Standard Operating Procedure for:

ANALYSIS OF CHLORDANE AND TOXAPHENE USING GC/MS WITH NEGATIVE CHEMICAL IONIZATION

DEPARTMENT OF ENVIRONMENTAL PROTECTION
CHEMISTRY SECTION
TALLAHASSEE FLORIDA

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

CONTENTS

1. SCOPE AND APPLICATION

2. SUMMARY OF THE METHOD

3. INTERFERENCES

4. DEFINITIONS

5. APPARATUS AND EQUIPMENT

    5.1. Apparatus
    5.2. Data System
    5.3. Gases

6. REAGENTS AND CHEMICALS

    6.1. Solvents
    6.2. Standards
    6.3. Stock and intermediate standards
    6.4. Working standard solutions
    6.5. Spiking Solutions
    6.6. Continous Calibration Verification (CCVs)

7. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

8. SAMPLE PREPARATION PROCEDURE

9. GENERAL DESCRIPTION OF GC/MS-NCI ANALYSIS

    9.1 GC/MS Maintenance and Tuning
    9.2 GC/MS system Initial Calibration
    9.3 Daily GC/MS Performance and Calibration Check
    9.4 GC/MS Sample Analysis
    9.5 Data processing using the Masshunter software
    9.6 Analysis of Chlordane and Toxaphene using GC/MS-NCI in the SIM mode

10. REPORTING AND DOCUMENTATION

11. QUALITY CONTROL

12. DATA ARCHIVAL

13. DETERMINATION OF METHOD DETECTION LIMITS

14. SAFETY

15. REFERENCES

16. TABLES

17. APPENDICES

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

1. SCOPE AND APPLICATION

1.1. This method is applicable to the determination of Chlordane technical and Toxaphene in water, soil, waste and tissue samples as listed in Table 1. Method Detection Limits and Practical Quantitation Limits yielded by this method are also provided in Tables 1.

1.2. This method is based on EPA 8276 revision 1 (ref 15.1) Laboratory Information Management System (LIMS) tests: W-CT-CI-R, S-CT-CI, WS-CT-CI, T-CT-CI, SPLP-CT-CI and TCLP-CT-CI.

1.3. This Standard Operation Procedure is restricted to use by an analyst experienced in the use of an Agilent 5977 GC/MS system, and in the use of appropriate computer programs. The minimum qualifications required:

- B.S. in Chemistry or Biochemistry with minimum one year of professional chemical or biochemical experience
- Knowledge of principles and practice of gas chromatography/mass spectrometry
- Ability to perform basic laboratory mathematics
- Knowledge of the Masshunter GC/MS software and DEP LIMS.

2. SUMMARY OF THE METHOD

Soil and waste samples are extracted using the appropriate procedures listed in the Preparation SOP- GC-002 (ref 15-2). Tissue samples are extracted according to SOP-GC-004 (ref 15-13). Water samples are extracted according to SOP-GC-036-1 (ref 15-14) The extracts are analyzed using an Agilent 5977 GC/MS system operating in the Negative Chemical Ionization mode (NCI) using methane as the reagent gas. Qualitative identification of the parameters in the extract is performed using the retention time and the relative abundance of the characteristic SIM ions. Quantitative analysis is performed using an internal standard technique.

3. INTERFERENCES

3.1. Raw GC/MS data from all blanks, samples, and spikes must be evaluated for interferences. Determine if the source of interference is in the preparation and/or cleanup of the samples and take corrective action to eliminate the problem.

3.2. Contamination by carryover can occur whenever high-level and low-level samples are analyzed sequentially. To reduce carryover, rinse the syringe several times with solvent between samples. It may be necessary to analyze one or more solvent blanks between samples and even change out the syringe in extreme cases.

3.3 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in gas chromatograms. All of these materials must routinely demonstrate to be free from interferences under the conditions of the analysis by running laboratory reagent blanks. Glassware must be scrupulously cleaned. Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. Solvent rinsing should be followed by rinsing with DI water. The glassware should then be drained dry and heated in a muffle furnace at 550 °C for 8 hours. Some thermally stable materials may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may supplement the muffle furnace heating. Volumetric glassware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil. The use of high purity reagents and solvents helps

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required or irradiation with UV light. Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the sample characteristics.

4. DEFINITIONS

4.1. ACCURACY: The closeness of agreement between an observed value and an accepted reference value. When applied to a set of observed values, accuracy will be a combination of a random component and of a common systematic error (or bias) component.

4.2. BATCH: A group of samples, which behave similarly with respect to the sampling or the testing procedures being employed, that are processed as a unit. For QC purposes, if the number of samples in a group is greater than 20, then each group of 20 samples or less will all be handled as a separate batch.

4.3. BIAS: The deviation due to matrix effects of the measured value (Xs - Xu) from a known spiked amount. Bias can be assessed by comparing a measured value to an accepted reference value in a sample of known concentration or by determining the recovery of a known amount of contaminant spiked into a sample (matrix spike). Thus, the bias (B) due to matrix effects based on a matrix spike is calculated as:

$$B = (Xs - Xu) - K$$

where:
Xs = measured value for spiked sample,
Xu = measured value for unspiked sample, and
K = known value of the spike in the sample.

Using the following equation yields the percent recovery (%R).

$$\%R = 100 (Xs - Xu) / K$$

4.4. PRACTICAL QUANTITATION LIMIT (PQL): The lowest concentration that can be reliably achieved within specified limits of precision and accuracy during routine laboratory operating conditions. The PQL is generally 3 to 5 times the MDL. However, it may be nominally chosen within these guidelines to simplify data reporting. For many analytes, the PQL concentration is selected as the lowest non-zero standard in the calibration curve. Sample PQLs are highly matrix dependent.

4.5. FIELD DUPLICATES: Independent samples which are collected as close as possible to the same point in space and time. They are two separate samples taken from the same source, stored in separate containers, and analyzed independently. These duplicates are useful in documenting the precision of the sampling process.

4.6. INTERNAL STANDARD: A pure compound added to a sample extract in known amounts and used to calibrate concentration measurements of other compounds that are sample components. The internal standard must be a compound that is not a sample component.

4.7. LABORATORY FORTIFIED BLANK/LABORATORY CONTROL SAMPLE (LFB/LCS): A known matrix spiked with compound(s) representative of the target analytes. The LFB is analyzed exactly as a sample. This is used to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

4.8. MATRIX: The component or substrate (e.g., surface water, drinking water) which contains the analyte of interest.

4.9. MATRIX SPIKE: An aliquot of sample spiked with a known concentration of target analyte(s). The spiking occurs prior to sample preparation and analysis. A matrix spike is used to document the bias of a method in a given sample matrix.

4.10. MATRIX SPIKE DUPLICATES: A second aliquot of the same sample used for matrix spike spiked with identical concentrations of target analyte(s). The spiking occurs prior to sample preparation and analysis. They are used to document the precision and bias of a method in a given sample matrix.

4.11. METHOD BLANK: An analyte-free matrix to which all reagents are added in the same volumes or proportions as used in sample processing. The method blank must be carried through the complete sample preparation and analytical procedure. The method blank is used to document contamination resulting from the analytical process. For a method blank to be acceptable for use with the accompanying samples, the concentration in the blank of any analyte of concern shall not be higher than the method detection limit

4.12. METHOD DETECTION LIMIT (MDL): The minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix type containing the analyte. For operational purposes, when it is necessary to determine the MDL in the matrix, the MDL should be determined by multiplying the appropriate one-sided 99% t-statistic by the standard deviation obtained from a minimum of seven analyses of a matrix spike containing the analyte of interest at a concentration one to five times the estimated MDL.

4.13. ORGANIC-FREE REAGENT WATER: All references to water in the method refer to water in which interference is not observed at the method detection limit of the compounds of interest. Organic-free reagent water can be generated by passing tap water through a carbon filter bed containing about 1 pound of activated carbon. A water purification system may be used to generate organic-free de-ionized water.

4.14. PRECISION: The agreement among a set of replicate measurements without assumption of knowledge of the true value. Precision is estimated by means of duplicate analyses. These samples should contain concentrations of analyte above the MDL, and involve the use of matrix spikes or laboratory fortified blanks. The estimates of precision are based on the relative percent difference (RPD).

$$RPD = 100 [2 (X1 - X2) / (X1 + X2)]$$

where X1 and X2 are the two measurements

4.15. INITIAL CALIBRATION VERIFICATION (ICV): A standard solution from a source, other than normal calibration standards, that is certified and traceable. These standards are used to check the accuracy of a calibration curve immediately after the calibration standards are run.

4.16. CONTINOUS CALIBRATION VERIFICATION (CCV): A mid-level calibration standard solution from a source that is certified and traceable. These standards are used to check the accuracy of a calibration curve on daily bases.

4.17. SPIKE SOLUTION (SS): A solution of method analytes of known concentrations that is used to fortify an aliquot of laboratory reagent water or sample matrix.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

4.18. STANDARD CURVE: A plot of concentrations of known analyte standards versus the instrument response to the analyte. Calibration standards are prepared by successively diluting a standard solution to produce working standards, which cover the working range of the instrument. Standards should be prepared at the frequency specified in section six (6) of this SOP. The calibration standards should be prepared using the same type of solvent as the final sample preparation solvent.

4.19. SURROGATE: An organic compound which is similar to the target analyte(s) in chemical composition and behavior in the analytical process, but which is not normally found in environmental samples. Surrogates are used to monitor the preparation and are added to each field and QC sample prior to extraction.

4.20. PQL SPIKE: also known as A Lower Limit of quantitation (LLOQ) check standard (spike). An aliquot of sample spiked with target analyte(s) at PQL concentration. Either clean control material or representative sample matrix may be used here. The spiking occurs prior to sample preparation and analysis. A PQL spike is used to document the bias of a method in a clean matrix or sample matrix at the quantitation limit.

5. APPARATUS AND EQUIPMENT

5.1 Apparatus.

Agilent 5977 GC/MS single quadrupole system or equivalent capable of operating in the chemical ionization mode. Must be temperature programmable with an injector port designed for split/splitless injection. A Multi-Mode Inlet (MMI) may be used, if available. The GC, GC Automatic Sampler and MSD operating parameters are illustrated in Tables 2, 3 and 4. The analytical column is an HP5MS UI or equivalent, fused silica capillary, 30 m x 0.25 mm x 0.25 um, operated in 5-25m backflash configuration.

5.2 Data system

5.2.1 A computer system is interfaced to the GC/MS that allows the continuous acquisition and storage on machine-readable media of all chromatographic measurements obtained throughout the duration of the chromatographic run sequence program.

5.2.2 This system currently uses the Masshunter software from Agilent Technologies Inc. For reporting data, the system currently uses DEP LIMS.

5.3 Gases

5.3.1 Helium, UPC Grade or higher, is used as the carrier gas. The helium is passed through an Agilent Gas Clean filter before it enters the GC.

5.3.2 Methane, UPC Grade or higher, is used as the reagent gas. The CH_4 is passed through a HP/Agilent Chemical Ionization Gas Filter before entering the MSD.

6. REAGENTS AND CHEMICALS

6.1. Solvents

6.1.1. Methylene Chloride, Fisher Scientific, Pesticide, HPLC or OPTIMA grade.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

6.1.2. Isooctane, Fisher Scientific, Pesticide, HPLC or OPTIMA grade.

6.2. Standards

6.2.1. For detailed standard preparation see APPENDIX 1

6.3. Stock and Intermediate Standards

6.3.1. Custom made stock standards and/or neat standards for individual analytes available) are purchased from commercial suppliers. The concentrations of (two different sources or two different lot numbers if only one vendor is available) currently purchased custom made standards are listed in the appendix 1: “Standard preparation procedure”.

6.3.2. Intermediate standard solutions are mixtures of individual standards used to prepare working standards. They are prepared in our lab from stock standards by mixing / diluting with isooctane or acetone. They can also be purchased from a certified manufacturer (ISO 9000 or EPA certified). The concentrations of the components in the intermediate standard are typically several mg/L. Intermediate standards are replaced once per year. New standard is checked vs. a second source (QC Standard), after diluting it to one of the working standard levels. The criterion of acceptance is +/- 10% difference. All standard preparations and verifications have to be recorded in the Standard Preparation Tracker located in DEP LIMS. A list containing all standards with their serial numbers used in the analysis must be part of analysis documentation folder. Currently standard serial numbers are entered in the analysis run sequence.

6.4. Working Standard Solutions (Calibration standards)

6.4.1. Working standard solutions are a series of diluted individual or mixture standards in isooctane; v/v, they are used for calibration and are prepared by diluting intermediate standard solutions. The internal standard listed below is added to every working standard at 100 µg/L.

Internal Standards for Chlordane/Toxaphene analysis:

2,3,3’,4,4’5-Hexachlorobiphenyl-2’,6,6’-d3 (HCB-d3)
Alpha-BHC-d6
Octachloronaphthalene (optional for Decachlorobiphenyl)

6.4.2. The concentrations for each analyte in the working (calibration) standards are listed in Table 5. The second source (ICV) and CCV standards are prepared at midpoint level. Since it is very difficult to obtain matching Toxaphene standards, two ICV standards may be run (three independent sources). The middle concentration one is used for calibration.

6.5. Spiking Solution

6.5.1. These solutions are used when fortifying sample matrix spikes or lab fortified blanks at extraction. These fortified samples are used to verify the accuracy and precision of the sample preparation and analysis.

6.5.2. Spike solutions are prepared by diluting stock or intermediate standards with acetone. Concentrations of the parameters in these solutions are listed in Table 5.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

6.5.3. Freshly prepared spike solutions are checked vs. valid working standards. The acceptance criterion is ± 20% difference.

6.6. Second Source (ICV) and Continuous Calibration Verification (CCV)

6.6.1 Second Source Standards are prepared from a source, other than calibration standards, that is certified and traceable. These standards are used to check the integrity of the calibration standards and are run and analyzed whenever an initial calibration curve is built. The acceptance criterion is ± 30% difference.

6.6.2 Continuous Calibration Verification (CCV). The Analyst must ensure that the calibration standards are checked with the corresponding CCV with every initial calibration and then in 12 hr intervals thereafter. The acceptance criteria is +- 20%.

7. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

7.1. Samples must be collected in glass containers with Teflon-lined screw caps.

7.2. All soil samples must be refrigerated at 6 degrees C or less from time of collection until extraction. All tissue samples must be refrigerated at less than -10 degrees C from the time of collection until extraction. All sample extracts must be kept at 6 degrees C or less.

7.3. All samples must be extracted within 7 days of collection (waters) or 14 days of collection (soils) and completely analyzed within 40 days of extraction. If the water samples are to be analyzed for Aldrin and residual Chlorine is present, then add 80 mg of Sodium Thiosulfate per 1 L of sample and mix well. Tissue samples must be extracted within 365 days of collection and completely analyzed within 40 days of extraction.

8. SAMPLE PREPARATION PROCEDURE

8.1. For soil samples see Preparation SOP GC-002: Extraction of pesticides and polychlorinated biphenyls (PCBs) from Water Wastes, soil/sediments PUFs and filters for subsequent analysis by GC or GC/MS techniques (ref 15.2). Also refer to Appendix 5 “Preparation Flowcharts” For soil samples 30 g of sample is extracted with 1:1 methylene chloride/acetone, solvent exchanged to isooctane and then concentrated to 2 mL of final volume. The soil extracts are further cleaned up by using GPC/Florisil or H2SO4/TBA methods. 1mL sample is typically vialed for GPC clean-up; 0.8 mL is injected and 0.7 ml is submitted to allow for the ISTD addition (100 uL of 800 ppb solution). If sulfuric acid is used TBA cleanup may be performed. 1mL of sample is used and blown down to 900 uL to allow for the ISTD addition (100 uL of 1000 ppb solution).

8.2. For tissue samples see preparation SOP GC-004-1 (ref 15-13). 5 g tissue samples are extracted with Methylene Chloride using DIONEX ASE 350 pressurized fluid extraction system. The final volume is 2 mL in isooctane. The extracts are further cleaned up by using GPC/Florisil or H2SO4 following the procedure described above for the soil samples. TBA cleanup is not necessary for tissue extracts.

8.3. For water samples see SOP GC-036-1 (ref 15-14). 500 g pre-filtered water samples are extracted using C-18 SPE cartridges and eluted with Methylene Chloride. The final volume is 0.25 mL in Iso-8. Internal standard is added before the final blowdown.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

8.4. Extreme care has to be exercised by all laboratory staff involved in preparation and analysis to limit background contamination due to ultra-low detection limits achievable by using the Negative Chemical Ionization techniques.

9. GENERAL DESCRIPTION OF GC/MS-NCI ANALYSIS

9.1. GC/MS-NCI Maintenance and Tuning

9.1.1. Initial checking and maintenance of the instrument consists mainly of the following steps:

9.1.1.1. Check the vacuum operation and periodically make sure there is no air/water leak. This is determined in PCI mode by checking for m/z 32 after the methane pretune. Air/water leak test should be run each time the vacuum system is vented. It may take several hours (even overnight) of pumping down to bring the air/water content below 5%!

9.1.1.2. Perform the GC/MS CI autotune before every time the initial calibration is run. There are no tune performance criteria. If autotune completes, it passes. The EM Volts should not go above 2600V. Print the autotune report and include with the documentation. The typical autotune report is shown in the appendix 2. Save the current settings in the autotune file NCICH4.U. If the autotune fails, the ion source may have to be cleaned.

9.1.1.3. Each time after the ion source was cleaned the instrument may have to be tuned for CI operation starting with the methane gas adjustment. Those steps include methane pretune, methane flow adjustment and PCI autotune preceding the NCI autotune. For details refer to the MSD operating manual (ref 15.12)

9.1.1.4. Replace the injector liner (Agilent 4 mm single taper liner for splitless injection), cut 1-3 inch of the column after running contaminated samples or encountering unusually heavy matrix. Occasional cutting the column is still necessary even if backflush technology is used.

9.1.1.5. Run an Instrument Blank and look for instrument contamination.

9.1.1.6. In case the instrument is contaminated raise the furnace temperature to 260 ^{o}C and leave it overnight.

9.1.1.7. To perform maintenance of the split/splitless inlet replace the liner and septum and clean the inside of the inlet body using a steel brush and/or with cotton swabs soaked with methanol and methylene chloride.

9.1.1.8. The autosampler syringe has to be frequently and thoroughly cleaned to avoid injection repeatability problems. A dirty plunger will cause reproducibility problems, bad injections occurring randomly, and poor peak shapes and calibration problems. Replacing the syringe on regular, frequent basis is highly recommended.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

9.1.1.9. Re-check for instrument contamination and if needed repeat the procedure until the instrument is contamination free.

9.2. GC/MS-NCI System Initial Calibration

9.2.1. Analyze the calibration standards at levels listed in Table 5. At each level, the internal standards should be present at 100 µg/L. The linearity of the calibration curve is defined by an RSD of <20% of the Response Factors of the calibration curve, or by a correlation coefficient not smaller than 0.99. If a higher order curve is to be used the correlation coefficient has to be at least 0.99. When new calibration is inspected linear fit is usually tried first, followed by average response factor (%RSD which is equivalent to weighted linear forced through origin), then followed by a quadratic fit. Do not use the "include" option for the origin. "Ignore origin" should be used preferentially though forcing through origin may have to be used under some circumstances. "Force" origin option typically works better whenever the lowest calibration level is considerably higher than the MDL. When calibration curve fit is selected in the Masshunter software the % calibration drift has to be monitored in order to have all the calibration levels within 70-130% of the expected value. Special care has to be taken to carefully fit the curves at the lower end of the calibration range where the vast majority of problems occur. All calibration curves should be weighted curves (1/x or 1/x2); do not use equal weighing. It is permissible to remove individual analyte calibration levels from either the lowest and/or highest levels of the curve (the PQL may have to be adjusted). Removal of interior levels is forbidden. An entire single standard calibration level may be removed from the interior of the curve whenever the standard was not correctly injected or a wrong standard was injected. If more than 10% of the total number of analytes fail the calibration fit criteria or more than 20% of analytes fail the % calibration drift criteria (CCV), then the instrument system must be corrected. Some possible problems include standard mixture degradation, injection port inlet contamination, analytical column contamination, and active sites in the column or chromatographic system.

9.2.2. Each time the column length is trimmed due to contamination or a new column is installed the retention times for each compound will shift to some degree. Therefore, it may be necessary to readjust the acquisition start window for the second segment containing only Decachlorobiphenyl surrogate. The first acquisition window containing all the other SIM ions covers the rest of the chromatogram and does not need to be adjusted. Both the analysis end of the method (Masshunter Quant) as well as the data-gathering end of the method (Masshunter - Acquisition) must be adjusted. Acquisition method parameters such as SIM ions, the acquisition time segments and dwell times are given in table 4A. Table 4B shows quantifier and qualifier ions for all analytical peaks. All the secondary ions should all be present and the relative intensities should agree within ±30% (absolute) of the reference mass spectrum after background subtraction is performed.

9.2.3 Initial calibration curve is always immediately checked with the second source initial calibration verification standard (ICV). The acceptance criterion is ±30%.

9.3 Daily GC/MS Performance and Calibration Check

The initial calibration curve and instrument performance must be verified on each day of analysis by the measurement of one or more check standards.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

9.3.1 Analyze midpoint level standards as continuing calibration check (CCV) standards. These calibration check standards must be analyzed at the start of each run and at 12-hour intervals thereafter. The acceptance criteria for all components are 80-120% recovery. If the CCV fails corrective action must be taken prior to the analysis of samples. When compounds fail, they may still be reported as non-detects (with a "J" qualifier) if it can be demonstrated that there was adequate sensitivity to detect the compounds at the applicable quantitation limit. For situations when the failed compound is present, the concentrations in the affected samples must be reported as estimated.

9.3.2 Calibration verification standard must also meet the following criteria:

9.3.2.1 Each of the target analytes in the calibration verification standard should meet the appropriate minimum RFs. The minimum RF of the most common target analytes must be within 50% of the original calibration. If minimum RFs are not met, the system should be evaluated, and corrective action taken before sample analysis begins.

9.3.2.2 Internal standard responses: the peak area for each of the internal standards may not change beyond the factor of two (-50% to +100%) from that of the average internal standard response of all the levels in the most recent initial calibration.

9.3.2.3 Internal standards retention times- the retention times of the internal standards in the calibration verification standard must not drift by more than 30 seconds from that of the mid-point standard level of the most recent initial calibration.

9.3.2.4 If any of the requirements mentioned in 9.3.3 are not met, the system must be evaluated, and corrective action must be taken before sample analysis begins. Some possible problems are standard mixture degradation, injection port inlet contamination, contamination at the front end of the analytical column, active sites in the column or chromatographic system, and a contaminated MS source.

9.3.2.5 NOTE: the decrease in the response area of the internal standards and/or target analytes may be due to the use of a hydrocarbon reagent gas (methane) in the negative ionization mode and a decreased source operating temperature. These conditions make the ion source susceptible to a buildup of a hydrocarbon film on its surface. This in turn may lead to lower signal. If this decrease of signal is gradual, it should not signify anything worse than hydrocarbon buildup. If the signal drops rapidly over one or two runs, something else is happening and measures should be taken immediately to remedy the problem.

9.4 GC/MS-NCI Sample Analysis.

9.4.1 Quantitative analysis of all analytes is accomplished by the internal standard calibration method. All target component positives found have to be quantified using at least a five-point calibration curve (for linear fit), at least six points for quadratic and at least seven points for higher order curves. Each sample extract MUST contain 100 µg/L of each internal standard. Any extract that is determined, in initial analysis, to contain high concentrations of any of

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

the target analytes should be diluted as deemed necessary. **The concentration of internal standard must remain at 100 µg/L when diluting the samples.**

9.4.2 Analysis Run Sequence.

The analyst will set up an analysis run sequence by placing the GC vials containing the sample extracts and standards on the autosampler tray in the following order or similar:

- isooctane (to clean the system)
- Instrument Blank (containing internal standards at 100 ug/L)
- pesticides calibration set (when initial calibration is run, levels 1-8)
- ICV second source initial calibration check
- CCV continuing calibration check
- extraction blank
- laboratory fortified blank (LFB)
- laboratory fortified blank duplicate
- sample matrix spike,
- sample matrix spike duplicate
- samples (for up to 12 hrs)
- CCV continuing calibration check
- samples (for up to 12 hours)

9.4.3 Print a hard copy of the run sequence and place it in the final data folder.

9.5 Data Processing using the Masshunter software.

9.5.1 Examine the quantitation reports soon after the samples have been analyzed. Check all of the calibration check standards analyzed during the run sequence and ensure that they met the minimum criteria given above.

9.5.2 The concentration reported for an identified hit in the sample extract, expressed in ug/L, is quantitated using the initial calibration curve. However, the analyst must ensure that all identification criteria are fulfiled and the peak area integration was correctly done. If not, the integration has to be re-done by choosing a new set of integration parameters or by manual integration (see SOP CM-018.1 for lab manual integration, ref 15.10).

9.5.4 If the response for any of the compounds exceeds the calibration curve, the extract must be diluted so that the response will be within the limits of the curve, and then reanalyzed. Make sure when performing dilutions to use isooctane spiked with the appropriate levels of internal standard so that their response will be the same in the dilution as it was in the original extract.

9.5.5 The internal standard responses and retention times in the calibration check standard (CCV) must be evaluated immediately after data acquisition. If the retention time for any internal standard changes by more than 30s from the last calibration, then the chromatographic system must be evaluated for either a) chromatographic matrix induced shift or inspected for malfunctions (such as leaks) and corrections must be made. The m/z abundances for all of the internal standards must be within a factor of two (50% to 200%) from the abundance in the daily calibration standard check. Corrective action is indicated if the m/z abundance is outside of these limits. If the holding time for a sample has expired and the internal standard areas are out of the acceptance range, then the

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

final report must be comented and any positives detected must be qualified with a "J" to indicate that the result is an estimate.

9.5.6 Analysis of surrogates.

9.5.6.1 The analyst should monitor the performance of the extraction, cleanup, analytical system, and effectiveness of the method by spiking each sample, QC samples and Method Blanks with the PSCL- surrogate spike solution currently containing Decachlorobiphenyl and PCNB at 200 ug/L The surrogate compound is expected to be found at 20 µg/L in all the sample extracts, Blanks, LFBs, and Matrix Spikes for soil and tissue samples and at 40 ug/L for water samples..

9.5.6.2 To determine the acceptance accuracy limits for a surrogate the following procedure should be used (ref 15.3): (1) for each sample analyzed, calculate the percent recovery of each surrogate in the sample, (2) once a minimum of thirty samples of the same matrix has been analyzed, calculate the average percent recovery (p), and standard deviation of the percent recoveries (s), for each of the surrogates, (3) for a given matrix, calculate the upper and lower control limit for method performance for each surrogate standard as follows:

Upper Control Limit (UCL) = p + ts
Lower Control Limit (LCL) = p – ts
Where “t” stands for Student’s coefficient for n-1 degrees of freedom (n = number of measurements).

The control limits are supposed to be checked once per year if enough measurements have been conducted. The acceptance limits of 50-150 % should be used until enough statistical data is acquired to perform the necessary calculations.

9.5.6.3 If a surrogate recovery fails, the sample has to be re-extracted and re-analyzed. If the sample exceeded the holding time for extraction, and the recovery is biased high, the re-extraction is not required and the reported hits have to be qualified as estimated (J), with the following comment entered in the Sample Report: “Estimated values for parameter” are due to surrogate recovery out of control limits”. If the recovery is biased low the sample must be re-extracted and reanalyzed.

9.5.7 Analysis of matrix spikes and LFBs

Check the Matrix Spike and Lab Fortified Blank recoveries. They must be within the current acceptance ranges. A comment must be added to the final report explaining any deviation from the routine limits whenever the spikes or surrogates are out of range. Matrix spikes and LFBs recoveries should be calculated based on available in-house data once enough data points are collected. The limits are established as in 9.5.6.2. LFB and matrix spikes recovery limits are calculated independently; the limits for LFBs may sometimes be applied to both LFBs and matrix spikes if not enough matrix spike data is yet available..

9.5.8 Blank contamination

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

In the event a positive identification is found in both the sample(s) and the instrument blank, the instrument must be cleaned and the sample(s) rerun/reanalyzed.
In the event a positive identification is found in both the sample(s) and the extraction blank then the sample set has to be re-extracted and reanalyzed. If samples passed the holding time, the results will be reported with a "V" qualifier up to ten times the amount found in the blank.

9.5.9 Continuing Calibration

9.5.9.1 After initial calibration, the GC/MS-NCI response is immediately checked using second source Initial Calibration Verification (ICV) standard (acceptance criteria 70-130%). Afterwards the instrument's response is checked on a continuing basis (every 12 hours) by using a continuing calibration check (CCV), middle range pesticides standard. Criterion of acceptance for continuing calibration check is +/-20 % of initial calibration. If a CCV fails, all the samples run after this standard, have to be re-run for all positive hits found at concentrations higher than PQL level. If the samples have exceeded the analysis holding time then all hits must be flagged as estimated.

9.5.9.2 If CCVs do not meet method requirements but the associated analytical results are below the detection limit or below the quantifiable threshold (PQL) and no systematic failure is observed (i.e., the failure is deduced to be due to a statistical outlier in a multi-component analysis where results from other CCV components and LFB results do not indicate a systematic bias in the analysis or detector response), an appropriate test comment and result qualifier (J) shall be provided to indicate the CCVs are outside control limits. However, when a systematic or system failure is detected or the affected analytical data are above the quantifiable threshold, the analysis shall be re-run if possible. If re-analysis is not an option, the analytical results shall be qualified as estimates (see 15.5 for more details).

9.6 Analysis of Chlordane and Toxaphene using GC/MS-NCI in the SIM mode

9.6.1 The acquisition method for Chlordane and Toxaphene (CHLDTOX_yymmdd) contains, in the first acquisition segment, ions for internal standard, alpha-BHC-d6. In the second acquisition segment all 14 ions characteristic for the Chlordane technical components and 15 ions commonly found in Toxaphene are included. Additionally, one ion (m/z=329) is used to quantify the internal standard 2,3,3',4,4'5-Hexachlorobiphenyl-2',6,6'-d3 (HCB-d3). The second acquisition segment covers all analytes of interest and extends to shortly before the Decachlorobiphenyl surrogate peak. Ions for the surrogate, Decachlorobiphenyl, are found in the third acquisition segment. The first surrogate, PCNB, elutes during the first acquisition window. The use of single acquisition window for analytes of interest ensures that the entire Chlordane and Toxaphene patterns can be recreated by summing up all the different SIM ions.

All the individual ions used in the acquisition method are listed in Table 4A. The individual SIM ions listed in the acquisition method each reconstruct parts of the respective analyte's pattern, over certain RT spans; they will each show up as clusters of peaks at different RTs in different parts of the chromatogram. Therefore, correlation between the acquisition ions and the analytical Chlordane and Toxaphene peaks is not straightforward. Sums of most abundant isotopic ions are used for

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

quantitation of individual analyte peaks, while the same individual isotopic ions serve as qualifiers. This approach, rather than Total Ion Chromatograms for analyte peaks, allows for better distinguishing of Toxaphene from Chlordane peaks. The Chlordane and Toxaphene analytical peaks with their quant and qual ions are listed in Table 4B.

9.6.2 Identification of Chlordane and Toxaphene patterns is accomplished by visual comparison of the sample chromatogram to the standard. Masshunter "Qual" software is used for this purpose. To extract either Toxaphene or Chlordane from a complex chromatogram use "extract Toxaphene" or "extract Chlordane" processing methods respectively. Refer to appendix 3 for Chlordane and Toxaphene patterns and analytical peaks.

9.6.3 For severely weathered Toxaphene patterns the analysis is further aided by comparison with special standard mixes containing pure individual Toxaphene components (aka parlars). The specific parlars present in those mixes were selected for their environmental persistence. Matching the Toxaphene peaks present in the severely weathered samples with some (at least four) or all of the individual parlars provides additional evidence for the presence of Toxaphene. Two mixes, called Toxa-4 and Toxa-S, containing four individual parlars each, are run periodically. They must be run, however, each time after the column was trimmed or replaced.

9.6.3 Once the analyte pattern has been identified the quantitation of Chlordane and Toxaphene is accomplished by using four major peaks characteristic Chlordane and seven major peaks for Toxaphene. (see table 4B). Four Chlordane technical peaks are identical with 4 major Chlordane technical components: (Alpha-Chlordane and Gamma-Chlordane, Trans-Nonachlor and Cis-Nonachlor). For best results the seven major Toxaphene peaks are chosen in such a way that they are evenly spread all over the entire Toxaphene pattern. A separate calibration curve is build for each of the individual analytical peaks. The concentration for the total analyte is then assigned to each analytical peak. The amount of the analyte is calculated using the individual calibration factors for each of the analytical peaks and the calibration model established (linear or non-linear) from the multi-point calibration. Different calibration models may be used for each selected peak. A seven point calibration is typically run for each analyte. The concentrations obtained for all the analytical peaks are then, finally, averaged to determine the concentration of that analyte.

9.6.4 Weathering of analyte in the environment and changes resulting from waste treatment processes may significantly alter the patterns, especially for Toxaphene. Averaging of several, carefully chosen, analytical peaks provides for some ability to overcome weathering effects and still obtain acceptable quantitation results. To accomplish that, it is essential not to attempt to" reverse match" the analytical peaks used for Toxaphene calibration to those identified in the degraded sample pattern. Instead, the individual concentrations calculated for the analytical Toxaphene peaks are summed up and still divided by seven, even if some peaks are missing in the sample.

9.6.5 In some cases, it may be advisable to use the total area approach to calculate the concentration in a weathered Toxaphene sample. Great care must be exercised, however, to ensure that no interfering compounds elute in the same portion of the chromatogram.

9.6.6 Positives are quantified based on their peak area. The concentration reported for an identified hit in the sample extract, expressed in ug/L, is based on calibration curve of the initial calibration. The Masshunter software used to process data will do the

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

interpolation for the sample extract. However, the analyst must ensure that all identification criteria are fulfiled and the peak area integration was correctly done. If not, the integration has to be corrected by choosing a new set of integration parameters or by manual integration (for manual integration procedure see 15.10). Only extract concentrations are calculated by the processing software. The final analyte concentrations in sample are calculated in the QC Manager during the data upload process software. This is done as follows:

- C_{sample} (ug/kg) = (C_x) × ($V_{extract}$) × (DF)/(W_{sample}) for sediment and waste and tissue

- C_{sample} (ug/L) = (Cx) × ($V_{extract}$) × (DF)/(V_{sample}) for water samples

  where:

  - C_x -The concentration, in ug/L, of the analyte in the sample extract determined by the processing software.

  - $V_{extract}$ -The volume of the extract, in mLs (usually 0.25 or 10 or 2 mLs) recorded in the preparation report.

  - DF -The dilution of the sample extract recorded in the dilution report.

  - W_{sample} - For sediment it is the dry weight of the sample that was extracted. For waste and tissue, it is the weight of the sample taken for extraction. The weights are expressed in grams and are recorded in the preparation report.

  - V_{sample} -This is the volume of the water sample taken in extraction, expressed in mLs (usually 1000mL).

10. REPORTING AND DOCUMENTATION

10.1 When analysis is complete, generate an upload file for all the reported samples, QC samples and CCVs. The QC Manager is then used to download the final sample results into the Laboratory Information Management System (LIMS). Sample volume/weight/dry weight as well as final extract volumes are entered at this stage. If a sample has been diluted then make sure that the information was entered properly into the QC Manager. The following convention should be used in QC calculator:

CCV for CCVs

SURS_1234567 or SURT_1234567 or SURW_1234567 for surrogate in sample.

10.2 The QC Manager converter will add MDLs for all analytes not included in the text file (non-detects). It will also average the analytical peaks for both Chlordane and Toxaphene and transfer the average to QC Manager as the raw results for both analytes.

10.3 Add the appropriate qualifiers and comments to the sample data in the QC Manager after performing a complete review of the sample data and QC data. Follow DEP SOP OG-003-Data Review/Authorization Checklist (ref 15-4) for details of data review and reporting, See DEP SOP LB-027, Standard Operating Procedure for Reporting Qualified Data (ref 15-5) for guidelines for qualifying data when quality control results are outside established

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

control limits and re-analysis is not a viable or desired option. Definitions of the available qualifying codes are listed in DEP SOP LB-027.

10.4 All quantitation reports for samples and QC should be placed in a results folder labeled with the job number, the sample numbers, and the type of analysis.

10.5 Enter the results for each sample in the analysis results form. Print out QC results from the QC Manager. Include a copy of dilutions page if any. Also include a printout of sample positives from the data review (a scrollable report) and file it in the data folder.

10.6 Submit the result folder to the GC pesticides section supervisor. The supervisor will review results and then authorize the sample. The supervisor will then file the folders in the GC-pesticides filing cabinets.

11. QUALITY CONTROL

11.1 See DEP SOP GC-001, Quality Assurance/Quality Control in the GC Pesticides Laboratory (ref 15.6) for detailed information on quality control, definitions and interferences. See DEP SOP LB-027, Standard Operating Procedure for Reporting Qualified Data (ref 15.5) for guidelines for qualifying data when quality control results are outside established control limits and re-analysis is not a viable or desired option.

11.2 All data generated by a chemistry analyst are subjected to supervisory review and authorization. The supervisor is assessing the validity of data generated by assuring that all QA/QC criteria are met by checking the following review/authorization checklist:

- Check sample preparation report
  - procedure
  - surrogate and spiking solutions
  - preparation holding time
- Check project log-in folder for special requests
- Check initial calibration for linearity and fit
- Check continuing calibration check standards
- Check surrogate recoveries
- Check accuracy and precision for LFB and Matrix Spikes
- Check internal standard recoveries
- Check retention time drift
- Check for blanks contamination
- Check for positives:
  - Proper integration of the peaks (baseline)
  - Retention time confirmation
  - Amount confirmation
  - If response is within the calibration range
  - Calculations
  - Qualifiers
- Check information in the Masshunter Report Header:
  - SOP #
  - Instrument ID
  - Analyst name

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

- Evaluate the data against any field information
- Check field preservation record
- Check LIMS report:
  - Preparation and analysis dates
  - MDLs
  - Amounts reported for positives
  - Qualifiers
  - Comments

12. DATA ARCHIVAL

12.1 All raw data are retained in electronic or hard copy format. All paper formats are maintained either in the laboratory office or in a State warehouse for at least ten years. Electronic chromatographic raw data are archived by analysis file ID and instrument ID on dual CDs by the Laboratory Support Section.

12.2 Move the valid data files (tune, ICALS, CCVs, QC and sample data files) soon after collection from the hard disk of the instrument computers to the network drive \\TLHLAB3\GC\GCMSD2\Backup_GC_MSD2. Raw data files on the hard disk of each instrument may be deleted after 3 to 4 months or whenever they are no longer needed.

13. DETERMINATION OF METHOD DETECTION LIMITS

13.1 The MDLs were established using procedures given in EPA Chapter 40 Part 136, Appendix B of the Federal Register (ref 15.11). Also refer to Reference 15.7 and 15.8**.**

13.2 at least seven replicate samples are analyzed near the MDL. The MDL is the concentration of analyte at which there is analyte present with 99% confidence. The validation exercise is split into at least three extraction batches performed on three separate days. The samples are run in all the instruments that are considered identical for the purpose of this analysis. Along with the spikes an equal number of blanks is extracted.

13.3 The results of the (at least) seven replicate data sets are tabulated and the statistical calculations are performed electronically to establish or update the MDLs and PQLs for each analyte and matrix. The MDL results calculated based on the blanks and spikes data are compared and the MDL is set at the higher value (at least).

13.4 MDLs are verified quarterly and recalculated annually. At least one spike is run in each instrument per quarter in which samples are analyzed. The spike is prepared at concentration between MDL and PQL and it is also considered a PQL verification. The MDLs are recalculated annually (at least) based on the last 24 months of data. Both spikes and blanks are used in calculations. If the calculated MDL is within a factor of 2 of the current MDL, the current MDL may be kept unchanged. Otherwise, the MDL has to be changed to the new calculated value. MDL/PQL verification spikes are reported to LIMS as QC type SRM. The LIMS statistics module is used for the annual re-calculation of MDLs.

14. SAFETY/HAZARDOUS WASTE MANAGEMENT

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

See SOP OG-001, Safety and Waste Management Practices in the Organic Analysis Groups (Volatile, Semi-Volatile and Pesticide Laboratories) (ref 15.9) for specific details on safety and hazardous waste management.

.

15. REFERENCES

15.1 Test Methods for Evaluating Solid Wastes, Third Edition, SW-846, Method 8276, revision1, Toxaphene and Toxaphene Congeners By Gas Chromatography/Negative Ion Chemical Ionization Mass Spectrometry (GC-NICI/MS).

15.2 GC SOP GC-002: Extraction of pesticides and polychlorinated biphenyls (PCBs) from Water Wastes, soil/sediments PUFs and filters for subsequent analysis by GC-ECD/NPD or GC/MS/MS or GC/MS-NCI.

15.3 EPA 8000D Determinative Chromatographic Separation; Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods, SW 846; 3rd Edition, Update 5, revision 4, July, 2014.

15.4 DEP SOP OG-003. Data Review/Authorization Checklist

15.5 DEP SOP LB-027. Standard Operating Procedure for Reporting Qualified Data.

15.6 DEP SOP GC-001. Quality Assurance/Quality Control in the GC Pesticides Laboratory

15.7 Florida Department of Environmental Protection, Standard Operating Procedure LB-007 *Procedure and Policy for Method and Instrument Validation.*

15.8 Florida Department of Environmental Protection, Standard Operating Procedure LB-031 *Limit of Detection Verification.*

15.9 DEP SOP OG-001. Safety and Waste Management Practices in the Organic Analysis Groups (Volatile, Semi-Volatile and Pesticide Laboratories).

15.10 DEP SOP CM-018-1 Laboratory Policy Regarding Manual Chromatographic Peak Integration.

15.11 Code of Federal Regulations, Title 40, Part 136, Appendix B.

15.12 "Agilent 5977 series MSD" Operation Manual, Agilent Technologies, 2013.

15.13 DEP SOP GC-004-1. Extraction of Organophosphorus, Organonitrogen and Organohalide pesticides and PCBs from Fish Tissue for Subsequent analysis by GC-NPD/ECD.

15.14 DEP SOP GC-036-1. Analysis of Chlorinated Pesticides and PCBs in Water Samples Using Solid Phase Extraction and GC/MS-SIM.

16. TABLES

TABLE 1: Internal standard associations, MDLs, PQLs, approximate RTs and RRTs for pesticides, surrogate and internal standard included in LIMS test S-CT-CI, WS-CT-CI, T-CT-CI and W-CT-CI-R. LIMS reporting units are ug/kg for soils wastes and tissues and ng/L for waters

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

Analyte	**Approximate Retention Time (min)**	**Associated internal standard**	**RRT**	**MDL (ug/L) s/ws/t/w***	**MDL (ug/Kg) or ng/L s/ws/t/w***	**PQL (ug/L) s/ws/t/w***	**PQL (ug/Kg) or ng/L s/ws/t/w***
				extract	sample	extract	sample
PCNB	18.67	Alpha-BHC-d6	1.12				
Decachlorobiphenyl surrogate	49.0	HCB-d3	1.26				
Chlordane				10.0/10.0/NA/10	0.67/100/NA/5.0	40/00/NA/40	2.7/400/NA/20
Chlordane-1	27.44	HCB-d3	0.74				
Chlordane-2	28.31	HCB-d3	0.76				
Chlordane-3	28.60	HCB-d3	0.77				
Chlordane-4	32.13	HCB-d3	0.86				
Toxaphene				60/60/25/60	4.0/600/10/30	240/240/100/240	16/2400/40/120
Toxaphene-1	30.76	HCB-d3	0.83				
Toxaphene-2	31.99	HCB-d3	0.86				
Toxaphene-3	34.11	HCB-d3	0.92				
Toxaphene-4	34.32	HCB-d3	0.92				
Toxaphene-5	36.55	HCB-d3	0.98				
Toxaphene-6	37.60	HCB-d3	1.01				
Toxaphene-7	39.73	HCB-d3	1.07				
Alpha-BHC-d6	16.73	Internal Standard					
Octachloronaphthalene	48.1	Internal standard					
HCB-d3	37.26	Internal standard					

s/ws/t/w*- soil/waste/tissue/water

TABLE 2: GC/MS Operating Conditions: (METHOD CHLDTOX_ -yymmdd)

Parameter	**Setting**
Mass spec:	70 eV. Peak width 0.7s.Negative Chemical Ionization. Acquisition mode – SIM. Tune file Ncich4.u.Source temp. 150 C, Quad temperature 150 C. Methane flow 40. Resolution- high. Gain factor 15.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

Scan Time:	About 3.3 cycles/s. SIM mode dwell times 10 ms, summing up to about 300 ms per time segment.
GC Oven Temperature Program:	60 °C for 1.0 min, to 150 °C at 10 °C /min, hold 0 min, to 260 °C at 3 °C /min hold 0 min, to 310 °C at 20 °C /min, hold 3 min
Injector operating conditions:	Temp: 220 °C for 40 min then at 600 C /min to 260, hold 9 min; then at 600 C/min to 310 C hold until end of run. Mode: splitless; Constant Flow ON, (1.2 mL/minCryo – off.
Column	HP5MS UI, 30m-0.25mm-0.25um; 5+25 backflash configuration; see column 1 and column 2 below.
Column 1 configuration	HP-5MS UI 5m-0.25mm-0.25um; in: front MMI inlet, out aux EPC3
Column 1 flow	1.1 mL/min for 40 min then at 100 mL/min to -2.5 mL/min for 9 min, then at 100 mL/min to -5 mL/min until end of run.
Column 2 configuration	HP-5MS UI 25m-0.25mm-0.25um; in: aux EPC3, out vacuum
Column 2 flow	1.2 mL/min for 49 min, then at 100 mL/min to 2.5 mL/min until end of run
MS interface temperature:	280 °C
Injection Type:	Pulsed splitless. 30 psi until 1.4 min purge to split vent 50 mL/min at 15 min. Total flow 64.5 mL/min. Gas saver ON. Saver time – 2 min, saver flow 20.0 mL/min.
Injection Volume:	1 µL
Total Run Time	52.17 min.

TABLE 3: GC AUTOSAMPLER OPERATING PARAMETERS FOR GC/MS-NCI ANALYSIS

Autosampler Parameter	**Setting**
Syringe size	10 uL
Injection volume	1 uL
Last Bottle	100
Solvent A washes	2 pre- 8 post (acetone)
Solvent B washes	2 pre- 8 post (iso-octane)
Sample washes	3
Sample wash volume	8 uL
Sample pumps	6
Dwell time pre-inj	0
Dwell time post-inj	0
Plunger speed	Fast
Solvent wash draw speed	300 uL/min

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

Solvent wash dispense speed	3000 uL/min
Sample wash draw speed	300 uL/min
Sample wash dispense speed	3000 uL/min
Injection dispense speed	6000 uL/min
Viscosity delay	2 sec

TABLE 4A. SIM method acquisition parameters for Chlordane and Toxaphene

Compound Name	ISTD	SIM Ions	Time segment (min)	Dwell (ms)
PCNB **SURROGATE**	FALSE	249.0, 251.0, 265.0	TS-1 12.00	50
Alpha-BHC-d6	TRUE	261.0, 259.0, 263.0	TS-1 12.00	50
Decachlorobiphenyl **SURROGATE**	FALSE	497.0, 464.0, 430.0	TS-3 45.00	50
Octachloronaphthalene	TRUE	403.8, 333.90, 367.80	TS-3 45.00	50
HCB-d3	TRUE	329.9, 362.9, 364.9	TS-2 20.00	10
Chlordane	FALSE	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	TS-2 20.00	10
Toxaphene	FALSE	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	TS-2 20.00	10

TABLE 4B. Quantifier and Qualifier SIM ions for analytical Chlordane and Toxaphene peaks.

Analyte	**Quantifier Ions**	**Qualifier Ions**
HCB-d3 istd	329.9	362.9, 364.9
Alpha-BHC istd	261	259, 263
Octachloronaphthalene	403.8	333.9. 367.8
PCNB surrogate	249.0	251.0, 265.0
Decachlorobiphenyl surrogate	464	430, 497
Chlordane-1	410 sum all	408, 412
Chlordane-2	410 sum all	408, 412
Chlordane-3	444 sum all	442, 446
Chlordane-4	444 sum all	442, 446
Toxaphene-1	343 sum all	341, 345
Toxaphene-2	377 sum all	379, 381
Toxaphene-3	377 sum all	379, 381

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

Toxaphene-4	377 sum all	379, 381
Toxaphene-5	377 sum all	379, 381
Toxaphene-6	413 sum all	411, 415
Toxaphene-7	413 sum all	411, 415

Table 5: Analyte concentrations in calibration standards for Chlordane and Toxaphene (ug/L).

	Interm	**spike**	**L8**	**L7**	**L6**	**L5**	**L4**	**L3**	**L2**
	NCI								
CHTX									
Chlordane	**10000**	**2500**	**1000**	**750**	**500**	**200**	**100**	**50**	**20**
Toxaphene	**40000**	**10000**	**5000**	**3000**	**2000**	**1000**	**500**	**250**	**100**
PCNB	**10000**	**200**	**100**	**75**	**50**	**20**	**10**	**5**	**2**
Decachlorobiphenyl	**10000**	**200**	**100**	**75**	**50**	**20**	**10**	**5**	**2**

CCV and ICV concentrations are equal to L4
Expected surrogate concentration in all spikes and samples is 20 ug/L
Expexted concentrations in LFBs/MSs are 100 ug/L for Chlordane and 1000 ug/L for Toxaphene
10xdil spike solution is used to prepare the PQL spikes
Expexted concentrations in PQL spikes are 20 ug/L for Chlordane and 200 ug/L for Toxaphene for soil/waste samples and 10 ug/L for Chlordane and 100 ug/L for toxaphene for tissue samples.

TABLE 6: QC MEASUREMENTS AND ACCEPTACE CRITERIA

#	QC MEASUREMENT	ACCEPTANCE CRITERION	CORRECTIVE ACTION
1	LFB, Matrix Spike precision	≤ 30%	Reanalyze/Re-extract/Flag
2	LFBrecoveries	LFB statistics for water and soil 70-130% Tissue	Reanalyze/Re-extract/Flag
	MS recoveries	MS staistics for water and soil 70-130% tissue	
	ICV second source	± 30%	Rerun Ical, Reanalyze, if discrepancy persists, compare with a third independent source.
3	CCV	± 20%	Re-run and recalibrate/Flag
4	PQL	QC statistics for water and soil 70-130% tissue	Re-run /Re-extract/Flag
5	Surrogate PCNB recovery	38-141 % water 37-128% soil not used in tissue	Reanalyze/Re-extract/Flag
	Surrogate Decachlorobiphenyl recovery	26-97% water 43-123% soil 68-111% tissue	

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

6	Linearity criterion	20% RSD; 0.99 CC, not more than 10% analytes can fail ICal	Use appropriate cal fit / Re-run/ Recalibrate
7	Internal Std. recovery	50-200%	Reanalyze/recalibrate/flag
8	% cal drift	± 30% of expected value, 50% maybe acceptable for the lowest point.	Change fit/weighing/recalibrate/flag

* Statistical recovery limits for LFB/MS, PQLs and surrogates recoveries are typically based on QC Manager statistics for the last one or two calendar years (if available). The limits are recalculated at least annually.The results of statistical calculations are saved at FLDEP1\DEAR\LABS\LAB5\CHEMISTRY\ORGANIC\QC LIMITS STATS.

The statistical recovery limits are then entered into QC Manager limits tables. The initial QC limits are replaced with statistical ones whenever enough data points are accumulated. At least 20 points are required, but it is preferable to wait for about 50 points or more. If enough data points is available for LFBs but not for matrix spikes then LFB statistical limits may be applicable for both categories on temporary basis.

16. APPENDICES

17.1 APPENDIX 1

Standard Preparation Procedure

Stock solutions usually come in 1mL glass vials. Take the vials out of the freezer at least 30min before preparation. Open vials and transfer the entire solution into 1mL GC vials (using Pasteur pipettes). Record the transfer date and keep the vial label.

The Intermediate Standard is prepared by diluting the Stock solution. The Working Level Standards are prepared by diluting the Intermediate Standard.

All standard preparations are recorded in the Standard/Reagent Preparation Tracker. In order to prepare fresh standards go to LIMS-Technical-Standard/Reagent Preparation Tracker. Select GC from the pull down list. Hit “Reports” and then “History”. When prompted enter the standard tracking number for the standard you are going to replace. Examine the “History Tree”. An example of a “History Tree” is shown below:

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

```
History of Serial # 63208
-----------------------------------------
63208-S_CL_TQ ICV L6
   59861-ECD B  INT
      59860-ECD B STOCK
   59859-DICOFOL-STOCK-
      59858-DICOFOL-STOCK-
         53378-DICOFOL-NEAT
   59735-ECD A INT
      59609-TMX-DCB SURR INT
         59608-TMX SURR STOCK
            59559-TMX ECD SURR
         59607-DCB SURR STOCK
            59601-DCB ECD SURR
      53666-ECD A  STOCK
      53665-ECD A STOCK
   59151-CLTQ ISTD INT
      59058-GCMS CL ISTD INT1
         50162-DDT-D8
         49705-A-BHC-D6 NEAT
      59055-HCB-D3 1000 PPM
         53297-HCB-D3 1000 PPM
            49722-HCB-D3 NEAT
      59054-TCB-D5 1000 PPM
         53296-TCB-D5 1000 PPM
            49720-TCB-D5 NEAT
      59053-DCB-D5 1000 PPM
         53298-DCB-D5 1000 PPM
            49716-DCB-D5 NEAT
```

In order to get exact information on how the standard was prepared look at “history data sheets” that will show exact information on standards preparations. Before preparing new standards consult with the GC group supervisor whether any changes to standards preparation procedures should be made. Those may include different prepared volumes, concentrations, new, additional concentration levels, etc.

All freshly prepared standards need to be entered in the Standard Preparation Tracker.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

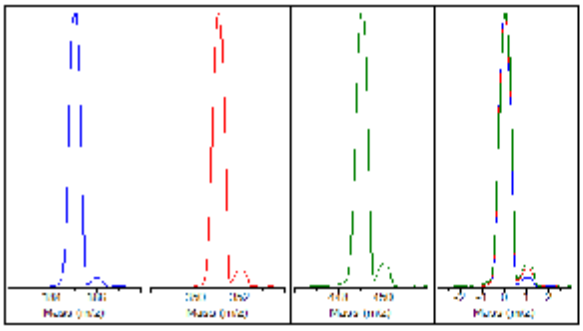
17.2 APPENDIX 2

NCI autotune report

Methane@40 CI Autotune - 5977

9/22/2014 9:32:17 AM (UTC-4:00) GC_MSD_2

D:\MASSHUNTER\GCMS\1\5977\NCICH4-106.U US1419N302



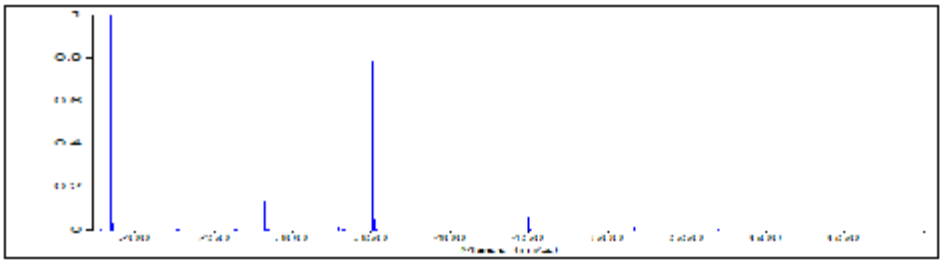
Ion Polarity	Neg	Mass Gain	-600
Emission	50.0	Mass Offset	-31
Electron Energy	189.9	Amu Gain	1341
Filament	1	Amu Offset	123.25
Repeller	3.99	Width219	0.000
Ion Focus	144.9	DC Polarity	Pos
Entrance Lens	19.9	HED Enable	On
Ent Lens Offset	13.48	EM Volts	1058.8
Ion Body	0.00	Extractor Lens	0.00
		Scan Speed	3
		Averages	3
PFDTD	Open	Step Size	0.10

Actual m/z	Abund	Rel Abund	Pw50
185.00	840,592	100.0%	0.61
351.00	658,677	78.4%	0.63
449.00	48,682	5.8%	0.63

Temperatures and Pressures

MS Source	150	Turbo Speed	100.0
MS Quad	106	Hi Vac	1.76e-05

Low	High	Step	Speed	Threshold	Peaks	Base	Abundance	Total Ion
175.00	700.00	0.10	3	100	97	185.00	811,712	1,748,148



Target m/z	Actual m/z	Abund	Rel Abund	Iso m/z	Iso Abund	Iso Ratio
185.00	185.00	811,712	100.0%	186.00	26,736	3.3%
351.00	351.00	635,584	78.3%	352.00	41,424	6.5%
449.00	449.10	46,456	5.7%	450.10	3,982	8.6%

Air/Water Check: H20 ~N/A% N2 ~N/A% O2 ~N/A% CO2 ~N/A% N2/H20 ~N/A%

Column(1) Flow: 1.10 Column(2): 1.20 ml/min Interface Temp: 280

Ramp Criteria:

Ion Focus maximum N/A volts using ion N/A; Electron Multiplier Gain 45176.107

Repeller maximum N/A volts using ion N/A; Gain Factor 0.4518

Mass Gain Values(Scan Speed): -601(3) -601(2) -601(1) -601(0) -601(FS1) -601(FS2)

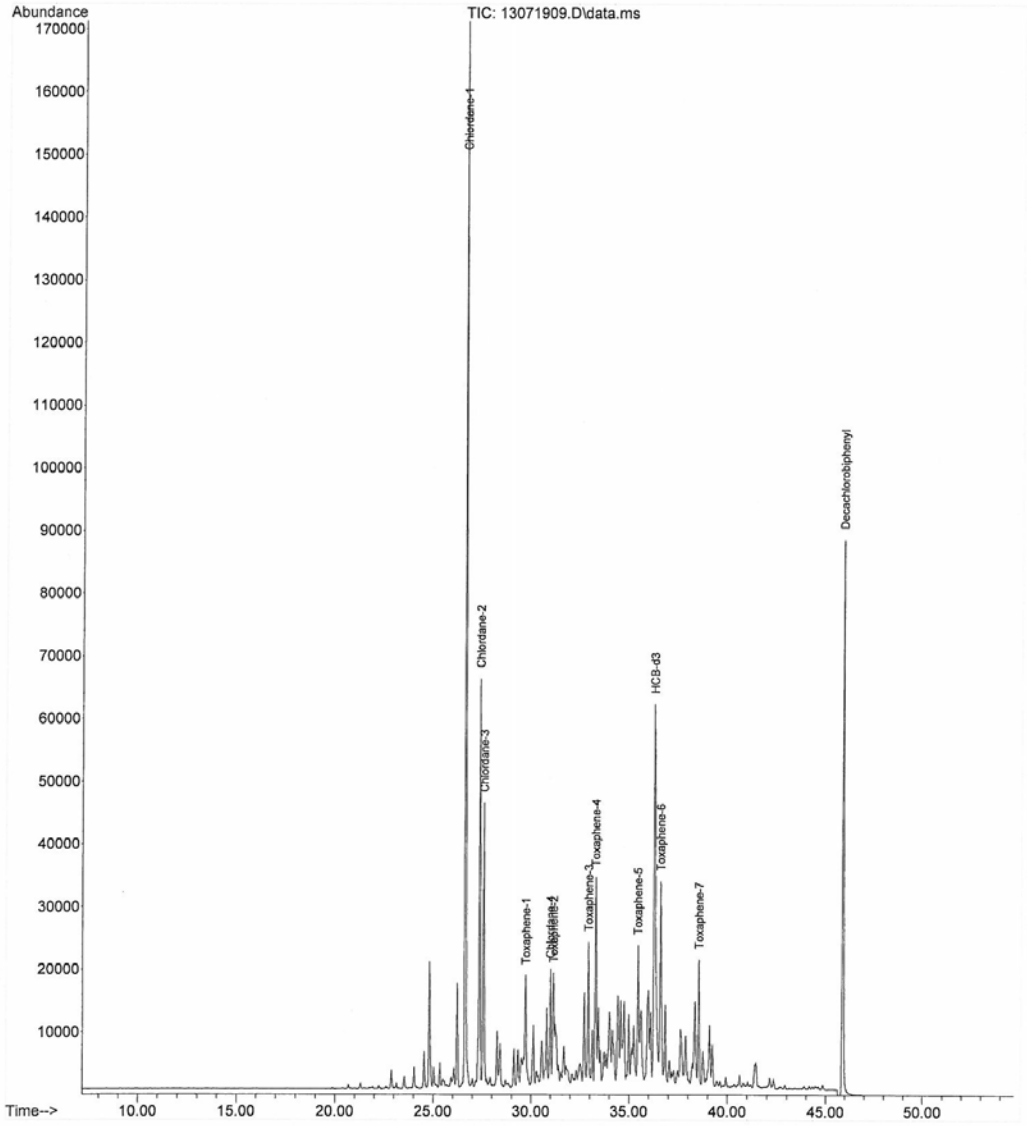
TARGET MASS:	50	69	131	219	414	502	1050
Amu Offset	123.3	123.3	123.3	123.3	123.3	123.3	123.3
Entrance Lens Offset	13.5	13.5	13.5	13.5	13.5	13.5	13.5

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

17.3 APPENDIX 3

Chromatographic patterns for Chlordane and Toxaphene

```
File       :C:\msdchem\1\DATA\130719bNCI\13071909.D
Operator   : Nagar_V
Acquired   : 19 Jul 2013   7:29 pm using AcqMethod CHLDTOX-130618.M
Instrument :   Instrument #1
Sample Name: CHTX-L5
Misc Info  : GC_MSD_1 SN:US00022270 SOP GC-034-1
Vial Number: 7
```



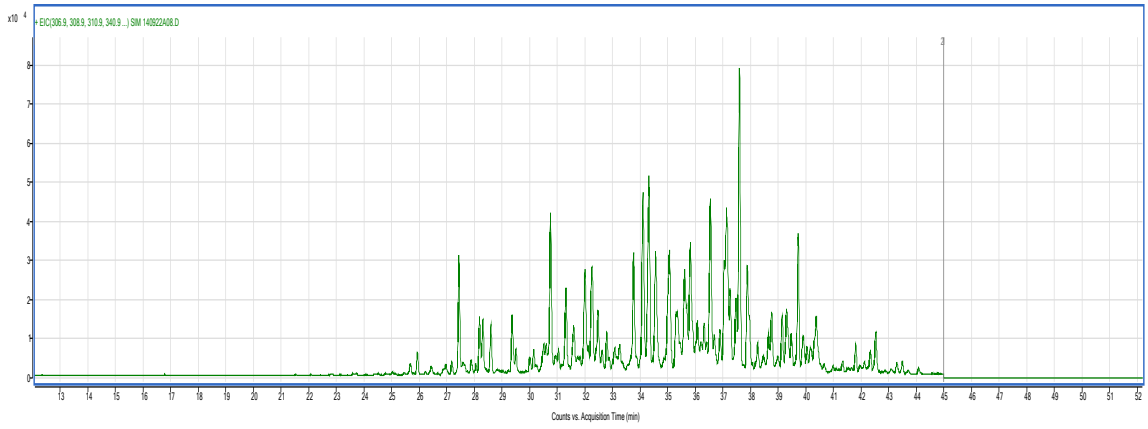
The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

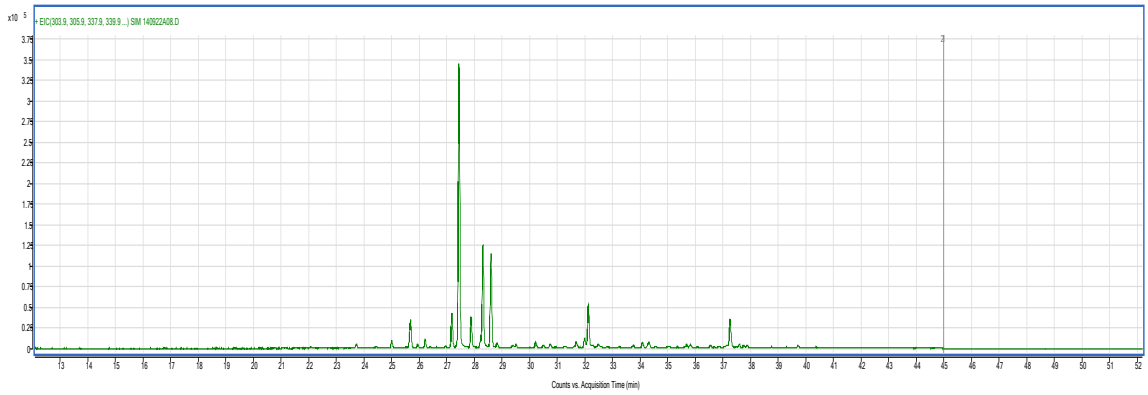
17.4 APPENDIX 4

Chlordane and Toxaphene chromatographic patterns (EIC extraction in Qual)

Toxaphene pattern obtained using Qual "extractToxaphene" EIC extraction method; file 140922A08.D - CHTX-L4 standard –upper picture

Chlordane pattern obtained using Qual "extract Chlordane" EIC pattern extraction method; file 140922A08.D - CHTX-L4 standard – lower picture





The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

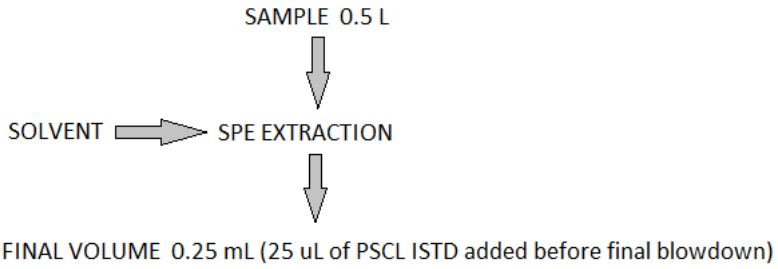
Appendix 5 Preparation flowcharts

Water samples GC/MS SIM PREP ID: USGS-O-1126-95

Chlorinated pesticides and PCBs in water

Tests: W-PCL-SQ-R, W-PCL-SQ, W-PCB-SQ-R, W-PCB, W-CT-CL-R, W-DIELDRIN

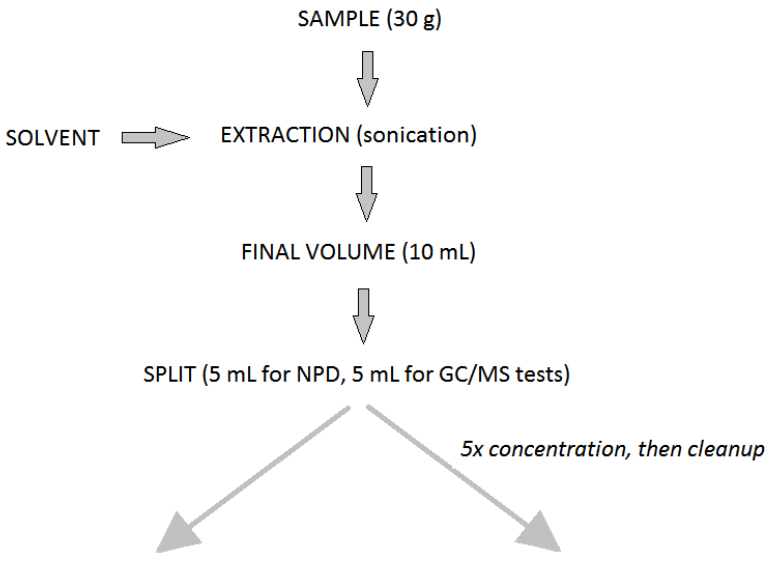
The USGS and RCRA tests follow the same procedure



Soil samples TQ/Cl/NPD Prep ID: 3550C (TQ) / 3550C (GC)

Chlorinated pesticides, PCBs and NP pesticides in soil

Tests: S-PSCL-TQ, S-PCB-TQ, S-CT-CL and S-PEST-NP



Aliquot 1

S-PEST-NP Final Vol = 10 mL

Submitted volume = 2 mL
+ 10 uL of NPD ISTD

3 mL kept as backup

Aliquot 2

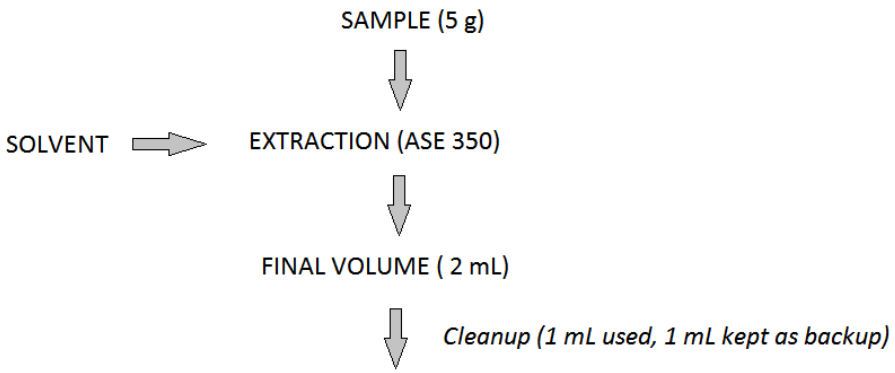
S-PSCL-TQ, S-PCB-TQ, S-CT-CL Final Vol = 2 mL

1 mL vial for GPC, 0.8 mL injected on GPC, followed by Florisil cleanup.
Submitted volume = 0.7 mL + 100 uL of 800 ppb PSCL ISTD

Alternatively, for PCBs and some chlorinated pesticides,
1 mL cleaned with sulfuric acid/TBA
Submitted volume = 0.9 mL + 100 uL of 1000 ppb PSCL ISTD

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

Tissue TQ/NCl/NPD Prep ID: SOP-GC-004 (TQ) / SOP GC-004
Chlorinated pesticides, PCBs and NP pesticides in fish tissue
Tests: T-PCBCL-TQ, T-CT-CL, T-PEST-NP



Aliquot
T-PCBCL-TQ, T-CT-CL, T-PEST-NP Final Vol = 2 mL

1 mL vial for GPC, 0.8 mL injected on GPC followed by Florisil cleanup.
Two fractions collected.

FR1: Submitted vol = 0.7 mL + 100 uL of 800 ppb PSCL ISTD + 4 uL of NPD ISTD

FR2: Submitted vol = 0.8 mL + 4 uL of NPD ISTD (NP analytes only)

Alternatively, for PCBs and some chlorinated pesticides,
1 mL is cleaned with sulfuric acid/TBA
Submitted vol = 0.9 mL + 100 uL of 1000 ppb PSCL ISTD

Appendix of Significant Changes

11/07/2013	Analysis of Chlordane and Toxaphene in tissues was added (test ID T-CT-CI). Modifications introduced throughout the text; Waste test was added (WS-CT-CI) and paragraph 9.6.6 describing the quantification of positives was added.
09/26/2014	Validation and all other changes due to the new instrument : Agilent 5977A GC/MS
09/25/2015	Updated QC control limits for surrogate recoveries.
01/06/2016	Updated QC control limits and calibration standards names. Modified the standards preparation appendix.
07/12/2016	Added test W-CT-CI-R throughout the text. Include origin prohibited for calibration fits. Ignore origin preferred.
01/09/2017	Updated QC control limits. Added Appendix 5 Preparation Flowcharts for soils and tissues

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

01/08/2017 Updated QC recovery limits. Added surrogate PCNB for water and soil tests. Updated tables. Added description for the use of Toxaphene parlars for weathered Toxaphene patterns.

02/27/2018 Updated MDL for Toxaphene in water. Updated surrogate recovery limits for PCNB in water.

02/06/2019 Updated MDLs/PQLs, standard concentration levels and QC limits (surrogate recoveries). Update initial calibration requirements.

02/10/2020 Updated MDL determination chapter. Updated QC limits.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.