike duplicate 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 matrix spike and matrix spike duplicate pair. Consult Method 8000 for information on developing acceptance criteria for the matrix spike/matrix spike duplicate.

9.6.2 A LCS should be included with each analytical batch. The LCS 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 the 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. Consult Method 8000 for information on developing acceptance criteria for the LCS.

9.6.3 The laboratory should establish the lower limit of quantitation (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 or at a minimum on a quarterly basis to validate quantitation capability at low analyte concentration levels. This verification may be accomplished either with 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 or equal to the desired regulatory action levels based on the stated project-specific requirements.

9.6.4 The determination of LLOQs using spiked clean control material represents a best-case scenario, and does not evaluate the 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.

9.6.4.1 A matrix-free LLOQ check standard is prepared by spiking a clean control material with the analyte(s) of interest at the predicted LLOQ concentration level(s). This LLOQ ckeck is carried through the same preparation procedures as the environmental samples and other QC. Recovery should be ±50% (or other such project-required acceptance limits for accuracy and precision) of the true value to verify the data reporting limit(s).

9.6.4.2 Alternatively, a representative sample matrix may be spiked with the analytes of interest at the predicated LLOQ concentration levels. This LLOQ check is carried through the same preparation procedures as the environmental samples and other QC. Individual LLOQs are verified when each respective analyte is recovered at ±50% of the predicted LLOQ concentration or established DQO criteria. This check may also be applied towards establishing the individual analyte reporting limit(s).

9.6.5 In-house limits may be calculated when sufficient data points exist.

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

9.8 The experience of the analyst performing GC/NIMS 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 the guidance in Method 8000 regarding whether recalibration of the system must take place.

9.9 It is recommended that the laboratory adopt additional QA practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

10.0 CALIBRATION AND STANDARDIZATION

See Sec. 11.0 for information on calibration and standardization.

11.0 PROCEDURE

11.1 Sample extraction - Refer to Chapter Two of SW-846 and Method 3500 (organic extraction and sample preparation) for guidance in choosing the appropriate extraction procedure. In general, water samples are extracted at a neutral pH with methylene chloride using Method 3510 (separatory funnel), Method 3520 (continuous liquid-liquid extraction), Method 3535 (solid-phase extraction), or other appropriate technique. Solid samples are extracted with hexane-acetone (1:1) or methylene chloride-acetone (1:1) using Method 3540 or 3541 (Soxhlet extraction methods), Method 3545 (pressurized fluid extraction), Method 3546 (microwave extraction), Method 3550 (ultrasonic extraction), or other appropriate technique. Solid samples may also be extracted using Method 3562 (supercritical fluid extraction).

NOTE: Hexane-acetone (1:1) may be more effective than methylene chloride-acetone (1:1) as an extraction solvent for organochlorine pesticides in some environmental and waste matrices. Relative to the methylene chloride-acetone mixture, the use of hexane-acetone generally reduces the amount of extracted interferences and improves the signal-to-noise ratio.

The choice of extraction solvent will depend on the analytes of interest. No single solvent or extraction procedure is universally applicable to all analyte groups and sample matrices. The analyst *must* demonstrate adequate performance for the selected analytes at the levels of interest for any solvent system employed, *including* those specifically listed in this method. At a minimum, such a demonstration will encompass the initial demonstration of proficiency described in Method 3500 using a clean reference matrix. Each new sample matrix should be spiked with the compounds of interest to determine the percent recovery. Method 8000

describes procedures that may be used to develop performance criteria for such demonstrations as well as for matrix spike and laboratory control sample results.

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 cleanup procedure(s) used will depend on the nature of the sample to be analyzed, expected interferences coextracted from the sample, and the DQOs for the measurements. General guidance for sample extract cleanup is provided in this section 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 If a sample is of biological origin or contains high molecular weight materials, Method 3640 (GPC - pesticide option) is recommended. Frequently, one of the adsorption chromatographic cleanups (Secs. 11.2.2 - 11.2.4) may also be necessary following GPC cleanup.

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

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

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

NOTE: Toxaphene and toxaphene congeners behave as relatively non-polar substances on silica gel and Florisil® and therefore would be found in fractions eluted with hexane and hexane/methylene chloride (e.g., 90:10) eluants. The volumes of solvents necessary to properly separate the target compounds from the interferences and obtain adequate recovery will depend on the amount of adsorbent used and its activity, which would be established using standards.

11.2.5 Method 3665 (sulfuric acid/permanganate) is a rigorous extract cleanup technique that may be used prior to toxaphene analysis to remove more fragile organic contaminants.

11.2.6 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 NIMS ion source during coelution.

11.3 GC conditions - This method uses a single column in conjunction with an electron capture NIMS detector. If an autosampler is not used, an internal standard is required. It is highly recommended to include an internal standard in all samples even if an autosampler is used. The GC column list in Sec. 6.0 indicates the columns used to develop the method performance data in the tables of this method and is not intended to exclude the use of other columns that are available or may be developed. Laboratories may use these or other capillary columns or 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/NIMS operating conditions according to the recommendations in Table 1 (toxaphene and toxaphene congeners). Alternative operating conditions may be acceptable provided that appropriate compound sensitivity and selectivity can be achieved.

11.4.1 Prior to the analysis of samples or calibration standards, the instrument must be tuned for NCI conditions. If possible, it is recommended to 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. 7.9 should permit most of the components of interest in a chromatogram to have retention times of 0.80-1.20 relative to the internal standard. 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 and 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. 7.7 and Method 8000). Alternate injection volumes may be used if applicable QC requirements are met. The injection volume must be the same for all standards and sample extracts. Figure 1 is a chromatogram of mixed standards showing all congeners.

NOTE: Toxaphene will require a calibration curve separate 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 area (or height) of the analyte or surrogate.
A_{is} = Peak area (or height) of the internal standard.
C_s = Concentration of the analyte or surrogate in µg/L.
C_{is} = Concentration of the internal standard in µg/L.

11.4.3.2 Calculate the average RF and the relative standard deviation (RSD) of the RFs for each target analyte using the equations below. The RSD should be ≤ 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 also 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, then 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.

Alternately, either of the two procedures described in Secs. 11.4.3.3.1 and 11.4.3.3.2 may be used to determine calibration function acceptability. These include re-fitting 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.

11.4.3.3.1 Re-fitting the calibration data back to the model or calculating the % difference is determined by using the following equation:

$$\% \text{ Difference} = \frac{C_c - C_e}{C_e} \times 100$$

where:

C_c = Calculated amount of standard, in mass or concentration units.
C_e = Expected amount of standard, in mass or concentration units.

The absolute value of the percent difference between these two amounts should be ±50% for the lowest calibration point and ±20% for all other standards.

11.4.3.3.2 RSE is calculated by using the following equation:

$$\%RSE = 100 * \sqrt{\sum_{i-1}^{n} \frac{[\hat{y}_i - y_i]^2}{y_i} \Big/ n - p}$$

where:

y_i = Actual response of the calibration level i
$\hat{y}_i$ = Predicted response at level i
p = Number of terms in the fitting equation (average = 1, linear = 2, quadratic 3)
n = Number of calibration points

The RSE acceptance limit criterion for the calibration model is ≤20%.

11.4.4 Evaluation of retention times - The RRT of each target analyte in each calibration standard should agree within 0.06 RRT units. Late-eluting target analytes usually have much better agreement. The RRT equation follows.

$$\text{RRT} = \frac{\text{Retention time of the analyte}}{\text{Retention time of the internal standard}}$$

11.4.5 Linearity of target analytes - If the RSD of any target analyte is ≤ 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).

11.4.5.1 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.

11.4.5.2 When the RSD exceeds 20%, the 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, the presence of active sites in the chromatographic system, analytes that exhibit poor chromatographic behavior, etc.

11.4.5.3 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 any 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.

11.4.5.4 The method of linear regression analysis has the potential for a significant bias to the lower portion of a calibration curve, while the relative percent difference 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 using the linear regression model, the analyst should perform a minimum quantitation check on the viability of the lowest calibration point by re-fitting 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 ±50% 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 re-fitting criteria should be considered "out-of-control." Corrective actions may be appropriate such as redefining the LLOQ and/or reporting those "out-of-control" target analytes as estimated when the concentration is at or near the lowest calibration point.

11.4.6 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 30% of the expected value(s). 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 - The 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/NIMS 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 Secs. 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 the minimum RFs are not met, the system should be evaluated, and corrective action should be 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; and active sites in the column or chromatographic system.

11.5.1.3 All target compounds of interest must be evaluated using a 20% criterion. Use percent difference when performing the average RF model calibration. Use 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 $\leq \pm$ 20%, then the initial calibration for that compound is assumed to be valid. Due to the 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 (i.e., > 20% difference or drift) for more than 20% of the compounds included in the initial calibration, then corrective action must be taken prior to the analysis of samples. In cases where 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. The calibration verification criteria must be met before sample analysis begins.

11.5.2 Method blank - A method blank should be analyzed prior to sample analyses in order to demonstrate that the total system (introduction device, transfer lines and GC/NIMS 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 - The 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 more

than 30 sec from that in the midpoint standard level of the most recent initial calibration sequence, then 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 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.

11.6 GC/NIMS 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/NIMS system. This pre-screening analysis 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/NIMS 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, equivalent to that added to the calibration standards, to the concentrated sample extract obtained from sample preparation. 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 the response for any quantitation ion exceeds the initial calibration range of the GC/NIMS system, 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/µL, 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, usually < 0.2 amu, in their spectra. These mass defects should be accounted for during data acquisition. The dwell time may be automatically calculated by the laboratory's GC/NIMS software or manually calculated using the formula below. The total scan time should be < 1000 msec 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.

$$\text{Dwell Time for the Group} = \frac{\text{Scan Time (msec)}}{\text{Total Ions in the Group}}$$

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 less 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 The RRT of the sample component is within ± 0.06 RRT units of the RRT of the standard component.

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. (Example: For an ion with an abundance of 50% in the reference spectrum, the corresponding abundance in a sample spectrum may range between 20% and 80%.) Use professional judgment in interpretation 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, using this criterion to decrease 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.

11.8.1.2.1 While toxaphene contains a large number of compounds that will produce well-resolved peaks in a GC/NIMS 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.

11.8.1.2.2 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, manually or under the data system control, 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.

11.8.1.2.3 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 disproportionally larger or smaller in the sample compared to the standard.

11.8.1.2.4 The heights or areas of the selected 4 to 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 to 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 Structural isomers that produce very similar mass spectra should be quantitated 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. 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.

11.8.5 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 Secs. 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. Therefore, performance data provided in this method are not intended to be and must not be used as absolute QC acceptance criteria for purposes of laboratory accreditation. Additionally, all performance data included in this method version 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, and optional cleanup techniques, and calibration procedures used.

13.4 Tables 2 through 4 represent SIM for method analytes using GC/NIMS.

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. These data are provided for guidance purposes only.

13.7 Table 7 contains single-laboratory performance data on spiked soil for toxaphene

congeners. These data are provided for guidance purposes only.

13.8 Table 8 contains single-laboratory performance data on spiked soil for organochlorine pesticides. These 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. The extracts from this study were further subjected to copper, acid, and silica gel celanup 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 celanup methods.

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

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

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

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

13.18 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* available from the American Chemical Society, Department of Government Relations and Science Policy, 1155 16th Street, NW, Washington, DC, 20036, (202) 872-4477), http://www.acs.org.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult *The 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/MS OPERATING CONDITIONS FOR TOXAPHENE AND TOXAPHENE CONGENERS USING A NARROW-BORE CAPILLARY COLUMN[1]

Column - 30-m x 0.25-mm ID fused silica capillary column J&W DB-XLB, 0.25-µm 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 minute
Injector mode	Splitless
Injector liner	4-mm I.D. splitless
Transfer line temperature	280°C
Initial oven temperature	60°C, hold 1 minute
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 minute followed by 320°C to 330°C at 50°C/min, hold 3.0 minutes
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 minutes
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

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

Table 2

SELECTED ION MONITORING FOR ANALYTES BY GC/NIMS

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		

[1] Total Ion Current

Table 3

SELECTED ION MONITORING FOR OTHER COMPOUNDS BY GC/NIMS

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

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

[2] Total Ion Current.

Table 4

SELECTED ION MONITORING FOR ANALYTES BY GC/NIMS

Compound	Quantitation Ion[1]	Other Ions	Comments
BHC	254.9	252.9, 256.9	M-Cl
Heptachlor[2]	299.9	297.9, 301.9, 234.9, 236.9, 238.9	M-Cl_2
Aldrin[2]	329.9	327.9, 331.9, 234.9, 236.9, 238.9	M-Cl+H
Dieldrin[2], endrin[2], endrin aldehyde[2]	379.8	377.9, 381.9, 234.9, 236.9, 238.9	M
Heptachlor epoxide[2]	387.8	385.9, 389.9, 234.9, 236.9, 238.9	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

[1] Ions 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 NIMS conditions.

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

[1] Data provided courtesy of Bill Brumley from US EPA ORD.

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	1,266	772
Avg	788	958	744
% RSD	16.1	17.8	13.4

[1] Data provided courtesy of Bill Brumley from US EPA ORD.

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

[1] Data provided courtesy of Bill Brumley from US EPA ORD and are included for informational purposes only to indicate that quantitation and acceptable recoveries of other organochlorine pesticide compounds using GC/NIMS is possible.

Table 9

PHASE I MULTI-LABORATORY VALIDATION DATA FOR UNKNOWN SPIKED STANDARDS

Toxaphene

ID	No. of Labs	True Value (pg/µL)	Grand Mean (pg/µL)	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/µL)	Grand Mean (pg/µL)	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/µL)	Grand Mean (pg/µL)	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/µL)	Grand Mean (pg/µL)	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/µL)	Grand Mean (pg/µL)	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/µL)	Grand Mean (pg/µL)	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/µL)	Grand Mean (pg/µL)	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/µL)	Grand Mean (pg/µL)	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/µL)	Grand Mean (pg/µL)	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%

Table 10

PHASE II MULTI-LABORATORY VALIDATION DATA USING SPIKED EXTRACTS OF UNCONTAMINATED REAL-WORLD SAMPLE MATRICES[1]

Toxaphene

ID	No. of Labs	True Value (pg/µL)	Grand Mean (pg/µL)	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/µL)	Grand Mean (pg/µL)	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/µL)	Grand Mean (pg/µL)	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%

[1] Liquid samples were solvent-extracted with methylene chloride according to Method 3510 using a sample volume of 1 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 MATRICES[1]

P26

ID	No. of Labs	True Value (pg/µL)	Grand Mean (pg/µL)	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/µL)	Grand Mean (pg/µL)	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/µL)	Grand Mean (pg/µL)	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 1 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 MATRICES[1]

P44

ID	No. of Labs	True Value (pg/µL)	Grand Mean (pg/µL)	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/µL)	Grand Mean (pg/µL)	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/µL)	Grand Mean (pg/µL)	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 1 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 11

PHASE II MULTI-LABORATORY VALIDATION DATA USING REAL-WORLD SAMPLES FROM TERRY CREEK IN BRUNSWICK, GEORGIA[1]

SEDIMENT

ID	No. of Labs	Grand Mean (pg/µL)	Grand Mean (mg/kg)	Std. Deviation (pg/µL)	Reproducibility (RSD)
Toxaphene	9	173,269	36,300	91,028	53%
Hx-Sed	9	3,599	754	366	10%
Hp-Sed	9	3,703	776	526	14%
P26	8	1,247	261	163	13%
P40	9	2,659	557	951	36%
P41	9	1,305	273	1530	117%
P44	8	902	189	172	19%
P50	9	1,775	372	231	13%
P62	8	3,835	803	1,412	37%

SOIL

ID	No. of Labs	Grand Mean (pg/µL)	Grand Mean (mg/kg)	Std. Deviation (pg/µL)	Reproducibility (RSD)
Toxaphene	9	2,980	624	1,727	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%

[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 isooctane.

Table 12

PHASE II MULTI-LABORATORY VALIDATION DATA USING REAL-WORLD SAMPLES FROM TERRY CREEK IN BRUNSWICK, GEORGIA WITH EXTRACTS SUBJECTED TO CLEANUP [1]

Technical Toxaphene

ID	No. of Labs	True Value (pg/µL)	Grand Mean (pg/µL)	Grand Mean (mg/kg)	% Rec.	Std. Dev. (pg/µL)	Reproducibility (RSD)
TC Sed	4	---	304,896	63,876	---	47,233	15%
TC Soil	4	---	15,559	3,260	---	4,574	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/µL)	Grand Mean (pg/µL)	Grand Mean (mg/kg)	% Rec.	Std. Dev. (pg/µL)	Reproducibility (RSD)
TC Sed	4	---	3,684	772	---	183	5%
TC Soil	4	---	1,382	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/µL)	Grand Mean (pg/µL)	Grand Mean (mg/kg)	% Rec.	Std. Dev. (pg/µL)	Reproducibility (RSD)
TC Sed	4	---	4,217	883	---	993	24%
TC Soil	4	---	1,104	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%

[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 12 (cont.)

PHASE II MULTI-LABORATORY VALIDATION DATA USING REAL-WORLD SAMPLES FROM TERRY CREEK IN BRUNSWICK, GEORGIA WITH EXTRACTS SUBJECTED TO CLEANUP[1]

P26

ID	No. of Labs	True Value (pg/µL)	Grand Mean (pg/µL)	Grand Mean (mg/kg)	% Rec.	Std. Dev. (pg/µL)	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/µL)	Grand Mean (pg/µL)	Grand Mean (mg/kg)	% Rec.	Std. Dev. (pg/µL)	Reproducibility (RSD)
TC Sed	4	---	4,201	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/µL)	Grand Mean (pg/µL)	Grand Mean (mg/kg)	% Rec.	Std. Dev. (pg/µL)	Reproducibility (RSD)
TC Sed	4	---	1,234	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%

[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.

PHASE II MULTI-LABORATORY VALIDATION DATA USING REAL-WORLD SAMPLES FROM TERRY CREEK IN BRUNSWICK, GEORGIA WITH EXTRACTS SUBJECTED TO CLEANUP[1]

P44

ID	No. of Labs	True Value (pg/µL)	Grand Mean (pg/µL)	Grand Mean (mg/kg)	% Rec.	Std. Dev. (pg/µL)	Reproducibility (RSD)
TC Sed	3	---	1,394	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/µL)	Grand Mean (pg/µL)	Grand Mean (mg/kg)	% Rec.	Std. Dev. (pg/µL)	Reproducibility (RSD)
TC Sed	4	---	2,923	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/µL)	Grand Mean (pg/µL)	Grand Mean (mg/kg)	% Rec.	Std. Dev. (pg/µL)	Reproducibility (RSD)
TC Sed	3	---	8,836	1,851	---	1,548	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 isooctane.

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] Extraction only	No. of Labs[2] Cleanup	Grand Mean (pg/µL)[1] Extraction only	Grand Mean (pg/µL)[2] Cleanup	Grand Mean (mg/kg)[1] Extraction only	Grand Mean (mg/kg)[2] Cleanup
Toxaphene	9	4	173,269	304,896	36,300	63,876
Hx-Sed	9	4	3,599	3,684	754	772
Hp-Sed	9	4	3,703	4,217	776	883
P26	8	4	1,247	1,870	261	392
P40	9	4	2,659	4,201	557	880
P41	9	4	1,305	1,234	273	258
P44	8	3	902	1,394	189	292
P50	9	4	1,775	2,923	372	612
P62	8	3	3,835	8,836	803	1,851

SOIL

ID	No. of Labs[1] Extraction only	No. of Labs[2] Cleanup	Grand Mean (pg/µL)[1] Extraction only	Grand Mean (pg/µL)[2] Cleanup	Grand Mean (mg/kg)[1] Extraction only	Grand Mean (mg/kg)[2] Cleanup
Toxaphene	9	4	2,980	15,559	624	3,260
Hx-Sed	9	4	153	1,382	32	290
Hp-Sed	9	4	129	1,104	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 isooctane.

[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 isooctane.

Table 14

PHASE II MULTI-LABORATORY VALIDATION DATA USING MULTIPLE TECHNICAL TOXAPHENE SPIKING PROTOCOLS FOR UNCONTAMINATED REAL-WORLD SAMPLE MATRICES[1]

Technical Toxaphene
Spiked Prior to Extraction and Clean-up

ID	No. of Labs	True Value (pg/µL)	Grand Mean (pg/µL)	% Recovery	Std. Deviation (pg/µL)	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/µL)	Grand Mean (pg/µL)	% Recovery	Std. Deviation (pg/µL)	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 MATRICES[1]

Technical Toxaphene
Spiked in the Final Extract

ID	No. of Labs	True Value (pg/µL)	Grand Mean (pg/µL)	% Recovery	Std. Deviation (pg/µL)	Reproducibility RSD
Sed 3L	4	75	86	114%	10	12%
PCB 209 Surr	4	100	105	105%	5	5%
eHCH Surr	4	250	276	111%	21	7%
Sed 3M	4	150	157	105%	21	13%
PCB 209 Surr	4	100	105	105%	5	5%
eHCH 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 isooctane.

FIGURE 1

EXAMPLE GAS CHROMATOGRAM OF A MIXED TOXAPHENE CONGENERS STANDARD

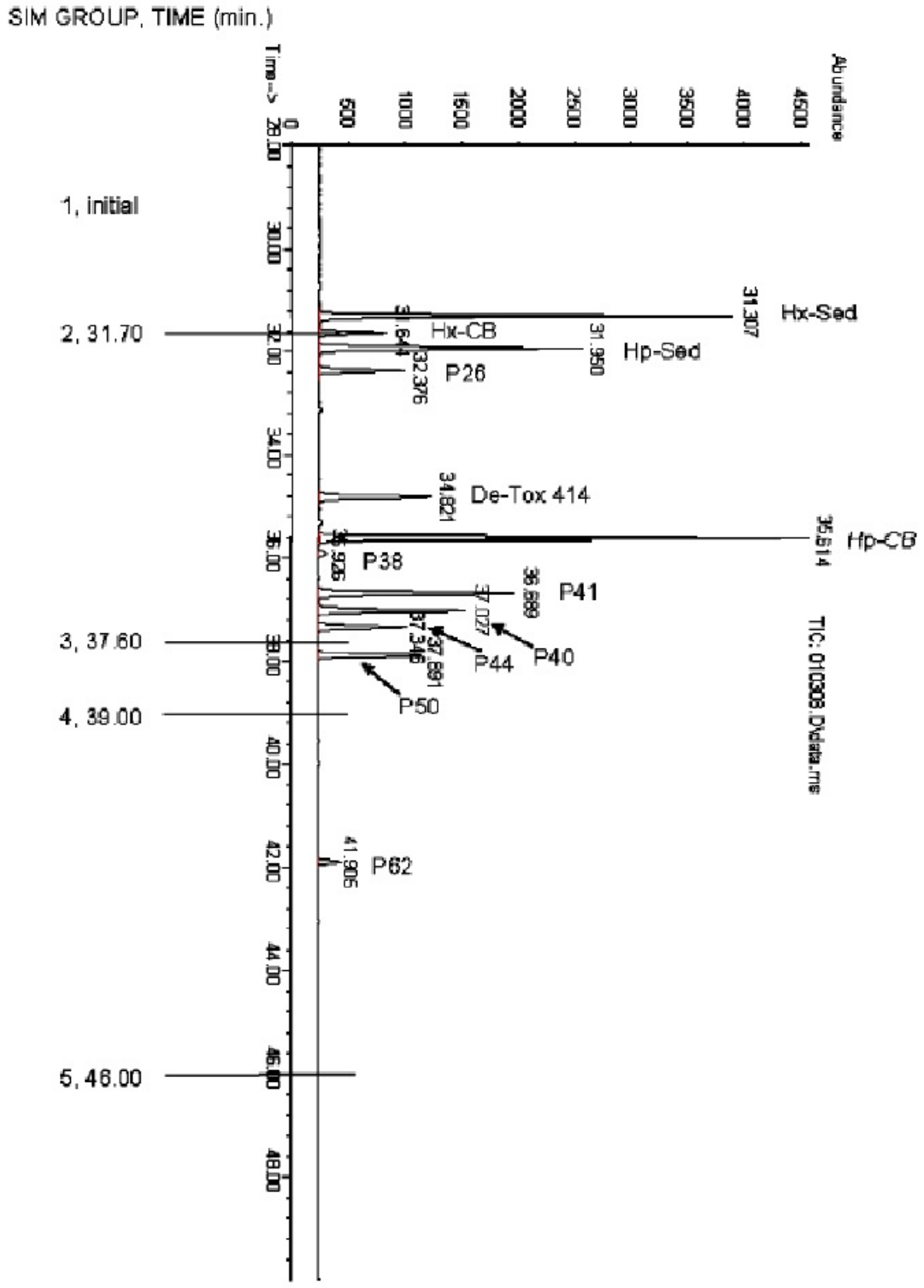

FIGURE 2

EXAMPLE GAS CHROMATOGRAM OF A FISH TISSUE EXTRACT
WITH WEATHERED TOXAPHENE AND SPIKED USING DECACHLOROBIPHENYL

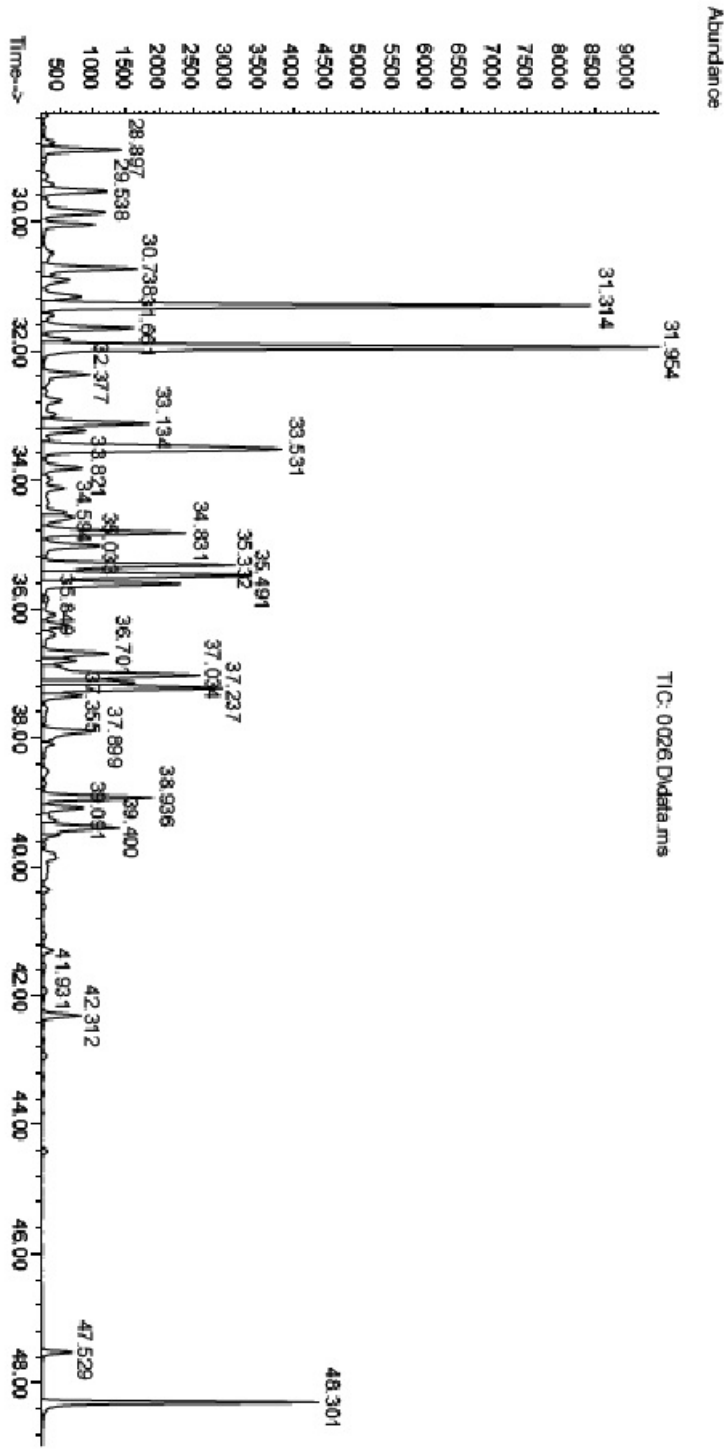

FIGURE 3

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

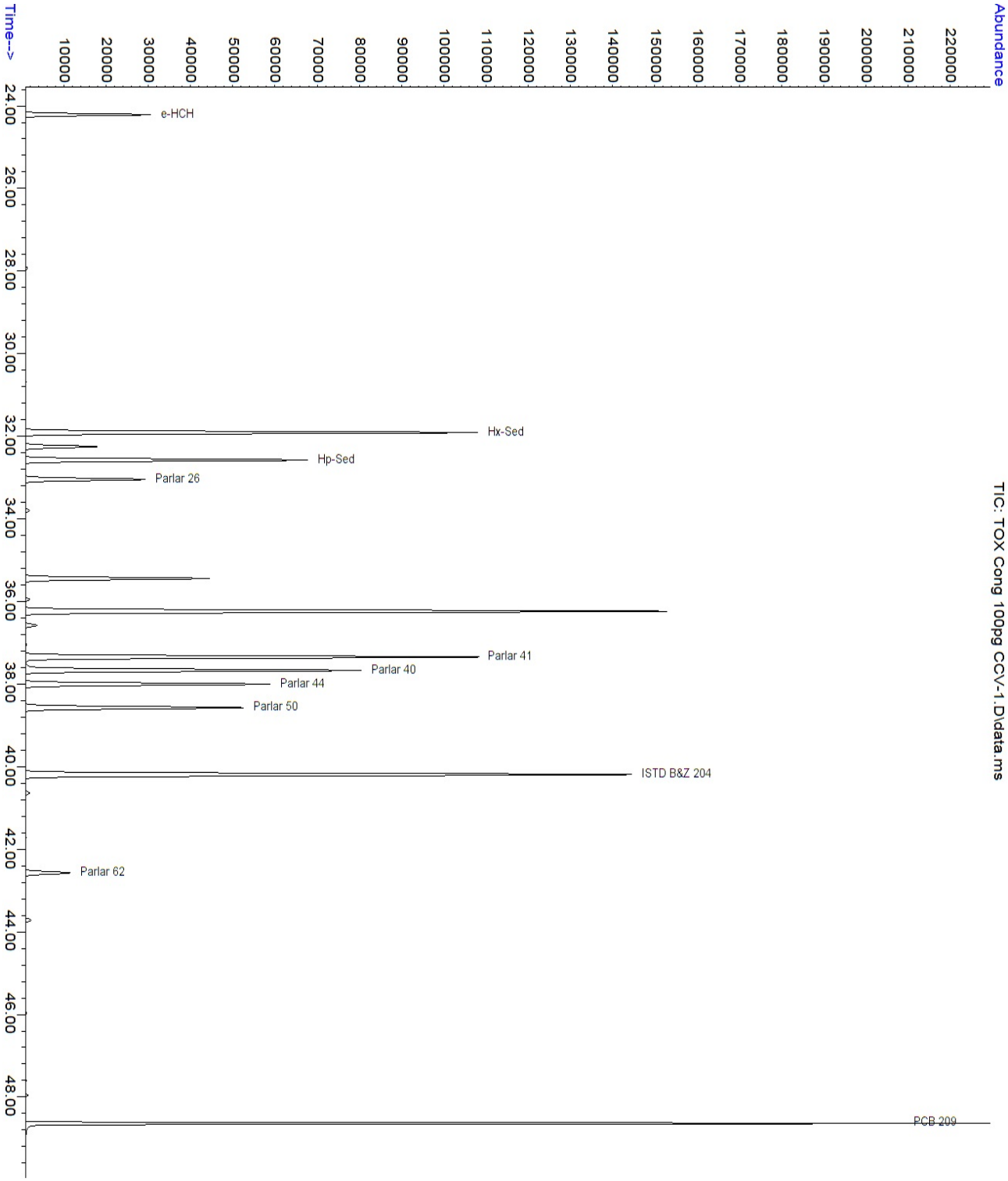

FIGURE 4

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