State Of Connecticut
Department of Environmental Protection

Recommended Reasonable Confidence Protocols

Quality Assurance and Quality Control Requirements

Pesticides by Method 8081, SW-846

Version 2.0
July 2006

Written by the Connecticut DEP QA/QC Workgroup

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1.0	First version for publication	7/11/2005
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1.0 QA/QC Requirements for Method 8081

1.1 Method Overview

Method 8081 is gas chromatography procedure used to determine chlorinated pesticides in a variety of matrices including waters, soils, sediments, wastes, etc. This procedure requires an experienced GC analyst familiar with the QA/QC requirements of the method. The sample introduction procedure requires the use of a solvent extraction procedure. All method references are to the latest promulgated version of the method found in Test Methods for Evaluating Solid Waste, SW-846.

Open-tubular, capillary columns are employed with electron capture detectors (ECD) or electrolytic conductivity detectors (ELCD). When compared to packed columns, these fused-silica, open-tubular columns offer improved resolution, better selectivity, increased sensitivity, and faster analysis. The target analytes may be determined with either a single- or dual-column chromatographic system. The method also may be applied to other matrices such as oils and wipe samples, if appropriate sample extraction procedures are employed.

1.1.1 Reporting Limits for Method 8081

The reporting limit (RL) for a compound is dependent on the concentration of the lowest standard in the initial calibration, sample weight/volume, extraction procedure, and moisture content. The following table lists approximate reporting limits for various matrices utilizing a gas chromatograph with an electrolytic conductivity detector (GC/ECD). Electrolytic conductivity detectors will have slightly higher reporting limits. Solid matrices in this table assume 100% solids.

Table 1.0 Typical Reporting Limits

Matrix	Typical Reporting Limit
Water	0.05 to 0.5 ug/L
Soil	1.7 to 17 ug/Kg

Moisture content of soils and sediments will raise the RL, as all results must be reported on a dry weight basis for these two matrices. Sample dilution or lower sample weight/volume will also cause the RLs to be raised.

Sample container type, preservation requirements, and holding times for waters, soils, and sediments are presented in Table 2A of this document.

1.1.2 General Quality Control Requirements

Each laboratory is required to operate a formal quality assurance program and be certified by the Connecticut Department of Public Health for the analysis performed. The minimum requirements include initial demonstration of laboratory proficiency, ongoing analysis of standards and blanks to confirm acceptable continuing performance, and analysis of laboratory control samples (LCS) to assess precision and accuracy. The use of site specific matrix spikes and matrix spike duplicates is highly recommended. Evaluation of sample matrix effects on compound recovery is key to making good decisions.

Laboratories must document and have on file an Initial Demonstration of Proficiency for each combination of sample preparation and determinative method being used. These data must meet or exceed the performance standards as presented in Section 1.5 and Table 1A. See Section 8.4 of Method 8000 in SW-846 for the procedure. The Initial Demonstration of Proficiency must include the following elements:

Table 1.1 IDOC Requirements

QC Element	Performance Criteria
Initial Calibration	Table 1A
Continuing Calibration	Table 1A
Method Blanks	Table 1A
Average Recovery	Table 1A
% Relative Standard Deviation	Table 1A
Surrogate Recovery	Table 1A
Internal Standards	Table 1A

Note: Because of the extensive analyte list and number of QC elements associated with the Initial Demonstration of Proficiency, it should be expected that one or more analytes might not meet the performance standards for one or more QC elements. The laboratory should make every effort to find and correct the problem, and repeat the analysis. All non-conforming analytes along with the laboratory acceptance criteria should be noted in the Initial Demonstration of Proficiency data.

Laboratories are required to generate laboratory specific performance criteria for LCS compound recovery limits, matrix spike/matrix spike duplicate compound recovery and

precision (RPD) limits, and surrogate recovery limits. These limits must meet or exceed the limits specified in Table 1A.

1.2 Summary of Method 8081

1.2.1 Sample Extraction and Cleanup

Samples for analysis by Method 8081 require extraction by one of the following methods. The use of a hydrophilic solvent mixture, either 1:1 Acetone/Hexane or 1:1 Acetone/Methylene chloride, is recommended for soil and sediment samples.

SW-846 Method	Matrix	Description
3542	Air Samples	Extraction of Analytes Collected Using a Modified Method 5 Sampling Train
3510C	Aqueous	Separatory Funnel liquid-Liquid Extraction
3520C	Aqueous	Continuous Liquid-Liquid Extraction
3511	Aqueous	Organic Compounds in Water by Microextraction
3540C	Soil/Sediment	Soxhlet Extraction
3541	Soil/Sediment	Automated Soxhlet Extraction
3545A	Soil/Sediment	Pressurized Fluid Extraction (PFE)
3546	Soil/Sediment	Microwave Extraction
3570	Soil/Sediment	Microscale Solvent Extraction (MSE)
3550C	Contaminated Solids [1]	Ultrasonic Extraction
3580A	NAPL	Solvent Dilution

Note:

1. ***Sonication may only be used for the extraction of highly contaminated (free product) non-soil/sediments (debris). Any other use of ultrasonic extraction is not allowed.***
2. ***Air drying of samples is not allowed.***

Extracts may be cleaned up, as required, by any of the following methods prior to GC/MS analysis by SW-846 Method 8081.

SW-846 Method	Description
3600	General Cleanup Selection
3610	Alumina
3620	Florisil
3630	Silica Gel
3640	Gel Permeation Chromatography
3660	Sulfur Cleanup

1.2.2 GC Analysis

The chlorinated pesticides are extracted from the sample using the appropriate method. The solvent extract is concentrated and then aliquots are injected onto the GC column in the gas chromatograph. The gas chromatograph (GC) oven is temperature programmed to facilitate separation of the analytes which are then detected by an ECD or ELCD interfaced to the column

Preliminary identification of target analytes is accomplished by comparing the retention time of the chromatographic peaks of the sample to known pesticides analyzed under the exact same conditions. Confirmation is accomplished either by analysis of the same extract on a dissimilar column, again comparing the retention times of the chromatographic peaks of the sample to known pesticides analyzed under the exact same conditions, or by using at least one other independent qualitative technique such as GC/MS. Quantitation is accomplished by constructing a calibration curve of pesticide concentration vs. peak area. Confirmation is not required in the case where pesticides are not detected above their specific reporting limit.

1.3 Method Interferences

Refer to SW-846 Methods 3500 (Sec. 3.0, in particular), 3600, and 8000 for a detailed discussion of interferences. Interferences co-extracted from the samples will vary considerably from matrix to matrix. While general cleanup techniques are referenced or provided as part of this method, unique samples may require additional cleanup approaches to achieve desired degrees of discrimination and quantitation. Sources of interference in this method can be grouped into four broad categories.

- Contaminated solvents, reagents, or sample processing hardware;
- Contaminated GC carrier gas, parts, column surfaces, or detector surfaces;
- Non-target compounds simultaneously extracted from the sample matrix which cause a detector response; and
- Co-elution of target analytes

An in depth discussion of the causes and corrective actions for all of these interferences is beyond the scope of this guidance document. A brief discussion of the more prevalent interferences is presented below.

1.3.1 Chemical Contaminants

Major contaminant sources for Method 8081 include, but are not limited to, plastics, impurities in laboratory chemicals, contaminated laboratory ware, etc. The use of non-polytetrafluoroethylene (PTFE) thread sealants, plastic tubing, or flow controllers with rubber components should be avoided, since such materials may contaminate the analytical system.

Analysis of blanks provides information about the presence of contaminants. When potential interfering peaks or high levels of target compounds are detected in blanks, the laboratory should try and find the source of the contamination and eliminate it. **Subtracting blank values from sample results is not permitted.** Any method blank exceedances should be fully documented in the laboratory report narrative.

Interferences by phthalate esters introduced during sample preparation can pose a major problem in chlorinated pesticide determinations by SW-846 Method 8081A. Common flexible plastics contain varying amounts of phthalate esters, as plasticizers, which are easily extracted or leached from such materials during laboratory operations. Interferences from phthalate esters can best be minimized by avoiding contact with any plastic materials and checking all solvents and reagents for phthalate contamination. Exhaustive cleanup of solvents, reagents and glassware may be required to eliminate background phthalate ester contamination. These materials may be removed prior to analysis using Method 3640 (Gel Permeation Cleanup) or Method 3630 (Silica Gel Cleanup).

1.3.2 Cross-contamination/ Carryover

Cross-contamination can occur when any sample is analyzed immediately after a sample containing high concentrations of pesticides or other compounds which cause a detector response, such as PCB's. Syringes on the autosampler may also become contaminated in the same manner. If a high sample is inadvertently analyzed, the system must be demonstrated to be clean by analysis of solvent blanks. Laboratories should be aware that carryover from high boiling point compounds may not appear until a later run (ghost peaks).

1.3.3 Sulfur Interferences

The presence of elemental sulfur (S) will result in broad peaks that interfere with the detection of early-eluting chlorinated pesticides. Sulfur contamination should be expected with sediment samples and can be removed through the use of SW-846 Method 3660.

1.3.4 Co-elution

As described in Section 3.8 and 3.9 of SW-846 Method 8081, co-elution among the many target analytes or other compounds can cause interference problems. The GC analyst should experiment with varying chromatographic conditions to obtain the most efficient compound separation.

1.3.5 Special Precautions

DDT and endrin are easily degraded in the injection port. Breakdown occurs when the injection port liner is contaminated with high boiling residue from sample injection or when the injector contains metal fittings. The potential for DDT and endrin breakdown should be evaluated before samples are analyzed and at the beginning of each 12-hour shift as described in Section 8.4.6 of SW-846 Method 8081.

1.4 Quality Control Requirements for SW-846 Method 8081

1.4.1 General Quality Control Requirements for Determinative Chromatography Methods

Refer to SW-846 Method 8000 for general quality control requirements for all chromatographic methods, including SW-846 Method 8081. These requirements insure that each laboratory maintain a formal quality assurance program and records to document the quality of all chromatographic data. Quality Control procedures necessary to evaluate the GC system operation may be found in SW-846 Method 8000, Section 7.0, and include evaluation of retention time windows, initial and verification of instrument calibrations and chromatographic performance of sample analyses. Instrument quality control and method performance requirements for the GC system may be found in SW-846 Method 8081, Sections 8.0 and 9.0, respectively.

1.4.2 Specific QA/QC Requirements and Performance Standards for SW-846 Method 8081

Specific QA/QC requirements and performance standards for SW-846 Method 8081 are presented in Table 1A. Strict compliance with the QA/QC requirements and performance standards for this method, as well as satisfying other analytical and reporting requirements will provide the environmental professional (EP) with “Reasonable Confidence” regarding the usability of analytical data to support DEP decisions.

While optional, parties electing to utilize these protocols will be assured that "Reasonable Confidence" data, will be generally accepted by agency reviewers. In order to achieve "Reasonable Confidence" parties must:

1. Comply with the applicable QC analytical requirements prescribed in Table 1A for this test procedure;

2. Evaluate and narrate, as necessary, compliance with performance standards prescribed in Table 1A for this test method; and

3. Adopt the reporting formats and elements specified in Section 1.7 of this method.

1.4.3 Site Specific Matrix Spike (MS), Matrix Spike Duplicate (MSD) Samples

It is strongly recommended that site specific MS/MSD samples be analyzed from each site, and each matrix type sampled. Percent recovery data from site specific samples allow the EP to make informed decisions regarding contamination levels at the site. Batch MS/MSD results do not give any indication of site specific matrix interferences or analytical problems related to the specific site matrices and are in general discouraged. Non-site specific MS/MSD's should not be reported for the RCP's. Additionally trip blanks, field blanks, rinsate blanks, etc. should not be used for MS/MSD's.

1.4.4 Special Analytical Considerations for Multi-Response Pesticides

The identification of multi-component mixtures (i.e., chlordane or toxaphene) is not based on a single peak, but rather on the characteristic peaks that comprise the "fingerprint" of the mixture, using both the retention times and shapes of the indicator peaks. If, based on site history, multi-component chlorinated pesticides are contaminants of concern; it is the responsibility of the EP to request that these multi-component analyte spikes be included in the LCS and MS/MSD's. Multi-component mixtures are not routinely included in LCS or MS/MSD's.

Because of the variable solubility, extraction efficiency and analytical sensitivity of the different compounds that are potentially analyzable by SW-846 Method 8081, the recovery ranges presented in Table II B-1 for laboratory control samples, matrix spikes, and surrogates should be considered general upper/lower acceptance limits when a single extraction procedure is utilized to prepare the extract for subsequent analysis. It is essential that laboratory-specific performance criteria for LCS and surrogate recoveries also be calculated and documented as described in SW-846 Method 8000B, Section 8.7. When experience indicates that the criteria recommended in specific methods are frequently not met for some analytes and/or matrices, the in-house performance criteria

will be a means of documenting these repeated exceedances. Laboratories are encouraged to actively monitor pertinent quality control performance standards described in Table II B-1 to assess analytical trends (i.e., systematic bias, etc) and improve overall method performance by preempting potential non-conformances.

Table 1A Specific QA/QC Requirements and Performance Standards for Method 8081*

Required QA/QC Parameter	Data Quality Objective	Required Performance Standard	Required Deliverable	Recommended Corrective Action	Analytical Response Action
Retention Time Windows	Accurate identification of Pesticides	1) Prior to or during an initial calibration when a new column is installed. 2) Calculate according to Method 8000, Section 7.6.	NO	N/A	N/A
Endrin and DDT Breakdown	Laboratory Analytical Accuracy	1) Before samples are analyzed and at the beginning of each 12-hour clock. 2) Breakdown must be ≤15% for each compound. Must be evaluated using Section 8.4.6 of Method 8081.	YES	Perform injection port/ column maintenance. Recalibrate if necessary.	Report exceedances in narrative.
Initial Calibration	Laboratory Analytical Accuracy	1) Minimum of 5 stds for single response pesticides. (Note 1) 2) Low std at reporting limit 3) % RSD must be ≤20% or if linear regression used "r" ≥ 0.990 4) For multi-response pesticides analysis of single std at mid-point of calibration range. 5) If curves are used, curve must NOT be forced through origin. Must use additional stds as specified in Method 8000, Section 7.5 6) Curves must be verified with independent ICV prior to sample analysis.	NO	Recalibrate as required by the method.	Sample analysis cannot proceed without a valid initial calibration. Report non-conforming compounds in narrative. If avg Rf or linear regression not used (e.g. quadratic equation), must note list of affected compounds in narrative
Continuing Calibration (CCAL)	Laboratory Analytical Accuracy	1) Prior to samples, every 12-hours or 20 samples, whichever is more frequent, and at the end of the analytical sequence. 2) Concentration near mid-point of curve. 3) Multi-response pesticides must be verified within 12-hours of analysis of any detects. 4) Percent difference or drift ≤15%. 5) Verify all analytes fall in retention time windows.	NO	1) Perform instrument maintenance, reanalyze CCAL and/or recalibrate. 2) Reanalyze associated samples if beginning or closing CCAL exhibited low response and associated pesticides not detected in samples. 3) Reanalyze associated samples if beginning or closing CCAL high and associated pesticides were detected in samples.	Report exceedances in narrative. Note: Associated samples means all samples analyzed since the last acceptable CCAL.

Table 1A Specific QA/QC Requirements and Performance Standards for Method 8081* (continued)

Required QA/QC Parameter	Data Quality Objective	Required Performance Standard	Required Deliverable	Recommended Corrective Action	Analytical Response Action
Method Blanks	Laboratory Contamination Evaluation	1) Extracted every 20 samples or every batch, whichever is greater. 2) Matrix specific 3) Target analytes must be <RL	YES	Locate source of contamination and correct problem. Reanalyze method blank. Re-extract samples if method blank contaminated	1) Report non-conformances in case narrative. 2) All results for compounds present in method blank must be "B" flagged if detected in samples associated with the method blank. 3) If re-extraction performed within holding time, report only compliant data. If re-extraction performed outside holding time report all data.
Laboratory Control Sample (LCS)	Laboratory Method Accuracy	1) Every 20 samples or each batch, whichever is more frequent. 2) Standard source different from initial calibration source. 3) Concentration level must be near or at the mid-point of the initial calibration. 4) Must contain all single response pesticides. 5) Matrix specific. 6) Laboratory determined percent recovery limits must be between 40-140% except for difficult analytes, which must be between 30-140% recovery.	YES	Recalculate the percent recoveries Reanalyze the LCS If MS/MSD in same batch compare to determine if problem isolated to LCS Re-extract LCS and samples if >10% compounds outside acceptance criteria and no MS/MSD with acceptable criteria Locate & correct problem, reanalyze associated samples	1) Report non-conformances in case narrative. 2) Individual laboratories must identify and document problem analytes that routinely fall outside the limits. Any exceedances must be noted in narrative. Data to support laboratory problem compounds kept on file at lab for review during audit 3) If re-extraction performed within holding time, report only compliant data. If re-extraction performed outside holding time report all data.

Table 1A Specific QA/QC Requirements and Performance Standards for Method 8081* (continued)

Required QA/QC Parameter	Data Quality Objective	Required Performance Standard	Required Deliverable	Recommended Corrective Action	Analytical Response Action
Site Specific Matrix Spike/Matrix Spike Duplicate	Precision and Accuracy in Sample Matrix	1) Every 20 samples per matrix* 2) Spike concentration in lower part of calibration curve. 3) Must contain all single response pesticides. 4) Laboratory determined percent recovery limits must be between 30-150% 5) RPD's ≤ 30% for single response pesticides.	YES (*If requested by EP)	If compounds out compare to LCS; if LCS recoveries in note in narrative; if LCS compounds out note in narrative probable lab error	Note outliers in narrative
Surrogates	Accuracy in Sample Matrix	1) Minimum 2 compounds across retention times of GC run. Recommended compounds Tetrachloro-m-xylene and decachlorobiphenyl. 2) Recovery limits lab generated and within 30-150% for both compounds on both columns. 3) Labs must develop own in-house limits, which fall within 30-150% limits.	YES	If the same surrogate outside limits on both columns, re-extract sample. If both surrogates outside limits on one column only, reanalyze sample. If surrogate diluted out below lowest calibration std, no recovery criteria.	1) Note exceedances in narrative. 2) If re-extraction or reanalysis confirms matrix interference or if re-extraction outside holding times report all results. 3) If re-extraction or reanalysis results in criteria and in holding time, report only compliant data.

Table 1A Specific QA/QC Requirements and Performance Standards for Method 8081* (continued)

Required QA/QC Parameter	Data Quality Objective	Required Performance Standard	Required Deliverable	Recommended Corrective Action	Analytical Response Action
Identification and Quantitation	Inter-laboratory Consistency	1) Laboratory should use the average calibration factor of each single response pesticide for quantitation. 2) Second column analysis: Laboratory must utilize a second dissimilar column to confirm all positive results above the RL. Report the higher of the two analyses. The QA/QC parameters in this document must be met for both columns 3) For multi-response pesticides quantitate as per Section 7.6 of Method 8081. Note: If a high RPD between the two columns can be definitely attributed to matrix interference, report the lower value and note in the narrative with an explanation.	NO	N/A	1) If the RPD between the results for the two columns exceeds 40%, the laboratory must flag the results with a "P" suffix and note in narrative. 2) If avg CF or RF for linear regression not used (e.g. quadratic equation), must note list of affected compounds in narrative.
General Reporting Issues	N/A	1) The laboratory should report only concentrations detected above the sample specific RL. 2) Concentrations below the reporting limit (RL) should be reported as "ND" with the sample specific RL also reported 3) Dilutions: If diluted and undiluted analyses are performed, the laboratory should report results for both sets of data. Compounds that exceed the linear range should be flagged ("E" flag). Do not report more than 2 sets of data/sample. 4) If a dilution is performed, the highest detected analyte must be in the upper 60% of the calibration curve, unless there are non-target analytes whose concentrations are so high as to cause damage to the instrumentation	N/A	N/A	Performance of dilutions must be documented in the case narrative

Notes for Table 1A:

* Refers to latest promulgated version of SW-846 Method 8081.
GC/MS = Gas Chromatography/Mass Spectrometry
CCC = Calibration Check Compound
%RSD = Relative Percent Standard Deviation
EP = Environmental Professional
r = Correlation Coefficient
RPD = Relative Percent Difference
N/A = Not Applicable

Note 1: Six standards are required for a quadratic equation calibration curve, and seven are required for a polynomial fit. In either case the correlation coefficient must be ≥ 0.990

1.5 Analyte List for SW-846 Method 8081

The Connecticut DEP (DEP) analyte list for SW-846 Method 8081 is presented in Table 1B. The compounds listed are readily determined by Method 8081. Most of the compounds listed have Connecticut Remediation Standard Criteria or are listed in the Approved Criteria for Additional Polluting Substances.

1.5.1 Additional Reporting Requirements for SW-846 Method 8081

While it is not necessary to request and report all the analytes listed in Table 1B to obtain Reasonable Confidence status, it is necessary to document such a limitation, for site characterization and data representativeness considerations. DEP strongly recommends that full list of analytes be reported during the initial stages of a site investigation and/or at sites with an unknown or complicated history of chemical usage or storage.

In cases where a shortened list of analytes is selected, the laboratory must still meet the method specific quality control requirements and performance standards associated with the requested analytes list to obtain Reasonable Confidence.

The Reporting Limit (RL) is based upon the lowest standard in the initial calibration. It is the responsibility of the environmental professional or EP to specify to the laboratory the detection limits required for the samples. In order to meet the detection limits it may be necessary to modify the analytical method by using increased sample volume or mass. In such cases the modifications must be noted in the narrative.

1.6 Routine Reporting Deliverables for Method 8081

The following table (Table 1.2) lists the routine report deliverables. Note that while laboratories are not required to report certain items, they must keep the data on file and may be required to report all items in special circumstances.

1.6.1 Reporting and Flagging of Results

The following rules apply to reporting results:

Non-Detects: Report all non-detects and results below the reporting limit as “ND” (Not Detected at the specified reporting limit). The reporting limit for each compound in each sample must be listed on the report and take into account the exact sample mass, any dilution factors, percent moisture, etc.

Compounds detected above the reporting limit in blanks and found in samples, also above the reporting limit, shall be flagged with a "B" suffix (e.g. 25B).

All soil/sediment results shall be reported on a dry weight basis.

Table 1.2 Report Deliverables

PARAMETER	DELIVERABLE	COMMENTS
Retention Time Windows	NO	
Endrin/DDT Breakdown	YES	Note non-conformances in narrative
Initial Calibration	NO	Note non-conformances in narrative
Continuing Calibration	NO	Note non-conformances in narrative
Method Blanks	YES	Note non-conformances in narrative. Flag all positive sample results above RL with "B" flag.
Lab Control Sample (LCS)	YES	Note non-conformances in narrative
Site Specific Matrix Spike/ Matrix Spike Duplicate	YES (If analyzed)	Note non-conformances in narrative
Surrogate Recoveries	YES	Note non-conformances in narrative
General Reporting Issues	YES	Note non-conformances in narrative
QA/QC Certification Form	YES	Signed by laboratory director or his/her designee.

Table 1B Analyte List for SW-846 Method 8081

ANALYTE	CAS Number	NOTES
Alachlor	15972608	
Aldrin	309002	
α-BHC	319846	
β-BHC	319857	
γ-BHC (Lindane)	58899	
δ-BHC	319868	
Chlordane (technical)	57749	
4,4'-DDD	72548	
4,4'-DDE	72559	
4,4'-DDT	50293	
Dieldrin	60571	See 1
Endosulfan I	959988	
Endosulfan II	33213659	
Endosulfan Sulfate	1031078	
Endrin	72208	
Endrin Aldehyde	7421934	
Endrin Ketone	53494705	
Heptachlor	76448	
Heptachlor Epoxide	1024573	
Methoxychlor	72435	
Toxaphene	8001352	

Notes:
1 – Aqueous RSR limit for Dieldrin (0.002 ug/L) may not be achievable.

Table 2A Sample Containers, Preservation, and Holding Times

MATRIX	CONTAINER	PRESERVATIVE	HOLDING TIME
Aqueous with no chlorine present	1-liter amber glass bottle with Teflon line cap	Store at 4 ± 2º C.	7 days to extraction. 40 days from extraction to analysis.
Aqueous with chlorine present	(1-liter amber glass bottle with Teflon line cap	Neutralize chlorine with either 25 mg ascorbic acid or 3 mg sodium thiosulfate. Store at 4 ± 2º C.	7 days to extraction. 40 days from extraction to analysis.
Soil/Sediment samples.	250 mL amber glass jar with Teflon lined cap.	Cool to 4 ± 2º C	14 days to extraction. 40 days from extraction to analysis. Up to one year for samples frozen within 48 hours of collection. (Note 1)
High Concentration Waste Samples	Collect in amber glass jar with Teflon lined cap.	Cool 4 ± 2º C.	14 days to extraction. 40 days from extraction to analysis.

Notes:
Note 1: If the freezing option is selected, the sample must be frozen within 48 hours of collection. The holding time recommences when thawing begins. The total holding time is calculated from the time of collection to freezing plus the time allowed for thawing. The total elapsed time must be less than 14 days.

The number of sample containers is optional. Laboratories should supply enough containers to allow for any reanalysis or breakage.