ANALYSIS OF ORGANONITROGEN AND ORGANOPHOSPHORUS PESTICIDES IN FISH TISSUE BY GAS CHROMATOGRAPHY WITH NITROGEN-PHOSPHORUS DETECTION (GC/NPD).



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1. SCOPE AND APPLICATION

1.1. This method is applicable to the determination of 11 organonitrogen and organophosphorus pesticides in fish tissue as listed in Table 3. Method Detection Limits and Practical Quantitation Limits yielded by this method are also provided in Table 3.

1.2. This method is based on EPA 8141B (see 13.1). Laboratory Information Management System (LIMS) tests: t-pest-np.

1.3. This Standard Operation Procedure is restricted to use by an analyst experienced in the use of a gas chromatograph, in the interpretation of gas chromatograms, 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
- Ability to perform basic laboratory mathematics
- Knowledge of EZChrom Chromatography Software, TARGET Chromatography Software and DEP LIMS.

2. SUMMARY OF THE METHOD

2.1. Fish tissue samples are extracted using the appropriate procedures listed in the Preparation SOPs Ref 13.4). The extract is analyzed using a Gas Chromatograph (GC) equipped with two capillary GC columns and two Nitrogen-Phosphorus Detectors (NPDs) to provide confirmatory analyses for all positive hits.

3. INTERFERENCES

3.1. 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 as described in Section 6.1. 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 detergent washing with hot water, and rinses with tap water and distilled water. The glassware should then be drained dry, and heated in a muffle furnace at 550 °C for at least 8 hours. Some thermally stable materials may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may be substituted for 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. 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 nature and diversity of the industrial complex or municipality being sampled.

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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.

4.1. 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.

4.2. 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).

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

4.3. 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.

4.4. 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.

4.5. 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.

4.6. 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.

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

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4.8. 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.

4.9. 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.

4.10. 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.

4.11. 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.

4.12. 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 deionized water.

4.13. 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).

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

where:
X1 and X2 are the two measurements

4.14. QUALITY CONTROL CHECK STANDARD (QCS): 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.

4.15. 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.

4.16. 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.

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4.17. 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. Gas Chromatograph: Shimadzu GC 2010 or other gas chromatograph capable of four steps temperature programming, with dual nitrogen phosphorus detectors, dual capillary split/splitless injectors and a dual Automatic Sampler. The GC and GC Automatic Sampler operating parameters are illustrated in Table 1 and Table 2 of the Appendix.

5.1.1. The first analytical column is a DB-5, J & W fused silica capillary, 30 m x 0.32 mm x 0.25 um or similar column.

5.1.2. The other analytical column is a DB-17, J & W fused silica capillary, 30 m x 0.32 mm x 0.25 um or similar column.

5.2. Data system

5.2.1. A computer system is interfaced to the Chromatograph 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 EZChrom software (see 13.2) from Agilent Technologies Inc., for data acquisition, and Target software (see 13,3), from Thermo Fisher Scientific Inc., which is used for processing and reporting of chromatographic data.

5.2.3. For reporting data, the system currently uses DEP LIMS.

5.3. Gases

5.3.1. Helium, UPC Grade, is used as the carrier and make up gas. The helium is passed through an oxygen and a hydrocarbon filter before it enters the GC.

5.3.2. Hydrogen, UPC Grade, is used as a combustion gas for the NPD detectors. The H_2 is passed through a hydrocarbon filter before entering the GC.

5.3.3. Air, UPC Grade is used as combustion gas for the NPD detectors. Is passed through a hydrocarbon filter prior entering the GC.

6. REAGENTS AND CHEMICALS

6.1. Solvents

6.1.1. ISO-Octane, Fisher Scientific, Pesticide, HPLC or OPTIMA grade.

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

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6.2. Standards

6.2.1. For detailed standard preparation see APPENDIX 1

6.3. Stock and Intermediate Standards

6.3.1. Individual stock standards are concentrated standards prepared from neat standards (approximately 100% purity) once a year. The concentration for all individual stock standards is 1,000 ng/uL. Stock Standards mixtures prepared from neat standards, are usually multiples of 1,000 ng/uL to match the ratios in the working standards. Standards may be prepared in the DEP lab or purchased from a certified manufacturer (ISO 9000 or EPA certified). Purchased custom made stock- standards, may have different concentrations than the ones prepared in house from neat.

6.3.2. Intermediate standard solutions are mixture or individual standards used to prepare working standards. They are prepared in our lab from stock standards by mixing / diluting with iso-octane and acetone or other appropriate solvents or can be purchased from a certified manufacturer (ISO 9000 or EPA certified). There is one standard mixture used in this procedure: NPD A. The concentrations of the components in the intermediate standard are listed in Table 4. Standard is 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 typically +/- 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.

6.4. Working Standard Solutions (Calibration standards)

6.4.1. Working standard solutions are a series of diluted individual or mixture standards in iso-octane and 20% acetone. They are used for calibration and are prepared by diluting intermediate standard solutions with iso-octane/acetone. Two internal standards, triphenyl phosphate (TPP) and 5-Nitroquinoline (NQ), are added to every working standard. (See Table 4 for amount).

6.4.2. The concentrations for each analyte in the intermediate and diluted standards are listed in Table 4.

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 with acetone stock or intermediate standards . Concentrations of the parameters in these solutions are listed in Table 4.

6.5.3. Freshly prepared spike solutions are checked vs. valid working standards. The acceptance criterion is +/- 10 % difference.

6.6. Quality Control Standards (QCS)

6.6.1. Quality Control Check Standards (QCS) 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 +/- 15% difference.

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6.6.2. The Analyst must insure that calibration standards routinely used, are checked with the corresponding QCS with every initial calibration.

7. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

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

7.2. For sample preservation refer to DEP SOP GC-004-1 (Ref 13.4).
7.3. All samples must be extracted within 365 days of collection and completely analyzed within 40 days of extraction.

8. SAMPLE PREPARATION PROCEDURE

8.1. See Preparation SOP: "EXTRACTION OF ORGANOPHOSPHORUS, ORGANONITROGEN, ORGANOHALIDE PESTICIDES and PCBs FROM FISH TISSUE FOR SUBSEQUENT ANALYSIS BY GC OR GC/MS TECHNIQUES" (see 13.4).
8.2. For distribution of parameters between the Florisil clean-up fractions refer to TABLE 6.

9. GENERAL DESCRIPTION OF GC/NPD ANALYSIS

9.1. Pre-Run GC Maintenance

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

9.1.1.1. Check the detector signals to be in the accepted range

9.1.1.2. Clean and refill solvent vials in the autosampler turret.

9.1.1.3. Replace the injector liner and cut 3-5 inches of the column after running contaminated samples or displaying unusual heavy matrix.

9.1.1.4. Run and Instrument Blank and look for instrument contamination.

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

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

9.1.2. Troubleshooting following runs producing poor data quality.

If root cause analysis of runs with poor data quality indicates an instrument performance problem then more detailed troubleshooting may be needed to address the issue. The following are the steps that may need to be taken to restore the performance of an NPD instrument:

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9.1.2.1. If after the second column trimming the chromatographic performance is not restored the column may need to be replaced.

9.1.2.2. Clean or replace the autosampler syringe. Surprising numbers of chromatographic problems are caused by dirty autosampler syringes.

9.1.2.3. Clean the NPD jet (dirty jet may cause irregular baseline and poor response).

9.1.2.4. Check inlet and detector assemblies for leaks using electronic leak detector. Fix any leaks discovered.

9.1.2.5. If the detector's response changes significantly between consecutive injections re-condition the beads.

9.1.2.6. If excessive noise is observed in the baseline, check the gas lines for leaks and verify the purity of gases used (helium, medical air and hydrogen). Replace the gas lines filters, if needed.

9.1.2.7. Baseline noise and other "electronic" baseline issues are frequently caused by impurities and/or electrical contact problems in the jet/bead assemblies). Disconnect the parts, clean/blow with compressed gas and reconnect.

9.1.2.8. Baseline noise/ sensitivity problems can sometimes be rectified by either conditioning the bead for 2 hrs at higher background current (2.5 pA) or at higher hydrogen flow (5 mL/min; "burning off" the bead).

9.1.2.9. If the detector performance still cannot be improved the bead needs to be recoated or replaced. Replacement beads are supposed to be stored in a dessicator due to their high sensitivity to moisture. The new bead, after installation, should be conditioned overnight at its operating temperature (280C) before being activated for the first time. Activating a bead too early, before the moisture is removed, may lead to irreversible damage.

9.1.2.10. If the problem persists with the new bead installed and properly conditioned contact Shimadzu service engineer for further assistance.

9.2. Summary of GC/NPD Analysis

9.2.1. The analysis of samples is accomplished by using two dissimilar fused silica capillary columns, usually a DB-5 and a DB-17 or similar column (usually 0.32mm ID/30m/0.25-0.50 um).

9.2.2. One of the two columns, based on the best performance, is chosen by the analyst to be "primary". The "primary column" is used to report the results. Important criteria for choosing the "primary" column include:

9.2.2.1. Stable retention time windows during the entire run sequence
9.2.2.2. The best chromatography (no tailing or fronting, no impurity interference with surrogates of target components)
9.2.2.3. The best calibration curves especially for target positives
9.2.2.4. The best LCS/Matrix Spikes results
9.2.2.5. The smallest number of CCV failures
9.2.2.6. The best surrogates results

9.2.3. Identification of positives requires all of the following QA/QC criteria to be meet:

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9.2.3.1. Use of an updated five point calibration (updated Retention Times and Response Factors).
9.2.3.2. Stable retention time windows (all calibration check standards are within the calibration windows).
9.2.3.3. Recoveries for surrogates and spikes are within the control limits.

9.2.4. All the samples are analyzed in two columns. The MBLK, LFB, Matrix Spikes and Sample reported results are based on the primary column (usually DB-17). If the results found in this column are not acceptable due to matrix interference or other problems, the results will be reported using the values found in the confirmatory column (usually DB-5).

9.2.5. A single peak target component is identified as positive if its peak is detected within its appropriate retention time window in both columns. The amount of the prospective hit found in one column has to be confirmed in the other column. Criterion for the amount confirmation is equal to or less than +/- 30% difference between the two columns responses. If besides retention time, there is some other evidence that the target analyte is a positive, and the amount in two column does not match, the result will be reported based on the smallest value found in the two columns, and will be qualified as estimated (J).

9.2.6. Quantitative analysis of pesticides is accomplished by the internal calibration method. All target component positives found have to be quantified using at least a five-point calibration curve (six points for the quadratic curve). 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 0.995. Initially RSD of the Response Factors is examined. If the RSD exceeds 20% then linear and finally quadratic curves are tried to establish the best fit for each parameter. Do not use the "include" option for treatment of the origin in calibration curves. Each calibration curve is additionally checked by re-fitting the lowest calibration point (PQL) back to the model. The calculated amounts for each of the standard components should be within 20% of the expected values. After identification, all positives are quantified. If out of calibration range, the samples are diluted, re-run and reanalyzed. The prospective hits, found below reported method detection limit, are reported as non-detect unless the customer requests otherwise.

9.2.7. The analyst should monitor the performance of the extraction, cleanup, analytical system, and effectiveness of the method by spiking each sample, QC sample and Method Blank with pesticide surrogate. The surrogate is nitro-m-xylene (NMX). To determine acceptance accuracy limits for surrogate the following procedure should be used (See 13.5): (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 coefficient for n-1 degrees of freedom (n=number of measurements).

The control limits should be checked once per year if enough measurements have been conducted.

9.2.8. If the surrogate recovery failed, 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 results have to be qualified as estimated (J).. If the recovery is biased low the sample must be re-extracted and reanalyzed.

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9.2.9. Absolute Retention times (Rts) are used for the identification of targets. The absolute retention time window is assessed during initial calibration as +/- 0.035 minute of the mean RT of the analyte in the initial calibration set of standards.

9.2.10. 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.

9.2.11. 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 affected samples' results will be reported with a "V" qualifier up to ten times the amount found in the blank.

9.3. Instrument Initial Calibration

9.3.1. Quantitation of every hit at PQL or higher level for all target components has to be accomplished by using a calibration curve with at least five calibration levels.

9.3.2. To create an initial calibration curve using internal standard procedure, inject five (or more) concentration levels of the calibration standard. One of them should be at a concentration equal of below PQL. The other concentrations should correspond to the expected range of concentrations in the real samples. The TARGET will calculate the ratio of response ratio (target analyte/internal standard) to the amount injected (relative response factor).

9.3.3. The system is considered to be in initial linear calibration if the Relative Standard Deviation (%RSD) for the analyte response factors (using peak area calculation) is less than or equal to 20% or the Correlation Coefficient is not lower than 0.995 for other fit types.

9.3.4. Every initial calibration should be checked vs. QCS standard. They have to match within +/- 15% difference. If a positive hit is quantified outside of this acceptance criterion the sample has to be rerun and re-quantified with a valid calibration curve. If sample has expired the result should be qualified as estimated.

9.4. Continuing Calibration

9.4.1. After initial calibration, the GC/NPD response is checked on a continuing basis (every 12 hours by using a continuing calibration check (CCV), middle range NPD A standard. Criterion of acceptance for continuing calibration check is +/-20 % of initial calibration. If a calibration check standard fails in both channels, all the samples run immediately 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.

9.4.2. In order to quantify positives in the re-run samples, the calibration curve has to be re-made by re-running a fresh calibration set together with the samples to be quantified. The GC-NPD system is also subjected to any maintenance that is deemed necessary to correct the calibration problem.

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 13.6 for more details).

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9.5. Analysis Run Sequence.

9.5.1. 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:

- iso-octane blank (imstrument blank, containing internal standards)
- NPD A calibration set (Levels 1, 2, 3, 4,5 or more)
- NPD A QCS
- NPD A MDL standard (instrument detection limit)
- NPD A CCV
- laboratory fortified blank (LFB) Florisil Fraction 1
- sample matrix spike, Florisil fraction 1
- sample matrix spike duplicate, Florisil fraction 1
- laboratory fortified blank (LFB) Florisil Fraction 2
- sample matrix spike, Florisil fraction 2
- sample matrix spike duplicate, Florisil fraction 2
- extraction blank, Florisil fraction 1
- samples, Florisil fraction 1
- NPDA CCV (not more than 12 hrs since the last CCV
- extraction blank, Florisil fraction 2
- samples Florisil fraction 2
- NPDA CCV (nto more than 12 hrs since the last CCV
- samples (up to 12 hrs), Florisil fraction 2
- Etc.

9.6. Data Processing/Reporting

9.6.1. Analytes are identified by matching retention times with those in the standard chromatograms. Typical retention times are listed in Table 1. Retention times will vary depending on the length of the column, but the elution sequence will be the same.

9.6.2. The concentration reported for an identified hit in the sample extract, expressed in ug/L, is quantitated on the initial calibration curve. The TARGET software used to process data will do the interpolation for the sample extract (see the TARGET SOP 13.3, for details). 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 for lab manual integration policy 13.7) . Target software does not currently perform the calculations to determine the concentration of the analyte in the sample. That calculation is performed by the QC manager. The result is referred to wet weight of the sample extracted. This is done as follows:

- C_{sample} (ug/kg) = (C_X) x ($V_{extract}$) x (DF)/(W_{sample})

  where:

  C_X - The concentration, in ug/L, of the analyte in the sample extract determined by TARGET.

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

  DF - The appropriate dilution recorded in the dilution report.

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W_{sample} - The wet weight of the sample that was extracted (usually 5 grams). The weights are expressed in grams and are recorded in the preparation report.

9.7. Reporting results in DEP LIMS.

9.7.1. QA/QC results, MDLs, and positives are reported in the DEP LIMS.

10. QUALITY CONTROL

10.1. For Quality Control refer to GC SOP GC-001-2 "Quality Assurance/Quality Control in the GC Pesticides Laboratory"(see 13.8).

10.2. 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/fit
- Check QCS standard recoveries
- 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 TARGET Report Header:
  - SOP #
  - Instrument ID
  - Column ID
  - Temperature program
- 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

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11. DATA ARCHIVAL

11.1. 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. SAFETY

12.1. The toxicity or carcinogeneity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Primary standards should be prepared in a hood. A NIOSH/MESA approved toxic gas respirator should be worn when the analyst handles high concentrations of these toxic compounds.

12.2. Lab cloth

12.2.1. Lab coats and safety eyeglasses must be worn at all times when in any laboratory.

12.2.2. Use gloves when handling samples, standards, or solvents, or when washing glassware.

12.3. Chemicals involved

12.3.1. Even though the toxicity or carcinogeneity of some of the reagents used in this method have not been precisely defined, each of them should be treated as a potential health hazard. From this point of view exposure to these chemicals must be reduced to the lowest possible level by whatever means available.

12.3.2. A reference file of Material Safety Data Sheets for each reagents used, is kept in the GC room for documentation. Refer to this folder for any materials you are handling. Update this folder upon receiving new chemicals or reagents.

12.3.3. Primary standards of these toxic compounds should be prepared in a hood (See “Hood Usage”). A NIOSH/MESA approved toxic gas respirator should be worn when handling highly concentrated solutions of these compounds.

12.4. Waste disposal

12.4.1. Neat standards should be sealed and labeled.

12.4.2. All stock standards, working standards, and un-used sample extracts must be emptied into two separate waste containers for Pesticides and PCBs. The containers must be labeled properly with hazard warning labels indicating the container contents.

12.5. Chlorinated and non-chlorinated solvents must be stored separately and labeled properly.

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12.5.1. GC vials containing, standards, and sample extracts, must be stored in two separate waste containers for Pesticides and PCBs. These containers should be labeled properly as to their contents and stored in the hood.

12.6. Flammable solvents must be stored in a flammables cabinet.

12.7. Hood Usage

12.7.1. Handle all sample extraction, solvent transfers, concentrations, sample preps, and cleanups in the hood at all times.

12.7.2. At any time, no more than two people can work at each hood.

12.7.3. Turn the hood on and wait for adequate airflow. If there is no flow, DO NOT USE THE HOOD!

12.8. Glassware

12.8.1. Inspect every piece of glassware to be used. Do not use any that are chipped, cracked, etched, or scratched.

12.8.2. Dispose of all unwanted, broken glassware in the blue "GLASS" box. One should discriminate between "unwanted, broken" glassware and that which is only slightly damaged and reasonably easily repairable.

12.9. For more details see DEP SOP OG-001-3 (see 13.9)

13. REFERENCES

13.1. EPA 8141B Organophosphorus Compounds by Gas Chromatography; Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods, SW 846; 3rd Edition, Update 4, January 3, 2008.
13.2. DEP SOP CM-015-1 EzChrom Elite Client/Server Chromatography Data System.
13.3. DEP SOP CM-016-1 Target Chromatography software.
13.4. DEP SOP GC-004-1 Extraction of Organophosphorus, Organonitrogen, Organohalide Pesticides and PCBs from Fish Tissue for Subsequent Analysis by GC or GC/MS Techniques.
13.5. EPA 8000B Determinative Chromatographic Separation; Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods, SW 846; 3rd Edition, Update 4, January 3, 2008.
13.6. DEP SOP CM-008-1 Standard Operating Procedure for Reporting Qualified Data.
13.7. DEP SOP CM-018-1 Laboratory Policy Regarding Manual Chromatographic Peak Integration
13.8. DEP SOP GC-001-2 Quality Assurance/Quality Control in the GC Pesticides Laboratory.
13.9. DEP SOP OG-001-3; Safety and Waste Management Practices in the Organic Analysis Groups (Volatile, Semi-Volatile and Pesticide Laboratories)

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14. APPENDICES

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 10mL centrifuge 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.

Surrogate:
The individual surrogate stock solution is prepared from purchased neat material.

Neat	Components	Source
1000mg	NMX	Chem Service

Individual Stock solution:
Solvent: Acetone

Component	Neat added mg	Stock V_f mL	Conc. Stock ug/mL
NMX	50	50	1000

Surrogate spike solution (Mix):
Solvent: Acetone

Component	Amount Stock added mL	Dilution Factor	Surr. spike V_f mL	conc.ug/mL
TMX*	3.125	80	250 ml	2.5
DCB**	6.250	40		5
NMX***	12.5	20		50

*Tetrachloro-M-Xylene. (ECD surrogate also present in the mix)
**Decachlorobiphenyl. (ECD surrogate also present in the mix)
***Nitro-M-Xylene.

Internal standard:

The individual internal standard stock solution is prepared from purchased neat material.

Neat	Components	Source
100mg	TPP****	Chem Service
100 mg	NQ*****	Acros Organics

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Individual Stock solution:
Solvent: Acetone

Component	Neat added mg	Stock V_f mL	Conc. Stock ug/mL
TPP	50	50	1000
NQ	100		2000

Internal standard intermediate solution
Solvent: Acetone

Component	Amount Stock added mL	Dilution Factor	Surr. spike V_f mL	conc.ug/mL
TPP/NQ	10	20	200	50/100

Isooctane+Internal standard solution
Solvent: isooctane

Component	Amount spike added mL	Dilution Factor	Surr. spike V_f mL	conc.ug/L
TPP/NQ	1	200	200	250/500

**** Triphenyl phosphate
***** 5-Nitroquinoline

NPDA Custom Mix:

Solvent: ethyl acetate

Stock conc. ug/mL	Components	Source
62.5	Disulfoton, Ethoprop, Phorate, Terbufos	1.o2si 2.Crescent Chem.
125	Ametryn, Atrazine, Atrazine Desethyl, Chlorpyrifos Ethyl, Chlorpyrifos Methyl, Ethion, , Simazine, , Malathion, Mevinphos, Fipronil Molinate	
250	Metribuzin, Prometryn, EPTC, Pendimethalin	
375	Norflurazon, Hexazinone	

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Intermediate NPDA Standard Solution:

Amount **o2siNPDA** Stock added (**mL)**	Dilution Factor	**INT** in acetone V_f **mL**	conc. ug/mL
2	25	**50**	2.5-12
Amount **NMX** Stock added mL			
1.25	40		25

Amount **Crescent Chemical NPDA** Stock added (mL)	Dilution Factor	**INT QCS** in acetone V_f **mL**	conc. ug/mL
1	25	25	2.5-15

NPDA Working Level Standards:
Solvent: ISO-8:ACETONE 4:1 V/V

	V_f mL	Amount NPDA INT added (mL)	Amount INTERNAL STD SPIKE added (mL)	Dilution Factor	Conc. ug/L
L1	100	10	0.5	10	250-1500
L2	100	4	0.5	25	100-600
L3	200	4	1.0	50	50-300
L4	100	1	0.5	100	25-150
L5	100	0.7	0.5	175	17.5-105
L6	100	0.4	0.5	250	10-60
MDL	100	0.1	0.5	1000	2.5-15
L3 QCS	100	2	0.5	100	50-300
SPK	100	20		5	500-3000

* NPDA INT added, spike solution in acetone.

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15. TABLES

TABLE 1: OPERATING PATAMETERS FOR GC/NPD ANALYSIS (METHOD NPDCV35)

GC PARAMETER	SETTING	GC PARAMETER	SETTING
Temperature 1	80° C	Injector Temperature	220° C
Time 1	1 minute	Detector Temperature	280° C
Ramp 1	9° C per minute	Carrier Gas	Helium
Temperature 2	180° C	Column Head Pressure	23.8 psi, 18psi
Time 2	2.0 minutes	Column Flow Rate	5.83, 3.93 mL/ min
Ramp 2	2.0° C per minute	Septum Purge Flow Rate	3.0 mL/ min
Temperature 3	195° C	Make up Gas	Helium
Time 3	0.0 minute	Make up Gas Flow Rate	20 mL/min
Ramp 3	10.0° C per minute	Hydrogen Gas Flow Rate	1.0 mL/min
Temperature 4	275° C	Air Flow Rate	150 mL/min
Time 4	5.39 minutes	Injection Mode	Splitless for 1 minute
		Injection Volume	1.0 uL
		control mode	constant velocity

TABLE 2: GC AUTOSAMPLER OPERATING PARAMETERS FOR GC/NPD ANALYSIS (OPTION-SAMPLER-ALLOC-MODE4))

Autosampler Parameter	Setting	Autosampler Parameter	Setting
Inj/Bottle	1	Volume	1 uL
First Bottle	1	# of pre-solvent washes	6 (Acetone)
Last Bottle	150	# of solvent washes	6 (Iso-Octane)
# of Sample washes	2	Priority Sample (1=Yes)	No
# of Pumps	6	Capillary On-Column (1=Yes)	No
Viscosity	0.2		

TABLE 3: RETENTION TIMES, METHOD DETECTION LIMITS AND PRACTICAL QUANTITATION LIMITS and internal standard associations

Target Component	Internal standard	RT DB-5	RT DB-17	MDL in extract	MDL in sample	PQL in extract	PQL in sample
		minute	minute	ug/L	ug/kg	ug/L	ug/kg
Atrazine	NQ	13.1	13.8	10	4.0	40	16
Chlorpyrifos Ethyl	NQ	17.8	18.5	10	4.0	40	16
Disulfoton	NQ	14.1	14.4	5.0	2.0	20	8.0
Ethion	TPP	24.6	25.7	10	4.0	40	16
Ethoprop	NQ	11.6	11.7	5.0	2.0	20	8.0
Metribuzin	NQ	15.3	17.8	15	6.0	60	24
Norflurazon	TPP	25.5	27.3	30	12	120	48
Phorate	NQ	12.3	12.5	5.0	2.0	20	8.0
Simazine	NQ	12.9	14.1	10	4.0	40	16
Chlorpyrifos Methyl	NQ	15.6	16.7	10	4.0	40	16
Malathion	NQ	17.4	19.2	10	4.0	40	16

NQ- 5-Nitroquinoline; TPP Triphenyl Phosphate

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TABLE 4: STANDARD CONCENTRATION LEVELS

Parameters		Standard Calibration Solutions (ug/L)						Spike Solution (ug/L)
	Intermediate	L-1	L-2	L-3	L-4	L-5	L=-6	
Atrazine	5000	500	200	100	50	35	20	800
Chlorpyrifos Ethyl	5000	500	200	100	50	35	20	800
Disulfoton	2500	250	100	50	25	17.5	10	400
Ethion	5000	500	200	100	50	35	20	800
Ethoprop	2500	250	100	50	25	17.5	10	400
Metribuzin	10000	1000	400	200	100	70	40	1600
Norflurazon	15000	1500	600	300	150	105	60	2400
Phorate	2500	250	100	50	25	17.5	10	400
Chlorpyrifos Methyl	5000	500	200	100	50	35	20	800
Simazine	5000	500	200	100	50	35	20	800
Malathion	5000	500	200	100	50	35	20	800

- Add 5 uL of 50 ug/mL TPP + 100 ug/mL NQ ISTD to every 1.0 mL of sample or standard.

TABLE 5: 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	50-130%	Reanalyze/Re-extract/Flag
3	CCV	± 20%	Re-run and recalibrate/Flag
4	QCS verification	± 15%	Re-run and recalibrate/Flag
5	Surrogate recovery NMX	24-111%	Reanalyze/Re-extract/Flag
6	Linearity criterion	20% RSD, 0.995 CC	Use appropriate Cal fit / Re-run / Recalibrate
7	Internal standard recovery	50-200%	Reanalyze/Flag/Comment
8	Lowest calibration point refitting	± 20% of expected value	Use appropriate Cal fit / Rerun / Recalibrate / Flag / Comment

TABLE 6: FLORISIL CLEAN-UP FRACTIONS

Florisil clean-up Fraction 1	Florisil clean-up Fraction 2
Atrazine Chlorpyrifos Ethyl Disulfoton Ethion Ethoprop Metribuzin Phorate Simazine Chlorpyrifos Methyl Malathion	Norflurazon

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APPENDIX OF CHANGES

08/27/2012	MDL and PQL revisions made to Table 3. Analytes Malathion and Chlorpyrifos Methyl added to the method.
01/07/2014	Requirement for six levels for quadratic calibration have been added. The statistical limits formula for surrogate recoveries have been updated. The standard preparation appendix has been updated. The QC recovery limits have been updated.
01/05/2015	Chapter 9.1.2 was added describing troubleshooting after runs with poor data quality. QC control limits updated.
12/29/2016	Updated surrogate recovery limits; prohibition for “include origin” in calibration fits added. Standards concentration table updated.

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