Standard Operating Procedure for:

ANALYSIS OF CHLORINATED PESTICIDES IN WATER SAMPLES USING SOLID PHASE EXTRACTION AND GC/MS-SIM

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1. SCOPE AND APPLICATION

This method is applicable to the determination of selected organochlorine pesticides in water as listed in Table 1. Method Detection Limits and Practical Quantitation Limits yielded by this method are also provided in Table 1.

1.1. This method is based on USGS-Method-O-1126-95 (Under Clean Water Act) as well as EPA 8270D mod. (under RCRA)(ref 15.1) Laboratory Information Management System (LIMS) tests: W-PCL-SQ and W-PCL-SQ-R. To achieve compliance with the two methods mentioned above This SOP uses the strictest of the specific QC requirements between the two methods (see appendix 4).

1.2. This Standard Operation Procedure is restricted to use by an analyst experienced in the use of an Agilent 5977A 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

2.1. General description.

Water samples are extracted using C-18 solid phase extraction (as per USGS-Method-O-1126-95) and analyzed using GC/MS with selected –ion monitoring (SIM mode). The extracts are analyzed using an Agilent 5977A GC/MS/ system. 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.

2.2 Prep method modifications as compared to USGS-Method-O-1126-95:

2.2.1 Filtered samples' pH is adjusted to 2 as opposed to neutral. pH 2 improves recoveries of some analytes with no negative effects on others.

2.2.2 Methylene chloride is used as eluting solvent rather than hexane-isopropanol 3:1. Methylene chloride is better suited for chlorinated pesticides; eliminates water from the extracts and rather laborious drying procedure.

2.2.3 Final volume 0.25 mL rather than "around" 100 uL). 100 uL is hard to measure with the existing test tubes; Greater sensitivity of Agilent 5977A allows for higher final volumes, which also mean less matrix injected on the instruments (less maintenance and less interferences).

2.2.4 Sample volume 500 mL rather than 1 L. 1L sample volumes are notoriously difficult to aspire through SPE cartridges, even if pre-filtered. Due to increased sensitivity of Agilent 5977 GC/MS sample volume reduction was made.

2.3 Modifications to analytical methods as compared to USGS-Method-O-1126-95 and EPA 8270D:

2.3.1 DFTPP tune/tune check (EPA 8270D only) is not used. As allowed by the method 8270D, manufacturer's recommendations regarding instrument tuning are followed. Extractor Ion Source was not designed to tune to DFTPP.

2.3.2 The analysis uses analyte protectants (Ethoxyglycerin, Gulonolactone and Sorbitol). Those compounds are injected using sandwich technique at 1000ppm/1000ppm/2000ppm respectively. Analyte protectants are necessary to suppress matrix induced response enhancement effects. Agilent 5977 GC/MS system with Extractor Ion Source seem to be especially sensitive to those kind of effects.

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2.3.3 The degradation /tailing factor mix (EPA 8270D only) is run at lower concentration (5 ppm instead of 50 ppm to reduce carry-over effects with DDT-p,p'. Only pentachlorophenol peak is used to determine the tailing factors. Benzidine peak is not detected in the presence of the analyte protectants. Since the absence of Benzidine peak does not have any bearing on the analysis of the target analytes it is considered inconsequential for the purpose of this analysis.

2.3.4 The method uses different internal standards and a different surrogate that suggested in USGS-Method-O-1126-95 and EPA 8270D due to their closer similarity to the target analytes.

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 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

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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 material spiked with compound(s) representative of the target analytes. The LFB is analyzed exactly the same way like other sample. This is used to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

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

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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 standard solution from a source that is certified and traceable. These standards are used to check the accuracy of a calibration curve.

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.

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.

5. APPARATUS AND EQUIPMENT

5.1 Apparatus.

Agilent 5977A GC/MS system with an extractor ion source or equivalent instrumentation. Extractor source on Agilent 5977A is necessary to achieve the detection limits as described in this SOP. The instrument must be temperature programmable with an injector port designed for cold splitless injection. A Multi-Mode Inlet (MMI) may be used if available. The GC, GC Automatic Sampler and MSD operating parameters are summarized in Appendix 1. The

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analytical column is an HP-5MS UI or equivalent, fused silica capillary, 30 m x 0.25 mm x 0.25 um or similar column with Ultimate Union placed after the initial 5 m of the column (5m-25m mid-column 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 and collision cell gas. The helium is passed through an oxygen and a hydrocarbon filters before it enters the GC.

5.3.2 Nitrogen, UPC Grade or higher, is used as a collision cell gas. The N_2 is passed through a hydrocarbon filter before entering the GC.

6. REAGENTS AND CHEMICALS

6.1. Solvents

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

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

6.1.3. Acetone, Fisher Scientific, Pesticides, HPLC or Optima grade.

6.1.4. Hydrochloric acid, Fisher Scientific.

6.2. Standards

6.2.1. For descriptionof standard preparations see APPENDICES 2 and 3

6.3. Stock and Intermediate Standards

6.3.1 Custom made stock standards and/or neat standards for individual analytes are purchased from commercial suppliers. Their concentrations are recorded in the standard preparation tracker (part of LIMS). Two different sources of stock standards are normally purchased from two different suppliers; if not available then two different lots are purchased from one supplier.

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 in the low mg/L (ppm) range. 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 typical criterion of acceptance is +/- 10% difference. All standard preparations and verifications have to be recorded in the Standard

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Preparation Tracker located in DEP LIMS. A list containing all working standards used in the analysis with their serial numbers must be part of analysis documentation folder.

6.4. Working Standard Solutions (Calibration standards)

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

Internal Standard Mixture for CL analytes and PCBs:

Alpha-BHC-d6
2,5-Dichlorobiphenyl-2',3',4',5',6'-d5 (DCB-d5)
2,3,5,6-Tetrachlorobiphenyl-2',3',4',5',6'-d5 (TCB-d5)
2,3,3',4,4'5-Hexachlorobiphenyl-2',6,6'-d3 (HCB-d3)
DDT-p,p'-d 8

6.4.2. The concentrations for each analyte in the working (calibration) standards are listed in Table 2 for chlorinated pesticides. The second source/CCV standard is prepared at a midpoint level.

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.

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

6.6. 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. The acceptance criterion is ± 30%.

6.7. Continuous 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.

6.7.1 The acceptance criterion is ± 20% difference.

6.7.2 The Analyst must insure that the calibration standards are checked with the corresponding CCV with every initial calibration and then after each batch of 10 samples (or 12 hrs intervals thereafter for RCRA samples). Practically the 10 samples requirement is used as more stringent.

6.8 Analyte protectants.
The use of analyte protectants was proposed by Anastassiades et al. (ref 15-3 and 15-4) and it has become an established practice for pesticides analysis in food products analysis, where triple quad GC/MS/MS technology has found the widest use. Agilent 5977C GC/MS instrument is quite sensitive

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to matrix induced response enhancements which makes exact quantitation problematic, especially for some analytes. Analyte protectants are generally very polar organic compounds injected at very high concentrations along with every standard and sample in the analytical run sequence. Due to their polarity, they are thought to deactivate all the active sites in the system, and, due to their very high concentration, to render all the other matrices in the samples basically insignificant. In effect all the samples and standards injected into the GC/MS system have effectively the same matrix – the analyte protectants – and therefore matrix induced response differences are greatly reduced. This method uses a mixture of three specific analyte protectant compounds: Ethoxyglycerin, Gulonolactone and Sorbitol at 1250 ppm, 1250 ppm and 2500 ppm respectively. Please note that this formulation (named APs 5977) is two times diluted as compared to the formulation used in this laboratory for GC/MS triple quad analysis. Due to solubility considerations the working solution is prepared in MeOH containing 5% of DI water. It is injected along with every standard and sample using a "sandwich injection" technigue. 0.4 uL of the above analyte protectants mixture is injected, which corresponds to 0.5 ug, 0.5 ug and 1 ug of the corresponding analyte protectants on column.

7. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

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

7.2. All samples must be refrigerated at 4 degrees C or less from time of collection until extraction.

7.3. All samples must be extracted within 7 days of collection 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.

8. SAMPLE PREPARATION PROCEDURE

8.1. PREP METHOD SUMMARY. The water samples are filtered at the collection site using glass-fiber filters with 0.7 um pore diameter to remove suspended particulate matter. The pH of water samples is then adjusted to 2 with Hydrochloric acid and the filtered water samples are pumped through 500 mg C-18 SPE columns, preconditioned with DI water at pH=2. The SPE columns are dried using a gentle stream nitrogen to remove residual water. The absorbed pesticides are then eluted from the columns by using methylene chloride. 50 ul of 500 ppb internal standards mix is then added and the eluent is further evaporated using gentle stream of nitrogen followed by solvent exchange to iso-octane. The final volume is 0.25 mL.

8.2. Extreme care has to be exercised by all the laboratory staff involved in preparation and analysis to limit background contamination due to very low detection limits achievable with the Agilent 5977A GC/MS system. DI water should be used to perform both method blanks and Laboratory Fortified Blanks due to low level pesticide contamination usually found in both tap water and well water.

8.3. Glassware and consummables:

8.3.1. General use organic preparations glassware and supplies are listed in SOP GC-002-2 (ref 15.15).
8.3.2. JTBaker vaccum manifold
8.3.3. Luer lock connectors and stopcocks
8.3.4. 75 mL reservoir tubes.

8.4. SPE procedure:

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8.4.1 Water samples should be pre-filtered in the field. If they were not, pre-filter the samples using 0.7 glass-fiber filter to remove particulate matter. The entire 1 L bottle of sample should be filtered at this stage. Dispense 500 g of the pre-filtered sample into pre-weighted 600 mL glass beaker. Weigh the beaker with the sample to determine sample volume. Adjust the pH of the sample in the beaker to pH=2 with 1M hydrochloric acid.

8.4.2 Pre-dissolve 50 uL of 200 ppb chlorinated surrogates spike (for all blanks and samples) in 10 mL of acetone and add to each sample and blank. For Laboratory fortified Blanks and Matrix Spikes pre-dissolve 50 uL of 200 ppb chlorinated surrogates spike as well as 50 uL of 200/1000 ppb chlorinated pesticides spike in 10 mL of acetone and add to each LFB/MS. Mix the samples in the beakers thoroughly. Acetone serves as conditioner for the sample; pre-diluting the spikes greatly improves the recoveries for some analytes poorly soluble in water.

8.4.3 SPE column pre-cleaning: pre-clean the 500 mg C-18 SPE columns by rinsing with 2x3 mL of methylene chloride. The columns should be installed on the JTBaker vaccum manifold. Allow the solvent to drain by gravity, then completely remove all solvent from column by slight vacuum.

8.4.4 SPE column conditioning: Immediately before sample extraction, add 2x3 mL of methanol to the SPE column and allow the methanol to partially drain through the column by gravity. Conditioning is needed to solvate the C-18 phase attached to silica particles in the SPE column. This conditioning ensures maximum interaction of the C-18 phase with the sample. **Note: do not allow the columns to go dry once conditioning has started. Maintain levels of fluids by adding additional fluid or by closing Luer lock fittings.**

8.4.5 SPE column equilibration: replace the methanol in the SPE column with DI water at pH=2 to equilibrate the column with the sample matrix. Add 2x3 mL of that water at pH=2 and allow the water and methanol to partially drain through the column by gravity or slight vacuum. About five minutes is required for each volume of water and methanol to drip through the column.

8.4.6 Sample extraction: Attach 75 mL reservoir tubes to the SPE columns using Luer connectors. Pour the sample to the upper container and make sure that the entire system is under vaccum. Depending on the sample it may take even several hours to filter a 500 mL sample.

8.4.7 SPE column drying: Dry the SPE columns by using slight vacuum. Do not dry the columns for excessive periods of time. Some low boiling pesticides might evaporate with longer drying times, resulting in low recoveries.

8.4.8 Elution of target analytes: Label 15 mL, pre-calibrated to 250 uL, culture tubes with sample identifications and place in a holding rack. Place the dried SPE columns in the appropriate culture tubes. The open end of the SPE column rests on the edge of the culture tube, keeping the male Luer end of the SPE column raised a few centimeters above the bottom of the culture tube. Add small amount of Na_2SO_4 to the SPE column to remove residual water. Add a small amount of Na_2SO_4 to beaker, and then rinse beaker, glass stir rod, and reservoir tube with methylene chloride. Add that methylene chloride aliquot to SPE column and allow the solvent to drain by slight vacuum into the culture tube. Collect 12 mL total volume of extract. Add 25 uL of 1000 ppb chlorinated internal standard solution to each culture tube. **Note:** Rinsing the beaker with the eluting solvent improves recoveries for some target analyte that tend to adsorb on glass surfaces.

8.4.9 Evaporation of solvent/solvent exchange: Setup a Nitrogen Evaporator (N-Evap^{TM}) by turning on nitrogen-gas, heater (30 °C bath temperature), and attaching clean needles. Blow down to 0.25 mL, vortexing several times during initial blow down to remove any residual water. Solvent exchange to isooctane three times. Submit 0.25 mL in isooctane for analysis.

8.4.10 Each sample preparation must be recorded by the preparation team in the electronic Prep Worksheet Manager module of LIMS. For

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chlorinated pesticides extractions using SPE technique there is a ready-made, partially prefilled template in LIMS. The template name is identical with LIMS prep ID: USGS-O-1126-95. For exact instructions how to fill an electronic prep worksheet please refer to Sop GC-002-2 (ref 15.15).

9. GENERAL DESCRIPTION OF GC/MS-SIM ANALYSIS

9.1. GC/MS Maintenance and Tuning

9.1.1 USGS-method-O-1126-95 requires autotuning the instrument before each run of samples. DFTPP tune recommended by EPA 8270D is not applicable for Agilent 5977A GC/MS with the Extractor Ion Source. Instead, as recommended by 8270D paragraph 11.3.1, this SOP modifies the method to follow manufacturer's tuning instructions, which, in this case, is PFTBA autotune. Therefore PFTBA autotune is performed before each run to fulfill the requirements of both methods.

9.1.2 Initial checking and maintenance of the instrument consists mainly of the following steps

9.1.2.1 Check the vacuum operation and periodically run an air/water leak test. The air/water leak check is now included in the autotune procedure, so it is performed anyway every time the instrument is tuned.

9.1.2.2 Before each run perform the EI high sensitivity autotune of the GC/MS system and save the current settings in the autotune file. The report is printed automatically and it should be placed in the autotune reports log. If the autotune criteria are not met repeat the autotune. If still failing, run the autotune from the default values. If that fails the ion source may have to be cleaned.

9.1.2.3 Replace the injector liner (Agilent 2 mm dimpled liner part no: 5190-2297 is recommended for cold splitless injection), cut 1-3 inch of the column after running contaminated samples or encountering unusually heavy matrix. Occasional trimming of the column is still necessary even if backflush technology is used.

9.1.2.4 Run an Instrument Blank and look for instrument contamination.

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

9.1.2.6 To perform maintenance of the MMI inlet replace the liner and septum and clean the inside of the inlet body with cotton swabs soaked with methanol and methylene chloride. **Do not use a harsh brush to clean the inside of the inlet body!** To complete the MMI inlet cleaning perform an inlet bake out procedure. Set the inlet mode to split (split ratio 200:1) and let the gas flow for a couple of minutes. Then raise the inlet temperature to 330 ^{o}C and the oven temperature to 300 ^{o}C. Bake for 1-2 hrs. It is beneficial and recommended to bake the ion source at 350 ^{o}C at the same time.

9.1.2.7 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.

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

9.2 GC/MS System Initial Calibration

9.2.1. Analyze the calibration standards at the following levels listed in Tables 2. At each level, the internal standards should be present at 100 µg/L. The linearity of the calibration curve is defined by an RSD that is less than or equal to 20% of the Response Factors of the calibration curve, or by a correlation coefficient not smaller than 0.995. If a higher order curve is to be used the correlation coefficient has to be at least 0.995. 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 quadratic fit and finally higher order curves. 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 80-120% 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. Weighting of the calibration curve is frequently necessary to accomplish that goal. 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, 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 have to be determined from the scan mode or special SIM methods with no time segments. The SIM mode time segments (acquisition windows) have to be then adjusted accordingly. A mid-level standard should be then run to confirm that all the peaks are fully within the acquisition windows. Both the analysis end of the method (Masshunter) as well as the data-gathering end of the method (Acquisition) must be adjusted. Alternatively, RT locking procedure can be used to keep the retention times unchanged after the column is trimmed. Approximate retention times, the primary quantitation ions and confirmatory, secondary ions are given along with SIM acquisition time segments start/stop times in Table 1. The primary and secondary ions should all be present and the relative intensities should agree within ±20% (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%. If the Initial Calibration is not run with the batch, the CCV acceptance criterium is +/- 20% (see 9.3.2)

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.

9.3.1 Degradation/tailing factor check (required by EPA 8270D)
A modified GC/MS tuning standard is used to perform this task. The standard contains 5 mg/L each of DDT-p,p' and Pentachlorophenol. Benzidine, which is also recommended by method 8270D, is usually not detectable in the presence of analyte protectants. Since the absence of benzidine peak in the chromatograms

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has no bearing on the chromatographic behavior of the target analytes and surrogates, only pentachlorophenol is used for the tailing factor determination. The degradation check/tailing factor standard is run at the beginning of the analytical run sequence to verify injection port inertness and GC column performance. A full scan version of the acquisition method is used for this purpose. Degradation of DDT to DDD and DDE should not exceed 20%. The percent breakdown calculation is performed using the following formula (EPA method 8081B)

%Dec. DDT = Sum Peak Area (DDE + DDD) / Sum Peak Area (DDE + DDD + DDT) × 100%

The following are the Relative Retention Times for DDD-p,p' and DDE-p,p' (relative to DDT-p,p'):
DDE-p,p' RRT = 0.880
DDD-p,p' RRT = 0.945

Usually it is necessary to perform spectral deconvolution of the chromatograms to remove the analyte protectants "hump" and obtain correct integration values and clean spectra for target analytes.

The tailing factor for pentachlorophenol should not be higher than 2 according to the following equation:

Tailing Factor = BC / AB

Where the peak is defined as follows: AC is the width at 10% height; DE is the height of peak and B is the height at 10% of DE. This equation compares the width of the back half of the peak to the width of the front half of the peak at 10% of the height. If DDT-p,p' degradation and or tailing factors are excessive the injection port maintenance has to be performed until proper chromatographic performance is restored.

9.3.2 Analyze midpoint level standards as continuing calibration check (CCV) standards. These calibration check standards must be analyzed at the start of each run and then after each batch of 10 samples. The acceptance criteria for all components are 80-120% recovery. If multiple CCV failures are observed 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.

9.3.3 Calibration verification standard must also meet the following criteria:

9.3.3.1 Minimum response factors: The minimum RF of the most common target analytes must be within 50% of the original calibration.

9.3.3.2 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.

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

9.3.3.4 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.

9.3.4 MDL or close to MDL level standard has to be run at least once a week to verify that the detection limits are being achieved.

9.4 GC/MS-SIM 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 target analytes should be diluted as deemed necessary. **The concentration of internal standard must remain at 100 µg/L when diluting samples by adding additional internal standard to the extract.**

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)
- degradation/performance check
- Instrument Blank (containing internal standards at 100 ug/L)
- pesticides calibration set (when initial calibration is run, levels 3-10)
- PSCL ICV L-6 initial calibration verification check (when initial calibration is run)
- PSCL CCV L-6 continuing calibration check
- extraction blank
- laboratory fortified blank (LFB)
- laboratory fortified blank duplicate
- sample matrix spike,
- sample matrix spike duplicate
- samples (for up to 10 total, including QC samples)
- CCV continuing calibration check
- samples (up to 1o total)

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.

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9.5.1 Using the Qual software examine the degradation/performance check sample. Calculate DDT decomposition and the tailing factor for pentachlorophenol.

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

9.5.3 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 fulfilled 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 15.12).

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 must be evaluated immediately after data acquisition. If the retention time for any internal standard changes by more than 0.05 minutes 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 final report must be commented 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 CLPEST surrogate spike solution currently containing Decachlorobiphenyl and Tetrachloro-m-xylene at 200 ug/L. The surrogate compounds are expected to be found at 40 µg/L in all the sample extracts, Blanks, LFBs, and Matrix Spikes.

9.5.6.2 To determine the acceptance accuracy limits for a surrogate the following procedure should be used (ref 15.5): (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).

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The control limits are supposed to be checked once per year if enough measurements have been conducted. The acceptance limits of 50-150 % for decachlorobiphenyl and 40-140% for Tetrachloro-m-xylene will 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 "analyte" 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.

9.5.8 Blank contamination

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 response is checked immediately by running an Initial Calibration Verification standard (ICV) and then, on a continuing basis (every 10 samples), 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.

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If re-analysis is not an option, the analytical results shall be qualified as estimates (see 15.6 for more details).

9.5.10. Quantitation of positives.
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 interpolation for the sample extract. However, the analyst must ensure that all identification criteria are fulfilled 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 15.12). 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} (ng/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) recorded in the preparation report.

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

- V_{sample} -This is the volume of the water sample taken in extraction, expressed in mLs (usually 500 mL).

10 REPORTING AND DOCUMENTATION

10.1 When analysis is complete, generate a .txt file in the Masshunter software 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). 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:

PSCL_CCV
SURPSCL_CCV for surrogate in CCV
SURPSCL_1234567 for surrogate in sample

10.2 The QC manager will convert the data from ug/L to ng/L (LIMS reporting units, water samples only; not applicable for soils and tissues) and also it will add MDLs for all analytes not included in the text file (nondetects). The converter will also average the PCB aroclors result and report the average as the corresponding aroclor.

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 15-6) for details of data review and reporting, See DEP SOP LB-027, Standard Operating Procedure for Reporting Qualified Data (ref 15-7) for guidelines for qualifying data when quality control results are outside established

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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 print out 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 15.8) for detailed information on quality control, definitions and interferences. See DEP SOP LB-027, Standard Operating Procedure for Reporting Qualified Data (ref 15.7) 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
  - Proper identification of Aroclor patterns
- Check information in the MassHunter Report Header:
  - SOP #
  - Instrument ID

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  - Analyst name
- 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\BACKUP_GC_MSD-3 (or MSD-4). 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.

12.3 Copy the processed data files Quantitation reports (Masshunter .txt files) into \\TLHLAB3\GC\UPLOAD_GC_MSD-3(or MSD-4) to convert in QC Manager for reporting in LIMS. These files may be deleted after all results are authorized.

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 15.3). Also refer to Reference 15.9 and 15.10**.**

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 spikes and blanks analysis 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).

14. SAFETY/HAZARDOUS WASTE MANAGEMENT

See SOP OG-001, Safety and Waste Management Practices in the Organic Analysis Groups (Volatile, Semi-Volatile and Pesticide Laboratories) (ref 15.11) for specific details on safety and hazardous waste management.

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15. REFERENCES

15.1 Test Methods for Evaluating Solid Wastes, Third Edition, SW-846, Method 8270D, Semi-volatile Organic Compounds By Gas Chromatography/Mass Spectrometry (GC/MS).

15.2 USGS-Method-O-1126-95." Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory – Determination of Pesticides in Water by C-18 Solid-Phase Extraction and Capillary Column Gas Chromatography/Mass Spectrometry with Selected Ion Monitoring". Denver, Colorado 1995.

15.3 Anastassiades, M., Mastovska, K., Lehotay, s.J., "Evaluation of analyte protectants to improve gas chromatographic analysis of pesticides" Journal of Chromatography A, 1015 (2003) 163-184.

15.4 Mastovska, K., Lehotay, S.J., Anastassiades, M., "Combination of analyte protectants to overcome matrix effects in routine GC analysis of pesticides residues in food matrices" anal. Chem. **2005**, 77, 8129-8137.

15.5 EPA 8000C Determinative Chromatographic Separation; Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods, SW 846; 3rd Edition, Update 4, January 3, 2008.

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

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

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

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

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

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

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

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

15.14 Test Methods for Evaluating Solid Wastes, Third Edition, SW-846, Method 8082A, Polychlorinated Biphenyls (PCBs) By Gas Chromatography

15.15 DEP SOP GC-002-2. Extraction of Pesticides and Polychlorinated Biphenyls (PCBs) from Water, Wastes, Soil/Sediment, PUFs and Filters for Subsequent Analysis by GC or GC/MS Techniques.

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16. TABLES

TABLE 1: Quantifier and qualifier Ions, Internal standard associations, MDLs, PQLs and approximate RTs for Chlorinated Pesticides included in LIMS tests W-PCL-SQ and W-PCL-SQ-R **LIMS reporting units are ng/L.**

Analyte	Approximate Retention Time (min)	Associated internal standard	Quantifier ion	Qualifier ions	MDL ug/L extract	PQL ug/L extract	MDL ng/L sample	PQL ng/L sample
Tetrachloro-m-xylene surrogate	7.747	DCB-d5	242.0	209.0 244.0				
Decachlorobiphenyl surrogate	18.843	HCB-d3	498.0	428.0 214.0				
Trifluralin	8.101	DCB-d5	264.0	306.0 290.0	2.0	8.0	1.0	4.0
Alpha-BHC	8.524	Alpha-BHC-d6	183.0	217.0 219.0	4.0	16	2.0	8.0
Beta-BHC	9.063	Alpha-BHC-d6	219.0	221.0 254.0	8.0	32	4.0	16
Gamma-BHC	9.209	Alpha-BHC-d6	219.0	183.0 217.0	8.0	32	4.0	16
Delta-BHC	9.706	Alpha-BHC-d6	219.0	183.0 217.0	8.0	32	4.0	16
Chlorothalonil	9.820	DCB-d5	266.0	264.0 268.0	4.0	16	2.0	8.0
Heptachlor	10.817	TCB-d5	337.0	339.0 372.0	3.0	12	1.5	6.0
Aldrin	11.635	TCB-d5	293.0	291.0 265.0	8.0	32	2.0	8.0
Dicofol	11.790	TCB-d5	252.0	250.0 139.0	60	240	30	120
Heptachlor Epoxide	12.591	TCB-d5	353.0	351.0 263.0	4.0	16	2.0	8.0
Gamma-Chlordane	13.174	TCB-d5	373.0	375.0 377.0	2.0	8.0	1.0	4.0
Endosulfan I	13.478	TCB-d5	277.0	337.0 339.0	8.0	32	4.0	16
Alpha-Chlordane	13.543	TCB-d5	373.0	375.0 377.0	2.0	8.0	1.0	4.0
DDE-p,p’	14.055	TCB-d5	246.0	318.0 316.0	6.0	24	3.0	12
Dieldrin	14.123	TCB-d5	380.0	263.0 279.0	8.0	32	4.0	16

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Endrin	14.661	TCB-d5	263.0	265.0 261.0	4.0	16	2.0	8.0
Endosulfan II	14.892	TCB-d5	337.0	339.0 277.0	8.0	32	4.0	16
DDD-p,p’	15.101	TCB-d5	235.0	237.0 212.0	4.0	16	2.0	8.0
Endrin Aldehyde	15.366	TCB-d5	345.0	349.0 347.0	3.0	12	1.5	6.0
Carbophenothion	15.757	TCB-d5	342.0	344.0 296.0	8.0	32	4.0	16
Endosulfan Sulfate	15.922	HCB-d3	272.0	235.0 387.0	4.0	16	2.0	8.0
DDT-p,p’	15.983	DDT-p,p’-d8	235.0	237.0 318.0	6.0	24	3.0	12
Endrin Ketone	16.695	HCB-d3	317.0	319.0 345.0	4.0	16	2.0	8.0
Methoxychlor	16.872	DDT-p,p’-d8	227.0	228.0 212.0	8.0	32	4.0	16
Mirex	17.400	HCB-d3	272.0	274.0 237.0	4.0	16	2.0	8.0
Permethrin	17.952	HCB-d3	183.0	184.0 165.0	20	80	10	40
Cypermethrin	18.428	HCB-d3	181.0	163.0 165.0	40	160	20	80
Dicofol-conf*	16.885	DDT-p,p’-d8	139.0	251.0 141.0				
DCB-d5	8.141	Internal standard	227.0	157.0 229.0				
Alpha-BHC-d6	8.450	Internal standard	222.0	224.0 226.0				
TCB-d5	11.498	Internal standard	297.0	225.0 295.0				
DDT-p,p’-d8	15.933	Internal standard	243.0	245.0 173.0				
HCB-d3	16.833	Internal standard	363.0	365.0 293.0				

*****Dicofol –conf is used for confirmation purposes only due to its erratic chromatographic behavior. The first Dicofol peak is used for quantitation**.**

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Table 2: Analyte concentrations in calibration standards and /QCS/ICV for Chlorinated pesticides (ug/L) (Test ID W-PCL-SQ and W-PCL-SQ-R). The expected spike concentrations in LFBs/MSs are equal to 40/200 ug/L (in extract)

PSCL	L10	L9	L8	L7	L6	L5	L4	L3	L6
Aldrin	400	300	200	100	50	20	10	4	50
Alpha-BHC	400	300	200	100	50	20	10	4	50
Cis/Alpha-Chlordane	400	300	200	100	50	20	10	4	50
Beta-BHC	400	300	200	100	50	20	10	4	50
DDD-p,p'	400	300	200	100	50	20	10	4	50
DDE-p,p'	400	300	200	100	50	20	10	4	50
Delta-BHC	400	300	200	100	50	20	10	4	50
Dieldrin	400	300	200	100	50	20	10	4	50
Endosulfan I	400	300	200	100	50	20	10	4	50
Endosulfan II	400	300	200	100	50	20	10	4	50
Endosulfan Sulfate	400	300	200	100	50	20	10	4	50
Endrin Aldehyde	400	300	200	100	50	20	10	4	50
Endrin Ketone	400	300	200	100	50	20	10	4	50
Gamma-BHC	400	300	200	100	50	20	10	4	50
Trans/Gamma-Chlordane	400	300	200	100	50	20	10	4	50
Heptachlor	400	300	200	100	50	20	10	4	50
Heptachlor Epoxide	400	300	200	100	50	20	10	4	50
Methoxychlor	400	300	200	100	50	20	10	4	50
Carbophenothion	400	300	200	100	50	20	10	4	50
Chlorothalonil	400	300	200	100	50	20	10	4	50
Cypermethrin	2000	1500	1000	500	250	100	50	20	250
Dicofol	400	300	200	100	50	20	10	4	50
Mirex	400	300	200	100	50	20	10	4	50
Permethrin	2000	1500	1000	500	250	100	50	20	250
Trifluralin	400	300	200	100	50	20	10	4	50
DDT-p,p'	400	300	200	100	50	20	10	4	50
Endrin	400	300	200	100	50	20	10	4	50
TMX	400	300	200	100	50	20	10	4	50
DCB	400	300	200	100	50	20	10	4	50

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TABLE 3: QC MEASUREMENTS AND ACCEPTACE CRITERIA

#	QC MEASUREMENT	ACCEPTANCE CRITERION	CORRECTIVE ACTION
1	LFB, Matrix Spike precision	≤ 30%	Reanalyze/Re-extract/Flag
2	LFB, Matrix Spike - recoveries	60-150% except for 40-120% for Aldrin, Heptachlor and Mirex 30-150 for Dicofol	Reanalyze/Re-extract/Flag
3	ICV	± 30%	Reanalyze/Flag
4	CCV	± 20%	Re-run and recalibrate/Flag
5	Surrogate recovery	Tetrachloro-m-xylene 40-140% Decachlorobiphenyl 50-150%	Reanalyze/Re-extract/Flag
6	Linearity criterion	20% RSD; 0.995 CC	Use appropriate cal fit / Re-run/ Recalibrate
7	Internal Std. recovery	50-200%	Reanalyze/recalibrate/flag
8	% cal drift	± 20% of expected value	Change fit/weighing/recalibrate/flag

17. APPENDICES

APPENDIX 1

GC/MS Acquisition Method – Method: PSCL_SIM_yymmdd

INSTRUMENT CONTROL PARAMETERS: GC_MSD-4

D:\MassHunter\GCMS\1\methods\PSCL_SIM_150914.M
Mon Sep 14 13:22:50 2015

Control Information
------- -----------

Sample Inlet : GC
Injection Source : GC ALS
Injection Location: Front
Mass Spectrometer: Enabled

No Sample Prep method has been assigned to this method.

GC Oven Temperature
Setpoint On
(Initial): 50 °C
Hold Time: 1.5 min
Post Run: 280 °C
Program:
#1 Rate 55 °C/min
#1 Value 160 °C
#1 Hold Time 0 min
#2 Rate 7 °C/min

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#2 Value	245 °C
#2 Hold Time	0 min
#3 Rate	50 °C/min
#3 Value	310 °C
#3 Hold Time	5 min

Equilibration Time	0.5 min
Max Temperature	325 °C
Maximum Temperature Override	Disabled
Slow Fan	Disabled
Cryo	Off

ALS
Front Injector

Syringe Size	10 µL
Injection Volume	1 µL
Solvent A Washes (PreInj)	3
Solvent A Washes (PostInj)	15
Solvent A Volume	8 µL
Solvent B Washes (PreInj)	3
Solvent B Washes (PostInj)	15
Solvent B Volume	8 µL
Sample Washes	1
Sample Wash Volume	4 µL
Sample Pumps	7
Dwell Time (PreInj)	0 min
Dwell Time (PostInj)	0 min
Solvent Wash Draw Speed	300 µL/min
Solvent Wash Dispense Speed	6000 µL/min
Sample Wash Draw Speed	50 µL/min
Sample Wash Dispense Speed	6000 µL/min
Injection Dispense Speed	68 µL/min
Viscosity Delay	7 sec
Sample Depth	Disabled
Injection Type	3-layer Sandwich
L1 Airgap	0 µL
L2 Volume	0.2 µL
L2 Airgap	0 µL
L3 Volume	0.4 µL
L3 Airgap	0 µL
Solvent Wash Mode	A-A6,B-B4

Sample Overlap

Mode	Sample overlap is not enabled

ALS Errors	Pause for user interaction

Front MM Inlet He
Temperature

Setpoint	On
(Initial)	60 °C
Hold Time	0.35 min
Post Run	0 °C

Program

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#1 Rate	900 °C/min
#1 Value	280 °C
#1 Hold Time	17 min
#2 Rate	900 °C/min
#2 Value	310 °C
#2 Hold Time	0 min

Mode	Pulsed Splitless
Heater	On 60 °C
Pressure	On 9.6147 psi
Total Flow	On 54.1 mL/min
Septum Purge Flow	On 3 mL/min
Gas Saver	Off
Injection Pulse Pressure	30 psi Until 1.4 min
Purge Flow to Split Vent	50 mL/min at 1.5 min
Cryo	Off

Back SS Inlet He

Mode	Splitless
Heater	Off
Pressure	Off
Total Flow	Off
Septum Purge Flow	On 2 mL/min
Purge Flow to Split Vent	60 mL/min at 2 min

Thermal Aux 2 (MSD Transfer Line)

Temperature	
Setpoint	On
(Initial)	300 °C
Post Run	0 °C

Column
Column #1

Flow	
Setpoint	Off
(Initial)	1.1 mL/min
Hold Time	17 min
Post Run	-4.3283 mL/min
Program	
#1 Rate	100 mL/min per min
#1 Value	-2.5 mL/min
#1 Hold Time	2.5 min
#2 Rate	100 mL/min per min
#2 Value	-5 mL/min
#2 Hold Time	0 min

Agilent 19091S-431UI: 2197.57912
HP-5MS UI
0 °C—325 °C (325 °C): 5 m x 250 µm x 0.25 µm

Column lock	Unlocked
In	Front MM Inlet He
Out	Aux EPC 3

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(Initial)	50 °C
Pressure	9.6147 psi
Flow	1.1 mL/min
Average Velocity	25.587 cm/sec
Holdup Time	0.32569 min

Column #2

Flow	
Setpoint	Off
(Initial)	1.2 mL/min
Hold Time	19.536 min
Post Run	0.21034 mL/min
Program	
#1 Rate	100 mL/min per min
#1 Value	2.5 mL/min
#1 Hold Time	0 min

Agilent 19091S-431UI
0 °C—325 °C (325 °C): 25 m x 250 µm x 0.25 µm

Column lock	Unlocked
In	Aux EPC 3 He
Out	MSD
(Initial)	50 °C
Pressure	7.6522 psi
Flow	1.2 mL/min
Average Velocity	43.734 cm/sec
Holdup Time	0.95274 min

Column Outlet Pressure 0 psi

Aux EPC 1,2,3
Aux EPC 1 N2

Pressure	
Setpoint	Off
(Initial)	10 psi
Post Run	0 psi

Excluded from Affecting GC's Readiness State

Aux EPC 2 N2

Pressure	
Setpoint	Off
(Initial)	10 psi
Post Run	0 psi

Excluded from Affecting GC's Readiness State

Aux EPC 3 He
Excluded from Affecting GC's Readiness State
Aux EPC 3 He Supplies Column 2

Aux EPC 4,5,6
Aux EPC 4 N2

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Pressure
Setpoint Off
(Initial) 10 psi
Post Run 0 psi

Excluded from Affecting GC's Readiness State

Aux EPC 5 N2
Pressure
Setpoint Off
(Initial) 10 psi
Post Run 0 psi

Excluded from Affecting GC's Readiness State

Aux EPC 6 He
Pressure
Setpoint Off
(Initial) 10 psi
Post Run 0 psi

Excluded from Affecting GC's Readiness State

Signals
Signal #1: Test Plot
Description Test Plot
Details
Save Off
Data Rate 50 Hz
Dual Injection Assignment Front Sample

Signal #2: Test Plot
Description Test Plot
Details
Save Off
Data Rate 50 Hz
Dual Injection Assignment Back Sample

Signal #3: Test Plot
Description Test Plot
Details
Save Off
Data Rate 50 Hz
Dual Injection Assignment Back Sample

Signal #4: Test Plot
Description Test Plot
Details
Save Off
Data Rate 50 Hz
Dual Injection Assignment Back Sample

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MS Information
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General Information
------- -----------

Acquisition Mode : SIM
Solvent Delay (minutes) : 5
Tune file : D:\MassHunter\GCMS\1\5977\etune.u
EM Setting mode Gain : 5.000000

Number of SIM Groups : 15
Run Time (if MS only) : 650 minutes

[SIM Parameters]
Group 1 Group ID : 2
Resolution : 0
Group Start Time : 5
Number of Ions : 9
Ions
Dwell In Group :(Mass, Dwell) (Mass, Dwell) (Mass, Dwell)
(157.00,35) (209.00,35) (227.00,35)
(229.00,35) (242.00,35) (244.00,35)
(264.00,35) (290.00,35) (306.00,35)

Group 2 Group ID : 3
Resolution : 0
Group Start Time : 8.33
Number of Ions : 6
Ions
Dwell In Group :(Mass, Dwell) (Mass, Dwell) (Mass, Dwell)
(183.00,50) (217.00,50) (219.00,50)
(222.00,50) (224.00,50) (226.00,50)

Group 3 Group ID : 4
Resolution : 0
Group Start Time : 8.9
Number of Ions : 5
Ions
Dwell In Group :(Mass, Dwell) (Mass, Dwell) (Mass, Dwell)
(183.00,60) (217.00,60) (219.00,60)
(221.00,60) (254.00,60)
Group 4 Group ID : 5
Resolution : 0
Group Start Time : 9.45
Number of Ions : 6
Ions
Dwell In Group :(Mass, Dwell) (Mass, Dwell) (Mass, Dwell)
(183.00,50) (217.00,50) (219.00,50)
(264.00,50) (266.00,50) (268.00,50)

Group 5 Group ID : 6
Resolution : 0
Group Start Time : 10.25

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Number of Ions : 3
Ions
Dwell In Group :(Mass, Dwell) (Mass, Dwell) (Mass, Dwell)
(337.00,100) (339.00,100) (372.00,100)

Group 6 Group ID : 7
Resolution : 0
Group Start Time : 11.15
Number of Ions : 9
Ions
Dwell In Group :(Mass, Dwell) (Mass, Dwell) (Mass, Dwell)
(139.00,35) (225.00,35) (250.00,35)
(252.00,35) (265.00,35) (291.00,35)
(293.00,35) (295.00,35) (297.00,35)

Group 7 Group ID : 8
Resolution : 0
Group Start Time : 12.15
Number of Ions : 3
Ions
Dwell In Group :(Mass, Dwell) (Mass, Dwell) (Mass, Dwell)
(263.00,100) (351.00,100) (353.00,100)

Group 8 Group ID : 9
Resolution : 0
Group Start Time : 12.85
Number of Ions : 6
Ions
Dwell In Group :(Mass, Dwell) (Mass, Dwell) (Mass, Dwell)
(277.00,50) (337.00,50) (339.00,50)
(373.00,50) (375.00,50) (377.00,50)

Group 9 Group ID : 10
Resolution : 0
Group Start Time : 13.75
Number of Ions : 7
Ions
Dwell In Group :(Mass, Dwell) (Mass, Dwell) (Mass, Dwell)
(246.00,45) (263.00,45) (277.00,45)
(279.00,45) (316.00,45) (318.00,45)
(380.00,45)
Group 10 Group ID : 11
Resolution : 0
Group Start Time : 14.4
Number of Ions : 12
Ions
Dwell In Group :(Mass, Dwell) (Mass, Dwell) (Mass, Dwell)
(212.00,25) (235.00,25) (237.00,25)
(261.00,25) (263.00,25) (265.00,25)
(277.00,25) (337.00,25) (339.00,25)
(345.00,25) (347.00,25) (349.00,25)

Group 11 Group ID : 12
Resolution : 0
Group Start Time : 15.6

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Number of Ions : 12
Ions
Dwell In Group :(Mass, Dwell) (Mass, Dwell) (Mass, Dwell)
(173.00,25) (235.00,25) (237.00,25)
(243.00,25) (245.00,25) (272.00,25)
(274.00,25) (296.00,25) (318.00,25)
(342.00,25) (344.00,25) (387.00,25)

Group 12 Group ID : 13
Resolution : 0
Group Start Time : 16.35
Number of Ions : 12
Ions
Dwell In Group :(Mass, Dwell) (Mass, Dwell) (Mass, Dwell)
(139.00,25) (141.00,25) (212.00,25)
(227.00,25) (228.00,25) (251.00,25)
(293.00,25) (317.00,25) (319.00,25)
(345.00,25) (363.00,25) (365.00,25)

Group 13 Group ID : 14
Resolution : 0
Group Start Time : 17.15
Number of Ions : 3
Ions
Dwell In Group :(Mass, Dwell) (Mass, Dwell) (Mass, Dwell)
(237.00,100) (272.00,100) (274.00,100)

Group 14 Group ID : 15
Resolution : 0
Group Start Time : 17.7
Number of Ions : 3
Ions
Dwell In Group :(Mass, Dwell) (Mass, Dwell) (Mass, Dwell)
(165.00,100) (183.00,100) (184.00,100)

Group 15 Group ID : 16
Resolution : 0
Group Start Time : 18.2
Number of Ions : 6
Ions
Dwell In Group :(Mass, Dwell) (Mass, Dwell) (Mass, Dwell)
(163.00,50) (165.00,50) (181.00,50)
(214.00,50) (428.00,50) (498.00,50)

[MSZones]

MS Source : 300 C maximum 350 C
MS Quad : 150 C maximum 200 C

Timed Events
----- ------
Number Events= 1

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[Timed MS Detector Table Entries]

Time (min)	State (MS On/off)
19.30	off

END OF MS ACQUISTION PARAMETERS

TUNE PARAMETERS for SN: US1523M404

Trace Ion Detection is ON.

EMISSION : 34.593
ENERGY : 70.007
REPELLER : 4.979
IONFOCUS : 89.822
ENTRANCE_LE : 22.672
EMVOLTS : 986.266
Actual EMV : 1249.8
GAIN FACTOR : 5.01
AMUGAIN : 1370.000
AMUOFFSET : 120.750
FILAMENT : 1.000
DCPOLARITY : 0.000
ENTLENSOFFS : 9.945
Ion_Body : 7.002
EXTLENS : 0.122
MASSGAIN : -355.000
MASSOFFSET : -36.000

END OF TUNE PARAMETERS

END OF INSTRUMENT CONTROL PARAMETERS

APPENDIX 2

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:

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```
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.

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APPENDIX 3

ANALYTE PROTECTANTS

Follow the instructions in appendix 2 to re-prepare analytes protectants solutions.

Notes:
Neat D-sorbitol is hydroscopic and should be stored in a dessicator.D-Sorbitol and, especially, gulonolactone are difficult to dissolve in MeOH. To achieve complete dissolution of these components, 1 – 3 hours of hot sonication and repeated vigourous shaking (vortexing) may be needed. To avoid extensive sonication, required quantities of analyte protectants may be initially dissolved in 5 mL of water. The resulting solution may then be brought up to the final volume of 100 mL by adding methanol.The resulting solution is saturated with gulonolactone and therefore should be kept at room temperature to avoid excessive precipitation. Some precipitation, however, appears to be unavoidable.
AP solution used for GC/MS 5977 analysis is a two times diluted version of a standard used for GC/MS triple quad analyses.

APPENDIX 4

Comparison of QC requirements between method USGS-O-1126-95 and EPA 8270D

QC requirement	USGS-O-1126-95	EPA 8270D	This SOP
Tune/tune check	Autotune daily		Autotune daily
Qualifier ions acceptance %	20%	30%	20%
Degradation check	Not required	Tailing factors not more than 2, DDT degradation not more than 20%	Tailing factors not more than 2, DDT degradation not more than 20%
Initial calibration	RSD<35% other fits allowed, other fits allowed if cal drift below 20%	RSD<20% or CC>0.995	RSD<20% or CC>0.995
Calibration drift	<20%	<30%	<20%
Initial Calibration Verification (ICV) recovery limits	Not required	70-130%; second source standard	70-130%; second source standard
Continuous Calibration Verification (CCV) recovery limits	80-120%	80-120%	80-120%
Continuous Calibration Verification (CCV) frequency	Every 10 samples	Every 12 hrs	Every 10 samples as it is more strict
MDL check	Near or at MDL level standard run at least weekly	Not required	Near or at MDL level standard run at least weekly.

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Appendix of significant changes.

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