# STANDARD ANALYTICAL PROTOCOL FOR EXTRACTABLE CHEMICAL AGENTS USING GAS CHROMATOGRAPHY/MASS SPECTROMETRY

FLORIDA DEPARTMENT OF ENVIRONMENTAL PROTECTION

CHEMISTRY PROGRAM

2600 BLAIR STONE ROAD

TALLAHASSEE, FLORIDA 32399


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

# TABLE OF CONTENTS

**1. Scope and Application**

**2. Summary of Protocol**

**3. Interferences**

**4. Safety/Hazardous Waste Management**

**5. Equipment and Supplies**

**6. Reagents and Standards**

6.1 Reagents

6.2 Standards

**7. Sample Preservation, Storage, and Technical Holding Times**

7.1 Sample Preservation

7.2 Sample Storage

7.3 Sample Extract Storage

7.4 Technical Holding Times

**8. Quality Control (QC)**

8.1 Methods

8.2 Matrix Spike and Matrix Spike Duplicate (MS/MSD)

8.3 Laboratory Control Sample (LCS)

8.4 Instrument Detection Limit (IDL) Determination and Limit of Quantitation (LOQ)

**9. Calibration and Standardization**

9.1 Instrument Operating Conditions

9.2 GC/MS Mass Calibration (Tuning) and Ion Abundance

9.3 Initial Calibration

9.4 Continuing Calibration Verification

**10. Analytical Procedure**

10.1 Sample Preparation

10.2 Preparation of Solid Samples Using Micro-Scale Extraction

10.3 Waste Dilution

10.4 Wipe Sample using Pressurized Fluid Extraction

10.5 Sample Clean-Up

10.6 Final Concentration of Extract


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

10.7 Sample Analysis by GC/MS

**11. Calculations and Data Analysis**

11.1 Qualitative Identification of Target Compounds

11.2 Data Analysis and Calculations of Target Compounds

11.3 Technical Acceptance Criteria for Sample Analysis

11.4 Corrective Action for Sample Analysis

**12. Standard Analytical Procedure Performance**

12.1 Estimated Detection Levels, RT, and Quantitation Ion Determinations

12.2 Quantitation Levels (QLs)

12.3 Precision and Recovery in Reference Sample

**13. Pollution Prevention**

**14. References**

**15. Tables**

1. MDLs/PQLs, Spike Recovery Range and Precision in Water and Soil for CWAs
2. MDLs/PQLs, Spike Recovery Range and Precision in Wipes and Air for CWAs
3. Surrogate Recovery Limits
4. DFTPP Criteria
5. Retention Times, Characteristic Ions for Surrogates, Internal Standards, and Target Compounds
6. Working Calibration Standard Concentration for Full Scan
7. Working Calibration Standard Concentration for SIM mode
8. Dilution Scheme to Prepare Calibration Solution for Full Scan
9. Dilution Scheme to Prepare Calibration Solution for SIM
10. Internal standards and Surrogates for use with each CWA

**16. Appendix**

A. Daily Checklist

B. Glassware Cleaning Procedure

C. Decontamination Procedure

D. Waste Handling Procedure

E. Hazardous Waste Container

F. Transfers Into and Out of Agent Hoods

**17. SOP Update Revision Summary**



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

# ACRONYMS AND DEFINITIONS:

| Acronym | Definition of Acronym |
|---|---|
| AR | Army Regulation |
| CA | Chemical Agent |
| CAHO | Chemical Agent Hygiene Officer |
| CAM | Chemical Agent Manager |
| CASARM | Chemical agent standard analytical reference material |
| CHP | Chemical Hygiene Plan |
| CWA | Chemical Warfare Agent |
| Dilute Agent | General term for either RDTE or Ultra-dilute chemical agent solution |
| DoD | The Department of Defense |
| DSC | Dilute Solution Custodian |
| DSHP | Dilute Solution Hygiene Plan for Chemical Agent operations |
| DWL | U.S. Army Drinking Water Limits (Standards) for chemical agent solutions |
| ECBC | U.S. Army Edgewood Chemical and Biological Center and Engineering Center |
| EPA | The U.S. Environmental Protection Agency |
| FDEP | Florida Department of Environmental Protection |
| FSO | Facility Security Officer |
| Guidelines | ECBC "Guidelines for Managing a Research Development Testing Evaluation (RDTE) Dilute Solution Laboratory," dated September 2006 |
| IAA | Interagency agreement (between EPA and DoD, dated 11/22/2006) |
| ID | Identification by a unique number or code |
| MOU | Memorandum of Understanding |
| MSDS | Material Safety Data Sheet |
| NEMC | National Exposure Measurement Center (EPA) |
| OSHA | Occupational Safety & Health Administration |
| PPE | Personnel Protective Equipment |
| PSP | Physical Security Plan |
| RDTE | Research Development Testing Evaluation |
| RRT | Relative Retention Time |
| RRF | Relative Response Factor |
| QA/QC | Quality Assurance/Quality Control |
| QAO | Quality Assurance officer |
| RCRA | Resource Conservation and Recovery Act |
| RDTE | Research Development Test Evaluation dilute chemical agent solution |
| SIM | Selected Ion Monitoring |
| SOP | Standard Operating Procedure |
| TD | Technical Director |
| TOF | Time-of-Flight Mass Spectrometer |
| UD | Ultra-Dilute Chemical Agent solution |


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

# 1. SCOPE AND APPLICATION

**1.1.** The Florida Department of Environmental Protection (FDEP), in collaboration with the US Environmental Protection Agency (USEPA), National Homeland Security Center (NHSRC) and other federal agencies, has identified analytical methods for the analysis of extractable semi-volatile chemical agents in response to a homeland security incident. This SOP provides details to develop standard analytical protocols for laboratory identification and quantitation of target agents during site remediation. These methods are used to assist in determining the presence of contamination, effectiveness of decontamination, and site clearance following decontamination.

**1.2.** This SOP is for the determination and the measurement of the following chemical agents (CAs): GA, GB, GF, HD, GD1, GD2, and VX. This SOP's is based on USEPA (i.e., SW-846) Methods 8270D, 3511, 3545A, and 3570 for the sample preparation and analytical determinative techniques for solid, wipe, and water samples.

**1.3.** This SOP covers the following FDEP Laboratory tests:

| | |
|---|---|
| **W-CW-SQSM**: | GC/MS Analysis of Water Matrix Samples by SIM mode |
| **S-CW-SQSM**: | GC/MS Analysis of Sediment Matrix Samples by SIM mode |
| **SA-CW-SQSM**: | GC/MS Analysis of Wipe Matrix Samples by SIM mode |
| **W-CW-SQFS**: | GC/MS Analysis of Water Matrix Samples by full scan |
| **S-CW-SQFS**: | GC/MS Analysis of Sediment Matrix Samples by full scan |
| **SA-CW-SQFS**: | GC/MS Analysis of Wipe Matrix Samples by full scan |

**1.4.** Target Analytes

| Acronym | Chemicals | Registry # |
|---|---|---|
| **GA** | Tabun, Ethyl N,N-dimethylphosphoramidocyanidate | [77-81-6] |
| **GB** | Sarin, Isopropyl methylphosphonofluoridate | [107-44-8] |
| **GD** | Soman, Pinacolyl methylphosphonofluoridate | [541-25-3] |
| **GF** | Cyclohexyl methylphosphonofluoridate | [993-13-5] |
| **HD** | Mustard gas, Bis(2-chloroyethyl)sulfide | [111-48-8] |
| **VX** | Ethyl S-2-(diisopropylamino)ethyl methylphosphonothiolate | [50782-69-9] |

**1.5.** Supporting Standard Operating Procedures (SOPs)

Refer to the following Standard Operating Procedures (SOPs) for additional procedural information with regard to sample custody, safety, analytical methods, and non-conformance reporting. These SOPs contain critical details not fully described within this Accountability SOP. The chemical agent operators must be completely familiar with all of these SOPs.

**1.5.1.** Florida Department of Environmental Protection, *Health and Safety Plan*.

**1.5.2.** Florida Department of Environmental Protection, Emergency Action Plan - Bob Martinez Center (BMC)

**1.5.3.** Florida Department of Environmental Protection, *Standard Operating Procedure for Handling Perceived Threat Materials* (SOP **LB-029**).


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

**1.5.4.** Florida Department of Environmental Protection, *Standard Operating Procedure for Receiving Packages and Freight at the Loading Dock (SOP* **LB-022**).

**1.5.5.** Florida Department of Environmental Protection, *Laboratory Support Section Receiving Room: Standard Operating Procedure for the Receipt and Log-in of Samples by the DEP Central laboratory* (SOP **LB-016**).

**1.5.6.** Florida Department of Environmental Protection, *Standard Operating Procedure for Maintaining the Custody and Storage of Environmental Samples in the DEP Central Laboratory* (SOP **LB-023**).

**1.5.7.** Florida Department of Environmental Protection, *Laboratory Accountability, Storage and Hazard Communications Procedures* (SOP **ERLN-001**).

**1.5.8.** Florida Department of Environmental Protection, *Standard Operating Procedure for Receipt and Log-in of Chemical Agent Samples and Standards* (SOP **ERLN-005**).

**1.5.9.** Florida Department of Environmental Protection, *Chemical Agent Laboratory Training Module.*

**1.5.10.** Florida Department of Environmental Protection*, Non-Conformance Reporting System* (SOP **LB-002**).

**1.5.11.** Florida Department of Environmental Protection, *Standard Operating Procedure for Records Maintenance and Storage* (SOP **LB-006**)

**1.5.12.** Florida Department of Environmental Protection, *Standard Operating Procedure for Reporting Qualified Data* (SOP **LB-027**)

## 2. SUMMARY OF PROTOCOL

**2.1.** This SOP involves solvent extraction of the sample followed by gas chromatography/ mass spectrometry (GC/MS) analysis to determine the identity and concentration of semi-volatile Chemical Agents.

**2.2.** Prior to analysis, samples must be prepared using sample preparation techniques appropriate for each matrix type. Aqueous samples, solid samples and wipe samples are extracted by micro-scale extraction. Extracts may require a concentration step using nitrogen evaporation to achieve appropriate levels of quantitation (LOQs).

## 3. INTERFERENCES

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


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

**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. In extreme cases, it may be necessary to analyze a solvent blank between samples.

**3.3** Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or interferences to sample analysis. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing method blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be necessary.

## 4. SAFETY/HAZARDOUS WASTE MANAGEMENT

See the *Laboratory Health and Safety Plan* and the *Safety and Waste Management Practices in the Organic Analysis Groups* (**SOP OG-001**) for specific details on safety and hazardous waste management.

## 5. EQUIPMENT AND SUPPLIES

**5.1** Brand names, suppliers, catalog, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using equipment and supplies other than those specified; however, demonstration of equivalent performance meeting the requirements of this SOP must be provided. The laboratory shall document any use of alternate equipment or supplies in the deliverables narrative.

**5.2** General Equipment

**5.2.1** Vials – Amber glass, with polytetrafluoroethylene (PTFE) lined screw or crimp top (2.0 mL capacity for gas chromatograph [GC] auto sampler).

**5.2.2** Water Bath – Heated, with concentric ring cover, capable of heating to 80 °C and maintaining temperature control (±5 °C). The bath should be used in a hood.

**5.2.3** Syringes – Gas tight, contaminant-free, 1.0 mL, 2.0 mL, 10 µL, 25 µL, 500 µL with Luer-lok™ fitting.

**5.2.4** Pasteur glass pipettes – 1.0 mL, disposable

**5.2.5** Eppendorf 1-10 mL, Fisher Scientific. Accu-jet Pipet Controller from BrandTech, fits 0.10 to 100 mL pipets.

**5.2.6** Eppendorf 0.10 -1.0 mL, Fisher Scientific.

**5.2.7** Eppendorf 0.01 - 0.10 mL, Fisher Scientific.

**5.2.8** Disposable tips for Eppendorf pipette, 0.20, 1.0, 10 mL.

**5.2.9** Rack for 15, 50 mL tubes and Eppendorf pipette.


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

**5.2.10** Balances – Analytical, capable of accurately weighing ±0.0001 g, and one capable of weighing 100 g (±0.01 g). The balances must be calibrated with Class S weights or known reference weights once per each 12-hour work shift. The balances must be calibrated with Class S weights at a minimum of once per month. The balances also must be checked annually by a certified technician.

**5.2.11** Spatula – Stainless steel or PTFE

**5.2.12** Vortexer – Glas-Col DPM12 Digital Pulse Mixer

**5.2.13** Centrifuge – IEC HN-S11 Benchtop Centrifuge with 12-position, 50-mL conical tube rotor.

**5.2.14** Nitrogen Evaporation Device – Equipped with temperature control that can be maintained at 35 – 40 °C, a RapidVap™ or equivalent. To prevent the release of solvent fumes into the laboratory, this device must be used in a hood.

**5.2.15** Vacuum Filtration Apparatus

**5.2.15.1** Buchner Funnel

**5.2.15.2** Filter Paper - Whatman No. 41 or equivalent

**5.2.16** Borosilicate Glass Wool - Oven-cleaned (muffled)

**5.2.17** Silicon Carbide Boiling Chips - Approximately 10/40 mesh. Heat to 400 °C for 30 min. PTFE boiling chips solvent rinsed prior to use are acceptable.

**5.2.18** pH Paper - Including narrow range capable of measuring a pH of 2.0

**5.2.19** pH Meter - With a combination glass electrode. Calibrate prior to each use according to manufacturer's instructions

**5.2.20** Ottawa sand - Oven muffled in a 500 mL wide mouthed amber bottle. (Oven is muffled to 450 °C – held for 4 hours – then ramped back to room temperature).

**5.2.21** Class A Graduated Cylinder – 50, 100, and 1000 mL

**5.2.22** Class A Volumetric Flasks – 1,2,5,10, and 25 mL

## 5.3 Micro-Scale Extraction Apparatus

### 5.3.1 Solid Samples

**5.3.1.1** 100 mL PTFE tubes and caps – Bergh of, or equivalent

**5.3.1.2** Shaker – Glas-Col or equivalent


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

**5.3.1.3** Sonicator – Branson or equivalent

**5.3.1.4** Apparatus for determining percent dry weight (if required, this must be performed in a hood)

**5.3.1.4.1** Drying oven – Capable of maintaining 103 – 105 °C

**5.3.1.4.2** Desiccator

**5.3.1.4.3** Crucibles – Disposable aluminum

**5.3.1.5** Glass beads – Solvent-rinsed, baked in 400 °C oven for approximately 1 hour

**5.3.1.6** Volatile organic analysis (VOA) vials – 40 mL capacity, disposable, pre-cleaned with PTFE-lined caps

### 5.3.2 Water Samples

**5.3.2.1** Volatile organic analysis (VOA) vials – 40 or 60 mL capacity, disposable, pre-cleaned with PTFE-lined caps

**5.3.2.2** Glass Scintillation Vials – At least 20 mL with screw-cap and PTFE or aluminum foil liner

**5.3.2.3** Drying Column – 19 mm ID chromatographic column with coarse frit. Substitution of a small pad of borosilicate glass wool for the frit will help prevent cross contamination of sample extracts.

## 5.4 Gas Chromatograph/Mass Spectrometer (GC/MS) System

**5.4.1** Gas Chromatograph – Agilent 7890 or equivalent. The GC system must be capable of temperature programming and have a flow controller that maintains a constant column flow rate throughout the temperature program operations. The system must be suitable for splitless injection and have all required accessories including syringes, analytical columns, and gases. All GC carrier gas lines must be constructed from stainless steel or copper tubing. Non-PTFE thread sealants or flow controllers with rubber components are not to be used. Note: Due to the potential hazards associated with the analysis of CWAs, it is recommended that a split vent trap is used for every GC system (Agilent RD-1020 Universal/External Split Vent Trap or equivalent).

**5.4.2** Gas Chromatography Column – Minimum length 30 m x 0.25 mm ID (or 0.32 mm) bonded phase silicon coated fused silica capillary column DB-5 (J&W Scientific); RTX-5, RTX-5Sil MS (Restek); Zebron ZB-5(Phenomenex); SPB-5 (Supelco); AT-5 (Alltech); HP-5 (Agilent); CP-Sil 8 CB (Chrompack); 007-2 (Quadrex); BP-5 (SGE); or equivalent. Note that this is a minimum requirement for column length. Longer columns may be used. Although a film thickness of 1.0 micron is recommended because of its larger capacity, a film thickness of 0.25 micron may be used. A description of the GC column used for analysis shall be provided in the deliverables narrative. A capillary column is


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

considered equivalent if:

**5.4.2.1** The column does not introduce contaminants that interfere with the identification and quantification of the compounds listed in Table 1

**5.4.2.2** The analytical results generated using the column meet the initial and continuing calibration verification technical acceptance criteria listed in the SOP and the quantitation levels (QLs) listed in Table 1. (Note: Quantitation levels listed in Table 1 are based on method development in a single laboratory. Quantitation limits will be added once developed).

**5.4.2.3** The column provides equal or better resolution of the compounds listed in Table 1 than columns listed in Section 5.4.2

**5.4.3** Mass Spectrometer – Agilent 5975, 5973, or equivalent. Must be capable of scanning from 35 – 500 atomic mass unit (amu) every second or less, using 70 volts (nominal) electron energy in the electron impact ionization mode, and producing a mass spectrum that meets the tuning acceptance criteria when 50 ng of decafluorotriphenylphosphine (DFTPP) is injected through the GC inlet. The instrument must be vented to the outside of the facility or to a trapping system that prevents the release of contaminants into the instrument room.

**5.4.4** GC/MS Interface – The laboratory may use any GC/MS interface that provides acceptable sensitivity at QLs. However, direct insertion of the GC column into the MS source is the recommended interface.

## 6. REAGENTS AND STANDARDS

**NOTE: Record Lot Numbers of all the reagents and solvents used in the Note Book**

### 6.1 Reagents

**6.1.1** Organic-free Reagent Water – Defined as water in which an interferant is not observed at or above the QL for each analyte of interest. Reagent water may be generated by passing tap water through a filter bed containing activated carbon.

**6.1.2** Acetone, ethyl acetate, methanol, methylene chloride and toluene – pesticide residue analysis grade or equivalent. Whenever possible, anhydrous solvents should be used or solvents should be pre-dried by adding sodium sulfate until no clumping occurs and the solution becomes clear (not cloudy).

**6.1.3** Sodium Sulfate – Powdered or granular anhydrous reagent grade, heated at 400 °C for 4 hours in a shallow tray, cooled in a desicator, and stored in a glass bottle OR Hydromatrix – Diatomaceous earth-based material rinsed with methylene chloride and dried at 400 °C for 4 hours in a shallow tray, cooled in a desiccator, and stored in a glass bottle.


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

**6.1.4** Triethylamine – reagent grade or equivalent

**6.1.5** Tris (hydroxymethyl)aminomethane, Acros, Cat.# 14050-5000

**6.1.6** 1M HCL: 45.5 mL of conc. HCl diluted to 500 mL with DI water

**6.1.7** 0.2M Tris buffer: 48.4 g of Tris (hydroxymethyl)aminomethane (sec. 5.2.23) dissolved with DI water in 2L of volumetric flask, add 32 mL of 1M HCl (sec. 5.2.24) and make it to final volume 2L. The final pH is in the range of 8.5 – 9.0.

## 6.2 Standards

The laboratory must be able to verify that the standards are certified. Manufacturer's certificates of analysis must be retained by the laboratory and presented upon request. Standard solutions purchased from a chemical supply house as extracts in sealed, glass ampules may be retained and used until the expiration date provided by the manufacturer. If no manufacturer's expiration date is provided, the standard solutions, if in unopened ampules, may be retained and used for 2 years from the preparation date. The expiration date of the standards, upon the breaking of the glass seal of the ampule, is 6 months (or sooner, if the standard has degraded or evaporated). Note: For many of the target compounds, neat standards may be unavailable; therefore, the laboratory may need to purchase diluted standards.

### 6.2.1 Stock Standard Solutions

Stock standard solutions are defined as standards that are to be used to produce working standards. They may be in the form of single compounds or mixtures. They may be purchased or prepared in methylene chloride or another suitable solvent. These are the primary standards.

### 6.2.2 Working Standards

Working standards, or secondary standards, are made by diluting primary standards. These standards are used in instrument calibration and in surrogates, as well as to check the calibration after samples have been run.

**6.2.2.1** Surrogate Standard Spiking Solution – The surrogate standard spiking solution contains the appropriate surrogates for the target compounds. Surrogate standards are added to all samples and calibration solutions. Additional surrogates may be added at the laboratory's discretion.

**6.2.2.2** Matrix Spiking Solution – This solution contains all target analytes.

**6.2.2.3** Instrument Performance Check Solution – This is a solution of DFTPP such that a 1 µL injection will contain 50 ng of DFTPP. The DFTPP may also be included in the calibration standards at this level.

**6.2.2.4** Internal Standard Solution – An internal standard mix solution can be purchased from commercial sources (e.g., Ultra Scientific, Supelco P/N 861238, or


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

equivalent). The mixture contains the following compounds: 1,4-dichlorobenzene-d4, naphthalene-d8, acenaphthene-d10, phenanthrene-d10, chrysene-d12 and perylene-d12, in a concentration of 4000 ug/mL of each internal standard solution. Intermediate solution of internal standard mix is prepared. A sufficient portion of this solution will be added to each sample extract just prior to analysis by GC/MS to result in a concentration of 0.5 ng/µL

**6.2.2.5** Initial and Continuing Calibration Solutions

**6.2.2.5.1** Prepare calibration standards at a minimum of five concentration levels. Each calibration standard should contain each target compound of interest, associated surrogate and internal standard.

*Note 1: 1.0 µL injections of all calibration standards must be used. All samples analyzed must be injected at the same volume (1.0 µL) as the calibration standard.*

*Note 2: The concentrations listed in Table 6 and 7 were used during initial method evaluation in a single laboratory. For most analytes, the low calibration standard is set at the expected quantitation level (e.g., LOQ). The remaining calibration standards should be prepared at concentrations that meet the specifications in Section 9.3.2.*

**6.2.2.5.2** The continuing calibration standard is prepared at or near the midpoint of the calibration curve.

### 6.2.3 Storage of Standard Solutions

**6.2.3.1** Store the stock standard solutions at 4 °C (± 2 °C) in PTFE lined screw-cap amber bottles. Fresh standards should be prepared every 6 months at a minimum (or sooner if the expiration date has elapsed).

**6.2.3.2** Store the working standards at ≤ 6 °C in PTFE sealed containers. Certain analytes may degrade in as little as 2 weeks; therefore, the working standard solution should be checked at least weekly for stability. These solutions must be replaced after 12 months (or sooner if the stock standard solutions have expired), or if comparison with quality control (QC) check samples indicates a problem.

**6.2.3.3** Protect all standards from light. Store samples, extracts, and standards separately.

**6.2.3.4** The laboratory is responsible for maintaining the integrity of standard solutions and verifying solutions prior to use. Standards must be brought to room temperature prior to use, checked for losses, and checked that all components have remained in solution.

## 7.0 SAMPLE PRESERVATION, STORAGE, AND TECHNICAL HOLDING TIMES

### 7.1 Sample Preservation.

Samples must be iced or refrigerated at 4 °C (±2 °C) from the time of collection until extraction.


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

### 7.2 Sample Storage

**7.2.1** Samples must be protected from light and refrigerated at 4 °C (±2°C) from the time of receipt until 60 days after delivery of results to the reference agency. After 60 days, the samples may be disposed of in a manner that complies with all applicable regulations.

**7.2.2** Samples must be stored in an atmosphere demonstrated to be free of all potential contaminants.

### 7.3 Sample Extract Storage

**7.3.1** Sample extracts must be protected from light and stored at ≤ 6 °C until 1 year after delivery of results to the reference agency.

**7.3.2** Samples, sample extracts, and standards must be stored separately.

### 7.4 Technical Holding Times

**7.4.1** Certain analytes will start to degrade immediately after sample collection; therefore, it is recommended that samples be extracted immediately upon receipt in the laboratory.

**7.4.2** Extracts must be analyzed within 14 days following extraction.

## 8.0 QUALITY CONTROL (QC)

### 8.1 Method Blanks

**8.1.1** A method blank is a volume of a clean reference matrix (e.g., reagent water for water samples, clean inert sand along with purified sodium sulfate or Hydromatrix drying agent for solid samples, clean absorbent for air samples, or clean wipe for wipe samples) spiked with sufficient amount of surrogate standard spiking solution such that the same amount of surrogate is added as for the associated samples and carried through the entire analytical procedure. Internal standard solution is added just prior to full scan analysis by GC/MS to give a concentration of 1 ng/µL for each internal standard. The volume or weight of the reference matrix must be approximately equal to the volume or weight of the samples associated with the blank.

#### 8.1.2 Frequency of Method Blanks

**8.1.2.1** A method blank must be extracted each time samples are extracted. The number of samples extracted with each method blank shall not exceed 20 field samples [excluding MS/MSD and Performance Evaluation (PE) samples]. In addition, a method blank shall:

**8.1.2.2** Be extracted by the same procedure used to extract samples; and

**8.1.2.3** Be analyzed on each GC/MS system used to analyze associated samples and conditions (i.e., GC/MS settings).

#### 8.1.3 Method Blank Preparation


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

**8.1.3.1** A method blank for water samples consists of a 35 mL volume of reagent water spiked with a sufficient amount of the surrogate standard spiking solution to result in the addition of 0.5 µg of each surrogate. For solid samples, a method blank consists of 10 grams of clean inert sand and 2.5 grams of sodium sulfate spiked with sufficient amount of the surrogate spiking solution to result in the addition of 0.5 µg of each surrogate. A method blank for air samples consists of a clean unused XAD-2 spiked with a sufficient amount of the surrogate standard spiking solution to result in the addition of 10 µg of each surrogate. A method blank for wipe samples consists of a clean, unused wipe spiked with a sufficient amount of the surrogate standard spiking solution to result in the addition of 0.5 µg of each surrogate. Extract, concentrate, and analyze the blank according to procedure.

**8.1.3.2** Under no circumstances should method blanks be analyzed at a dilution.

### 8.1.4 Technical Acceptance Criteria for Method Blank Analysis

**8.1.4.1** All blanks must be extracted and analyzed at the frequency described in Section 8.1.2 on a GC/MS system meeting the DFTPP in Section 9.2.2, initial calibration in Section 9.3.2, and continuing calibration verification (CCV) technical acceptance criteria in Section 9.4.

**8.1.4.2** The Percent Recovery (%Recovery) of each of the surrogates in the blank must be within the acceptance limits listed in Table 3.

**8.1.4.3** A method blank for solid, water, air, and wipe samples must contain less than the QL of target compounds.

*Note: In cases where a blank has detects above the quantitation limit, but associated samples have detects greater than 10 times the blank, consult your supervisor to determine if re-extraction is required.*

### 8.1.5 Corrective Action for Method Blanks

**8.1.5.1** If a method blank does not meet the technical acceptance criteria for method blank analysis, the laboratory shall consider the analytical system to be out of control.

**8.1.5.2** If contamination is the problem, then the source of the contamination must be investigated and appropriate corrective measures must be taken and documented before further sample analysis proceeds. It is the laboratory's responsibility to ensure that interferences caused by contaminants in solvents, reagents, glassware, and sample storage and processing hardware that lead to discrete artifacts and/or elevated baselines in the GC/MS be eliminated. If possible, samples associated with the contaminated blank must be re-extracted and re-analyzed.


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

**8.1.5.3** If surrogate recoveries in the method blank do not meet the acceptance criteria listed in Table 3, first re-analyze the method blank. If the surrogate recoveries do not meet the acceptance criteria after re-analysis, the method blank and all samples associated with that method blank must be re-extracted, if possible, and re-analyzed.

**8.1.5.4** If the method blank does not meet internal standard response requirements listed in Section 11.3.5, follow the corrective action procedure outlined in Section 11.4.4.1. The laboratory shall resolve and document the resolution of the problem before proceeding with sample analysis

**8.1.5.5** If the method blank does not meet the retention time (RT) requirements for internal standards (Section 11.3.6), check the instrument for malfunction and recalibrate. Reanalyze the method blank. Sample analyses cannot proceed until the method blank meets these requirements.

## 8.2 Matrix Spike and Matrix Spike Duplicate (MS/MSD)

To evaluate the effects of the sample matrix on the methods used for analyses, a mixture of target compounds must be spiked into two aliquots of a water or solid sample and analyzed in accordance with the appropriate method. Mixtures should be spiked at levels at a concentration near the midpoint of the calibration range.

### 8.2.1 Frequency of MS/MSD Analyses

**8.2.1.1** An MS/MSD shall be analyzed if requested by the reference agency or specified on the Traffic Report/Chain of Custody Record (TR/COC).

**8.2.1.2** As part of USEPA's quality assurance/quality control (QA/QC) program, water rinsate samples and/or field blanks (field QC) or PE samples may accompany solid, water, air and/or wipe samples that are delivered to the laboratory for analysis. The laboratory must not perform MS/MSD analysis on any of the field QC or PE samples.

**8.2.1.3** If the reference agency designates a sample to be used as an MS/MSD, then that sample must be used. If there is insufficient sample remaining to perform an MS/MSD, then the laboratory shall choose another sample on which to perform an MS/MSD analysis. At the time the selection is made, the laboratory shall notify the reference agency that insufficient sample was received and identify the reference agency sample selected for the MS/MSD analysis.

**8.2.1.4** If there is insufficient sample remaining in any of the samples in a batch to perform the requested MS/MSD, then the laboratory must immediately contact the reference agency to inform them of the problem. The reference agency will either approve that no MS/MSD be performed or require that a reduced sample aliquot be used for the MS/MSD analysis.

### 8.2.2 Procedure for Preparing MS/MSD


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

#### 8.2.2.1 Water Samples

Prepare two additional aliquots of the sample chosen for spiking. The volume should be equal to that of the associated samples. Add a sufficient amount of surrogate standard spiking solution and matrix spiking solution to each aliquot to result in the addition of 10 µg of each surrogate and 40 µg (or at the mid-point calibration range) of each matrix spike compound. Extract, concentrate, clean up, and analyze the MS/MSD according to the procedures for water samples (Section 10.2).

#### 8.2.2.2 Solid Samples

Prepare two additional aliquots of the sample chosen for spiking in the two PTFE tubes. The mass should be equal to that of the associated samples. If the sample contains moisture, add twice the weight anhydrous powdered sodium sulfate or equal weight of Hydromatrix to each aliquot (relative to sample). Mix well. Add a sufficient amount of the surrogate standard spiking solution and the matrix spiking solution to each aliquot, to result in the addition of 10 µg of each surrogate and 40 µg of each matrix spike compound, and then follow the appropriate extraction procedure in Section 10.3. Extract, concentrate, clean up, and analyze the MS/MSD according to the procedures for solid samples. Mixtures should be spiked at levels near the midpoint of the calibration curve.

### 8.2.3 Dilution of MS/MSD

The sample used for matrix spike preparation should be analyzed prior or with the MS/MSD analysis. When preparing the MS/MSD samples, they must be prepared at the same concentration as the most concentrated extract for which the original sample results are reported. This insures that any potential matrix effect is observed equally in the sample and matrix spikes.

### 8.2.4 Calculations for MS/MSD

**8.2.4.1** Calculate the percent recovery of each matrix spike compound in the MS/MSD sample using Equation 1 (see Section 11.2.8 for more information).

**Equation 1: Percent Recovery Calculation**

$$\boldsymbol{Recovery} = \%\boldsymbol{R} = \frac{\boldsymbol{Cs - Cu}}{\boldsymbol{Cn}} \times \mathbf{100}$$

*Where*:

Cs = Measured concentration of the spiked sample aliquot.
Cu = Measured concentration of the un-spiked sample aliquot (use 0 for the LCS).
Cn = Nominal (theoretical) concentration increase that results from spiking the sample, or the nominal concentration of the spiked aliquot (for LCS).


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

**8.2.4.2** Calculate the Relative Percent Difference (RPD) of the concentrations of each compound in the MS/MSD using Equation 2. Concentrations of the matrix spike compounds are calculated using the same equations as used for target compounds (Equation 6 for water samples and Equation 7 for solid samples in Section 11.2.6).

**Equation 2: Relative Percent Difference Calculation**

$$\boldsymbol{RPD = \frac{|C1 - C2|}{\frac{(C1 + C2)}{2}} \times 100}$$

*Where:*
C1 = Measured concentration of the first sample aliquot.
C2 = Measured concentration of the second sample aliquot.

### 8.2.5 Technical Acceptance Criteria for MS/MSD

**8.2.5.1** If requested, all MS/MSDs must be prepared and analyzed. on a GC/MS system meeting DFTPP, initial and continuing calibration verification technical acceptance criteria, and the method blank technical acceptance criteria.

**8.2.5.2** The MS/MSD must have an associated method blank meeting the Blank technical acceptance criteria.

**8.2.5.3** The MS/MSD must be extracted and analyzed within the technical holding time.

**8.2.5.4** The retention time (RT) shift for each of the internal standards must be within ±0.50 minutes (30 seconds) between the MS/MSD sample and the most recent CCV standard analysis.

**8.2.5.5** The limits for matrix spike compound recovery and RPD are given in Table 1 and 2. As these limits are only advisory, no further action by the laboratory is required.

### 8.2.6 Corrective Action for MS/MSD

Any MS/MSD that fails to meet the technical acceptance criteria in Sections 8.2.5.1 through 8.2.5.4 must be reanalyzed.

## 8.3 Laboratory Control Sample (LCS)

An LCS consists of an aliquot of clean reference matrix, of the same mass or volume as the corresponding field samples, and spiked with the same compounds at the same concentrations used to spike the MS/MSD. When the results of the MS/MSD analysis indicate matrix interference may be present, the LCS results are used to verify that the interferences are due to the sample matrix and not from artifacts introduced in the laboratory.


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

### 8.3.1 Preparation of LCS

Extract and analyze the LCS according to the procedures in Sections 10.2 (for water samples), Sections 10.3 for (solid samples), Section 10.4 (for waste dilution samples), 10.5 (for air filter samples) or Section 10.6 (for wipe samples).

### 8.3.2 Frequency of LCS Analyses

One LCS must be prepared, extracted, analyzed, and reported for every 20 field samples or fewer extracted in a batch of a similar matrix. The LCS must be extracted and analyzed concurrently with the samples, using the same extraction protocol, cleanup procedure (if required), and instrumentation.

### 8.3.3 Calculations for LCS

Calculate the recovery of each compound in the LCS using Equation 12 (Section 11.2.8.2).

### 8.3.4 Technical Acceptance Criteria for LCS Analysis

**8.3.4.1** All laboratory control samples must be extracted and analyzed on a GC/MS system meeting the tuning, initial and continuing calibration verification, and the method blank technical acceptance criteria.

**8.3.4.2** The limits for LCS compound recovery will be established upon laboratory validation of these procedures. Recovery limits of 50 -150% are applied until laboratory limits are established.

## 8.4 Instrument Detection Limit (IDL) Determination and Limit of Quantitation (LOQ)

Before any field samples are analyzed, an IDL for each target compound shall be determined on each instrument used for analysis. IDLs must be verified annually thereafter, or after major instrument maintenance. Major instrument maintenance includes, but is not limited to: cleaning or replacement of the mass spectrometer source, mass filters, electron multiplier, and installing a different GC column type. The IDL is instrument specific and independent of sample matrices.

**8.4.1** The IDL for each compound is determined as the concentration that produces an average signal-to-noise ratio of between 3:1 and 5:1 for at least 3 replicate injections.

**8.4.2** The LOQ for each compound is determined as approximately 3 times the IDL.

**8.4.3** All documentation for the IDL/LOQ determination shall be maintained at the laboratory and provided to the reference agency or the data user upon request.

**8.4.4** If project action levels are known, the LOQ determined can be used to demonstrate laboratory capability for achieving precision and recovery to address the action level.


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

# 9 CALIBRATION AND STANDARDIZATION

## 9.1 Instrument Operating Conditions

### 9.1.1 Gas Chromatograph (GC)

**9.1.1.1** The following are only suggested GC analytical conditions. The GC conditions may have to be modified if analysis for a specific class of compounds if required. Other conditions may be used, provided that all technical acceptance criteria in Sections 9.2.2, 9.3.2, 9.4. and 11.3 are met.

| Parameter | Setting |
|---|---|
| Initial column temperature | 40 °C for 1 minutes |
| Column temperature program | 40-290 °C at 23 °C/minute |
| Final column temperature hold | 290-320 °C at 15°C/minute; Hold Required: 1.13 minutes after all compounds listed in Table 1 have eluted |
| Injector temperature | 250 °C |
| Injector | pulsed, splitless |
| Sample volume | 1.0 µL |
| Carrier gas | Helium at 32 cm/sec |
| Injection pulse pressure | 30 psi until 0.40 min |
| Purge flow | 60 mL/min @ 0.40 min |

**9.1.1.2** Optimize GC conditions for analyte separation and sensitivity. Once optimized, the same GC conditions must be used for the analysis of all standards, samples, blanks, and MS/MSDs.

### 9.1.2 Mass Spectrometer (MS)

**9.1.2.1** The following are the required MS analytical conditions:

| Parameter | Setting |
|---|---|
| Mass range | 35 – 450 amu |
| Electron Energy | 70 eV |
| MS Source Temperature | 230 °C or according to manufacturer specifications |
| Ionization mode | Electron Ionization (EI) |
| Scan Rate | ~5 scan/sec (A minimum of 10 points across peaks) |
| MS transfer line temperature | 250 – 300 °C |

The standard MS conditions for Selected Ion Monitoring (SIM) analyses performed in electron ionization mode are:


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

| Parameters | Setting |
|---|---|
| MS transfer line temperature | 280 °C |
| MS source Temperature | 230 °C |
| MS quadrupole Temperature | 150 °C |
| Solvent delay time | 2.0 min |
| Electron energy | 70 eV |
| Ion dwell time | 100 msec per ion (each analyte was assigned its own SIM group; depending on the number of ions monitored, cycle times ranged from 1.44 – 2.86 cycles/sec |
| Ionization polarity | Positive |

## 9.2 GC/MS Mass Calibration (Tuning) and Ion Abundance

### 9.2.1 Summary of GC/MS Instrument Performance Check

**9.2.1.1** The GC/MS system must be tuned to meet the manufacturer's specifications, using a suitable calibration compound such as perfluoro-tri-n-butylamine (FC-43) or perfluorokerosene (PFK). The mass calibration and resolution of the GC/MS system are verified by the analysis of the instrument performance check solution. Prior to the analysis of any samples, including MS/MSDs, blanks, or calibration standards, the laboratory must establish that the GC/MS system meets the mass spectral ion abundance criteria for the instrument performance check solution (Table 4) containing DFTPP.

### 9.2.2 GC/MS Instrument Performance Check

**9.2.2.1** The instrument performance check solution must be analyzed once at the beginning of each 12-hour period during which samples or standards are analyzed.

**9.2.2.2** The 12-hour time period for a GC/MS system instrument performance check and calibration standards (initial or continuing calibration verification (CCV) criteria) begins at the moment of injection of the DFTPP. If an instrument performance check is not performed but the CCV meets all technical acceptance criteria, then the 12-hour time period begins at the moment of injection of the CCV.

**9.2.2.3** The analysis of the instrument performance check solution may be performed as an injection of 50 ng of DFTPP into the GC/MS or by adding a sufficient amount of DFTPP to the calibration standards to result in an on-column amount of 50 ng of DFTPP and analyzing the calibration standard.

### 9.2.3 Technical Acceptance Criteria for GC/MS Instrument Performance Check

**9.2.3.1** The abundance criteria listed in Table 4 must be met. The mass spectrum of DFTPP must be acquired in the following manner: three scans (the peak apex scan and the scans immediately preceding and following the apex) are acquired and averaged. Background subtraction is required and must be accomplished using a single scan acquired no more than 20 scans prior to the elution of DFTPP. The background subtraction should be designed only to eliminate column bleed or instrument background ions. Do not subtract


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

part of the DFTPP peak.

*Note 1: All subsequent standards, samples, MS/MSDs, and blanks associated with a DFTPP analysis must use the identical GC/MS instrument run conditions.*

*Note 2: The above tuning criteria are the "suggested" criteria when using DFTPP by Method 8270D. If alternative tunes are used, consult the method or manufacturer notes for guidance on criteria*

### 9.2.4 Corrective Action for GC/MS Instrument Performance Check

**9.2.4.1** If the GC/MS instrument performance check technical acceptance criteria are not met, re-tune the GC/MS system. It may be necessary to perform maintenance to achieve the technical acceptance criteria.

**9.2.4.2** The instrument performance check technical acceptance criteria must be met before any standards, samples, including MS/MSDs, or required blanks are analyzed.

## 9.3 Initial Calibration

Prior to sample analysis, and after instrument performance check technical acceptance criteria have been met, each GC/MS system must be calibrated at a minimum of five concentrations (Table 6,7) to determine instrument sensitivity and the linearity of GC/MS response for the target and surrogate compounds. If the curve is not linear (i.e., %RSD is outside the acceptance range), a quadratic curve may be used for that analyte. Each initial calibration standard contains all the target compounds, surrogates, and internal standards.

### 9.3.1 Frequency of Initial Calibration

**9.3.1.1** Each GC/MS must be calibrated whenever the laboratory takes corrective action that may change or affect the initial calibration criteria, or if the CCV technical acceptance criteria are not met.

**9.3.1.2** If time remains in the 12-hour period after meeting initial calibration acceptance criteria, samples may be analyzed. It is not necessary to analyze a continuing calibration standard within this period.

### 9.3.2 Procedure for Initial Calibration

**9.3.2.1** Prepare at least five calibration standards containing all the detected target compounds and associated surrogates at the concentrations described in Table 6-7.

**9.3.2.2** Add sufficient amount of internal standard solution to aliquots of calibration standards to result in 1 ng/µL of each internal standard. Standards should permit most of the target compounds to have relative retention times (RRTs) of approximately 0.60 to 1.70, using the assignments of internal standards to target compounds given in Table 10.


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

**9.3.2.3** Analyze each calibration standard with a 1.0 uL injection.

### 9.3.3 Calculations for Initial Calibration

**9.3.3.1** Calculate the relative response factors (RRFs) for each analyte and surrogate using Equation 3 and the primary characteristic ions found in Table 5. Assign target compounds and surrogates to internal standards according to Table 10. For internal standards, use the primary ion listed in Table 5 unless interferences are present. Note: Unless otherwise stated, the area response of the primary characteristic ion is the quantitation ion.

**Equation 3: Relative Response Factor Calculation**

$$RRF = \frac{Ax}{Ais} \times \frac{Cis}{Cx}$$

*Where:*
Ax = Area of the characteristic ion for the compound to be measured (Table 5).
Ais = Area of the characteristic ion for specific internal standard (Table 5).
Cis = Amount of the internal standard injected (ng).
Cx = Amount of the target compound or surrogate injected (ng).

**9.3.3.2** The Mean Relative Response Factor (RRF) for the Initial Calibration

**9.3.3.2.1** RRFs and mean RRFs must be calculated for all compounds. Calculate the percent relative standard deviation (%RSD) of the RRF values for the initial calibration. If linear regression or quadratic curve fitting is needed, consult SW-846 Method 8000C for guidance on the appropriate calculations.

### 9.3.4 Technical Acceptance Criteria for Initial Calibration

**9.3.4.1** Technical acceptance criteria for initial calibration have not yet been established.

### 9.3.5 Corrective Action for Initial Calibration

**9.3.5.1** If any technical acceptance criteria for initial calibration are not met, inspect the system for problems and take corrective actions to achieve the acceptance criteria.

**9.3.5.2** Initial calibration technical acceptance criteria must be met before any samples, including MS/MSDs or required blanks are analyzed.

## 9.4 Continuing Calibration Verification

### 9.4.1 Summary of Continuing Calibration Verification

**9.4.1.1** Prior to the analysis of samples, and after instrument performance check technical


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

acceptance criteria and initial calibration technical acceptance criteria have been met, each GC/MS system must be routinely checked by analyzing a CCV standard to ensure that the instrument continues to meet the instrument sensitivity and linearity requirements. The CCV standard contains all the target compounds, surrogates, and internal standards. The same injection volume must be used for all standards, samples, and blanks.

### 9.4.2 Frequency of Continuing Calibration Verification

**9.4.2.1** Each GC/MS used for analysis must be checked once every 12-hour time period of operation or after the analysis of 10 samples (whichever comes first). The 12-hour time period begins with the injection of DFTPP for full scan or the analysis of the CCV.

### 9.4.3 Procedure for Continuing Calibration Verification

**9.4.3.1** Add sufficient amount of internal standard solution to an aliquot of CCV standard to result in a concentration of 1.0 ng/µL for full scan analysis and 0.10 ng/µL for SIM analysis.

**9.4.3.2** Analyze the CCV standard with a 1.0 µL injection.

### 9.4.4 Calculations for CCV

**9.4.4.1** Calculate an RRF for each target compound and surrogate using Equation 3 and the primary characteristic ions found in Table 5.

**9.4.4.2** Calculate the Percent Difference (%Difference) between the RRF from the most recent initial calibration and the continuing calibration verification RRF for each target compound and surrogate using Equation 4.

**Equation 4: Relative Response Factor Percent Difference Calculation**

$$Percent\ Difference = \frac{RRFc - RRFi}{RRFi} \times 100$$

*Where:*

RRFi = Mean Relative Response Factor from the most recent initial calibration meeting technical acceptance criteria.

RRFc = Relative Response Factor from CCV standard.

### 9.4.5 Technical Acceptance Criteria for CCV

**9.4.5.1** The CCV standard must be analyzed at or near the mid-point concentration level on a GC/MS system meeting the instrument performance check and the initial calibration technical acceptance criteria.


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

**9.4.5.2** The RRF for each target compound and surrogate must be ≥ the compounds' inclusive range listed in Table 5.

**9.4.5.3** The RRF percent difference for each target compound must be within the inclusive range listed in Table 5. Note: If regression techniques are used for the initial calibration, the CCV should be evaluated in terms of percent drift using concentrations.(See Equation 4a).

**Equation 4a: Percent Drift (PD) Calculation for CCV**

$$PD = \frac{(\text{Calculated Concentration} - \text{Theoretical Concentration})}{\text{Theoretical Concentration}} \times 100\%$$

**9.4.5.4** The percent drift (PD) for each target compound must be within the inclusive range listed in Table 1-2.

**9.4.5.5** Excluding those ions in the solvent front, no quantitation ion may saturate the detector.

### 9.4.6 Corrective Action for CCV

**9.4.6.1** If the CCV technical acceptance criteria are not met, recalibrate the GC/MS instrument.

**9.4.6.2** CCV technical acceptance criteria must be met before any samples MS/MSDs, or required blanks, are analyzed. Any samples or required blanks analyzed when CCV criteria have not been met will require reanalysis.

## 10 ANALYTICAL PROCEDURE

### 10.1 Sample Preparation – General

**10.1.1** If an insufficient sample amount (less than 90% of the required amount) is received, the laboratory shall use a reduced volume or mass for the sample analysis.

**10.1.2** If multi-phase samples (e.g., a two-phase liquid sample, oily, sludge/sandy soil sample) are received by the laboratory, the laboratory shall contact the reference agency to apprise them of the type of sample received. If some or all phases of the sample are amenable to analysis, the reference agency may require the laboratory to do any of the following:

- Mix the sample and analyze an aliquot from the homogenized sample,
- Separate the phases of the sample and analyze each phase separately,
- Separate the phases and analyze one or more of the phases, but not all of the phases,
- Do not analyze the sample

### 10.2 Preparation of Water Samples using Microscale Extraction


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

**10.2.1** Approximately 35 mL of a water sample in a 60-mL VOA vial or centrifuge vial is required for this extraction. Remove any excess sample such that a total sample volume of 35 mL is obtained and recap the sample vial. Weigh the capped VOA vial. Record the weight to the nearest 0.1 grams. If control samples are being generated, exactly 35.00 mL of water can be measured into the vial and the weighing step can be eliminated for those samples.

**10.2.2** Spike 0.50 µg of each surrogate into the samples, blanks, etc. The final volume of the sample extracts will be 2.0 mL. For example, if the surrogate compounds in the spiking solution (prepared in methanol) are at a concentration of 25 ng/µL, add 40.0 µL of surrogate spiking solution. Add 10 µL of CWA and VX (5-10 µg/mL, supplied from EPA) to each sample.

**10.2.3** Add 8.75 g of sodium chloride and shake vigorously, or vortex each vial for 2 minutes or until the sodium chloride dissolves completely.

**10.2.4** Add 2.00 mL of methylene chloride (DCM), using a class A volumetric pipette or gas-tight syringe or Eppendorf pipette. Shake vigorously or vortex each vial for 2 minutes.

**10.2.5** Briefly allow the phases to settle by gravity or by centrifugation for several minutes. Note: Centrifugation (3 minutes at 500 g) is strongly recommended to facilitate separation of the phases and affords a greater recovery of the final sample extract.

**10.2.6** Using a pipette, transfer approximately 1.5 mL (or as much as possible) of the DCM (lower) layer to a 10-mL or 15-mL centrifuge tube with a PTFE lined screw cap, taking precautions to exclude any water from the syringe or pipette. Add a small amount (~50-100 mg) of anhydrous sodium sulfate to the tube, cap it, and shake.

**10.2.7** Using a 1.0 mL volumetric long pipette, transfer accurately 1.0 mL (or a known volume, if less than 1.0 mL of extract is collected) of the dried extract to a 2.0 mL GC vial (autosampler vial) with a PTFE lined screw cap. Cap the vials.

**10.2.8** Discard the remaining contents of the VOA vials according to laboratory waste disposal guidelines. Shake off the last few drops with short, brisk wrist movements. If needed, rinse the vial with a water-soluble solvent to ensure that the extraction solvent is removed. Weigh the vials with caps and record the weight. This will allow for determination of the volume of water extracted. Note: Extracts must be protected from light and stored at 4 °C (±2 °C).

**10.2.9** Proceed to Section 10.9 for sample analysis.

### 10.3 Preparation of Solid Samples using Microscale Extraction

**10.3.1** Decant and discard any water layer. Mix samples thoroughly, especially composite samples. Discard any foreign objects such as sticks, leaves and rocks.

**10.3.2** pH Determination – If a pH determination for solid samples is requested, transfer 50 g of sample to a 100-mL beaker. Add 50 mL of water and stir for 1 hour. Determine the pH of the sample with a pH meter or wide-range pH paper while stirring. Report pH value on appropriate data sheets. If the pH of the sample is greater than 11 or less than 5, document this in the deliverables narrative. Discard this portion of the sample. Note: If limited sample


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

weight (less than 50 g) is received, use a smaller 1:1 ratio of grams of solid sample to mLs of water for the pH determination.

**10.3.3** Percent Moisture Determination – Immediately after weighing the sample for extraction, weigh 5–10 g of the sample into a tared crucible. Determine the Percent Moisture (%Moisture) by drying overnight at 103-105 °C. Allow to cool in a desiccator before weighing.

**Equation 5: Percent Moisture Calculation**

$$Percent\ Moisture = \frac{Grams\ of\ Wet\ Sample - Grams\ of\ Dry\ Sample}{Grams\ of\ Wet\ Sample} \times 100$$

**10.3.4 Extraction**

**10.3.4.1.1** Weigh 10 g of sample into the tared extraction vial (i.e., 60 mL VOA vial or bottle). Wipe the lip and threads of the tube with a clean cloth (i.e., Kimwipe® or equivalent). Tightly cap, and record the weight to the nearest 0.01 g.

**10.3.4.1.2** Spike 0.50 µg of each surrogate into the samples, blanks, etc. The final volume of the sample extracts will be 1.0 mL. For example, if the surrogate compounds in the spiking solution (prepared in methanol) are at a concentration of 25 ng/µL, add 20 µL of surrogate spiking solution. Add 5.0 µL of CWA mix (5-10 µg /mL, supplied from EPA).

**10.3.4.1.3** Add approximately 2.5 g of anhydrous sodium sulfate to the sample and mix with spatula until the slurry is free flowing. Additional sodium sulfate shall be added if necessary.

**10.3.4.1.4** Add 25 mL of DCM to the tube, and cap tightly. Note: For certain sample types, 25 mL of solvent will not be sufficient to completely immerse the solid. For these situations, add the minimal amount of solvent such that the solid is completely immersed. Record the additional volume of solvent.

**10.3.4.1.5** Shake the sample for 30 min using shaker. Shake the tubes vigorously or vortex to mix. Allow the solids to settle or centrifuge for 5-10 minutes. Decant or pipette the solvent layer into pre-cleaned, 40 mL vial or test tube with PTFE screw cap.

**10.3.4.1.6** Repeat steps 10.3.4.1 – 10.3.4.2 using separate aliquot of soil for VX extraction. Add 30 mL of 0.2M tris buffer and shake for 15 min using shaker. Centrifuge the vial and transfer the extract to 40 mL vial. Add 5 mL of DCM, shake vigorously or vortex each vial for 2 minutes. Briefly allow the the phases to settle by gravity or by centrifugation for several minutes. Using a pipette, transfer DCM (lower layer) to


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

another 40 mL vial, taking precautions to exclude any water from the syringe or pipette. Repeat the extraction with another 5 mL of DCM. Combine both extracts, add a small amount of anhydrous sodium sulfate to the tube, cap it, and shake or vortex it. Transfer the DCM extract to a 10-mL or 15-mL centrifuge tube with a PTFE lined screw cap. Concentrate to 1 mL final volume in methylene chloride (DCM). Add 10 ul of internal standard (50 PPM) to the extract before analysis.

**10.3.4.1.7** Proceed to step 10.8.

## 10.4 Preparation of Waste Samples by Dilution

**10.4.1.** This procedure is designed for wastes (e.g. oils, non-aqueous liquids or organic solids) that may contain organic chemicals at a concentration greater than $2.0 \times 10^4$ mg/kg and that are soluble in the dilution solvent. Sample dilution may be performed in a 10 mL volumetric flask. If disposable glassware is preferred, the 20 mL scintillation vial may be calibrated for use. Pipette 10 mL of DCM into the scintillation vial and mark the bottom of the meniscus. Discard this solvent. Transfer approximately 1.0 g of each phase of the sample to separate 20 mL vials or 10 mL volumetric flasks (record weight to the nearest g). Add sufficient surrogate spiking solution to all samples and blanks to result in the addition of 10 µg of each surrogate compound. Immediately dilute to 10 mL with DCM. Add 2.0 g of anhydrous sodium sulfate to the sample and cap. Shake the sample for 2 minutes. Loosely pack disposable Pasteur pipets with 2 – 3 cm glass wool plugs. Filter the extract through the glass wool and collect 5.0 mL of the extract in a tube or vial. The extract is ready for analysis. Proceed to Section 11.9.

## 10.5 Preparation of Air Samples using Pressurized Fluid Extraction

**10.5.1** Transfer the XAD filter and sorbent into an appropriately sized extraction cell. Add sufficient amount of the surrogate standard spiking solution to result in the addition of 0.8 µg of each surrogate to the sample. If SIM analysis is required, spike 100 ng of each surrogate into the samples, blanks, etc.

**10.5.2** Place the extraction cell into the instrument (what instrument?) or autosampler tray, as described by the instrument manufacturer. Place a pre-cleaned collection vessel in the instrument for each sample, as described by the instrument manufacturer. The total volume of the collected extract will depend on the specific instrument and the extraction procedure recommended by the manufacturer, and may range from 0.5 -1.4 times the volume of the extraction cell. Ensure that the collection vessel is sufficiently large to hold the extract. The following are recommended extraction conditions:

| | |
|---|---|
| Oven temperature | 100 °C |
| Pressure: | 1500-2000 psi |
| Nitrogen purge: | 1 minute at 150 psi (purge time may be extended for larger cells) |
| Flush volume: | 60% of the cell volume |
| Static cycles: | 2 |
| Static time: | 10 minutes (after 5 minutes pre-heat equilibration) |

**10.5.3** Optimize the extraction conditions as needed, according to the manufacturer's instructions. An appropriate amount of 1:1(v/v) acetone/DCM should be used to achieve the extraction conditions


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

detailed in the preceding paragraph. Once established, the same pressure should be used for all samples in the same batch. Begin the extraction according to the manufacturer's instructions. Collect each extract in a clean vial. Allow the extracts to cool after the extractions are complete.

**10.6 Preparation of Wipe Samples using Microscale Extraction**:

**10.6.1** Place a dry wipe into an extraction vial (i.e., 40 -mL VOA vial). Rinse the wipe two times with 10 mL of DCM and discard the rinse.

**10.6.1.1.1** Spike the surrogate, CWA and VX as in step 10.3.4.2.

Note: the CWAs and surrogates should be spiked immediately or as soon as possible after transfer to the VOA vial to ensure that the wipe is still wet.

**10.6.2** Add 15 mL of DCM to the vial, and cap tightly. Note: If 15 mL of solvent will not be sufficient to completely immerse the wipe, add the minimal amount of solvent such that the wipe is completely immersed. Record the additional volume of solvent.

**10.6.3** Extract the samples by sonication in a water bath for at least 30 minutes.

**10.6.4** Remove the vial from sonicator, shake briefly by hand and transfer the solvent layer, by pipette or decanted into pre-cleaned, 40-mL, clear glass test tubes with PTFE screw cap.

**10.6.5** Repeat steps 10.6.2 – 10.6.4 with an additional 15 mL of DCM.

**10.6.6** Combine the two sample extracts and proceed to step 10.8 for extract concentration. Once the extract is concentrated proceed to section 10.9 for analysis.

**10.7 Sample Cleanup**

**10.7.1** The use of cleanup procedures is optional and the need for them will depend on the sample, matrix, and the class of compound to be detected. It is well known that certain cleanup procedures are not amenable for the analysis of certain classes of compounds. For example, gel permeation chromatography, Florisil, and activated silica gel cleanup are often used for the analysis of compounds, but these cleanup procedures may not be appropriate for organophosphorous based nerve agents. After completion of any cleanup procedure, proceed to Section 10.8 for final concentration.

**10.8 Final Concentration of Extract – Nitrogen Evaporation Technique (RapidVap or equiv.)**

**10.8.1** Nitrogen Evaporation Technique (Taken from ASTM Method D3086) -Place the VOA vial in a warm water bath (30 –35 °C recommended) and evaporate the solvent volume to just below 1.0 mL by blowing a gentle stream of clean, dry nitrogen (filtered through a column of activated carbon) above the extract. The internal wall of the vial must be rinsed down several times with DCM during the operation. During evaporation, the tube solvent level must be kept below the water level of the bath. The extract must never be allowed to become dry. If using a RapidVap or TurboVap $N_2$ system, follow manufacturer's guidelines. Adjust the final volume to 1.0 mL with the same solvent used for extraction. Transfer the extract to a 2-mL autosampler vial, cap and


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

label the vial. Store at 4 °C (±2 °C).

*CAUTION: Gas lines from the gas source to the evaporation apparatus must be stainless steel, copper, or PTFE tubing. Plastic tubing must not be used between the carbon trap and the sample since it may introduce interferences.*

**10.8.2** Final Extract Volumes – The final extract volumes in Sections 10.8.2.1 through 10.9.2.4 are recommended volumes. If more sensitive GC/MS systems are used, then the larger extract volumes (less concentrated extracts) may be used, provided that the LOQs for all target compounds can be achieved, and that all surrogates and internal standards have an expected extract concentration that is at the mid-point of the calibration curve.

**10.8.2.1** Water/Liquid –See step 10.2.7.

**10.8.2.2** Solid – Adjust the final volume for solid samples to 1.0 mL with DCM.

**10.8.2.3** Air – Adjust the final volume for air samples to 1.0 mL with /DCM.

**10.8.2.4** Wipe – Adjust the final volume for wipe samples to 1.0 mL with DCM.

**10.8.2.5** Before analysis add 10 µL of 10 µg/mL of internal standard to the extract vial.

## 10.9 Sample Analysis by GC/MS

**10.9.1** Analyze extracts only after the GC/MS system has met the instrument performance check initial calibration and CCV requirements. The same instrument conditions used for calibration must be used for the analysis of samples.

**10.9.2** The internal standard solution is added to an aliquot of each sample extract. Add 10 µL of 100 µg/mL of internal standard to accurately measured aliquot of sample extract to result in 1 ng/µL concentration of each internal standard for full scan. If SIM analysis is required, add 10 µL of 10 µg/mL of internal standard to result in 0.1 ng/µL concentration of each internal standard.

**10.9.3** If sample extracts are to be diluted, it is diluted with the solution of same concentration of internal standard. 1 ng/µL (full scan) or 0.10 ng/µL (SIM).

**10.9.4** Inject 1.0 µL of the sample extract into the GC/MS. Note: The same injection volume used for calibration standards must be used for sample extracts.

**10.9.5 Sample Dilutions.**

**10.9.5.1** If the response of any target compound in any sample exceeds the response of the same target compound in the highest standard of the initial calibration, that sample extract must be diluted. For dilution use the internal standard solution of the same concentration in the sample.

**10.9.5.2** Use the results of the original analysis to determine the approximate Dilution Factor


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

(DF) required for the largest analyte peak to fall within the initial calibration range. The DF chosen must keep the response of the largest peak for a target compound in the upper half of the calibration range of the instrument.

**10.9.6** If a target compound is suspected to be present, but is not detected by full scan mode, SIM analysis should be performed. Note: SIM analysis requires specific instrument conditions (Section 9.1.2), as well as lower amounts of surrogates and internal standards to be added to standards, samples and blanks. In addition, a separate calibration curve is required prior to analyzing any samples or blanks in the SIM mode.

# 11 CALCULATIONS AND DATA ANALYSIS

## 11.1 Qualitative Identification of Target Compounds

**11.1.1** The compounds listed in Table 1 must be identified by an analyst competent in the interpretation of mass spectra by comparison of the sample mass spectrum to the mass spectrum of the standard of the suspected compound. Two criteria must be satisfied to verify the identifications:

**11.1.1.1** Elution of the sample analyte within the GC RRT unit window established from the 12-hour calibration standard.

**11.1.1.2** Correspondence of the sample analyte and calibration standard component mass spectra.

**11.1.2** For establishing correspondence of the GC RRT, the sample component must compare within ±0.06 RRT units of the standard component. For samples analyzed during the same 12-hour time period as the initial calibration standards, compare the analyte RTs to those from the midpoint initial calibration standard. Otherwise, use the corresponding CCV standard. If coelution of interfering components prohibits accurate assignment of the sample component RRT from the total ion chromatogram, the RRT should be assigned by using response for ions unique to the component of interest.

**11.1.3** For comparison of standard and sample component mass spectra, mass spectra obtained from a calibration standard on a GC/MS meeting the daily instrument performance requirements for DFTPP are required.

**11.1.4** All ions present in the standard mass spectrum at a relative intensity greater than 10% (most abundant ion in the spectrum equals 100%) must be present in the sample spectrum. The relative intensities of ions specified in Table 5 must agree within ±20% between the standard and sample spectra (e.g., for an ion with an abundance of 50% in the standard spectra, the corresponding sample ion abundance must be between 30 – 70%). Ions greater than 10% in the sample spectrum, but not present in the standard spectrum, must be considered and accounted for by the analyst making the comparison. The verification process should favor false positives. All compounds meeting the identification criteria must be reported with their spectra. When target compounds are below LOQs but the spectrum meets the identification criteria, report the concentration with a “J”. For example, if the LOQ is 5.0 g/L and concentration of 3.0 g/L is calculated,


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

report as "3.0 J".

**11.1.5** If a compound is not verified automatically through the instrument software by the spectral identification criteria in Sections 11.1.1 – 11.1.4, but in the judgment of the Analyst is a positive detect, then the laboratory must report the identification and proceed with quantitation.

## 11.2 Data Analysis and Calculations of Target Compounds

**11.2.1** Positive target compounds must be quantitated by the internal standard method. The assigned internal standard for each analyte is provided in Table 10. The primary characteristic ions used for quantitation of each analyte are listed in Table 5.

**11.2.2** It is expected that situations will arise when the automated quantitation procedures in the GC/MS software provide inappropriate quantitation. This normally occurs when there is compound coelution, baseline noise, or matrix interferences. In these circumstances, the laboratory must perform a manual quantitation. Manual integrations are performed by integrating the area of the quantitation ion of the compound. This integration shall only include the area attributable to the specific target compound. The area integrated must not include baseline background noise. The area integrated must not extend past the point where the sides of the peak intersect with the baseline noise. Manual integration is not to be used solely to meet QC criteria, nor is it to be used as a substitute for corrective action on the chromatographic system.

**11.2.3** In all instances where the data system report has been edited or where manual integration or quantitation has been performed, the GC/MS operator must identify such edits or manual procedures by initialing and dating the changes made to the report, and shall include the integration scan range. The GC/MS operator must also mark each integrated area on the quantitation report. In addition, a hardcopy printout of the EICP of the quantitation ion displaying the manual integration shall be included in the raw data.

**11.2.4** The requirements listed in Sections 11.2.1 – 11.2.3 apply to all standards, samples, and blanks.

**11.2.5** The RRF from the initial calibration is used to calculate the concentration in the sample. Secondary ion quantitation is allowed only when there are sample interferences with the primary ion. If linear regression is used, a regression curve must be used to calculate the concentration in samples. Refer to SW-846 Method 8000C for guidance on how to use regression curves for quantitation.

**11.2.6** Calculate the concentration in the sample using the RRF and Equations 6 – 9.

**11.2.6.1** Water Calculations

**Equation 6: Concentration of Water Sample**


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

$$Conc.\left({}^{ug}/_{L}\right) = \frac{(Ax)(Is)(Vt)(DF)}{(Ais)(RRF)(Vo)(Vi)}$$

*Where:*

$A_x$ = Area of the characteristic ion for the compound to be measured.
$A_{is}$ = Area of the characteristic ion for the internal standard.
$I_s$ = Amount of internal standard injected in ng.
$V_o$ = Volume of water extracted in mL.
$V_i$ = Volume of extract injected in µL.
$V_t$ = Volume of the concentrated extract in µL.
RRF = Mean Relative Response Factor determined from the initial calibration standard.
DF = Dilution Factor. If no dilution is performed, DF = 1.0.

**11.2.6.1.1** Solid Calculations

**Equation 7: Concentration of Solid Sample**

$$Conc.{}^{ug}/_{Kg}\,(dry\ weight\ basis) = \frac{(A)(I)(V)(DF)}{(Ais)(Vi)(RRF)(Ws)(D)}$$

*Where:*
$A_x$, $I_s$, $A_{is}$, $V_i$, $V_t$ are as given for water, above.

$$D = \frac{100 - \%Moisture}{100}$$

Ws = Weight of sample extracted in g.

RRF = Mean Relative Response Factor determined from the initial calibration standard.

DF = Dilution Factor.

**11.2.6.1.2** Air Calculations

**Equation 8: Concentration of Air Sample**

$$Conc.{}^{ng}/_{m^3} = \frac{(Ax)(Is)(Vt)(DF)}{(Ais)(Vo)(Vi)(RRF)}$$

*Where:*
Ax = area response for the compound to be measured, counts
$A_{is}$ = area response for the internal standard, counts
Is = amount of internal standard, ng
RRF = the mean RRF from the most recent initial calibration, dimensionless
$V_o$ = volume of air sampled, std $m^3$


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

$V_t$ = volume of final extract, µL
$V_i$ = volume of extract injected, µL
DF = dilution factor for the extract. If there was no dilution, DF equals 1. If the sample was diluted, DF is greater than 1.

#### 11.2.6.1.3 Wipes

**Equation 9: Concentration of Wipe Sample**

$$Conc.^{ng}/_{std\ m} = \frac{(Ax)(Is)(Vt)(DF)}{(Ais)(Area)(Vi)(RRF)}$$

*Where:*
Ax = area response for the compound to be measured, counts
$A_{is}$ = area response for the internal standard, counts
Is = amount of internal standard, ng
RRF = the mean RRF from the most recent initial calibration, dimensionless
Area = area of surface wiped, m2
$V_t$ = volume of final extract, µL
$V_i$ = volume of extract injected, µL
DF = dilution factor for the extract. If there was no dilution, DF equals 1. If the sample was diluted, DF is greater than 1.

### 11.2.7 Adjusted LOQ Calculations

#### 11.2.7.1.1 Water Samples

**Equation 10: Aqueous Adjusted LOQ**

$$\text{Adjusted LOQ} = \text{Method LOQ} \times \frac{(V_x)(V_t)(DF)}{(V_o)(V_c)}$$

*Where:*
$V_t$, DF, and Vo are as given in Equation 6.
Vx = Method sample volume
$V_c$ = Method concentrated extract volume

#### 11.2.7.2 Solid Samples

**Equation 11: Solid Adjusted LOQ**

$$\frac{(W_x)(V_t)(DF)}{}$$


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

Adjusted LOQ = Method LOQ × ($W_s$)($V_c$)(D)

*Where:*
$V_t$ and DF are as given in Equation 6
$W_s$ and D are as given in Equation 7
$W_x$ = Method sample weight
$V_c$ = Method concentrated extract volume

**11.2.8 Surrogate Recoveries**

**11.2.8.1** Calculate surrogate recoveries for all samples, blanks, and MS/MSDs. Determine if recovery is within limits (Table 3).

**11.2.8.2** Calculate the concentrations of the surrogates using the same equations as used for the target compounds. Calculate the recovery of each surrogate using Equation 12.

**Equation 12: Percent Recovery**

$$\boldsymbol{Recovery = \%R = \frac{Cs - Cu}{Cn} \times 100}$$

*Where*:
Cs = Measured concentration of the spiked sample aliquot.
Cu = Measured concentration of the unspiked sample aliquot (use 0 for the LCS).
Cn = Nominal (theoretical) concentration increase that results from spiking the sample, or the nominal concentration of the spiked aliquot (for LCS).

**11.3 Technical Acceptance Criteria for Sample Analysis**:

**11.3.1** The samples must be analyzed on a GC/MS system meeting the instrument performance check, initial calibration, CCV, and blank technical acceptance criteria.

**11.3.2** The sample must be extracted and analyzed within the technical holding times.

**11.3.3** The sample must have an associated method blank meeting the blank technical acceptance criteria.

**11.3.4** The percent recoveries of the surrogates in a sample must be within the recovery limits listed in Table 3. Note: The surrogate recovery requirements do not apply to samples that have been diluted.

**11.3.5** The instrumental response for each of the internal standards in the sample must


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

be within the range of 50.0 – 200% of the response of the internal standard in the most recent CCV standard analysis.

**11.3.6** The RT shift for each of the internal standards must be within ±0.50 minute (30 seconds) between the sample and the most recent CCV standard analysis.

**11.3.7** Excluding those ions in the solvent front, no ion may saturate the detector. If a target compound concentration exceeds the upper limit of the initial calibration range, a more dilute aliquot of the sample extract must also be analyzed.

### 11.4 Corrective Action for Sample Analysis

**11.4.1** The sample technical acceptance criteria must be met before data are reported.

**11.4.2** Corrective action for failure to meet instrument performance checks and initial and continuing calibration verification must be completed before the analysis of samples.

**11.4.3** Corrective Action for Surrogate Recoveries that Fail to Meet Their Acceptance Criteria (Section 11.3.4, Table 3).

**11.4.3.1** If the surrogate recoveries in a sample fail to meet the acceptance criteria specified in Section 11.3.4, check calculations, sample preparation logs, surrogate standard spiking solutions, and the instrument operation.

**11.4.3.1.1** If the calculations were incorrect, correct them and verify that the surrogate recoveries meet their acceptance criteria.

**11.4.3.1.2** If the sample preparation logs indicate that the incorrect amount of surrogate standard spiking solution was added to the sample, then re-extract (if possible) and reanalyze the sample after adding the correct amount of surrogate standard spiking solution.

**11.4.3.1.3** If the surrogate standard spiking solution was improperly prepared, concentrated, or degraded, re-prepare the solution, and re-extract (if possible) and re-analyze the samples.

**11.4.3.1.4** If the instrument malfunctioned, correct the instrument problem and reanalyze the sample extract. Verify that the surrogate recoveries meet their acceptance criteria.

**11.4.3.1.5** If the instrument malfunction affected the calibrations, recalibrate the instrument before reanalyzing the sample extract.


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

**11.4.3.2** If the above actions do not correct the problem, then the problem may be due to a sample matrix effect. To determine if there was matrix effect, take the following corrective action steps:

**11.4.3.2.1** Re-extract (if possible) and reanalyze the sample. EXCEPTION: If surrogate recoveries in a sample used for a matrix spike/matrix spike duplicate (MS/MSD) were considered unacceptable, then it should be re-extracted/reanalyzed only if surrogate recoveries met (did not meet?) the acceptance criteria in both the MS/MSD analyses.

**11.4.3.2.2** If the surrogate recoveries meet acceptance criteria in the re-extracted/re-analyzed sample, then the problem was within the laboratory's control.

**11.4.3.2.3** Submit data from both analyses. Distinguish between the initial analysis and the extraction/reanalysis on all deliverables.

**11.4.4 Corrective Action for Internal Standard Compound Responses that Fail to Meet the Acceptance Criteria (Sections 11.3.5 and 11.3.6)**

**11.4.4.1** If the internal standards in a sample fail to meet their acceptance criteria, check calculations, internal standard solutions, and instrument operation.

**11.4.4.1.1** If the calculations were incorrect, correct them, and verify that the internal standard responses meet their acceptance criteria.

**11.4.4.1.2** If the internal standard solution was improperly prepared, concentrated, or degraded, re-prepare solutions and reanalyze another aliquot of the sample extract (if possible) after adding the correct amount of the freshly prepared internal standard solution.

**11.4.4.1.3** If the instrument malfunctioned, correct the instrument problem and reanalyze the sample extract.

**11.4.4.1.4** If the instrument malfunction affected the calibration, recalibrate the instrument before reanalyzing the sample extract.

**11.4.4.2** If the above actions do not correct the problem, then the problem may be due to a sample matrix effect. To determine if there was matrix effect, take the following corrective action steps:


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

**11.4.4.2.1** Reanalyze the sample extract.

*EXCEPTION: If internal standard responses in a sample used for an MS and/or MSD are outside the acceptance windows, then the sample should be reanalyzed only if internal standard compound recoveries met the internal standard acceptance criteria in the MS/MSD analysis.*

**11.4.4.2.2** If the internal standard responses meet acceptance criteria in the reanalyzed sample extract, then the problem was within the laboratory's control.

**11.4.4.2.3** Submit data from both analyses. Distinguish between the initial analysis and the reanalysis on all deliverables.

### 11.4.5 Corrective Action for Internal Standard Compound RTs Outside Acceptance Criteria (Section 11.3.6)

**11.4.5.1** If the internal standard compound RTs are not within their acceptance criteria, check the instrument for malfunctions. If the instrument malfunctioned, correct the instrument problem and reanalyze the sample extract. If the instrument malfunction affected the calibration, recalibrate the instrument before reanalyzing the sample extract.

**11.4.5.2** If the above actions do not correct the problem, then the problem may be due to a sample matrix effect. To determine if there was matrix effect, take the following corrective action steps:

**11.4.5.2.1** Reanalyze the sample extract.

**11.4.5.2.2** If the internal standard compound RTs are within the acceptance criteria in the reanalyzed sample extract, then the problem was within the laboratory's control.

**11.4.5.2.3** Submit data from both analyses. Distinguish between the initial analysis and the reanalysis on all deliverables.

## 12 STANDARD ANALYTICAL PROCEDURE PERFORMANCE

Method performance was evaluated for measurement of some of the analytes listed in Section 1.2 and Table 1 - 2 in a single laboratory, using reagent water, Georgia Bt2 red clay, surface wipes, and XAD/quartz filters. Resulting detection and quantitation levels, precision, and recovery from this evaluation are listed in Tables 1 and 2. Details regarding laboratory results are provided in the laboratory's SVOC method development summary reports (Reference 16.9).

### 12.1 Estimated Detection Levels, RT and Quantitation Ion Determinations


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

**12.1.1** Estimated detection levels listed in Tables 1- 2 were established as follows: 1.0 µL of a 1.0 ng/µL standard of each target analyte containing internal standards was injected into the GC/MS instrument. If a signal-to-noise ratio of at least 3 was achieved, then 1.0 ng/µL became the estimated detection level for that analyte. If this minimum ratio was not achieved, standards of 5.0, 10, and 50 ng/µL were analyzed until a ratio of at least 3 was reached. Only 1 replicate per concentration was analyzed. The mass spectrum of the analyte at this concentration was verified by comparison with the spectra in the NIST library. Retention times and quantitation ions listed in Table 4 were established using the instrument conditions listed in Section 10.1.

### 12.2 Quantitation Levels (QLs)

**12.2.1** QLs were determined by spiking reference samples (reagent water, Georgia Bt2 red clay, XAD/quartz filter, and 3"x 3" pre-cleaned wipes) using the sample preparation procedures described in this SOP. At first, a spiking level of 1x the estimated detection level was used. If this spiking level gave recoveries of between 40-160%, the concentration was selected by the laboratory as the QL. If recoveries were outside this range, the spiking level was adjusted until a recovery within this range was achieved. Spiking levels of 2x, 5x, 10x, and 60x the estimated detection level were used to establish QLs. Tables 1-2 lists resulting QLs selected by the laboratory.

### 12.3 Precision and Recovery in Reference Samples:

**12.3.1** Study samples were spiked at 1x, 2x, 5x, and/or 10x times the QL with either 2, 3 or 4 replicates at each concentration level. Results of precision and recovery are presented in Tables 1-2.

## 13 POLLUTION PREVENTION

**13.1** Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

## 14 REFERENCES

**14.1** US Environmental Protection Agency. Semi-volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS). SW-846 Method 8270E. Revision 6, June 2018.

**14.2** US Environmental Protection Agency. Organic Compounds in Water by Micro extraction. SW-846 Method 3511. Revision 1. July 1014.


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

**14.3** US Environmental Protection Agency. Pressurized Fluid Extraction (PFE). SW-846 Method 3545A. Revision 1. February 2007.

**14.4** US Environmental Protection Agency. Microscale Solvent Extraction. SW-846 Method 3570. Revision 0. November 2002.

**14.5** Environmental Protection Agency. Cleanup. SW-846 Method 3600C. Revision 3. December 1996.

**14.6** US Environmental Protection Agency. Silica Gel Cleanup. SW-846 Method 3630C. Revision 3. December 1996.

**14.7** US Environmental Protection Agency. Waste Dilution. SW-846 Method 3580A. Revision 1. July 1992.

**14.8** US Environmental Protection Agency. Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Ambient Air Using Gas Chromatography/Mass Spectrometry (GC/MS). Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air. Compendium Method TO-13A. January 1999.

**14.9** Battelle SVOC Study Data Reports (Reports available from EPA-NERL, contact Brian Schumacher: schumacher.brian@epa.gov ).

**14.10** Florida Department of Environmental Protection Emergency Action Plan - Bob Martinez Center (BMC), August 2021.

**14.11** Florida Department of Environmental Protection, *Standard Operating Procedure for Receipt and Log-in of Chemical Agent Samples and Standards* (SOP **ERLN-005**).

**14.12** Florida Department of Environmental Protection, Standard Operating Procedure **ERLN-001** *Laboratory Accountability, Storage, and Hazard Communication for Chemical Agent Dilute Solution Laboratories*.

**14.13** Florida Department of Environmental Protection Comprehensive Quality Assurance Plan, Chemistry Section, Bureau of Labs, 2017.

**14.14** Florida Department of Environmental Protection, Standard Operating Procedure **LB-029** *Standard Operating Procedure for Handling Perceived Threat Materials.*

**14.15** Florida Department of Environmental Protection, Standard Operating Procedure **LB-022** *Receiving Packages and Freight at the Loading Dock.*

**14.16** Florida Department of Environmental Protection, Standard Operating Procedure **LB-016** *Laboratory Support Section Receiving Room: Standard Operating Procedure for the Receipt and Log-in of Samples by the DEP Central laboratory.*

**14.17** Florida Department of Environmental Protection, Standard Operating Procedure **LB-023** *Standard Operating Procedure for Maintaining the Custody and Storage of Environmental*


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

*Samples in the DEP Central Laboratory.*

**14.18** Florida Department of Environmental Protection, Standard Operating Procedure **LB-002** *Non-Conformance Reporting System*.

**14.19** Florida Department of Environmental Protection, Standard Operating Procedure **LB-006** *Standard Operating Procedure for Records Maintenance and Storage.*

**14.20** Florida Department of Environmental Protection, *Chemical Agent Laboratory Training Module, May 2010.*

**14.21** Florida Department of Environmental Protection, Standard Operating Procedure **LB-027** *SOP for Reporting Qualified Data.*

REMAINDER OF PAGE INTENTIONALLY LEFT BLANK


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

## 15 TABLES

**Table 1**: **MDLs/PQLs, Spike Recovery Acceptance Range and Precision in Water and Sediment for CWAs.**

| Analyte | MDL (µg/L) | PQL (µg/L) | MDL (µg/kg) | PQL (µg/kg) | Accuracy Range Water (%) | Accuracy Range Soil (%) | Prcsn (% RPD) |
|---|---|---|---|---|---|---|---|
| Tabun(GA) | | | | | | | |
| Sarin (GB) | 2.86 | 11.44 | 5.0 | 20 | | | |
| Soman(total,GD1,GD2) | 2.86 | 11.44 | 5.0 | 20 | | | |
| Cyclohexyl sarin (GF) | 2.86 | 11.44 | 5.0 | 20 | | | |
| Mustard, sulfur (HD) | 2.86 | 11.44 | 5.0 | 20 | | | |
| VX | 2.86 | 11.44 | 5.0 | 20 | | | |

**Table 2: MDLs/PQLs, Spike Recovery Acceptance Range and Precision in Wipes and Air for CWAs.**

| Analyte | MDL (µg) | PQL (µg) | MDL (µg/) | PQL (µg/) | Accuracy Range Wipes (%) | Accuracy Range air (%) | Prcsn (% RPD) |
|---|---|---|---|---|---|---|---|
| Tabun(GA) | | | | | | | |
| Sarin (GB) | 0.050 | 0.20 | | | | | |
| Soman(total,GD1,GD2) | 0.050 | 0.20 | | | | | |
| Cyclohexyl sarin (GF) | 0.050 | 0.20 | | | | | |
| Mustard, sulfur (HD) | 0.050 | 0.20 | | | | | |
| VX | 0.050 | 0.20 | | | | | |

**Table 3. Surrogate Recovery Limits**

| Surrogate | Recovery range (%) |
|---|---|
| Nitrobenzene-d5 | 35 - 115 |
| Terphenyl-d14 | 33 - 140 |
| Tripheny phosphate | 30 - 150 |
| Phencyclidine-d5 | 30 - 150 |


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

**Table 4: Decafluorotriphenylphosphine (DFTPP) Key Ions and Ion Abundance Criteria.**

| Mass | Ion Abundance Criteria |
|---|---|
| 51 | 10-80% of mass 198 |
| 68 | less than 2% of mass 69 |
| 69 | Present |
| 70 | less than 2% of mass 69 |
| 127 | 10-80% of mass 198 |
| 197 | less than 2% of mass 198 |
| 198 | Base peak, or >50% of Mass 442 |
| 199 | 5-9% of mass 198 |
| 275 | 10-60% of mass 198 |
| 365 | Greater than 1% of mass 198 |
| 441 | Present but <24% of mass 442 |
| 442 | Base Peak, or greater than 50% of mass 198 |
| 443 | 15-24% of mass 442 |
| DDT | DDT breakdown should be less than 20% |
| TF | Tailing Factor (TF) for Benzidine (<3) and Pentachlorophenol (<5) |

**Table 5. Retention Times and Characteristic Ions for Target Compounds, Surrogates, and Internal Standards.**

| Analyte | Retention Times | Minimum RRF | Primary ions | Secondary Ions |
|---|---|---|---|---|
| Tabun (GA) | | | 70 | 162, 106, 133 |
| Sarin (GB) | **2.771** | | 99 | 125, 81 |
| Soman(total,GD1,GD2) | **4.408** | | 99 | 126, 69, 82 |
| Cyclohexyl sarin (GF) | **5.554** | | 99 | 81, 67 |
| Mustard, sulfur (HD) | **5.536** | | 109 | 158, 160, 63, 111 |
| VX | **8.366** | | 114 | 127, 79, 72 |
| Nitrobenzene-d5 **(S)** | **4.784** | | 82 | 128, 54, 98 |
| | | | | |
| Terphenyl-d14 **(S)** | **10.547** | | 244 | 122, 212 |
| Tripheny phosphate **(S)** | **11.328** | | 326 | 325, 215, 77, 233 |
| Phencyclidine-d5 **(S)** | **9.397** | | 205 | 246, 248, 190, 96 |
| 1,4-Dichlorobenzene-d14 **(IS)** | **4.125** | | 152 | 150, 115, 78, 52 |
| Naphthalene-d8 **(IS)** | **5.453** | | 136 | 68, 108 |
| Phenanthrene-d10 **(IS)** | **8.824** | | 188 | 94, 80 |
| Acenaphthene-d10 **(IS)** | | | 164 | 160, 162 |
| Chrysene-d12 **(IS)** | | | 240 | 120, 236 |
| Perylene-d12 **(IS)** | | | 264 | 260, 265, 132 |


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

**Table 6. Working Calibration Standard Concentration (µg/mL) for Full Scan**

| Analyte | CAS RN | Cal. 1 | Cal. 2 | Cal. 3 | Cal. 4 | Cal.5 | Cal. 6 | Cal. 7 |
|---|---|---|---|---|---|---|---|---|
| Tabun (GA) | | | | | | | | |
| Sarin (GB) | **107-44-8** | 0.050 | 0.10 | 0.20 | 0.30 | 0.40 | 0.80 | 1.0 |
| Soman(GD total, GD1, GD2) | | 0.050 | 0.10 | 0.20 | 0.30 | 0.40 | 0.80 | 1.0 |
| Cyclohexyl sarin (GF) | | 0.050 | 0.10 | 0.20 | 0.30 | 0.40 | 0.80 | 1.0 |
| Mustard, sulfur (HD) | | 0.050 | 0.10 | 0.20 | 0.30 | 0.40 | 0.80 | 1.0 |
| VX | **50782-69-9** | 0.05 | 0.10 | 0.20 | 0.30 | 0.40 | 0.80 | 1.0 |
| Nitrobenzene-d5 **(S)** | | 0.20 | 0.10 | 0.20 | 0.30 | 0.40 | 0.80 | 1.0 |
| p-Terphenyl-d14 **(S)** | | 0.20 | 0.10 | 0.20 | 0.30 | 0.40 | 0.80 | 1.0 |
| Triphenyl phosphate **(S)** | | 0.20 | 0.10 | 0.20 | 0.30 | 0.40 | 0.80 | 1.0 |
| Phencyclidine-d5 **(S)** | | 0.20 | 0.10 | 0.20 | 0.30 | 0.40 | 0.80 | 1.0 |
| 1,4-Dichlorobenzene-d4 **(IS)** | | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Naphthalene-d8 **(IS)** | | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Acenaphthene-d10 **(IS)** | | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Phenanthrene-d10 **(IS)** | | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Chrysene-d12 **(IS)** | | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Perylene-d12 **(IS)** | | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |

**Table 7. Calibration Standard Concentration (µg/mL) in SIM mode**

| Analyte | CAS RN | Cal. 1 | Cal. 2 | Cal. 3 | Cal. 4 | Cal. 5 | Cal. 6 | Cal.7 |
|---|---|---|---|---|---|---|---|---|
| Tabun (GA) | | | | | | | | |
| Sarin (GB) | | 0.010 | 0.050 | 0.080 | 0.10 | 0.25 | 0.50 | 1.0 |
| Soman(GD total, GD1, GD2) | | 0.010 | 0.050 | 0.080 | 0.10 | 0.25 | 0.50 | 1.0 |
| Cyclohexyl sarin (GF) | | 0.010 | 0.050 | 0.080 | 0.10 | 0.25 | 0.50 | 1.0 |
| Mustard, sulfur (HD) | | 0.0040 | 0.020 | 0.030 | 0.040 | 0.10 | 0.20 | 1.0 |
| VX | | 0.01 | 0.050 | 0.080 | 0.10 | 0.25 | 0.50 | 1.0 |
| Nitrobenzene-d5 **(S)** | | 0.010 | 0.050 | 0.080 | 0.10 | 0.25 | 0.50 | 1.0 |
| | | | | | | | | |
| p-Terphenyl-d14 **(S)** | | 0.010 | 0.050 | 0.080 | 0.10 | 0.25 | 0.50 | 1.0 |
| Triphenyl phosphate **(S)** | | 0.010 | 0.050 | 0.080 | 0.10 | 0.25 | 0.50 | 1.0 |
| Phencyclidine-d5 **(S)** | | 0.010 | 0.050 | 0.080 | 0.10 | 0.25 | 0.50 | 1.0 |
| 1,4-Dichlorobenzene-d4 **(IS)** | | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Naphthalene-d8 **(IS)** | | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Acenaphthene-d10 **(IS)** | | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Phenanthrene-d10 **(IS)** | | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Chrysene-d12 **(IS)** | | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Perylene-d12 **(IS)** | | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |


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

**Table 8.** Dilution scheme used to prepare calibration solution for **Full Scan,** GC/MS**.** Note: Prior to sample analysis, 10 µL of internal standard solution (50 µg/mL) was added to a 1 mL aliquot of calibration standard, resulting in a concentration of 0.5 µg/mL for each internal standard. **(I'm not sure that these schemes are correct… the levels aren't right or it isn't clear and I'm not reading it right.)**

| Calibration Level | Stock Standard | Stock Conc. (µg/L) | Amount (µL) | Final Conc. (µg/L) | Final Vol. (mL) |
|---|---|---|---|---|---|
| **7** | Sarin (GB) | 10 | 500 | 1.0 | **5.0** |
| | Soman (GD) | 5.0 | 250 | 0.50 | |
| | Cyclohexyl sarin (GF) | 10 | 500 | 1.0 | |
| | Mustard, sulfur (HD) | 5.0 | 250 | 0.50 | |
| | VX | 10 | 500 | 1.0 | |
| | Nitrobenzene-d5<br>p-Terphenyl-d14<br>Triphenyl phosphate<br>Phencyclidine-d5 | 100 | 50 | 1.0 | |
| **6** | Level 7 | See Above | 800 | Level 6 | **1.0** |
| **5** | Level 7 | See Above | 400 | Level 5 | **1.0** |
| **4** | Level 7 | See Above | 300 | Level 4 | **1.0** |
| **3** | Level 7 | See Above | 200 | Level 3 | **1.0** |
| **2** | Level 7 | See Above | 100 | Level 2 | **1.0** |
| **1** | Level 7 | See Above | 50 | Level 1 | **1.0** |

**Table 9.** Dilution scheme used to prepare calibration solution for **SIM,** GC/MS**.** Note: Prior to sample analysis, 10 µL of internal standard solution (50 µg/mL) was added to a 1 mL aliquot of calibration standard, resulting in a con centration of 0.5 µg/mL for each internal standard.

| Calibration Level | Stock Standard | Stock Conc. (µg/L) | Amount (µL) | Final Conc. (µg/L) | Final Vol. (mL) |
|---|---|---|---|---|---|
| **7** | Sarin (GB) | 10 | 500 | 1.0 | **5.0** |
| | Soman (GD) | 5.0 | 250 | 1.0 | |
| | Cyclohexyl sarin (GF) | 10 | 500 | 1.0 | |
| | Mustard, sulfur (HD) | 5.0 | 250 | 0.5.0 | |
| | VX | 10 | 500 | 1.0 | |
| | Nitrobenzene-d5<br>p-Terphenyl-d14<br>Triphenyl phosphate<br>Phencyclidine-d5 | 100 | 50 | 1.0 | |
| **6** | Level 7 | See Above | 500 | Level 6 | **1.0** |
| **5** | Level 7 | See Above | 250 | Level 5 | **1.0** |
| **4** | Level 7 | See Above | 100 | Level 4 | **1.0** |
| **3** | Level 7 | See Above | 80 | Level 3 | **1.0** |
| **2** | Level 7 | See Above | 50 | Level 2 | **1.0** |
| **1** | Level 7 | See Above | 10 | Level 1 | **1.0** |


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

**Table 10. Internal Standards and Surrogates for use with each CWA**

| CWA | Surrogate Compounds | Internal Standard |
|---|---|---|
| Tabun (GA) | Tripheny phosphate, p-Terphenyl-d14 | Phenanthrene-d10 |
| Sarin (GB) | Nitrobenzene-d5 | 1,4-Dichlorobenzene-d4 |
| Soman (GD1 and GD2) | Nitrobenzene-d5 | 1,4-Dichlorobenzene-d4 |
| Cyclohexyl sarin (GF) | Tripheny phosphate, p-Terphenyl-d14 | Naphthalene-d8 |
| Mustard, sulfur (HD) | Tripheny phosphate, p-Terphenyl-d14 | Naphthalene-d8 |
| VX | Phencyclidine-d5 | Phenanthrene-d10 |

# 15 APPENDICES

## APPENDIX A - Daily Checklist

The following checks and functions are performed daily prior to beginning work with a dilute agent solution:

- **PPE:** Wear dilute agent work ensemble as specified in the Technical SOP and have approved respiratory protection at the work station.
- **Decontaminating solutions (personal and agent):** Verify that the stock is sufficient, appropriate for the agent in use, and that it has not expired.
- **SOP:** Confirm that a current copy of the SOP associated with the current activity is present at the work station and that all operator(s) performing the work have signed the signature page.
- **Ventilation certifications:** Confirm that the certifications are current and systems are working properly. Recertification is required if changes to hood footprint, ducting, or blower/motor maintenance has occurred.
- **Emergency equipment:** Verify the location, serviceability, and a clear path to fire extinguishers, shower, eyewash stations, and all other emergency and safety equipment.
- **Laboratory fume hoods:** Perform the daily and working checks to ensure proper operation of the fume hoods. These checks include:
  - Record the magnehelic reading on the Laboratory Fume Hood Performance chart, ensuring it is within limits. If not, notify the laboratory manager and follow instructions on the chart.
  - Measure the airflow using a hanging vane anemometer.
  - Test the audible and visual alarms (at least once a week).

## APPENDIX B - Glassware Cleaning Procedure

After glass items have been properly decontaminated (24-hour immersion in decontamination solution) and removed from the hood, they must be thoroughly washed and solvent-rinsed using the following procedure:


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

- Rinse all glassware with ~ 5% HCl solution to ensure all decontaminant is removed. Capture all rinsate into the HCl waste jug for future disposal.
- Tap water rinse.
- Wash glassware in warm water and microsoap. Use the scrub brushes to clean the insides of beakers, etc. For volumetric glassware too small for a brush, fill it with soapy water, then invert and shake vigorously. Repeat this step several times.
- Rinse glassware with tap water until all visible soap suds are gone (at least three times).
- Rinse all glassware in deionized (DI) water three times or more to ensure all residues are removed. Allow glassware to air dry and then baked in high temperature oven for 4 hours.
- Return all equipment to the proper storage space once it is dry.
- Prior to use, always solvent-rinse glassware with the solvent of choice for each project.

## APPENDIX C – Decontamination Procedure

- **Decontamination Solutions:** A variety of oxidizing or caustic solutions are used for decontamination solutions. For each type of decontamination solution, ratios have been established so that it is possible to calculate the amount of decontamination required for a specific amount of agent. These ratios are given in Table below. It is important to note that these ratios were defined with respect to bulk agent decontamination and apply only peripherally to other decontamination tasks, especially where non-aqueous solvents are involved. In this case, rigid interpretation of these guidelines could result in dangerous situations. In any non-routine or uncertain application, consult with the Facility Manager and the CAHO before proceeding. The units of these entries are grams of decontaminant per gram of neat agent. Shaded entries indicate the preferred decontamination.

**Filter Use Record**

Filter installed by: _____

Project Number: _____

Installation date: _____

Date of first agent exposure _____

Note: Recommended due dates for media change:
12 months for maximum agent loading volumes below 1 mL
6 Months for maximum agent loading volumes 1 mL to 10 mL
1 month for maximum agent loading volumes > 10 mL

**Figure 1. Example Filter Use Record**


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

**Table 11: Ratios (w/w) of Decontamination to Agent**

| Decontamination solution | Preparation | Amount of decontamination solution (g) for 1 g of agent | | | |
|---|---|---|---|---|---|
| | | GB | GD, GF | HD | L |
| 5.25% Bleach | Purchase | 65 | 55 | **65** | |
| 10% NaOH | 378 g/gal $H_2O$ | **65** | **55** | | |
| 10% HTH | 378 g/gal $H_2O$ | | | 65 | |
| 10% alcoholic NaOH | 100 g EtOH + 900 g 10% NaOH | | | | **200** |
| 10% $Na_2CO_3$ | 100 g/L | 65 | 55 | | |

- **Personal Decontamination:** Personnel exposed to chemical agent vapor should be decontaminated by removing all clothing in a clean air environment, and shampooing or rinsing the hair to prevent vapor off-gassing. Personnel exposed to chemical agent liquid should be decontaminated by washing in warm or hot water at least three times. Use liquid soap, copious amounts of water, and mild to moderate friction with a single-use sponge or wash cloth in the first and second washes. Always wash from areas of lesser contamination to areas of greater contamination. The third wash should be a rinse with copious amounts of warm or hot water. Shampoo can be used to wash the hair. The rapid physical removal of a chemical agent is essential. If warm or hot water is not available, but cold water is, use cold water. Do not delay decontamination to obtain warm water. Rinse the eyes, mucous membranes, or open wounds with copious amounts of sterile saline or water.
- **Checking Expired Decontamination Solutions:** For non-chlorinated decontamination solutions, check the pH by using a hand-held pH meter (this needs to be first checked with a buffer solution) or pH indicating paper. If pH is greater than 10, the decontamination solution is within acceptable ranges and expiration date is changed to 6 months from test date. If solution is outside acceptable ranges, the decontamination solution is disposed of as hazardous waste.

  For the decontamination solutions 10% HTH, test pH as above in 1, and test for active chlorine by taking a drop of the solution and putting it on a piece of starch/iodide test paper. If a blue or black color appears, the solution is acceptable and the expiration date is changed to 6 months from the test date. If no color change is observed, the decontamination solution is disposed of as hazardous waste.

  Bleach is purchased as an unopened, unused 5.25% commercial bleach solution. The shelf life of unopened, unused bleach will be set to one year. After the one-year shelf life of the bleach solution has expired, the bleach solution will be disposed of as hazardous waste, and will be replaced with new bleach solutions.
- **Decontamination of Glassware:** Glassware used with dilute solution will be rinsed with large quantities of decontamination solution and then removed for washing. The decontamination solutions generated from the cleaning of glassware must be considered contaminated and treated as hazardous waste.
- **Decontamination of Syringes:** Syringes may not be removed from engineering control once contaminated. (They may be moved from laboratory fume hood to laboratory fume hood). Between uses they must be rinsed with solvent 10 times with the rinsate discarded into decontamination.


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

## APPENDIX D – Waste Handling Procedure

This section describes waste handling procedures for agent-contaminated waste and small amounts (<1 mL) of dilute agent solution. These waste-handling operations use reactive and toxic materials routinely used in decontamination solutions. Because the risks for violent reactions are real, these operations must be performed with extreme caution.

- Perform the normal preoperational check. Don gloves as per the glove policy.
- Label waste container with either tape or permanent marker indicating: Date, decontamination solution used, provide ID number, solvent (or residue) and other chemical expected to be greater than 1% of the waste when process is completed. Also include concentration of all regulated chemicals.
- If special steps are required or if larger quantities are discarded into a jug, affix a temporary tape indicating specific agents. (Remove the temporary label after decontamination steps are completed).
- **Fill the Container with Appropriate Decontamination Solution**. When filling the container, mix contents with a stir rod or large forceps at regular intervals. Exothermic and gas-releasing reactions are common especially with organic wastes. For this reason, the total organic waste contents should not be greater than 33% of the container capacity. If HTH is used as the decontamination solution, do not fill beyond 66% total capacity to allow for exothermic reaction and additional of ethanol to eliminate phasing. If any reaction is observed or expected, proceed with extreme caution. Do not cap container until the reaction ceases and the contents have cooled. After the reaction ceases, ensure HTH covers all material, but do not fill jug beyond shoulder.

When the container is full, record the expiration time, and date (24 hours after container is filled to allowable capacity) on the container. Leave some headspace in all waste containers.

- **Solid waste:** Treat waste containers holding both solid and liquid waste as solid waste. Perform mixing or stirring to ensure that all potentially exposed surfaces come in contact with decontamination solution. Prior to disposal, decontaminate these items with the proper decontamination solution depending upon the agent(s) to which they were exposed as indicated in the table below. The minimum reaction time from mixing with decontamination solution to disposal is also indicated.

**Decontamination Solutions**

| Chemical Agent | Decontamination Solution | Comments | Minimum Reaction Time |
|---|---|---|---|
| GB, GD, GF | 10% sodium hydroxide, or 10% potassium hydroxide | - | 3 hours |
| VX | 10% calcium hypochlorite (HTH) | - | 3 hours |
| HD | 5% sodium hypochlorite (bleach) or 10% HTH or monoethylamine (MEA) or conc. Nitric acid | MEA and conc. Nitric acid react vigorously. | 24 hours |


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

A check by analytical methods for residual intact agents after laboratory scale decontamination is not necessary if the agent is known to be in solution, appropriate decontaminants are used in calculated excess amounts, the time allowed for reaction is adequate, and no interferences are present.

For waste containers from RDTE dilute operations, measure the pH of the waste container using wide-range pH paper and record the pH on the lid of the container. Replace the lid and double bag the waste jug. Complete waste form and place inside the outer bag. Transfer processed waste jug to the waste cart. Call the Hazardous Waste Broker and follow directions to arrange for a waste pickup.

- **Final Waste Processing**: Before agent related waste can be removed for final disposition, measure the pH and record on the waste card. Additionally, some wastes must be tested for active chlorine. The following subsections describe these procedures.
- Place waste containers into the waste processing hoods and remove outer bags. Remove cap from the waste container.
- Ensure waste is not multi-phasic. If two or more phases are observed, add ethanol to container and stir for 1 hour to promote phase mixing. The solution must stand 24 hours before processing. If phases are still present, the solution must be stirred or rolled for 24 hours to allow decontamination solution to contact agents.
- Measure the pH of the waste container using wide range pH paper and record the pH on the lid of the container.
- Cap the waste container and close the inner plastic bag.
- Fill out manifest, recording pH in appropriate space on the form.
- Insert manifest between the inner and outer plastic bag.
- Close outer plastic bag and place container on waste cart to await transport to the hazardous staging area. Call the Hazardous Waste broker, (phone #), to arrange for pickup of the waste.
- **Specialized Waste Variations:** Process mixed agents and aqueous immiscible materials by the following steps:
- If decontaminating a G-agent solution, which is not miscible with water, use a 10% alcoholic sodium hydroxide solution.
- If decontaminating a VX or H-agent solution, which is not miscible with water, use 10% (wt) HTH mixture. If multiple phases are observed, add denatured ethanol equal to a 10% weight of the agent/decontamination mixture and stir 1 hour. Repeat as necessary until the mixture is homogeneous.
- Mixtures of G and V agents in a solution require the use of the VX procedure (10% HTH solution) in the ratios specified for the VX (e.g., for 1 g of VX and 1 g of GB, 160 g of the 10% HTH slurry would be used).
- If any of the items in an operation are contaminated with both HD and GD, a 5.25% sodium hypochlorite solution (bleach) is used to decontaminate the waste and the ratios of decontamination to agent and procedures for HD decontamination will be used as per solid waste section table.


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

## APPENDIX E – Hazardous Waste Container

Hazardous waste containers will be located in each laboratory fume hood with the appropriate decontamination solution and other solvents as required by the activity. Close bottles where open decontamination fumes will adversely affect results. Both solid and liquid waste containers will be one-third filled with decontamination solution before starting an activity. Additional decontamination solution may be added as required during operations. No waste container may be filled beyond the "shoulder" of the container.

- **Solid Waste Containers**: A 1-gallon plastic wide mouth "pickle" jar is preferred. A narrow plastic 2-quart container is occasionally used when the space available is limited.
- **Liquid Waste Container**: For larger volumes, a 1-gallon plastic wide mouth jar or a 1-gallon plastic jug fitted with a funnel is used. For smaller volumes, 8- to 32- ounce jars may be used.

## APPENDIX F – Transfers Into and Out of Agent Hoods

Since all of the operations with and storage of chemical agent dilute solutions take place in chemical agent laboratory fume hoods, this section is of particular importance.

- **Guidelines:** All dilute agent solutions must be double contained except when under engineering control. Both the primary (inner) and secondary containers must have the capacity to contain the sample (liquid and vapor) completely. The secondary container must be shatterproof.
- Potentially contaminated items (tools, clamps, supports, etc.) that are frequently used in different laboratory fume hoods may be transferred from one laboratory fume hood to another in a plastic container or double plastic bags individually closed.

- **Definition of Contamination:** Tools, supplies, and equipment meeting any one of the following conditions will be considered contaminated and require decontamination or disposal.
  - **6.2** Contacted dilute agent solution
  - **6.3** In a chemical agent vapor environment
  - **6.4** In a continuously controlled environment and exposed to above the Immediately Dangerous to Life and Health (IDLH) for any period of time
  - **6.5** In an uncontrolled environment where monitoring has indicated the environment exceeded the Short-Term Exposure Limit (STEL) concentration
  - **6.6** In an uncontrolled environment involving the storage, use, or presence of dilute agent solution and where physical factors (e.g., discoloration, stains, etc.) indicate possible exposure to chemical agent

Items placed into a dilute solution hood beyond the plane of the sash will be assumed to be conditionally clean (3X) if there has been no contact with dilute solution. If there has been no spill of "mother" level solutions in the hood, these items may be removed from the hood without being monitored, but must be treated as Conditionally Clean. Those items that are known or suspected to have come in contact with dilute solutions must remain in the hood until they are decontaminated and monitored to obtain Conditionally Clean (3X) status.


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

- **Vapor Containment and Surface Decontamination:** For items that will not be damaged by agent decontamination solutions: With the item remaining in ventilation control, wipe the exterior surfaces of the item with the appropriate decontaminant solution, discarding all wipes used to apply/remove the decontamination to the waste jug.
- For items that will be damaged by decontamination solutions:
  With the item remaining in ventilation control, wipe exterior surfaces of the item with isopropyl alcohol or appropriate solvent, then wipe dry. Repeat the solvent wipe and dry. Discard all wipes used to apply and remove the solvent to the waste jug.
- **Double bagging:** With the item remaining in ventilation control, completely wrap the item with the approved polyethylene. Seal all seams and openings with duct tape. Add a second layer of polyethylene, again sealing all seams and openings with duct tape.
- **Moving the Items:** Set a timer for 4 hours. This is the maximum time allowed for vapor containment using the approved polyethylene if the item is to be outside of ventilation control.
- Transfer the bagged item to a temporary location within the CA facility.
- At the end of the 4 hours (or before), return the bagged item to ventilation control, or add a second pair of bags following Step 2. If the item is over-bagged before the end of the 4 hours, the re-bagging can be performed outside ventilation control.

## 17. SOP Update Revision Summary

The following revisions were made to this SOP from the prior version:

- Incremented revision number and date.
- Typo corrections.
- Removed reference to use of a TOF instrument, which the lab no longer owns.
- Update references.


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