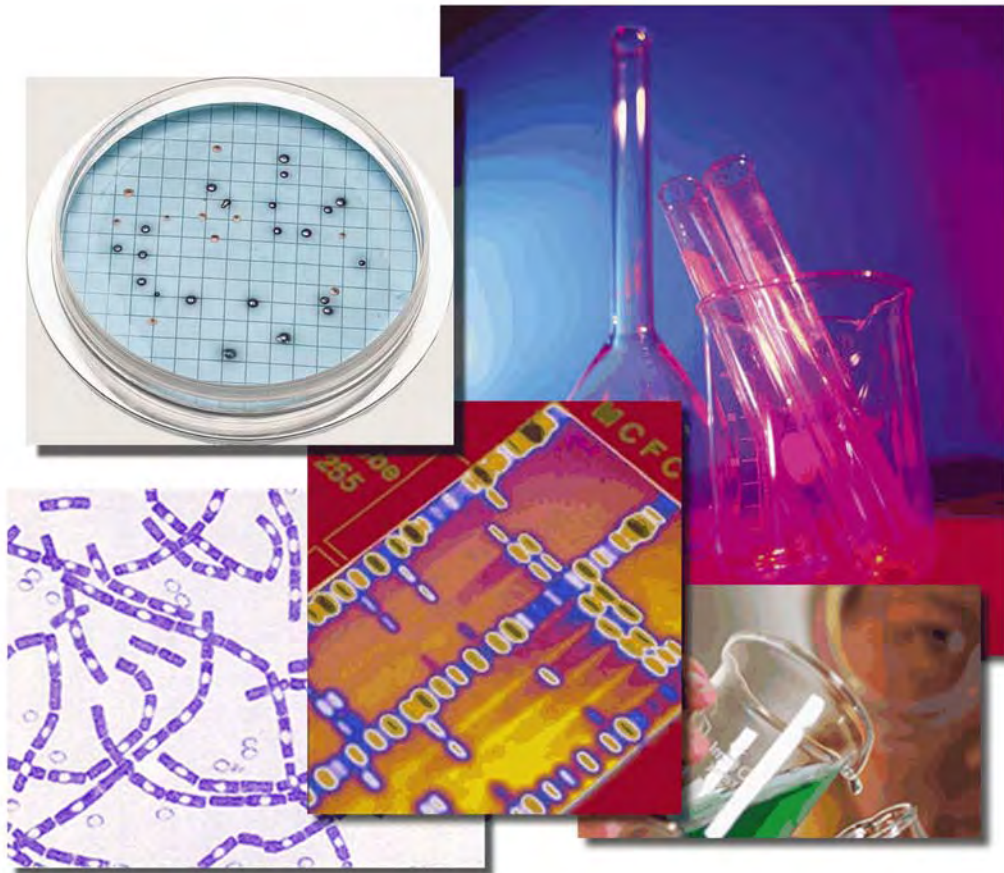




Standardized Analytical Methods for Use During Homeland Security Events

Revision 1.0

September 29, 2004



Standardized Analytical Methods for Use During Homeland Security Events

Revision 1.0

Prepared by

DynCorp Systems & Solutions LLC (A CSC Company)
Chantilly, VA 20151

EPA Contract No. 68-W-01-034

Work Assignment Manager

Oba Vincent
National Homeland Security Research Center
Office of Research and Development
Cincinnati, OH 45268

National Homeland Security Research Center
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Disclaimer

The U.S. Environmental Protection Agency through its Office of Research and Development funded and managed the research described here under Contract 68-W-01-034 to DynCorp Systems & Solutions LLC (A CSC Company). It has been subjected to the Agency's peer and administrative review and has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Questions concerning this document or its application should be addressed to:

Oba Vincent
National Homeland Security Research Center
Office of Research and Development (163)
26 West Martin Luther King Drive
Cincinnati, OH 45268
(513) 569-7456
vincent.oba@epa.gov

Foreword

The U.S. Environmental Protection Agency is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, EPA's research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The National Homeland Security Research Center (NHSRC) is the Agency's center for conducting research to facilitate protection and decontamination of structures and water infrastructure subject to chemical or biological terror attacks. NHSRC's research is designed to provide appropriate, affordable, effective, and validated technologies, methods, and guidance to understand the risk posed by potential chemical and biological terror attacks on buildings and water infrastructure, and to enhance our ability to detect, contain, and clean up in the event of such attacks. NHSRC will also provide direct technical assistance to response personnel in the event of future deliberate physical and radiological, chemical/biological attacks on buildings or water infrastructure and provide interagency liaison for EPA in Homeland Security research and technology.

This publication has been produced as part of the Center's long-term research plan. It is published and made available by EPA's Office of Research and Development to assist the user community and to link researchers with their clients.



E. Timothy Oppelt, Director
National Homeland Security Research Center

Acknowledgments

The contributions of the following persons and organizations to the development of this document are gratefully acknowledged:

EPA Homeland Security Laboratory Capability Workgroup Analytical Methods Subteam

Steve Allgeier, Office of Water

David Friedman, Office of Research and Development

Ben Hull, Office of Indoor Air and Radiation

Michael S. Johnson, Office of Solid Waste and Emergency Response

Alan Lindquist, Office of Research and Development

Oba Vincent, Office of Research and Development

Environmental Protection Agency

Linda Anderson-Carnahan, Region 10 - Manchester Laboratory

Don Betowski, National Exposure Research Laboratory

Isa Chamberlain, Region 10 - Manchester Laboratory

Joe Dorsey, Region 3 - Environmental Sciences Center

Diane Gregg, Region 6 Laboratory - Houston Branch

Ann C. Grimm, Office of Research and Development

Stephanie Harris, Region 10 - Manchester Laboratory

Jafrul Hasan, Health and Ecological Criteria Division

Bonita Johnson, Region 4 - Science and Ecosystems Support Division

Tammy Jones-Lepp, National Exposure Research Laboratory

Irwin Katz, Region 2

Peggy Knight, Region 10 - Manchester Laboratory

Barry Lesnik, Office of Solid Waste

John Nebelsick, Office of Solid Waste and Emergency Response

Ed O'Neill, Region 6 Laboratory - Houston Branch

Steve Reimer, Region 10 - Manchester Laboratory

Lavon Revells, Region 4 - Science and Ecosystems Support Division

Eugene Rice, Office of Research and Development

Fred Siegelman, Office of Pesticides Programs - Biological and Economic Analysis Division

Terry Smith, Office of Solid Waste and Emergency Response

Dave Stockton, Region 6 Laboratory - Houston Branch

Sue Warner, Region 3 - Environmental Sciences Center

Laura Webb, Region 7 - ENSVRLAB

Wayne Whipple, Region 5

Sen-yi Yang, Office of Solid Waste and Emergency Response

United States Geological Survey

Merle Shockey

Centers for Disease Control and Prevention

Matthew J. Arduino, Division of Health Quality Promotion

Jay E. Gee, Division of Bacterial and Mycotic Diseases

Dennis Juranek, Division of Parasitic Diseases

Laura J. Rose, Division of Health Quality Promotion

Department of Homeland Security

Lance Brooks

Federal Bureau of Investigation

Ben Garrett, Hazard Materials Response Unit

Paul Keller, Hazard Materials Response Unit

Jarrad Wagner, Hazard Materials Response Unit

Food and Drug Administration

David L. Craft, Molecular Biology and Microbiology

United States Army Edgewood Chemical Biological Center

Dennis Reutter, Edgewood Chemical Biological Center

John D. Wright, Dugway Proving Ground, Life Sciences Division

United States Department of Agriculture

Ronald Fayer

Page Intentionally Blank

Standardized Analytical Methods for Use During Homeland Security Events Revision 1.0

September 2004

Contents

Disclaimer	ii
Foreword	iii
Acknowledgments	iv
Section 1.0: Introduction	1
Section 2.0: Scope and Application	5
Section 3.0: Points of Contact	7
Section 4.0: Chemical Methods	9
4.1 General Guidance	9
4.1.1 Standard Operating Procedures for Identifying Chemical Methods	9
4.1.2 General Quality Control (QC) Guidance for Chemical Methods	10
4.1.3 Safety and Waste Management	11
4.2 Method Summaries	12
4.2.1 Laboratory Response Network (LRN)	12
4.2.2 CLP Method SOW ILM05.3 Cyanide: Analytical Methods for Total Cyanide Analysis	13
4.2.3 NERL Method 365.1, Revision 2: Determination of Phosphorus by Semi- Automated Colorimetry	13
4.2.4 EPA Method 200.2, Revision 2.8: Sample Preparation Procedure for Spectrochemical Determination of Total Recoverable Elements	14
4.2.5 EPA Method 300.1: Determination of Inorganic Anions in Drinking Water by Ion Chromatography	14
4.2.6 EPA Method 549.2: Determination of Diquat and Paraquat in Drinking Water by Liquid-Solid Extraction and High-Performance Liquid Chromatography with Ultraviolet Detection	15
4.2.7 EPA Method 3031 (SW-846): Acid Digestion of Oils for Metals Analysis by Atomic Absorption or ICP Spectrometry	15
4.2.8 EPA Method 3050B (SW-846): Acid Digestion of Sediments, Sludges, and Soils	15
4.2.9 EPA Method 3520C (SW-846): Continuous Liquid-Liquid Extraction	16
4.2.10 EPA Method 3535A (SW-846): Solid-Phase Extraction	18
4.2.11 EPA Method 3541 (SW-846): Automated Soxhlet Extraction	19
4.2.12 EPA Method 3545A (SW-846): Pressurized Fluid Extraction (PFE)	21
4.2.13 EPA Method 3580A (SW-846): Waste Dilution	23

4.2.14	EPA Method 3585 (SW-846): Waste Dilution for Volatile Organics	24
4.2.15	EPA Method 5030C (SW-846): Purge-and-Trap for Aqueous Samples	25
4.2.16	EPA Method 5035A (SW-846): Closed-System Purge-and-Trap and Extraction for Volatile Organics in Soil and Waste Samples	25
4.2.17	EPA Method 5050 (SW-846): Bomb Preparation Method for Solid Waste	26
4.2.18	EPA Method 6010C (SW-846): Inductively Coupled Plasma - Atomic Emission Spectrometry	26
4.2.19	EPA Method 6020A (SW-846): Inductively Coupled Plasma - Mass Spectrometry	27
4.2.20	EPA Method 7010 (SW-846): Graphite Furnace Atomic Absorption Spectrophotometry	27
4.2.21	EPA Method 7470A (SW-846): Mercury in Liquid Wastes (Manual Cold-Vapor Technique)	28
4.2.22	EPA Method 7471B (SW-846): Mercury in Solid or Semisolid Wastes (Manual Cold-Vapor Technique)	28
4.2.23	EPA Method 8015C (SW-846): Nonhalogenated Organics Using GC/FID	28
4.2.24	EPA Method 8260B (SW-846): Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)	29
4.2.25	EPA Method 8270D (SW-846): Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)	30
4.2.26	EPA Method 8315A (SW-846): Determination of Carbonyl Compounds by High Performance Liquid Chromatography (HPLC)	31
4.2.27	EPA Method 8318A (SW-846): <i>N</i> -Methylcarbamates by High Performance Liquid Chromatography (HPLC)	32
4.2.28	EPA Method 8321B (SW-846): Solvent-Extractable Nonvolatile Compounds by High Performance Liquid Chromatography/Thermospray/ Mass Spectrometry (HPLC/TS/MS) or Ultraviolet (UV) Detection	32
4.2.29	AOAC Official Method 994.08: Aflatoxin in Corn, Almonds, Brazil Nuts, Peanuts, and Pistachio Nuts	33
4.2.30	ASTM Method D5755-03: Standard Test Method for Microvacuum Sampling and Indirect Analysis of Dust by Transmission Electron Microscopy (TEM) for Asbestos Structure Number Surface Loading	33
4.2.31	ASTM Method D6480-99: Standard Test Method for Wipe Sampling of Surfaces, Indirect Preparation, and Analysis for Asbestos Structure Number Concentration by Transmission Electron Microscopy	34
4.2.32	ISO Method - 10312: Ambient Air - Determination of Asbestos Fibres - Direct-transfer Transmission Electron Microscopy Method (TEM)	34
4.2.33	NIOSH Method 6001: Arsine	35
4.2.34	NIOSH Method 6002: Phosphine	35
4.2.35	NIOSH Method 6004: Sulfur Dioxide	35
4.2.36	NIOSH Method 6010: Hydrogen Cyanide	36
4.2.37	NIOSH Method 6011: Bromine and Chlorine	36
4.2.38	NIOSH Method 6013: Hydrogen Sulfide	36
4.2.39	NIOSH Method 6015: Ammonia	37
4.2.40	NIOSH Method 6402: Phosphorus Trichloride	37
4.2.41	NIOSH Method 7903: Acids, Inorganic	37
4.2.42	NIOSH Method 7904: Cyanides, Aerosol and Gas	38
4.2.43	NIOSH Method 7906: Fluorides, Aerosol and Gas	38
4.2.44	NIOSH Method S301-1: Fluoroacetate Anion	39

4.2.45	OSHA Method ID-188: Ammonia in Workplace Atmospheres - Solid Sorbent .	39
4.2.46	OSHA Method ID-216SG: Boron Trifluoride (BF ₃)	39
4.2.47	Standard Method 4110 B: Ion Chromatography with Chemical Suppression of Eluent Conductivity	40
4.2.48	Standard Method 4500-NH ₃ B: Preliminary Distillation Step	40
4.2.49	Standard Method 4500-NH ₃ G: Automated Phenate Method	40
4.2.50	Standard Method 4500-Cl G: DPD Colorimetric Method	41
4.2.51	IO Compendium Method IO-3.1: Selection, Preparation, and Extraction of Filter Material	41
4.2.52	IO Compendium Method IO-3.4: Determination of Metals in Ambient Particulate Matter Using Inductively Coupled Plasma (ICP) Spectroscopy	42
4.2.53	IO Compendium Method IO-3.5: Determination of Metals in Ambient Particulate Matter Using Inductively Coupled Plasma/Mass Spectrometry (ICP/MS)	42
4.2.54	IO Compendium Method IO-5: Sampling and Analysis for Vapor and Particle Phase Mercury in Ambient Air Utilizing Cold Vapor Atomic Fluorescence Spectrometry (CVAFS)	43
4.2.55	EPA Air Toxics Method - 6 (TO-6): Method for the Determination of Phosgene in Ambient Air Using High Performance Liquid Chromatography . . .	43
4.2.56	EPA Air Toxics Method - 13A (TO-13A): Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Ambient Air Using Gas Chromatography/ Mass Spectrometry (GC/MS)	44
4.2.57	EPA Air Toxics Method - 15 (TO-15): Determination of Volatile Organic Compounds (VOCs) in Air Collected in Specially-Prepared (Summa) Canisters and Analyzed by Gas Chromatography/ Mass Spectrometry (GC/MS)	45
4.2.58	Journal of Analytical Atomic Spectrometry, 2000, 15, pp. 277-279: Boron Trichloride Analysis	46
4.2.59	Analytical Letters, 1994, 27 (14), pp. 2703-2718: <i>Screening-Procedure for Sodium Fluoracetate (Compound 1080) at Sub-Microgram/Gram Concentrations in Soils</i>	46
Section 5.0: Biological Methods		47
5.1	General Guidance	47
5.1.1	Standard Operating Procedures for Identifying Biological Methods	48
5.1.2	General Quality Control (QC) Guidance for Biological Methods	49
5.1.3	Safety and Waste Management	49
5.2	Method Summaries	50
5.2.1	Laboratory Response Network (LRN)	50
5.2.2	Biosafety Level 4 Viruses	51
5.2.3	U.S. EPA Method 1622: <i>Cryptosporidium</i> in Water by Filtration/IMS/FA	51
5.2.4	ICR Microbial Laboratory Manual Chapter XI	52
5.2.5	USDA/FSIS 4	52
5.2.6	Standard Methods 9260 E: <i>Shigella</i> spp.	53
5.2.7	Standard Methods 9260 F: Pathogenic <i>Escherichia coli</i>	53
5.2.8	Standard Methods 9260 G: <i>Campylobacter jejuni</i>	54
5.2.9	Standard Methods 9260 H: <i>Vibrio cholerae</i>	54

5.2.10	Literature Reference for Caliciviruses	55
5.2.11	Literature Reference for Enteroviruses	55
5.2.12	Literature Reference for Hepatitis A Viruses	56
5.2.13	Literature Reference for Venezeulan Equine Encephalitis (VEE) Virus	57
5.2.14	Literature Reference for <i>Toxoplasma gondii</i>	57
5.2.15	<i>Entamoeba histolytica</i> : Filtration/IMS/PCR	58
Section 6.0: Conclusions		59
Abbreviations and Acronyms		61
Appendices		
A	Chemical Methods	A-1
B	Biological Methods	B-1
Figures		
1-1.	Analytical Response Roadmap for Homeland Security Events	2

Section 1.0: Introduction

In the aftermath of the terrorist attacks of September 11, 2001, and the anthrax attacks in the Fall of 2001, Federal and State personnel successfully carried out their mission to provide response, recovery, and remediation under trying circumstances, including an unprecedented demand on their capabilities to analyze environmental samples. In reviewing these incidents, the Environmental Protection Agency's (EPA) *9/11 Lessons Learned Report* and its *Anthrax Lessons Learned* report identified several areas where the country could better prepare itself in the event of future incidents. One of the most important areas was the need to improve the nation's laboratory capacity and capability to respond to incidents requiring the analysis of large numbers of environmental samples in a short time.

In response, EPA formed the Homeland Security Laboratory Capacity Workgroup to identify and implement opportunities for near-term improvements and to develop recommendations for addressing longer-term, cross-cutting laboratory issues. The EPA Homeland Security Laboratory Capacity Workgroup consists of representatives from the Office of Research and Development, Office of Radiation and Indoor Air, Office of Water, Office of Solid Waste and Emergency Response, Office of Environmental Information, Office of Pesticide Programs, EPA Region 1, EPA Region 2, EPA Region 4, and EPA Region 6.

A critical area identified by the workgroup was the need for a list of pre-selected, pre-evaluated, standardized analytical methods to be used by all laboratories when analyzing homeland security incident samples. Having standardized methods would reduce confusion, permit sharing of sample load between laboratories, improve data comparability, simplify the task of outsourcing analytical support to the commercial laboratory sector, and improve the follow-up activities of validating results, analyzing data and making decisions.

To this end, workgroup members formed an Analytical Methods Subteam to address homeland security methods issues. **Figure 1-1** represents the analytical decision tree for responding to homeland security incidents.

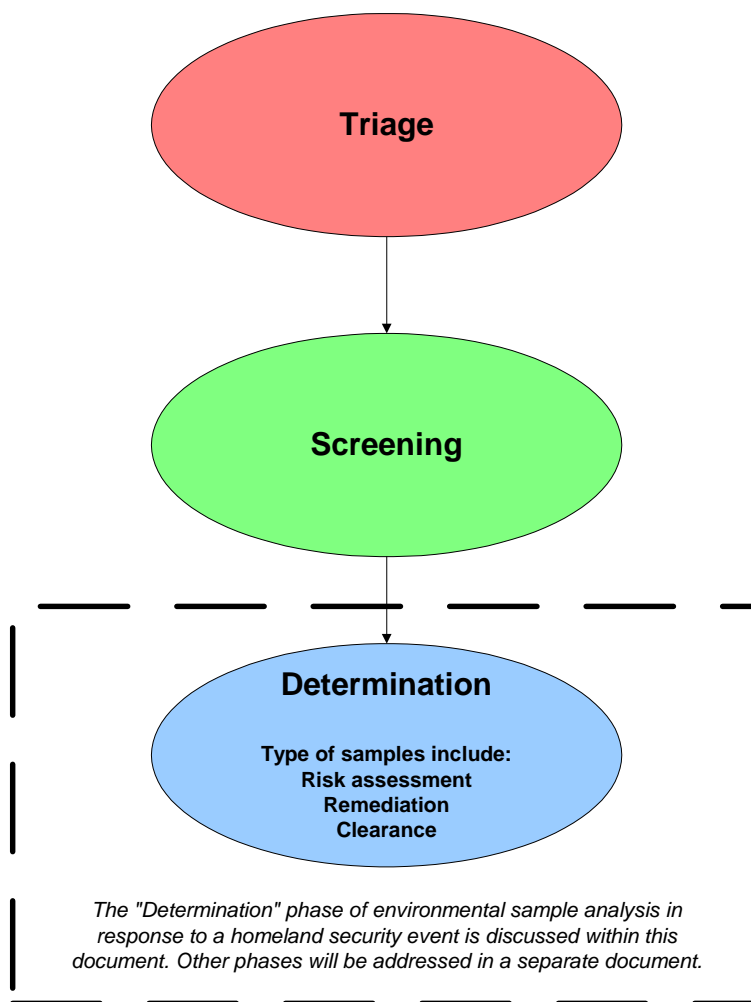


Figure 1-1. Analytical Response Roadmap for Homeland Security Events

The Subteam focused first on methods for use in assessing the extent of contamination and the efficacy of decontamination. A survey of available analytical methods for approximately 120 biological and chemical analytes was conducted using existing method resources including the following:

- National Environmental Methods Index (NEMI)
- Environmental Monitoring Method Index (EMMI)
- EPA Test Methods Index
- EPA Office of Solid Waste SW-846 Methods On-line
- EPA Microbiology Methods
- National Institute for Occupational Safety and Health (NIOSH) method index
- Occupational Safety and Health Administration (OSHA) method index
- AOAC International

- ASTM International
- International Organization for Standardization (ISO) methods
- Standard Methods for the Examination of Water and Wastewater
- PubMed Literature Database

EPA's National Homeland Security Research Center brought together experts from across EPA and from its sister agencies in a workshop setting to develop a compendium of analytical methods to be used when responding to future incidents. The methodology was considered for both chemical and biological agents of concern in the types of environmental sample matrices that were anticipated for analysis in homeland security incidents. The primary objective of this effort was not to identify the "best" method, but rather to have a balanced approach between leveraging existing determinative techniques and methodologies and providing consistent analytical results.

These EPA Homeland Security Biological and Chemical Method Review and Consolidation Workshops were held on April 13, 2004, and April 14, 2004, respectively. Participants included representatives from the EPA program offices, the EPA regions, the Centers for Disease Control and Prevention (CDC), the Food and Drug Administration (FDA), the Department of Homeland Security (DHS), the Federal Bureau of Investigation (FBI), the Department of Defense (DOD), the U.S. Department of Agriculture (USDA), and the U.S. Geological Survey (USGS).

During the workshops, participants were provided worksheets populated with as many known techniques as possible to select a single determinative technique and analytical method for each analyte. For biological methods, sample preparation techniques and/or sampling procedures were identified for water, dust, and air matrices. Workshop participants identified sample preparation procedures for chemical contaminants in solid, oily solid, aqueous, liquid, and gas phase matrices.

Not all methods identified and selected by workshop participants have been validated for the analyte/matrix combinations of concern. However, this is a living document, and the Agency anticipates that it will be updated periodically to reflect advances in analytical methodology, development of new technologies, and changes in analytes based on needs. The Agency also anticipates that standardized analytical protocols will be developed as needed, and that addendums may be generated to address materials that are not included in this document.

Page Intentionally Blank

Section 2.0: Scope and Application

The purpose of this document is to identify and briefly describe the specific methodologies that EPA and its contractors will employ when called upon to analyze environmental samples in times of national emergency. The methodologies presented in this document should be used to:

- Evaluate the nature and extent of contamination
- Evaluate decontamination effectiveness

The list of methods is limited to those methods that would be used to determine, to the extent possible within analytical limitations, the presence of chemical and biological agents of concern and to determine their concentrations in environmental media. The methods are not designed to be used for conducting an initial evaluation (triage) of suspected material to determine if it poses an immediate danger or if it needs to be analyzed in specially designed, highly secure facilities. Methods for addressing this analytical need are and will be the subject of other efforts. It is hoped that this document will also assist State and local governments in preparing for future emergencies.

Any deviations from the methods referenced in this document should be coordinated with the appropriate point(s) of contact identified in Section 3.

Participants in the EPA Homeland Security Biological and Chemical Method Review and Consolidation Workshops evaluated the suitability of existing methodologies and selected this set of methods for use by the EPA laboratories and contract laboratories if called upon to respond to an emergency. The Agency recognizes, however, that this advanced selection of such methods poses potential risks. These include the following:

- Selecting technologies that may not be the most cost-effective technologies that are currently available for addressing the particular situation at hand
- Selecting methodologies that may not be appropriate for use in responding to a particular emergency because the Agency did not anticipate having to analyze for a particular analyte or analyte/matrix combination
- Preventing development and adoption of new and better measurement technologies

To address these potential risks as soon as possible, the Agency plans to take several steps. These include the following:

- Developing and specifying measurement quality objectives (i.e., required minimum standards of accuracy (bias and precision) and sensitivity for the analysis of samples that are geared to the data quality needs of the particular stage of the emergency response/recovery process) for all analyte/matrix combinations listed in this document
- Specifying minimum measurement system verification (e.g., ASTM Standard D6956-03) and documentation standards for homeland security analyses
- Working with other government agencies and the private sector to establish a laboratory accreditation system to ensure that laboratories selected to assist the Agency and its Federal, State, and local partners in responding to homeland security incidents have the requisite expertise and systems to perform this type of testing

Page Intentionally Blank

Section 3.0: Points of Contact

Questions concerning this document should be addressed to the appropriate point(s) of contact identified below. As previously indicated, any deviations from the recommended method(s) should be reported immediately to ensure data comparability is maintained when responding to homeland security events. Prior to use of one of the methods listed in Appendix A (chemical) or Appendix B (biological) in response to a homeland security event, the appropriate point(s) of contact identified below should be consulted. Additionally, any deviations from the protocols should be coordinated with the appropriate point(s) of contact listed below.

General

Oba Vincent
National Homeland Security Research Center
Office of Research and Development (163)
26 West Martin Luther King Drive
Cincinnati, OH 45268
(513) 569-7456
vincent.oba@epa.gov

Chemical Methods

Michael S. Johnson
Office of Solid Waste and Emergency Response (5204G)
USEPA Headquarters Ariel Rios Building
1200 Pennsylvania Avenue, NW
Washington, DC 20460
(703) 603-0266
johnson.michaels@epa.gov

David Friedman
Office of Research and Development (8101R)
USEPA Headquarters Ariel Rios Building
1200 Pennsylvania Avenue, NW
Washington, DC 20460
(202) 564-6662
friedman.david@epa.gov

Biological Methods

Alan Lindquist
National Homeland Security Research Center
Office of Research and Development (163)
26 West Martin Luther King Drive
Cincinnati, OH 45268
(513) 569-7192
lindquist.alan@epa.gov

Eugene Rice
National Homeland Security Research Center
Office of Research and Development (163)
26 West Martin Luther King Drive
Cincinnati, OH 45268
(513) 569-7204
rice.eugene@epa.gov

Section 4.0: Chemical Methods

A list of analytical methods to be used in analyzing environmental samples for chemical contaminants during homeland security events is provided in Appendix A. Methods are listed for each analyte and for each sample matrix that potentially may need to be measured and analyzed when responding to an emergency. The methods table in Appendix A is sorted alphabetically by analyte and includes the following information:

- **Analyte(s).** The compound or compound(s) of interest.
- **CAS Number.** A unique identifier for chemical substances that provides an unambiguous way to identify a chemical or molecular structure when there are many possible systematic, generic, or trivial names.
- **Determinative technique.** An analytical instrument or technique used to determine the quantity and identification of compounds or components in a sample.
- **Determinative method identifier.** The unique identifier or number assigned to an analytical method by the method publisher.
- **Solid phase sample preparation procedure.** The recommended method/procedure for sample preparation to measure the analyte of interest in solid phase samples.
- **Oily solid sample preparation procedure.** The recommended method/procedure for sample preparation to measure the analyte of interest in oily phase samples.
- **Aqueous/Liquid phase sample preparation procedure.** The recommended method/procedure for sample preparation to measure the analyte of interest in aqueous and/or liquid phase samples.
- **Gas phase sample preparation procedure.** The recommended method/procedure for sample preparation and analysis to measure the analyte of interest in gas phase samples.

4.1 General Guidance

The guidance summarized in this section provides a general overview of how to identify the appropriate chemical method(s) for a given analyte-matrix combination as well as recommendations for quality control procedures.

For additional information on the properties of the chemicals listed in Appendix A, TOXNET (<http://toxnet.nlm.nih.gov/index.html>), a cluster of databases on toxicology, hazardous chemicals, and related areas maintained by the National Library of Medicine, is an excellent resource. Additional research on chemical contaminants is ongoing within EPA, and databases to manage this information are currently under development.

4.1.1 Standard Operating Procedures for Identifying Chemical Methods

To determine the appropriate method and sample preparation technique that is to be used on the environmental samples, locate analyte of concern in Appendix A: Chemical Methods under the “Analyte” column. After locating the analyte of concern, continue across the table to identify the determinative technique and determinative method for that particular compound. To determine the sample preparation

technique select the appropriate matrix column (Solid Phase, Oily Solid, Aqueous/Liquid Phase, or Gas Phase) for that particular analyte.

Sections 4.2.1 through 4.2.59 below provide summaries of the determinative and sample preparation methods listed in Appendix A. Where available, a direct link to the full text of the selected analytical method is provided in the method summary. For additional information on preparation procedures and methods available through consensus standards organizations, please use the contact information provided in Table 1.

Table 1. Sources of Chemical Methods

Name	Publisher	Reference
National Environmental Methods Index (NEMI)	EPA, USGS	http://www.nemi.gov
U.S. EPA Office of Water (OW) Methods	EPA Office of Water	http://www.epa.gov/safewater/methods/sourcalt.html
U.S. EPA SW-846 Methods	EPA Office of Solid Waste	http://www.epa.gov/epaoswer/hazwaste/test/main.htm
U.S. EPA Office of Research and Development Methods	EPA Office of Research and Development	http://www.epa.gov/nerlcwww/ordmeth.htm
U.S. EPA Air Toxics Methods	EPA Office of Air and Radiation	http://www.epa.gov/ttn/amtic/airtox.html
Occupational Safety and Health Administration Methods	OSHA	http://www.osha-slc.gov/dts/sltc/methods/toc.html
National Institutes for Occupational Safety and Health Methods	NIOSH	http://www.cdc.gov/niosh/nmam/
Standard Methods for the Examination of Water and Wastewater, 20 th Edition	American Public Health Association and American Water Works Association	http://www.apha.org http://www.awwa.org ISBN: 0875532357
Annual Book of ASTM Standards	ASTM International	http://www.astm.org
International Organization for Standardization Methods	ISO	http://www.iso.org
Official Methods of Analysis of AOAC International	AOAC International	http://www.aoac.org

4.1.2 General Quality Control (QC) Guidance for Chemical Methods

Having data of known and documented quality is critical in order for public officials to accurately assess how to respond to emergency situations. Having such data requires that laboratories: (1) conduct the necessary QC to ensure that measurement systems are in control and operating properly, (2) properly document results of the analyses, and (3) properly document measurement system evaluation of the analysis-specific QC. Ensuring data quality also requires that laboratory results are properly evaluated and the results of the data quality evaluation are transmitted to decision makers.

While method-specific QC requirements are described in many of the individual methods that are cited in this manual, and will be included in any standardized analytical protocols developed to address specific analytes and matrices of concern, the following describes a minimum set of QC procedures that shall be conducted for all chemical testing. Individual methods, sampling and analysis protocols, or contractual statements of work also should be consulted to determine any additional QC that may be needed. These QC requirements generally consist of analysis of laboratory control samples and or matrix spikes to identify and quantify measurement system accuracy at the levels of concern, blanks as a measure of freedom from contamination, and matrix spike duplicates (MSD) or sample replicates to assess data precision. QC tests should be run as frequently as necessary to ensure the reliability of analytical results.

In general, sufficient QC includes an initial demonstration of measurement system capability as well as ongoing analysis of standards and other samples to ensure the continued reliability of the analytical results. Examples of sufficient quality control includes:

- Demonstration that measurement system is operating properly
 - Initial calibration
 - Method blanks
- Demonstration of measurement system suitability for intended use
 - Precision and recovery (verify measurement system has adequate accuracy)
 - Analyte/matrix/level of concern-specific QC samples (verify that measurement system has adequate sensitivity at levels of concern)
- Demonstration of continued measurement system reliability
 - Matrix spike/matrix spike duplicates (recovery and precision)
 - QC samples (system accuracy and sensitivity at levels of concern)
 - Continuing calibration verification
 - Method blanks

Please note: The appropriate point of contact identified in Section 3 should be consulted regarding appropriate quality assurance and quality control procedures prior to sample analysis.

4.1.3 Safety and Waste Management

It is imperative that safety precautions are used during collection, processing, and analysis of environmental samples, particularly in emergency response situations that may include unknown hazards. Many of the methods summarized or cited in Section 4.2 contain specific requirements, guidance, or information regarding safety precautions that should be followed when handling or processing environmental samples and reagents. These methods also provide information regarding waste management. Other resources that can be consulted for additional information include the following:

- Occupational Health and Safety Administration's standard for Occupational Exposure to Hazardous Chemicals in Laboratories (29 CFR 1910.1450)
- Environmental Protection Agency's standards regulating hazardous waste (40 CFR parts 260 - 270)

4.2 Method Summaries

Method summaries for the analytical methods listed in Appendix A, including methods for sample preparation and determinative techniques, are provided in Sections 4.2.1 - 4.2.59. Information provided in these sections contains summary information only, extracted from the selected methods. The full version of the method should be consulted prior to sample analysis.

Each method summary contains a table identifying the contaminants in Appendix A to which the method applies, a brief description of the analytical method, and a link to the full version of the method or source for obtaining a full version of the method.

Please note that not all methods have been validated for the analyte/matrix combination listed in Appendix A. Please refer to the specified method to identify analyte/matrix combinations that have been validated. Any questions regarding information discussed in this section should be addressed to the appropriate contact(s) listed in Section 3.

4.2.1 Laboratory Response Network (LRN)

The agents listed below should be analyzed in accordance with the appropriate LRN protocols:

Contaminants	CAS Number
Alpha amanitin	NA
Botulinum toxin	NA
Microcystin	NA
Ricin	9009-86-3
Tetanus Toxin	NA

These agents will be analyzed using restricted procedures available only through the Laboratory Response Network (LRN). These procedures are not available to the general laboratory community and thus are not discussed within this document. For additional information on the LRN, please see contact information listed below or visit <http://www.bt.cdc.gov/lrn/>.

Centers for Disease Control and Prevention

Laboratory Response Branch
 Bioterrorism Preparedness and Response Program
 National Center for Infectious Diseases
 1600 Clifton Road NE, Mailstop C-18
 Atlanta, GA 30333
 Telephone: (404) 639-2790
 E-mail: lrn@cdc.gov

Local public health laboratories, private, and commercial laboratories with questions about the LRN should contact their State public health laboratory director or the Association of Public Health Laboratories (contact information provided below).

Association of Public Health Laboratories

2025 M Street NW, Suite 550

Washington, DC 20036

Telephone: (202) 822-5227

Fax: (202) 887-5098

Website: www.aphl.org

E-mail: info@aphl.org

4.2.2 CLP Method SOW ILM05.3 Cyanide: Analytical Methods for Total Cyanide Analysis

This method should be used for preparation of solid, oily-solid, and aqueous/liquid phase samples for the contaminant identified below and listed in Appendix A:

Contaminant	CAS Number
Cyanide	57-12-5

The method allows for either large volume (500 mL aqueous/liquid samples or 1 - 5 g solid samples mixed with 500 mL of reagent water) or medium volume (50 mL aqueous/liquid samples, or 1 g solid samples mixed with 50 mL reagent water) sample preparation. Aqueous/liquid samples are tested for sulfides and oxidizing agents prior to preparation. Sulfides are removed with cadmium carbonate or lead carbonate. Samples are treated with sulfuric acid and magnesium chloride and distilled into a sodium hydroxide solution. The solution is treated with color agents and the cyanide determined as an ion complex by visible spectrophotometry. The method quantitation limits are 10 µg/L or 2.5 mg/kg. Surfactants may also interfere with the distillation procedure.

Source: <http://www.epa.gov/superfund/programs/clp/download/ilm/ilm53d.pdf>

4.2.3 NERL Method 365.1, Revision 2: Determination of Phosphorus by Semi-Automated Colorimetry

This method should be used for preparation and analysis of aqueous/liquid phase samples for the contaminant identified below and listed in Appendix A:

Contaminant	CAS Number
Red Phosphorus	7723-14-0

This method measures all forms of phosphorus present in the sample, converting them to orthophosphate. The analyte is determined as a reduced antimony-phospho-molybdate complex. A 50-mL sample is digested with sulfuric acid and ammonium persulfate. The digestate is analyzed by automated spectrophotometry (colorimetry) in which the sample reacts with color agents. The range of the method is 0.01 - 1.0 mg P/L. Silica, arsenate, nitrite, and sulfide may cause interference.

Source: http://infotrek.er.usgs.gov/intermedia/nemi_port_read/mediaget/nemi_get_blob/23

4.2.4 EPA Method 200.2, Revision 2.8: Sample Preparation Procedure for Spectrochemical Determination of Total Recoverable Elements

This method should be used for preparation of aqueous/liquid phase samples for the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Arsenic (III) compounds	22569-72-8
Arsenic trichloride	7784-34-1
Arsine	7784-42-1
Cadmium	7440-43-9
Metals, NOS	NA

For aqueous/liquid samples, a 100-mL aliquot of the sample is digested with nitric and hydrochloric acids, the volume reduced to approximately 20 mL, and worked up to a final volume of 50 mL. Samples to be analyzed by Method 6020A (SW-846) require additional dilution to reduce chloride interference.

Source: “Methods for the Determination of Metals in Environmental Samples,” Supplement I, National Exposure Risk Laboratory-Cincinnati (NERL-CI), EPA/600/R-94/11, May 1994; and “Methods for the Determination of Inorganic Substances in Environmental Samples,” NERL-CI, EPA/600/R-93/100, August, 1993 are available from National Technical Information Service (NTIS), 5285 Port Royal Road, Springfield, VA 22161. Phone: 800-553-6847.

4.2.5 EPA Method 300.1: Determination of Inorganic Anions in Drinking Water by Ion Chromatography

This method should be used for the preparation and analysis of aqueous/liquid phase samples for the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Fluoracetate Salts	NA

A small volume of an aqueous/liquid sample (10 µL or 50 µL) is introduced into an ion chromatograph. The volume selected depends on the concentration of fluoroacetate ion in the sample. The anions of interest are separated and measured, using a system comprised of a guard column, analytical column, suppressor device, and conductivity detector. The separator columns and guard columns, as well as eluent conditions, are identical. To achieve comparable detection limits, an ion chromatographic system must utilize suppressed conductivity detection, be properly maintained, and be capable of yielding a baseline with no more than 5 nS noise/drift per minute of monitored response over the background conductivity. The method detection limit varies depending upon the nature of the sample and the specific instrumentation employed. The estimated calibration range is approximately 2 orders of magnitude.

Source: http://infotrek.er.usgs.gov/intermedia/nemi_port_read/mediaget/nemi_get_blob/159

4.2.6 EPA Method 549.2: Determination of Diquat and Paraquat in Drinking Water by Liquid-Solid Extraction and High-Performance Liquid Chromatography with Ultraviolet Detection

This method should be used for preparation and analysis of aqueous/liquid phase samples for the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Paraquat	4685-14-7

A 250-mL sample is extracted using a C-8 liquid/solid extraction (LSE) cartridge or a C-8 disk which has been specially prepared for the reversed-phase, ion-pair mode. The LSE disk or cartridge is eluted with acidic aqueous solvent to yield the eluate/extract. An ion-pair reagent is added to the eluate/extract. The concentrations of paraquat in the eluate/extract are measured using high performance liquid chromatography (HPLC) system equipped with a UV absorbance detector. A photodiode array detector is utilized to provide simultaneous detection and confirmation of the method analytes. The analytical range depends on the sample matrix and the instrumentation used.

Source: http://www.epa.gov/nerlcwww/m_549_2.pdf

4.2.7 EPA Method 3031 (SW-846): Acid Digestion of Oils for Metals Analysis by Atomic Absorption or ICP Spectrometry

This method should be used for preparation of oily-solid phase samples for the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Arsenic (III) compounds	22569-72-8
Arsenic trichloride	7784-34-1
Cadmium	7440-43-9
Metals, NOS	NA

A 0.5 g sample of oil, oil sludge, tar, wax, paint, or paint sludge is mixed with potassium permanganate and sulfuric acid. The mixture is then treated with nitric and hydrochloric acids, filtered and diluted to volume. Excess manganese may be removed with ammonium hydroxide. Digestates are analyzed by Method 6010C (SW-846).

Source: <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3031.pdf>

4.2.8 EPA Method 3050B (SW-846): Acid Digestion of Sediments, Sludges, and Soils

This method should be used for preparation of solid and oily-solid phase samples for the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Arsenic (III) compounds	22569-72-8
Arsenic Trichloride	7784-34-1

Contaminant	CAS Number
Arsine	7784-42-1
Cadmium	7440-43-1
Metals, NOS	NA

A 1 - 2 g sample is digested with nitric acid and hydrogen peroxide. Samples to be analyzed by Method 6010C (SW-846) for cadmium are also treated with hydrochloric acid. Sample volumes are reduced, then brought up to a final volume of 100 mL. Samples are analyzed for arsenic by Method 6020A (SW-846) and for cadmium by either Method 6010C or 6020A.

Source: <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3050b.pdf>

4.2.9 EPA Method 3520C (SW-846): Continuous Liquid-Liquid Extraction

This method should be used for preparation of aqueous/liquid phase samples for the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Bromadiolone	28772-56-7
Chloropicrin	76-06-2
Chlorosarin	1445-76-7
Chlorosoman	7040-57-5
Cyclohexyl Sarin (GF)	329-99-7
Dichlorvos	62-73-7
Dicrotophos	141-66-2
Diesel Range Organics	NA
Dimethylphosphite	868-85-9
Distilled Mustard (HD)/Mustard Gas (H)	505-60-2
Ethyldichloroarsine (ED)	598-14-1
Fenamiphos	22224-92-6
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3
Kerosene	64742-81-0
Lewisite 1 (L-1)	541-25-3
Lewisite 2 (L-2)	40334-69-8
Lewisite 3 (L-3)	40334-70-1

Contaminant	CAS Number
Methyl parathion	298-00-0
Mevinphos	7786-34-7
Nicotine	54-11-5
Nitrogen Mustard (HN-2) [unstable compound]	51-75-2
O-ethyl-S-(2-diisopropylaminoethyl)methyl phosphonothiolate (VX)	50782-69-9
Perfluoroisobutylene (PFIB)	382-21-8
Phencyclidine	60124-79-0
Phorate	298-02-2
Sarin (GB)	107-44-8
Semivolatile Organic Compounds, NOS	NA
Soman (GD)	96-64-0
Strychnine	57-24-9
Tabun (GA)	77-81-6
Tear Gas (CS), chlorobenzylidene malonitrile	2698-41-1
Tetraethyl pyrophosphate	107-49-3
Tetramethylenedisulfotetramine	80-12-6
Trimethyl phosphite	121-45-9
VE	21738-25-0
VG	78-53-5
VM	21770-86-5

This method describes a procedure for isolating organic compounds from aqueous/liquid samples. This method is applicable to the isolation and concentration of water-insoluble and slightly soluble organics in preparation for a variety of chromatographic procedures. A measured volume of sample, usually 1 liter, is placed into a continuous liquid-liquid extractor, adjusted, if necessary, to a specific pH (see Table 1 in Method 3520C), and extracted with organic solvent for 18 to 24 hours. The extract is filtered through sodium sulfate to remove residual moisture, concentrated, and exchanged as necessary into a solvent compatible with the cleanup or determinative procedure used for analysis.

Source: <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3520c.pdf>

4.2.10 EPA Method 3535A (SW-846): Solid-Phase Extraction

This method should be used for preparation of aqueous/liquid phase samples for the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Bromadiolone	28772-56-7
Chloropicrin	76-06-2
Chlorosarin	1445-76-7
Chlorosoman	7040-57-5
Cyclohexyl Sarin (GF)	329-99-7
Dichlorvos	62-73-7
Dicrotophos	141-66-2
Diesel Range Organics	NA
Dimethylphosphite	868-85-9
Distilled Mustard (HD)/Mustard Gas (H)	505-60-2
Ethyldichloroarsine (ED)	598-14-1
Fenamiphos	22224-92-6
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3
Kerosene	64742-81-0
Lewisite 1 (L-1)	541-25-3
Lewisite 2 (L-2)	40334-69-8
Lewisite 3 (L-3)	40334-70-1
Methyl parathion	298-00-0
Mevinphos	7786-34-7
Nicotine	54-11-5
Nitrogen Mustard (HN-2) [unstable compound]	51-75-2
O-ethyl-S-(2-diisopropylaminoethyl)methyl phosphonothiolate (VX)	50782-69-9
Perfluoroisobutylene (PFIB)	382-21-8
Phencyclidine	60124-79-0
Phorate	298-02-2
Sarin (GB)	107-44-8

Contaminant	CAS Number
Semivolatile Organic Compounds, NOS	NA
Soman (GD)	96-64-0
Strychnine	57-24-9
Tabun (GA)	77-81-6
Tear Gas (CS), chlorobenzylidene malonitrile	2698-41-1
Tetraethyl pyrophosphate	107-49-3
Tetramethylenedisulfotetramine	80-12-6
Trimethyl phosphite	121-45-9
VE	21738-25-0
VG	78-53-5
VM	21770-86-5

This method describes a procedure for isolating target organic analytes from aqueous/liquid samples using solid-phase extraction (SPE) media. Sample preparation procedures vary by analyte group. Following any necessary pH adjustment, a measured volume of sample is extracted by passing it through the solid-phase extraction medium (disks or cartridges), which is held in an extraction device designed for vacuum filtration of the sample. Target analytes are eluted from the solid-phase media using an appropriate solvent which is collected in a receiving vessel. The resulting solvent extract is dried using sodium sulfate and concentrated, as needed.

Source: <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3535a.pdf>

4.2.11 EPA Method 3541 (SW-846): Automated Soxhlet Extraction

This method should be used for preparation of solid and oily-solid phase samples for the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Bromadiolone	28772-56-7
Chloropicrin	76-06-2
Chlorosarin	1445-76-7
Chlorosoman	7040-57-5
Cyclohexyl Sarin (GF)	329-99-7
Dichlorvos	62-73-7
Dicrotophos	141-66-2
Diesel Range Organics	NA

Contaminant	CAS Number
Dimethylphosphite	868-85-9
Distilled Mustard (HD)/Mustard Gas (H)	505-60-2
Ethylchloroarsine (ED)	598-14-1
Fenamiphos	22224-92-6
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3
Lewisite 1 (L-1)	541-25-3
Lewisite 2 (L-2)	40334-69-8
Lewisite 3 (L-3)	40334-70-1
Methyl parathion	298-00-0
Mevinphos	7786-34-7
Nicotine	54-11-5
Nitrogen Mustard (HN-2) [unstable compound]	51-75-2
O-ethyl-S-(2-diisopropylaminoethyl)methyl phosphonothiolate (VX)	50782-69-9
Perfluoroisobutylene (PFIB)	382-21-8
Phencyclidine	60124-79-0
Phorate	298-02-2
Sarin (GB)	107-44-8
Semivolatile Organic Compounds, NOS	NA
Soman (GD)	96-64-0
Strychnine	57-24-9
Tabun (GA)	77-81-6
Tear Gas (CS), chlorobenzylidene malonitrile	2698-41-1
Tetraethyl pyrophosphate	107-49-3
Tetramethylenedisulfotetramine	80-12-6
Trimethyl phosphite	121-45-9
VE	21738-25-0
VG	78-53-5
VM	21770-86-5

Approximately 10 g of solid sample is mixed with an equal amount of anhydrous sodium sulfate, placed in an extraction thimble or between two plugs of glass wool, and after adding the appropriate surrogate amount, is extracted using an appropriate solvent in an automated Soxhlet extractor. The extract is filtered through sodium sulfate to remove residual moisture, concentrated and exchanged, as necessary into a solvent compatible with the cleanup or determinative procedure for analysis.

Source: <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3540c.pdf>

4.2.12 EPA Method 3545A (SW-846) Pressurized Fluid Extraction (PFE)

This method should be used for preparation of solid and oily-solid phase samples for the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Bromadiolone	28772-56-7
Chloropicrin	76-06-2
Chlorosarin	1445-76-7
Chlorosoman	7040-57-5
Cyclohexyl Sarin (GF)	329-99-7
Dichlorvos	62-73-7
Dicrotophos	141-66-2
Diesel Range Organics	NA
Dimethylphosphite	868-85-9
Distilled Mustard (HD)/Mustard Gas (H)	505-60-2
Ethyldichloroarsine (ED)	598-14-1
Fenamiphos	22224-92-6
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3
Kerosene	64742-81-0
Lewisite 1 (L-1)	541-25-3
Lewisite 2 (L-2)	40334-69-8
Lewisite 3 (L-2)	40334-70-1
Methyl parathion	298-00-0
Mevinphos	7786-34-7
Nicotine	54-11-5
Nitrogen Mustard (HN-2) [unstable compound]	51-75-2

Contaminant	CAS Number
O-ethyl-S-(2-diisopropylaminoethyl)methyl phosphonothiolate (VX)	50782-69-9
Perfluoroisobutylene (PFIB)	382-21-8
Phencyclidine	60124-79-0
Phorate	298-02-2
Sarin (GB)	107-44-8
Semivolatile Organic Compounds, NOS	NA
Soman (GD)	96-64-0
Strychnine	57-24-9
Tabun (GA)	77-81-6
Tear Gas (CS), chlorobenzylidene malonitrile	2698-41-1
Tetraethyl pyrophosphate	107-49-3
Tetramethylenedisulfotetramine	80-12-6
Trimethyl phosphite	121-45-9
VE	21738-25-0
VG	78-53-5
VM	21770-86-5

Approximately 10 to 30 g of soil sample is prepared for extraction either by air drying the sample, or by mixing the sample with anhydrous sodium sulfate or pelletized diatomaceous earth. The sample is then ground and loaded into the extraction cell. The extraction cell containing the sample is heated to the extraction temperature, pressurized with the appropriate solvent system, and extracted for 5 minutes (or as recommended by the instrument manufacturer). The extract may be concentrated, if necessary, and, as needed, exchanged into a solvent compatible with the cleanup or determinative step being employed. This method has been validated for solid matrices containing 250 to 12,500 µg/kg of semivolatile organic compounds, 250 to 2500 µg/kg of organophosphorus pesticides, 5 to 250 µg/kg of organochlorine pesticides, 50 to 5000 µg/kg of chlorinated herbicides, 1 to 1400 µg/kg of PCBs, and 1 to 2500 ng/kg of PCDDs/PCDFs.

Source: <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3545a.pdf>

4.2.13 EPA Method 3580A (SW-846): Waste Dilution

This method should be used for preparation of oily-solid phase samples for the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Bromadiolone	28772-56-7
Chloropicrin	76-06-2
Chlorosarin	1445-76-7
Chlorosoman	7040-57-5
Cyclohexyl Sarin (GF)	329-99-7
Dichlorvos	62-73-7
Dicrotophos	141-66-2
Diesel Range Organics	NA
Dimethylphosphite	868-85-9
Distilled Mustard (HD)/Mustard Gas (H)	505-60-2
Ethylchloroarsine (ED)	598-14-1
Fenamiphos	22224-92-6
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3
Kerosene	64742-81-0
Lewisite 1 (L-1)	541-25-3
Lewisite 2 (L-2)	40334-69-8
Lewisite 3 (L-3)	40334-70-1
Methyl parathion	298-00-0
Mevinphos	7786-34-7
Nicotine	54-11-5
Nitrogen Mustard (HN-2)	51-75-2
O-ethyl-S-(2-diisopropylaminoethyl)methyl phosphonothiolate (VX)	50782-69-9
Perfluoroisobutylene (PFIB)	382-21-8
Phencyclidine	60124-79-0
Phorate	298-02-2
Sarin (GB)	107-44-8

Contaminant	CAS Number
Semivolatile Organic Compounds, NOS	NA
Soman (GD)	96-64-0
Strychnine	57-24-9
Tabun (GA)	77-81-6
Tear Gas (CS), chlorobenzylidene malonitrile	2698-41-1
Tetraethyl pyrophosphate	107-49-3
Tetramethylenedisulfotetramine	80-12-6
Trimethyl phosphite	121-45-9
VE	21738-25-0
VG	78-53-5
VM	21770-86-5

This method describes a solvent dilution of a non-aqueous waste sample prior to cleanup and/or analysis. One gram of sample is weighed into a capped tube, and the sample is diluted to 10.0 mL with an appropriate solvent. The method is designed for wastes that may contain organic chemicals at a concentration greater than 20,000 mg/kg and that are soluble in the dilution solvent.

Source: <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3580a.pdf>

4.2.14 EPA Method 3585 (SW-846): Waste Dilution for Volatile Organics

This method should be used for preparation of oily-solid phase samples for the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Carbon disulfide	75-15-0
Cyanogen chloride	506-77-4
Ethylene Oxide	75-21-8
Gasoline Range Organics	NA
Phosgene	75-44-5
Volatile Organic Compounds, NOS	NA

This method describes a solvent dilution of a non-aqueous waste sample prior to direct injection analysis. It is designed for use in conjunction with GC or GC/MS analysis of wastes that may contain organic chemicals at a concentration greater than 1 mg/kg and that are soluble in the dilution solvent. Highly contaminated or highly complex samples may be diluted prior to analysis for volatiles using direct injection. One gram of sample is weighed into a capped tube or volumetric flask. The sample is diluted to 2.0 - 10.0 mL with *n*-hexadecane or other appropriate solvent. Diluted samples are injected into the GC or GC/MS for analysis.

Source: <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3585.pdf>

4.2.15 EPA Method 5030C (SW-846): Purge-and-Trap for Aqueous Samples

This method should be used for preparation of aqueous/liquid phase samples for the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Carbon disulfide	75-15-0
Cyanogen chloride	506-77-4
Ethylene oxide	75-21-8
Gasoline Range Organics	NA
Kerosene	64742-81-0
Volatile Organic Compounds, NOS	NA

This method describes a purge-and-trap procedure for the analysis of volatile organic compounds (VOCs) in aqueous/liquid samples and water miscible liquid samples. It also describes the analysis of high concentration soil and waste sample extracts prepared using Method 5035 (SW-846).

Aqueous/Liquid Samples: An inert gas is bubbled through a portion of the aqueous/liquid sample at ambient temperature, and the volatile components are efficiently transferred from the aqueous/liquid phase to the vapor phase. The vapor is swept through a sorbent column where the volatile components are adsorbed. After purging is completed, the sorbent column is heated and backflushed with inert gas to desorb the components onto a gas chromatographic column.

High Concentration Extracts from Method 5035 (SW-846): An aliquot of the extract prepared using Method 5035 is combined with organic-free reagent water in the purging chamber. It is then analyzed by purge-and-trap GC or GC/MS following the procedure used for the aqueous/liquid samples.

Source: <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/5030c.pdf>

4.2.16 EPA Method 5035A (SW-846): Closed-System Purge-and-Trap and Extraction for Volatile Organics in Soil and Waste Samples

This method should be used for preparation of solid phase samples for the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Carbon disulfide	75-15-0
Cyanogen chloride	506-77-4
Ethylene oxide	75-21-8
Gasoline Range Organics	NA
Kerosene	64742-81-0

Contaminant	CAS Number
Phosgene	75-44-5
Volatile Organic Compounds, NOS	NA

This method describes a closed-system purge-and-trap process for analysis of volatile organic compounds (VOCs) in solid samples containing low levels (0.5 to 200 µg/kg) of VOCs. The method also provides specific procedures for preparation of samples containing high levels (>200 µg/kg) of VOCs. For low-level VOCs, a 5 g sample is collected into a vial, that is placed into an autosampler device. Reagent water, surrogates, and internal standards are automatically added, and the vial heated to 40°C. The volatiles are purged into an appropriate trap using an inert gas combined with sample agitation. When purging is complete, the trap is heated and backflushed with helium to desorb the trapped sample components into a gas chromatograph for analysis. For high-level VOCs, samples are either collected into a vial that contains a water-miscible organic solvent or a portion of sample is removed from the vial and dispersed in a water-miscible solvent. An aliquot of the solvent is added to reagent water, along with surrogates and internal standards, then purged and analyzed using an appropriate determinative method (e.g., 8260B).

Source: http://www.epa.gov/epaoswer/hazwaste/test/pdfs/5035a_r1.pdf

4.2.17 EPA Method 5050 (SW-846): Bomb Preparation Method for Solid Waste

This method should be used for preparation of solid and aqueous/liquid phase samples for the contaminant identified below and listed in Appendix A:

Contaminant	CAS Number
Osmium tetroxide	20816-12-0

A 0.5 g sample is placed in a sample cup, which is placed in a bomb. The sample is combusted with pure oxygen. The bomb and cup are rinsed, and the rinse collected and analyzed using Method 6010C (SW-846).

Source: <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/5050.pdf>

4.2.18 EPA Method 6010C (SW-846): Inductively Coupled Plasma - Atomic Emission Spectrometry

This method should be used for determination of the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Arsenic (III) compounds	22569-72-8
Arsenic trichloride	7784-34-1
Cadmium	7440-43-9
Metals, NOS	NA
Osmium tetroxide	20816-12-0

Contaminant	CAS Number
Titanium tetrachloride	7550-45-0

This method determines arsenic (III) compounds and arsenic trichloride as arsenic, osmium tetroxide as osmium, and titanium tetrachloride as titanium. Any other metals are determined as the metal. Aqueous samples (prepared using OW Method 200.2 or SW-846 Method 5050), soil samples (prepared using SW-846 Methods 3050B or 5050), oily solid samples (prepared using SW-846 Methods 3050B or 3031), and air filter/particle samples (prepared using Inorganic (IO) Method 3.4) are analyzed by Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-AES). Detection limits vary with each analyte. The analytical range may be extended by sample dilution.

Source: <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/6010c.pdf>

4.2.19 EPA Method 6020A (SW-846): Inductively Coupled Plasma - Mass Spectrometry

This method should be used for determination of the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Arsenic (III) compounds	22569-72-8
Arsenic trichloride	7784-34-1
Cadmium	7440-43-9
Metals NOS	NA
Titanium tetrachloride	7550-45-0

This method determines arsenic (III) compounds, arsenic trichloride, and titanium tetrachloride as titanium. Any other metals are determined as the metal. Aqueous samples (prepared using OW Method 200.2 or SW-846 Method 5050), soil samples (prepared using SW-846 Methods 3050B or 5050), oily solid samples (prepared using SW-846 Methods 3050B or 3031), and air filter/particle samples (prepared using IO Method 3.5) are analyzed by Inductively Coupled Plasma - Mass Spectrometry. Detection limits vary with each analyte. The analytical range may be extended by sample dilution.

Source: <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/6020a.pdf>

4.2.20 EPA Method 7010 (SW-846): Graphite Furnace Atomic Absorption Spectrophotometry

This method should be used for determination of the contaminant identified below and listed in Appendix A:

Contaminant	CAS Number
Arsine	7784-42-1

This method determines arsine in environmental samples. Aqueous samples (prepared using OW Method 200.2) or soil samples (prepared using SW-846 Method 3050B) are analyzed by Graphite Furnace Atomic Absorption Spectrophotometry (GFAA). A representative aliquot of the sample is placed in the graphite

tube in the furnace, evaporated to dryness, charred, and atomized. Detection limits vary with each matrix and instrument used. The analytical range may be extended by sample dilution.

Source: <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/7010.pdf>

4.2.21 EPA Method 7470A (SW-846): Mercury in Liquid Wastes (Manual Cold-Vapor Technique)

This method should be used for preparation and analysis of aqueous/liquid samples for the contaminant identified below and listed in Appendix A:

Contaminant	CAS Number
Mercury	7439-97-6

A 100-mL aqueous or liquid waste sample is digested with acids, permanganate solution, persulfate solution, and heat. The sample is cooled and reduced with hydroxylamine - sodium chloride solution. Just prior to analysis, the sample is treated with Sn(II), reducing the mercury to Hg(0). The reduced sample is sparged and the mercury vapor is analyzed by cold vapor atomic absorption. The detection limit for the method is less than 0.2 µg/L. Chloride and copper are potential interferences.

Source: <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/7470a.pdf>

4.2.22 EPA Method 7471B (SW-846): Mercury in Solid or Semisolid Wastes (Manual Cold-Vapor Technique)

This method should be used for preparation of solid phase samples for the contaminant identified below and listed in Appendix A:

Contaminant	CAS Number
Mercury	7439-97-6

A 0.5 to 0.6 g sample is digested with aqua regia, permanganate solution and heat. The sample is cooled and reduced with hydroxylamine - sodium chloride solution. Just prior to analysis, the sample is treated with Sn(II), reducing the mercury to Hg(0). The reduced sample is sparged and the mercury vapor is analyzed by cold vapor atomic absorption. Chloride and copper are potential interferences.

Source: <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/7471b.pdf>

4.2.23 EPA Method 8015C (SW-846): Nonhalogenated Organics Using GC/FID

This method should be used for determination of the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Diesel Range Organics	NA
Gasoline Range Organics	NA
Kerosene	64742-81-0

This method provides gas chromatographic conditions for the detection of certain nonhalogenated volatile and semivolatile organic compounds. Depending on the analytes of interest, samples may be introduced into the GC by a variety of techniques including purge-and-trap, direction injection of aqueous/liquid samples, and solvent extraction. An appropriate column and temperature program are used in the gas chromatograph to separate the organic compounds. Detection is achieved by a flame ionization detector (FID). The method allows the use of packed or capillary columns for the analysis and confirmation of the non-halogenated individual analytes. The estimated method detection limits vary with each analyte and range between 2 and 48 µg/L for aqueous/liquid samples. The method detection limits in other matrices have not been evaluated for this method. The analytical range depends on the target analyte(s) and the instrument used.

Source: <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/8015c.pdf>

4.2.24 EPA Method 8260B (SW-846): Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)

This method should be used for determination of the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Carbon disulfide	75-15-0
Cyanogen chloride	506-77-4
Ethylene oxide	75-21-8
Phosgene	75-44-5
Volatile Organic Compounds, NOS	NA

Volatile compounds are introduced into a gas chromatograph by purge-and-trap or other methods (see Sec. 1.2 in Method 8260B). The analytes are introduced directly to a wide-bore capillary column or cryofocused on a capillary pre-column before being flash evaporated to a narrow-bore capillary for analysis. The column is temperature-programmed to separate the analytes, which are then detected with a mass spectrometer (MS) interfaced to the gas chromatograph (GC). Analytes eluted from the capillary column are introduced into the mass spectrometer via a jet separator or a direct connection. The estimated quantitation limit (EQL) of Method 8260B for an individual analyte is dependent on the instrument as well as the choice of sample preparation/introduction method. Using standard quadrupole instrumentation and the purge-and-trap technique, estimated quantitation limits are 5 µg/kg (wet weight) for soil/sediment samples and 5 µg/L for ground water (see Table 3 in Method 8260B). Somewhat lower limits may be achieved using an ion trap mass spectrometer or other instrumentation of improved design. No matter which instrument is used, EQLs will be proportionately higher for sample extracts and samples that require dilution or when a reduced sample size is used to avoid saturation of the detector.

Source: <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/8260b.pdf>

4.2.25 EPA Method 8270D (SW-846): Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)

This method should be used for determination of the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Chloropicrin	76-06-2
Chlorosarin	1445-76-7
Chlorosoman	7040-57-5
Cyclohexyl Sarin	329-99-7
Dichlorvos	62-73-7
Dicrotophos	141-66-2
Dimethylphosphite	868-85-9
Distilled Mustard (HD)/Mustard Gas (H)	505-60-2
Ethylchloroarsine (ED)	598-14-1
Fenamiphos	22224-92-6
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3
Lewisite 1 (L-1)	541-25-3
Lewisite 2 (L-2)	40334-69-8
Lewisite 3 (L-3)	40334-70-1
Methyl parathion	298-00-0
Mevinphos	7786-34-7
Nicotine	54-11-5
Nitrogen Mustard (HN-2) [unstable compound]	51-75-2
O-ethyl-S-(2-diisopropylaminoethyl)methyl phosphonothiolate (VX)	50782-69-9
Perfluoroisobutylene (PFIB)	382-21-8
Phencyclidine	60124-79-0
Phorate	298-02-2
Sarin (GB)	107-44-8
Semivolatile Organic Compounds, NOS	NA
Soman (GD)	96-64-0
Strychnine	57-24-9

Contaminant	CAS Number
Tabun	77-81-6
Tear gas (CS), chlorobenzylidene malonitrile	2698-41-1
Tetraethyl pyrophosphate	107-49-3
Trimethyl phosphite	121-45-9
VE	21738-25-0
VG	78-53-5
VM	21770-86-5

Samples are prepared for analysis by gas chromatography/mass spectrometry (GC/MS) using the appropriate sample preparation and, if necessary, sample cleanup procedures. Semivolatile compounds are introduced into the GC/MS by injecting the sample extract into a gas chromatograph (GC) with a narrow-bore fused-silica capillary column. The GC column is temperature-programmed to separate the analytes, which are then detected with a mass spectrometer (MS) connected to the gas chromatograph. Analytes eluted from the capillary column are introduced into the mass spectrometer. The estimated method detection limits vary with each analyte and range between 10 and 1000 µg/L for aqueous/liquid samples and 660 and 3300 µg/kg for soil samples. The analytical range depends on the target analyte(s) and the instrument used.

Source: <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/8270d.pdf>

4.2.26 EPA Method 8315A (SW-846): Determination of Carbonyl Compounds by High Performance Liquid Chromatography (HPLC)

This method should be used for preparation of solid and aqueous/liquid phase samples for the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Formaldehyde	50-00-0

A measured volume of aqueous/liquid sample (approximately 100 mL) or an appropriate amount of solids extract (approximately 25 g), is buffered to pH 3 and derivatized with 2,4-dinitrophenylhydrazine (DNPH). Using the appropriate extraction technique, the derivatives are extracted using methylene chloride and the extracts are exchanged with acetonitrile prior to HPLC analysis. HPLC conditions are described which permit the separation and measurement of various carbonyl compounds in the extract by absorbance detection at 360 nm. If formaldehyde is the only analyte of interest, the aqueous/liquid sample and/or solid sample extract should be buffered to pH 5.0 to minimize the formation of artifact formaldehyde. The method detection limit for formaldehyde varies depending on sample conditions and instrumentation but is approximately 6.2 µg/L for aqueous/liquid samples.

Source: <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/8315a.pdf>

4.2.27 EPA Method 8318A (SW-846): *N*-Methylcarbamates by High Performance Liquid Chromatography (HPLC)

This method should be used for preparation of solid, oily-solid, and aqueous/liquid phase samples for the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Aldicarb (Temik)	116-06-3
Carbofuran (Furadan)	1563-66-2
Oxamyl	23135-22-0

N-methylcarbamates are extracted from aqueous/liquid samples with methylene chloride, and from soils, oily solid waste, and oils with acetonitrile. The extract solvent is exchanged to methanol/ethylene glycol, and the extract is cleaned using a C-18 cartridge, filtered, and eluted on a C-18 analytical column. After separation, the target analytes are hydrolyzed and derivatized post-column, then quantitated fluorometrically. The sensitivity of the method usually depends on the level of interferences present, rather than on the instrumental conditions. Waste samples with a high level of extractable fluorescent compounds are expected to yield significantly higher detection limits. The estimated method detection limits vary with each analyte and range between 1.7 and 9.4 µg/L for aqueous/liquid samples and 10 and 50 µg/kg for soil samples.

Source: <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/8318a.pdf>

4.2.28 EPA Method 8321B (SW-846): Solvent-Extractable Nonvolatile Compounds by High Performance Liquid Chromatography/Thermospray/Mass Spectrometry (HPLC/TS/MS) or Ultraviolet (UV) Detection

This method should be used for determination of the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Bromadiolone	28772-56-7
Tetramethylenedisulfotetramine	80-12-6

This method provides reversed-phase high performance liquid chromatographic (RP/HPLC), thermospray (TS) mass spectrometric (MS), and ultraviolet (UV) conditions for detection of the target analytes. Sample extracts can be analyzed by direct injection into the thermospray or onto a liquid chromatographic-thermospray interface. A gradient elution program is used to separate the compounds. Primary analysis may be performed by UV detection; however, positive results should be confirmed by TS/MS. Quantitative analysis may be performed by either TS/MS or UV detection, using either an external or internal standard approach. TS/MS detection may be performed in either a negative ionization (discharge electrode) mode or a positive ionization mode, with a single quadrupole mass spectrometer. The analytical range and detection limits vary depending on the target analyte and instrument used.

Source: <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/8321b.pdf>

4.2.29 AOAC Official Method 994.08: Aflatoxin in Corn, Almonds, Brazil Nuts, Peanuts, and Pistachio Nuts

This method should be used for preparation of solid, oily-solid, and aqueous/liquid phase samples for the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Aflatoxin	1402-68-2
Brevetoxins	NA
Picrotoxin	124-87-8
Saxitoxin	35523-89-8
T-2 Mycotoxins	NA

Samples are extracted using an acetonitrile-water (9+1) solution. Sample extracts are then run through a multifunctional cleanup column. The purified extract and standards are derivatized with trifluoroacetic acid, and then analyzed using a liquid chromatography system with a fluorescence detector. Specific aflatoxins can be identified by their retention time and quantified using standard curves. Method performance was characterized using various commodities (e.g., corn) at aflatoxin levels over a range of 5-30 ng/g. Note: This method was originally designed for the analysis of aflatoxins in commodities where cleanup was necessary to remove other food components, like fats and proteins; the cleanup procedure may not be necessary with water analyses.

Source: AOAC International. 1998. *Official Methods of Analysis of AOAC International*. 16th Edition, 4th Revision; Vol II.

4.2.30 ASTM Method D5755-03: Standard Test Method for Microvacuum Sampling and Indirect Analysis of Dust by Transmission Electron Microscopy (TEM) for Asbestos Structure Number Surface Loading

This method should be used for preparation of solid phase samples for the contaminant identified below and listed in Appendix A:

Contaminant	CAS Number
Asbestos	1332-21-4

This method describes procedures to (a) identify asbestos in dust and (b) provide an estimate of the surface loading of asbestos reported as the number of asbestos structures per unit area of sampled surface. The sample is collected by vacuuming a known surface area with a standard 25 or 37 mm air sampling cassette using a plastic tube that is attached to the inlet orifice which acts as a nozzle. The sample is transferred from inside the cassette to an aqueous suspension of known volume. Aliquots of the suspension are then filtered through a membrane. A section of the membrane is prepared and transferred to a TEM grid using a direct transfer method. The asbestiform structures are identified, sized, and counted by TEM, using select area electron diffraction (SAED) and energy dispersive X-ray analysis (EDXA) at a magnification of 15,000 to 20,000X.

Source:

http://www.astm.org/cgi-bin/SoftCart.exe/STORE/filtrexx40.cgi?U+mystore+tavs3076+-L+D5755:03+/u sr6/htdocs/astm.org/DATABASE.CART/REDLINE_PAGES/D5755.htm

4.2.31 ASTM Method D6480-99: Standard Test Method for Wipe Sampling of Surfaces, Indirect Preparation, and Analysis for Asbestos Structure Number Concentration by Transmission Electron Microscopy

This method should be used for preparation of solid phase samples for the contaminant identified below and listed in Appendix A:

Contaminant	CAS Number
Asbestos	1332-21-4

This test method describes a procedure to identify asbestos in samples wiped from surfaces and to provide an estimate of the concentration of asbestos reported as the number of asbestos structures per unit area of sampled surface. A sample is collected by wiping a surface of known area with a wipe material. The sample is transferred from the wipe material to an aqueous suspension of known volume. Aliquots of the suspension are then filtered through a membrane filter. A section of the membrane filter is prepared and transferred to a TEM grid, using the direct transfer method. The asbestiform structures are identified, sized, and counted by TEM, using electron diffraction (ED) and EDXA at a magnification from 15,000 to 20,000X.

Source:

http://www.astm.org/cgi-bin/SoftCart.exe/STORE/filtrexx40.cgi?U+mystore+tavs3076+-L+D6480:99+/u sr6/htdocs/astm.org/DATABASE.CART/REDLINE_PAGES/D6480.htm

4.2.32 ISO Method - 10312: Ambient Air - Determination of Asbestos Fibres - Direct-transfer Transmission Electron Microscopy Method (TEM)

This method should be used for preparation and analysis of gas phase samples for the contaminant identified below and listed in Appendix A:

Contaminant	CAS Number
Asbestos	1332-21-4

This method determines the type(s) of asbestos fibers present but cannot discriminate between individual fibres of the asbestos and non-asbestos analogues of the same amphibole mineral. The method is defined for polycarbonate capillan/pore filters or cellulose ester (either mixed esters of cellulose or cellulose nitrate) filters through which a known volume of air has been drawn. The method is suitable for determination of asbestos in both exterior and building atmospheres. The range of concentrations that can be determined is 50 structures/mm² to 7,000 structures/mm² on the filter. In a 4000 L air sample with approximately 10 pg/m³ (typical of clean or rural atmospheres), an analytical sensitivity of 0.5 structure/L can be obtained. This is equivalent to a detection limit of 1.8 structure/L when an are of 0.195 mm of the TEM specimen is examined.

Source: <http://www.iso.org>

4.2.33 NIOSH Method 6001: Arsine

This method should be used for preparation and analysis of gas phase samples for the contaminant identified below and listed in Appendix A:

Contaminant	CAS Number
Arsine	7784-42-1

In this method, arsine is determined as arsenic. A volume of 0.1 to 10 L of air is drawn through a sorbent tube containing activated charcoal. The sorbent is extracted with a nitric acid solution. The arsine is determined by graphite furnace atomic absorption. The working range of the method is 0.001 to 0.2 mg/m³ for a 10-L sample. The method is subject to interferences from other arsenic compounds.

Source: <http://www.cdc.gov/niosh/nmam/pdfs/6001.pdf>

4.2.34 NIOSH Method 6002: Phosphine

This method should be used for preparation and analysis of gas phase samples for the contaminant identified below and listed in Appendix A:

Contaminant	CAS Number
Phosphine	7803-51-2

In this method, phosphine is determined as phosphate. One to 16 liters of air are drawn through a sorbent tube containing silica gel coated with Hg(CN)₂. The sorbent is extracted with a potassium permanganate/sulfuric acid solution and washed with reagent water. Following treatment with the color agent and extraction into organic solvent, the phosphate is determined by visible spectrometry. The working range of the method is 0.02 - 0.9 mg/m³ for a 16-L sample. The method is subject to interferences from phosphorus trichloride, phosphorus pentachloride, and organic phosphorus compounds.

Source: <http://www.cdc.gov/niosh/nmam/pdfs/6002.pdf>

4.2.35 NIOSH Method 6004: Sulfur Dioxide

This method should be used for preparation and analysis of gas phase samples for the contaminant identified below and listed in Appendix A:

Contaminant	CAS Number
Sulfur Dioxide	7446-09-5

In this method, sulfur dioxide is determined as sulfite plus sulfate. A volume of 40 to 200 liters of air is drawn through a sodium carbonate-treated filter that is preceded by a 0.8 µm filter to remove particulates and sulfuric acid. The treated filter is extracted with a carbonate/bicarbonate solution and the extract analyzed by ion chromatography for sulfite and sulfate. The sulfur dioxide is present as sulfite on the filter; however, because sulfite oxidizes to sulfate, both ions must be determined and the results summed. The working range of the method is 0.5 - 20 mg/m³ for a 100-L sample. The method is subject to interference from sulfur trioxide in dry conditions.

Source: <http://www.cdc.gov/niosh/nmam/pdfs/6004.pdf>

4.2.36 NIOSH Method 6010: Hydrogen Cyanide

This method should be used for preparation and analysis of gas phase samples for the contaminant identified below and listed in Appendix A:

Contaminant	CAS Number
Hydrogen Cyanide	74-90-8

Hydrogen cyanide is determined as a cyanide ion complex by this method. A volume of 2 to 90 liters of air is drawn through a soda lime sorbent tube. A glass-fiber filter is used to remove particulate cyanides prior to the sorbent tube. Cyanide is extracted from the sorbent with reagent water. The extract is pH adjusted and treated with the coupling-color agent. The cyanide ion is determined by visible spectrophotometry. The working range of the method is 3 - 260 mg/m³ for a 3-L sample. The method is subject to interference from high concentrations of hydrogen sulfide.

Source: <http://www.cdc.gov/niosh/nmam/pdfs/6010.pdf>

4.2.37 NIOSH Method 6011: Bromine and Chlorine

This method should be used for preparation and analysis of gas phase samples for the contaminant identified below and listed in Appendix A:

Contaminant	CAS Number
Chlorine	7782-50-5

In this method, chlorine is determined as chloride. A volume of 2 to 90 liters of air is drawn through a silver membrane filter. A prefilter is used to remove particulate chlorides. The filter is extracted with sodium hyposulfate solution, and the extract analyzed for chloride by ion chromatography. The working range of the method is 0.02 - 1.5 mg/m³ for a 90-L sample. The method is subject to positive interference by HCl and negative interference by hydrogen sulfide.

Source: <http://www.cdc.gov/niosh/nmam/pdfs/6011.pdf>

4.2.38 NIOSH Method 6013: Hydrogen Sulfide

This method should be used for preparation and analysis of gas phase samples for the contaminant identified below and listed in Appendix A:

Contaminant	CAS Number
Hydrogen Sulfide	7783-06-4

Hydrogen sulfide is determined as sulfate by this method. A volume of 1.2 to 40 liters of air is drawn through charcoal sorbent. A prefilter is used to remove particulates. The sorbent portions are extracted with an ammonium hydroxide/hydrogen peroxide solution and the extract is analyzed for sulfate by ion chromatography. The working range of the method is 0.9 - 20 mg/m³ for a 20-L sample. The method is subject to interference from sulfur dioxide.

Source: <http://www.cdc.gov/niosh/nmam/pdfs/6013.pdf>

4.2.39 NIOSH Method 6015: Ammonia

This method should be used for preparation and analysis of gas phase samples for the contaminant identified below and listed in Appendix A:

Contaminant	CAS Number
Ammonia	7664-41-7

Ammonia is determined as indophenol blue by this method. A volume of 0.1 to 96 liters of air is drawn through a sulfuric acid-treated silica gel sorbent. A prefilter is used to remove particulates. The sorbent is extracted with reagent water, the pH adjusted, and reagents are added to generate the indophenol blue compound in the presence of ammonium. The extract is analyzed by visible spectrophotometry. The working range of the method is 0.15 - 300 mg/m³ for a 10-L sample. No interferences have been identified.

Source: <http://www.cdc.gov/niosh/nmam/pdfs/6015.pdf>

4.2.40 NIOSH Method 6402: Phosphorus Trichloride

This method should be used for preparation and analysis of gas phase samples for the contaminant identified below and listed in Appendix A:

Contaminant	CAS Number
Phosphorus Trichloride	7719-12-2

In this method, phosphorus trichloride is determined as phosphate. A volume of 11 to 100 liters of air is drawn through a bubbler containing reagent water. The resulting H₃PO₃ solution is oxidized to H₃PO₄ and color agents are added. The solution is analyzed by visible spectrophotometry. The working range of the method is 1.2 - 80 mg/m³ for a 25-L sample. Phosphorus (V) compounds do not interfere. The sample solutions are stable to oxidation by air during sampling.

Source: <http://www.cdc.gov/niosh/nmam/pdfs/6402.pdf>

4.2.41 NIOSH Method 7903: Acids, Inorganic

This method should be used for preparation and analysis of gas phase samples for the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Hydrogen Bromide	10035-10-6
Hydrogen Chloride	7647-01-0
Hydrogen Fluoride	7664-39-3

Acids are analyzed as bromide, chloride, and fluoride, respectively, by this method. A volume of 3 to 100 liters of air is drawn through a silica gel sorbent. The sorbent portions are extracted with a buffered carbonate/bicarbonate solution and the extract is analyzed by ion chromatography. The working range of this method is 0.01 - 5 mg/m³ for a 50-L sample. Particulate salts of the acids are an interference (trapped

on the glass wool filter plug in the sorbent tube). Chlorine and bromine are also interferences. Acetate, formate, and propionate interferences may be reduced by use of a weaker eluent.

Source: <http://www.cdc.gov/niosh/nmam/pdfs/7903.pdf>

4.2.42 NIOSH Method 7904: Cyanides, Aerosol and Gas

This method should be used for preparation and analysis of gas phase samples for the contaminant identified below and listed in Appendix A:

Contaminant	CAS Number
Cyanide	57-12-5

In this method, cyanide(s) are determined as cyanide ion. A volume of 10 to 180 liters of air is drawn through a 0.8- μm PVC membrane filter and a bubbler containing 0.1N KOH solution. The filter collects aerosols of cyanide solutions and the bubbler collects HCN. The filters are extracted with the KOH solution. Sulfide must be removed from the solutions prior to analysis. Analyze the solutions by cyanide ion specific electrode (ISE). The working range of the method is 0.5 - 15 mg/m^3 for a 90-L sample. Sulfide, chloride, iodide, bromide, cadmium, zinc, silver, nickel, cuprous iron, and mercury are interferences.

Source: <http://www.cdc.gov/niosh/nmam/pdfs/7904.pdf>

4.2.43 NIOSH Method 7906: Fluorides, Aerosol and Gas

This method should be used for preparation and analysis of gas phase samples for the contaminant identified below and listed in Appendix A:

Contaminant	CAS Number
Hydrogen Fluoride	7664-39-3

Hydrogen fluoride is determined as fluoride ion by this method. A volume of 1 to 800 liters of air is drawn through a 0.8- μm cellulose ester membrane (to trap particulate fluorides) and a cellulose pad treated with sodium carbonate (to trap gaseous fluoride). The pad is extracted with reagent water and the extract is analyzed for fluoride by ion chromatography. The working range of the method is 0.04 - 8 mg/m^3 for 250-L samples. If other aerosols are present, gaseous fluoride may be slightly underestimated owing to adsorption onto or reaction with particles; with concurrent overestimation of particulate/gaseous fluoride ratio.

Source: <http://www.cdc.gov/niosh/nmam/pdfs/7906.pdf>

4.2.44 NIOSH Method S301-1: Fluoroacetate Anion

This method should be used for preparation and analysis of gas phase samples for the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Fluoroacetate salts	NA

This method was developed specifically for sodium fluoroacetate, but also may be applicable to other fluoroacetate salts. A known volume of air, 480 L, is drawn through a cellulose ester membrane filter to collect sodium fluoroacetate. Sodium fluoroacetate is extracted from the filter with 5 mL of deionized water, and the resulting sample is analyzed by ion chromatography using electrolytic conductivity detection. The analytical range of this method is estimated to be 0.01 - 0.16 mg/m³. The detection limit of the analytical method is estimated to be 20 ng of sodium fluoroacetate per injection, corresponding to a 100- μ L aliquot of a 0.2 μ g/mL standard.

Source: <http://www.cdc.gov/niosh/pdfs/s301.pdf>

4.2.45 OSHA Method ID-188: Ammonia in Workplace Atmospheres - Solid Sorbent

This method should be used for preparation and analysis of gas phase samples for the contaminant identified below and listed in Appendix A:

Contaminant	CAS Number
Ammonia	7664-41-7

In this method, ammonia is determined as ammonium ion. A volume of 7.5 to 24 liters of air is drawn through a sulfuric acid-treated carbon bead sorbent. The sorbent is extracted with reagent water and the extract analyzed for ammonium by ion chromatography. The detection limit for the method is 0.60 ppm for 24-L samples and the quantitation limit is 1.5 ppm for 24-L samples. Volatile amines, monethanolamine, isopropanolamine, and propanolamine may be interferences. Particulate ammonium salts can be a positive interference (trapped on the glass wool filter plug in the sorbent tube).

Source: <http://www.osha-slc.gov/dts/sltc/methods/inorganic/id188/id188.html>

4.2.46 OSHA Method ID-216SG: Boron Trifluoride (BF₃)

This method should be used for preparation and analysis of gas phase samples for the contaminant identified below and listed in Appendix A:

Contaminant	CAS Number
Boron Trifluoride	7637-07-2

Boron trifluoride is determined as fluoroborate by this method. A volume of 30 to 480 liters of air is drawn through a bubbler containing 0.1 M ammonium fluoride. The solution is diluted and analyzed with a fluoroborate ion specific electrode (ISE). The detection limit is 10 μ g in a 30-L sample.

Source: <http://www.osha-slc.gov/dts/sltc/methods/partial/id216sg/id216sg.html>

4.2.47 Standard Method 4110 B: Ion Chromatography with Chemical Suppression of Eluent Conductivity

This method should be used for preparation and analysis of aqueous/liquid phase samples for the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Hydrogen Bromide	10035-10-6
Hydrogen Chloride	7647-01-0

The contaminants are determined as bromide and chloride respectively by this method. Aqueous/liquid samples are pre-filtered and injected onto the ion chromatograph. The anions are identified on the basis of retention time and quantified by measurement of peak area or height. The method can detect bromide and chloride at 0.1 mg/L. Lower values can be achieved using a higher scale setting and an electronic integrator. Other salts of the anions are a positive interference. Low molecular weight organic acids may interfere with chloride.

Source: American Public Health Association, American Water Works Association, and Water Environment Federation. 1998. *Standard Methods for the Examination of Water and Wastewater*. 20th Edition. (<http://www.standardmethods.org/>)

4.2.48 Standard Method 4500-NH₃ B: Preliminary Distillation Step

This method should be used for preparation of aqueous/liquid phase samples for the contaminant identified below and listed in Appendix A:

Contaminant	CAS Number
Ammonia	7664-41-7

A 0.5 - 1 L water sample is dechlorinated, buffered, adjusted to pH 9.5, and distilled into a sulfuric acid solution. The distillate is brought up to volume and neutralized with sodium hydroxide. The distillate is analyzed by Method 4500-NH₃ G.

Source: American Public Health Association, American Water Works Association, and Water Environment Federation. 1998. *Standard Methods for the Examination of Water and Wastewater*. 20th Edition. (<http://www.standardmethods.org/>)

4.2.49 Standard Method 4500-NH₃ G: Automated Phenate Method

This method should be used for analysis of aqueous/liquid phase samples for the contaminant identified below and listed in Appendix A:

Contaminant	CAS Number
Ammonia	7664-41-7

Ammonia is determined as indophenol blue by this method. A portion of the neutralized distillate from procedure 4500-NH₃ B is run through the manifold described in the method. The ammonium in the distillate reacts with solutions of disodium EDTA, sodium phenate, sodium hypochlorite, and sodium

nitroprusside. The resulting indophenol blue is detected by colorimetry in a flow cell. The range of the method is 0.02 - 2.0 mg/L.

Source: American Public Health Association, American Water Works Association, and Water Environment Federation. 1998. *Standard Methods for the Examination of Water and Wastewater*. 20th Edition. (<http://www.standardmethods.org/>)

4.2.50 Standard Method 4500-Cl G: DPD Colorimetric Method

This method should be used for preparation of aqueous/liquid phase samples for the contaminant identified below and listed in Appendix A:

Contaminant	CAS Number
Chlorine	7782-50-5

A portion of aqueous/liquid sample is buffered and reacted with N,N-diethyl-p-phenylenediamine (DPD) color agent. The resulting free chlorine is determined by colorimetry. If total chlorine (including chloramines and nitrogen trichloride) is to be determined, KI crystals are added. Results for chromate and manganese are blank corrected using thioacetamide solution. The method can detect 10 µg/L chlorine. Organic contaminants and strong oxidizers may cause interference.

Source: American Public Health Association, American Water Works Association, and Water Environment Federation. 1998. *Standard Methods for the Examination of Water and Wastewater*. 20th Edition. (<http://www.standardmethods.org/>)

4.2.51 IO Compendium Method IO-3.1: Selection, Preparation, and Extraction of Filter Material

This method should be used for preparation and analysis of gas phase samples for the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Arsenic (III) compounds	22569-72-8
Arsenic trichloride	7784-34-1
Cadmium	7440-43-9

A subsample (one-ninth of the overall filter) is obtained by cutting a strip from the filter used to collect the sample. The filter strip is extracted using hydrochloric/nitric acid mix and microwave or hotplate heating. The extract is filtered and worked up to 20 mL. The extract is analyzed by compendium methods IO-3.4 or IO-3.5.

Source: <http://www.epa.gov/ttn/amtic/files/ambient/inorganic/mthd-3-1.pdf>

4.2.52 IO Compendium Method IO-3.4: Determination of Metals in Ambient Particulate Matter Using Inductively Coupled Plasma (ICP) Spectroscopy

This method should be used for preparation and analysis of gas phase samples for the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Arsenic (III) compounds	22569-72-8
Arsenic Trichloride	7784-34-1
Cadmium	7440-43-9
Osmium Tetroxide	20816-12-0

All analytes are determined as the metal by this method. Ambient air is sampled by high-volume filters using compendium method IO-2.1 (a sampling method). The filters are extracted by compendium method IO-3.1 and the extracts analyzed by Inductively Couple Plasma - Atomic Emission Spectroscopy (ICP-AES). Detection limits, ranges, and interference corrections are dependent on the analyte and the instrument used.

Source: <http://www.epa.gov/ttn/amtic/files/ambient/inorganic/mthd-3-4.pdf>

4.2.53 IO Compendium Method IO-3.5: Determination of Metals in Ambient Particulate Matter Using Inductively Coupled Plasma/Mass Spectrometry (ICP/MS)

This method should be used for preparation and analysis of gas phase samples for the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Arsenic (III) compounds	22569-72-8
Arsenic trichloride	7784-34-1
Arsine	7784-42-1
Cadmium	7440-43-9

All analytes are determined as the metal by this method. Ambient air is sampled by high-volume filters using compendium method IO-2.1 (a sampling method). The filters are extracted by compendium method IO-3.1 and the extracts analyzed by Inductively Couple Plasma/Mass Spectrometry (ICP/MS). Detection limits, ranges, and interference corrections are dependent on the analyte and the instrument used.

Source: <http://www.epa.gov/ttn/amtic/files/ambient/inorganic/mthd-3-5.pdf>

4.2.54 IO Compendium Method IO-5: Sampling and Analysis for Vapor and Particle Phase Mercury in Ambient Air Utilizing Cold Vapor Atomic Fluorescence Spectrometry (CVAFS)

This method should be used for preparation of analysis of gas phase samples for the contaminant identified below and listed in Appendix A:

Contaminant	CAS Number
Mercury	7439-97-6

Vapor phase mercury is collected using gold-coated glass bead traps at a flow rate of 0.3 L/min. The traps are directly desorbed onto a second (analytical) trap. The mercury desorbed from the analytical trap is determined by Atomic Fluorescence Spectrometry. Particulate mercury is sampled on glass-fiber filters at a flow rate of 30 L/min. The filters are extracted with nitric acid and microwave heating. The extract is oxidized with BrCl, then reduced with stannous chloride and purged from solution onto a gold-coated glass bead trap. This trap is desorbed onto a second trap, the second trap is desorbed, and the mercury is determined by Atomic Fluorescence Spectrometry. The detection limits are 30 pg/m³ for particulate mercury and 45 pg/m³ for vapor mercury. Detection limits, analytical range, and interferences are dependent on the instrument used. There are no known positive interferences at 253.7 nm wavelength. Water vapor will cause a negative interference.

Source: <http://www.epa.gov/ttn/amtic/files/ambient/inorganic/mthd-5.pdf>

4.2.55 EPA Air Toxics Method - 6 (TO-6): Method for the Determination of Phosgene in Ambient Air Using High Performance Liquid Chromatography

This method should be used for preparation and analysis of gas phase samples for the contaminant identified below and listed in Appendix A:

Contaminant	CAS Number
Phosgene	75-44-5

This method can be used to detect phosgene in air at the 0.1 ppbv level. Ambient air is drawn through a midjet impinger containing 10 mL of 2/98 aniline/toluene (by volume). Phosgene readily reacts with aniline to form carbanilide (1,3-diphenylurea), which is stable indefinitely. After sampling, the impinger contents are transferred to a screw capped vial having a Teflon-lined cap and returned to the laboratory for analysis. The solution is taken to dryness by heating, and the residue is dissolved acetonitrile. Carbanilide is determined in the acetonitrile solution using reverse-phase HPLC with an ultraviolet (UV) absorbance detector operating at 254 nm. Precision for phosgene spiked into a clean air stream is ±15-20% relative standard deviation. Recovery is quantitative within that precision, down to less than 3 ppbv.

Source: <http://www.epa.gov/ttn/amtic/files/ambient/airtox/to-6.pdf>

4.2.56 EPA Air Toxics Method - 13A (TO-13A): Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Ambient Air Using Gas Chromatography/Mass Spectrometry (GC/MS)

This method should be used for preparation and analysis of gas phase samples for the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Aldicarb (Temik)	116-06-3
Bromadiolone	28772-56-7
Carbofuran (Furadan)	1563-66-2
Chloropicrin	76-06-2
Chlorosarin	1445-76-7
Chlorosoman	7040-57-5
Cyclohexyl Sarin (GF)	329-99-7
Dichlorvos	62-73-7
Dicrotophos	141-66-2
Dimethylphosphite	868-85-9
Distilled Mustard (HD)/Mustard Gas (H)	505-60-2
Ethylchloroarsine (ED)	598-14-1
Fenamiphos	22224-92-6
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3
Lewisite 1 (L-1)	541-25-3
Lewisite 2 (L-2)	40334-69-8
Lewisite 3 (L-3)	40334-70-1
Methyl parathion	298-00-0
Mevinphos	7786-34-7
Nicotine	54-11-5
Nitrogen Mustard (HN-2) [unstable compound]	51-75-2
O-ethyl-S-(2-diisopropylaminoethyl)methyl phosphonothiolate (VX)	50782-69-9
Oxamyl	23135-22-0
Perfluoroisobutylene (PFIB)	382-21-8
Phencyclidine	60124-79-0
Phorate	298-02-2
Sarin (GB)	107-44-8

Contaminant	CAS Number
Semivolatile Organic Compounds, NOS	NA
Soman (GD)	96-64-0
Strychnine	57-24-9
Tabun (GA)	77-81-6
Tear Gas (CS), chlorobenzylidene malonitrile	2698-41-1
Tetraethyl pyrophosphate	107-49-3
Tetramethylenedisulfotetramine	80-12-6
Trimethyl phosphite	121-45-9
VE	21738-25-0
VG	78-53-5
VM	21770-86-5

Approximately 300 m³ of air is drawn through the filter and sorbent cartridge using a high-volume flow rate air sampler or equivalent. The filters and sorbent cartridge are extracted by Soxhlet extraction with appropriate solvent. The extract is concentrated by Kuderna-Danish (K-D) evaporator, followed by silica gel cleanup using column chromatography to remove potential interferences prior to analysis by GC/MS. With optimization to reagent purity and analytical conditions, the detection limits for the GC/MS method range from 1 ng to 10 pg based on field experience.

Source: <http://www.epa.gov/ttn/amtic/files/ambient/airtox/to-13arr.pdf>

4.2.57 EPA Air Toxics Method - 15 (TO-15): Determination of Volatile Organic Compounds (VOCs) in Air Collected in Specially-Prepared (Summa) Canisters and Analyzed by Gas Chromatography/Mass Spectrometry (GC/MS)

This method should be used for preparation and analysis of gas phase samples for the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Carbon disulfide	75-15-0
Cyanogen chloride	506-77-4
Ethylene oxide	75-21-8
Formaldehyde	50-00-0
Volatile Organic Compounds, NOS	NA

The atmosphere is sampled by introduction of air into a specially prepared stainless steel canister (summa). A sample of air is drawn through a sampling train comprised of components that regulate the rate and duration of sampling into the pre-evacuated and passivated summa canister. After the air sample is collected, the canister valve is closed, an identification tag is attached to the canister, and the canister is transported to the laboratory for analysis. To analyze the sample, a known volume of sample is directed

from the canister through a solid multisorbent concentrator. After the concentration and drying steps are completed, the VOCs are thermally desorbed, entrained in a carrier gas stream, and then focused in a small volume by trapping on a cryo-focusing (ultra-low temperature) trap or small volume multisorbent trap. The sample is then released by thermal desorption and analyzed by GC/MS. This method applies to ambient concentrations of VOCs above 0.5 ppbv and typically requires VOC enrichment by concentrating up to 1-L of a sample volume.

Source: <http://www.epa.gov/ttn/amtic/files/ambient/airtox/to-15r.pdf>

4.2.58 Journal of Analytical Atomic Spectrometry, 2000, 15, pp. 277-279: Boron Trichloride Analysis

This method should be used for preparation and analysis of gas phase samples for the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Boron trichloride	10294-34-5

An analytical procedure is described for the determination of boron trichloride by ICP-AES. A modified sampling and gas introduction system allows on-line monitoring of the investigated gas. The gas-phase sample is introduced into an aqueous mannitol solution, and the boron trichloride hydrolyzed to boric acid. Since this method is designed for measuring dichlorosilane, the detection limit was found to be 0.63 µg of boron/g dichlorosilane.

Source: Journal of Analytical Atomic Spectrometry, 2000, 15, pp. 277-279: “On-line monitoring of boron in dichlorosilan by means of inductively coupled plasma atomic emission spectrometry,” Royal Society of Chemistry. <http://pubs.rsc.org/ej/JA/2000/a908634j.pdf>

4.2.59 Analytical Letters, 1994, 27 (14), pp. 2703-2718: Screening-Procedure for Sodium Fluoroacetate (Compound 1080) at Sub-Microgram/Gram Concentrations in Soils

This method should be used for preparation of solid and oily-solid phase samples for the contaminants identified below and listed in Appendix A:

Contaminant	CAS Number
Fluoroacetate salts	NA

Sodium fluoroacetate (Compound 1080) is readily quantitated at sub-microgram per gram concentrations in small (ca. 1 g) soil samples. Samples are ultrasonically extracted with water, which is then partitioned with hexane, and acidified prior to re-extraction with ethyl acetate. The latter is taken to dryness in the presence of triethanolamine “keeper,” and the resulting acid is derivatized with pentafluorobenzyl bromide. Quantitation is performed using a gas chromatograph equipped with an electron-capture detector. A standardized statistical protocol is used to validate a screening level of 0.2 µg Compound 1080/g soil. Difluoroacetic, trifluoroacetic, and naturally occurring formic acids do not interfere with the determination. The recovery for Compound 1080 was 40% from soil fortified to 0.2 µg/g soil.

Source: Tomkins, B.A., “Screening-Procedure for Sodium Fluoroacetate (Compound 1080) at Sub-Microgram/Gram Concentrations in Soils,” *Analytical Letters*, 27(14), 2703-2718 (1994).

Section 5.0: Biological Methods

The purpose of this section is to provide analytical methods for the analysis of environmental samples for biological agents in response to a homeland security event.

Protocols from peer reviewed journal articles have been identified for analyte-matrix pairs where methods are not available. It should be noted that the limitations of these protocols are not the same as the limitations of the standardized methods that have been identified. Future steps include the development of standardized methods based on the journal protocols. The literature references will be replaced with standardized, validated protocols as they become available.

Although culture-based methods have been selected for the bacterial pathogens, for viruses, PCR techniques will be used because of the difficulty and time required to culture viruses. It should be noted that PCR techniques have limitations such as the inability to determine viability or infectivity of the analyte.

Sample collection and handling protocols are not available for all matrices included in this document. Future research will include the development and validation of sampling protocols.

A complete list of recommended biological methods for use in response to homeland security events is provided in Appendix B. The following information is included in Appendix B, organized by type of organism (e.g., bacteria, virus, protozoa):

- **Analyte(s).** The contaminant or contaminant(s) of interest.
- **Determinative technique.** An analytical instrument or technique used to determine the quantity and identification of compounds or components in a sample.
- **Determinative method identifier.** The unique identifier or number assigned to an analytical method by the method publisher.
- **Sample preparation procedure and/or sampling method.** The recommended sample preparation procedure and/or sample collection procedure for the analyte-matrix combination.

5.1 General Guidance

The guidance summarized in this section provides a general overview of how to identify the appropriate biological method(s) for a given analyte-matrix combination as well as recommendations for quality control procedures.

For additional information on the properties of the biological agents listed in Appendix B, TOXNET (<http://toxnet.nlm.nih.gov/index.html>), a cluster of databases on toxicology, hazardous chemicals, and related areas maintained by the National Library of Medicine is an excellent resource. Additional information also can be found on CDC's Emergency Preparedness and Response website (<http://www.bt.cdc.gov/>). Further research on biological agents is ongoing within EPA and databases to manage this information are currently under development.

5.1.1 Standard Operating Procedures for Identifying Biological Methods

To determine the appropriate method and sample preparation procedure and/or sampling method that is to be used on the environmental samples, locate the analyte of interest in Appendix B: Biological Methods under the “Analyte” column.

After locating the analyte of interest, continue across the table to identify the appropriate matrix (e.g., water, dust, aerosol). This will identify the determinative technique, determinative method, and sample preparation procedure and/or sampling method for the analyte of interest.

Sections 5.2.1 through 5.2.15 below provide summaries of the analytical methods listed in Appendix B. Where available, a direct link to the full text of the selected method is provided in the method summary. For additional information on preparation procedures and methods available through consensus standards organizations, please use the contact information provided in Table 2.

Table 2. Sources of Biological Methods

Name	Publisher	Reference
National Environmental Methods Index (NEMI)	EPA, USGS	http://www.nemi.gov
U.S. EPA Microbiology Methods	EPA	http://www.epa.gov/microbes/
USDA/FSIS Microbiology Laboratory Guidebook	USDA Food Safety and Inspection Service	http://www.fsis.usda.gov/OPHS/microlab/mlgbook.htm
ICR Microbial Laboratory Manual	EPA Office of Research and Development	http://www.epa.gov/nerlcwww/icrmicro.pdf
Occupational Safety and Health Administration Methods	OSHA	http://www.osha-slc.gov/dts/sltc/methods/toc.html
National Institutes for Occupational Safety and Health Methods	NIOSH	http://www.cdc.gov/niosh/nmam/
Standard Methods for the Examination of Water and Wastewater, 20 th Edition	American Public Health Association and American Water Works Association	http://www.apha.org http://www.awwa.org ISBN: 0875532357
Annual Book of ASTM Standards	ASTM International	http://www.astm.org
Applied and Environmental Microbiology	American Society for Microbiology	http://www.asm.org
Journal of Clinical Microbiology	American Society for Microbiology	http://www.asm.org

5.1.2 General Quality Control (QC) Guidance for Biological Methods

Having data of known and documented quality is critical in order for public officials to accurately assess how to respond to emergency situations. Having such data requires that laboratories: (1) conduct the necessary QC to ensure that measurement systems are in control and operating properly, (2) properly document results of the analyses, and (3) properly document measurement system evaluation of the analysis-specific QC. Ensuring data quality also requires that laboratory results are properly evaluated and the results of the data quality evaluation are transmitted to decision makers.

While method-specific QC requirements are described in many of the individual methods that are cited in this manual, and will be included in any standardized analytical protocols developed to address specific analytes and matrices of concern, the following describes a minimum set of QC procedures that shall be conducted for all biological testing. Individual methods, sampling and analysis protocols, or contractual statements of work also should be consulted to determine any additional QC that may be needed. These QC requirements generally consist of analysis of laboratory control samples and or matrix spikes to identify and quantify measurement system accuracy at the levels of concern, blanks as a measure of freedom from contamination, and matrix spike duplicates (MSD) or sample replicates to assess data precision. QC tests should be run as frequently as necessary to ensure the reliability of analytical results.

In general, sufficient QC includes an initial demonstration of measurement system capability as well as ongoing analysis of standards and other samples to ensure the continued reliability of the analytical results. Examples of sufficient QC includes:

- Demonstration that measurement system is operating properly
 - ▶ Initial calibration (for molecular techniques)
 - ▶ Method blanks
- Demonstration of initial and ongoing method performance
 - ▶ Precision and recovery
 - ▶ Matrix spike/matrix spike duplicates
 - ▶ QC samples
 - ▶ Method blanks

Please note: The appropriate point of contact identified in Section 3 should be consulted regarding appropriate quality assurance and quality control procedures prior to sample analysis.

5.1.3 Safety and Waste Management

It is imperative that safety precautions are used during collection, processing, and analysis of environmental samples, particularly in emergency response situations that may include unknown hazards. Many of the methods summarized or cited in Section 5.2 contain specific requirements, guidance, or information regarding safety precautions that should be followed when handling or processing environmental samples and reagents. These methods also provide information regarding waste management. Additional resources that can be consulted for additional information include the following:

- Environmental Protection Agency's standards regulating hazardous waste (40 CFR parts 260 - 270)
- Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, found at www.cdc.gov/od/ohs/biosfty/bmb14toc.htm

- Laboratory Security and Emergency Response Guidance for Laboratories Working with Select Agents, December 6, 2002 / 51 (RR19); 1-8, found at www.cdc.gov/mmwr/preview/mmwrhtml/rr5119a1.htm.

5.2 Method Summaries

Method summaries for the analytical methods listed in Appendix B are provided in Sections 5.2.1 - 5.2.15. Each method summary contains a table identifying the contaminants in Appendix B to which the method applies, provides a brief description of the analytical method, and includes a link to the full version of the method or source for obtaining a full version of the method. Any questions regarding information discussed in this section should be addressed to the appropriate contact(s) listed in Section 3.

5.2.1 Laboratory Response Network (LRN)

The agents identified below and listed in Appendix B should be analyzed in accordance with the appropriate LRN protocols:

Analyte(s)	Agent Category
<i>Bacillus anthracis</i> (Anthrax)	Bacteria
<i>Brucella</i> spp. (Brucellosis)	Bacteria
<i>Burkholderia mallei</i> (Glanders)	Bacteria
<i>Burkholderia pseudomallei</i> (Melioidosis)	Bacteria
<i>Francisella tularensis</i> (Tularemia)	Bacteria
<i>Coxiella burnetti</i> (Q-fever)	Bacteria
<i>Yersina pestis</i> (Plague)	Bacteria
Variola major (Smallpox)	Viruses

These agents will be analyzed using restricted procedures available only through the Laboratory Response Network (LRN). These procedures are not available to the general laboratory community and thus are not discussed within this document. For additional information on the LRN, please use the contact information provided below or visit <http://www.bt.cdc.gov/lrn/>.

Centers for Disease Control and Prevention

Laboratory Response Branch
 Bioterrorism Preparedness and Response Program
 National Center for Infectious Diseases
 1600 Clifton Road NE, Mailstop C-18
 Atlanta, GA 30333
 Telephone: (404) 639-2790
 E-mail: lrn@cdc.gov

Local public health laboratories, private laboratories, and commercial laboratories with questions about the LRN should contact their State public health laboratory director or the Association of Public Health Laboratories (contact information provided below).

Association of Public Health Laboratories

2025 M Street NW, Suite 550
 Washington, DC 20036
 Telephone: (202) 822-5227
 Fax: (202) 887-5098
 Website: www.aphl.org
 E-mail: info@aphl.org

5.2.2 Biosafety Level 4 Viruses

Samples to be analyzed for the viruses identified below and listed in Appendix B should be analyzed under biosafety level (BSL) 4 conditions at the Centers for Disease Control and Prevention (CDC):

Analyte(s)	Agent Category
Arenaviruses	Viruses
Nairovirus	Viruses
Hemorrhagic fever viruses	Viruses
Rift vally fever	Viruses
Variola major (Smallpox)	Viruses

For additional information on the LRN, please use the contact information provided below or visit <http://www.bt.cdc.gov/lrn/>.

Centers for Disease Control and Prevention

Laboratory Response Branch
 Bioterrorism Preparedness and Response Program
 National Center for Infectious Diseases
 1600 Clifton Road NE, Mailstop C-18
 Atlanta, GA 30333
 Telephone: (404) 639-2790
 E-mail: lrn@cdc.gov

5.2.3 U.S. EPA Method 1622: *Cryptosporidium* in Water by Filtration/IMS/FA

This method should be used for the contaminant identified below and listed in Appendix B:

Analyte(s)	Agent Category
<i>Cryptosporidium parvum</i> (Cryptosporidiosis)	Protozoa

A water sample is filtered and the oocysts and extraneous materials are retained on the filter. Materials on the filter are eluted, the eluate is centrifuged to pellet the oocysts, and the supernatant fluid is aspirated. The oocysts are magnetized by attachment of magnetic beads conjugated to

anti-*Cryptosporidium* antibodies. The magnetized oocysts are separated from the extraneous materials using a magnet, and the extraneous materials are discarded. The magnetic bead complex is then detached from the oocysts. The oocysts are stained on well slides with fluorescently labeled monoclonal antibodies and 4',6-diamidino-2-phenylindole (DAPI). The stained sample is examined using fluorescence and differential interference contrast (DIC) microscopy. Qualitative analysis is performed by scanning each slide well for objects that meet the size, shape, and fluorescence characteristics of *Cryptosporidium* oocysts. Quantitative analysis is performed by counting the total number of objects on the slide confirmed as oocysts.

Please note: This method was originally developed for water matrices; further research will be conducted to develop and standardize sample processing protocols for other matrices.

Source: USEPA. 2001. *Cryptosporidium* in Water by Filtration/IMS/FA (EPA-821-R-01-026). United States Environmental Protection Agency, Washington, D.C. (<http://www.epa.gov/nerlcwww/1622ap01.pdf>)

5.2.4 ICR Microbial Laboratory Manual Chapter XI

This method should be used for the contaminant identified below and listed in Appendix B:

Analyte(s)	Agent Category
<i>Clostridium perfringens</i>	Bacteria

An appropriate volume of water sample is passed through a membrane filter that retains the bacteria present in the sample. The membrane filter is placed on mCP agar and incubated anaerobically for 24 h at 44.5° C. Upon exposure to ammonium hydroxide, the yellow straw-colored *C. perfringens* colonies turn dark pink to magenta and are counted as presumptive *C. perfringens*. Biochemical confirmation includes anaerobic growth in thioglycollate, a positive gram stain reaction and stormy fermentation of iron milk.

Please note: This procedure has not been fully validated. At a minimum, the following sample processing quality control checks should be performed and evaluated before using this protocol: positive control, negative control, and blank. This method was originally developed for water matrices; further research will be conducted to develop and standardize sample processing protocols for other matrices.

Source: United States Environmental Protection Agency, Office of Research and Development. 1996. *ICR Microbial Laboratory Manual Chapter XI*. EPA/600/R-95/178. (<http://www.epa.gov/microbes/icrmicro.pdf>)

5.2.5 USDA/FSIS 4

This method should be used for the contaminant identified below and listed in Appendix B:

Analyte(s)	Agent Category
<i>Salmonella typhi</i> (Typhoid fever)	Bacteria

Samples are added to buffered peptone water and incubated for 24 hours. An aliquot of the broth is added to both tetrathionate broth and modified Rappaport-Vassiliadis broth and incubated for 24 hours. An aliquot of each broth is streaked onto Brilliant green sulfa agar, Xylose lysine Tergitol™ 4 agar , or

Double modified lysine iron agar and incubated for 24 hours. Isolates are inoculated into triple sugar iron and lysine iron agar slants. Additional biochemical tests are performed using commercially available biochemical test kits. Serological testing is done using polyvalent O antisera. The method addendum (4A) allows for the use of a rapid screening immunoassay kit.

Please note: This procedure has not been fully validated. At a minimum, the following sample processing quality control checks should be performed and evaluated before using this protocol: positive control, negative control, and blank.

Source: Isolation and Identification of *Salmonella* from Meat, Poultry, and Egg Products. 3rd Edition 1998. <http://www.fsis.usda.gov/Ophs/Microlab/MIg4.02.pdf>; <http://www.fsis.usda.gov/Ophs/Microlab/MIg4C01.pdf>

5.2.6 Standard Methods 9260 E: *Shigella* spp.

This method should be used for the contaminant identified below and listed in Appendix B:

Analyte(s)	Agent Category
<i>Shigella</i> spp.	Bacteria

This method contains two options for sample concentration: membrane filtration (liquid matrices) and centrifugation (liquid and solid matrices) for analyses. Both options include inoculation of an enrichment medium (Selenite F broth). Isolation of the target analyte is achieved by plating onto XLD and/or MacConkey agar. Biochemical identification consists of inoculating TSI and LIA slants. Serological grouping is done by slide agglutination tests using polyvalent and group specific antisera.

Please note: This procedure has not been fully validated. At a minimum, the following sample processing quality control checks should be performed and evaluated before using this protocol: positive control, negative control, and blank.

Source: American Public Health Association, American Water Works Association, and Water Environment Federation. 1998. *Standard Methods for the Examination of Water and Wastewater*. 20th Edition. (<http://www.standardmethods.org/>)

5.2.7 Standard Methods 9260 F: Pathogenic *Escherichia coli*

This method should be used for the contaminant identified below and listed in Appendix B:

Analyte(s)	Agent Category
<i>Escherichia coli</i> (<i>E. coli</i>) O157:H7	Bacteria

This method allows for two options, one being a modification of SM 9221B followed by plating and biochemical identification. The second option, modification of a food method, allows for the analysis of large sample volumes. A 200-mL water sample is centrifuged, resuspended in EHEC Enterohemorrhagic *E. coli* enrichment broth (EEB) and incubated for 6 hours. Tellurite Cefixime SMAC (TC SMAC) plates are inoculated with the EEB. Both the TC SMAC and EEB are incubated for up to 24 hours. Colorless colonies on TC SMAC are tested for indole production. Additional biochemical tests and serotyping are also done to confirm identification.

Please note: This procedure has not been fully validated. At a minimum, the following sample processing quality control checks should be performed and evaluated before using this protocol: positive control, negative control, and blank. This method was originally developed for liquid matrices; further research will be conducted to develop and standardize sample processing protocols for other matrices.

Source: American Public Health Association, American Water Works Association, and Water Environment Federation. 1998. *Standard Methods for the Examination of Water and Wastewater*. 20th Edition. (<http://www.standardmethods.org/>)

5.2.8 Standard Methods 9260 G: *Campylobacter jejuni*

This method should be used for the contaminant identified below and listed in Appendix B:

Analyte(s)	Agent Category
<i>Campylobacter jejuni</i>	Bacteria

Water samples are filtered using a cellulose nitrate membrane filter. Filters are placed face down on either Skirrow’s medium or Campy-BAP and incubated for 24 hours. Filters are then transferred to another selective medium face-down and incubated for a total of 5 days at 42°C under microaerophilic conditions. Identification is made by culture examination, microscopy, motility test, and biochemical testing. Biochemical tests include oxidase, catalase, nitrite and nitrate reduction, H₂S production, and hippurate hydrolysis. Serotyping is done using commercially available rapid test kits.

Please note: This procedure has not been fully validated. At a minimum, the following sample processing quality control checks should be performed and evaluated before using this protocol: positive control, negative control, and blank. This method was originally developed for water matrices; further research will be conducted to develop and standardize sample processing protocols for other matrices.

Source: American Public Health Association, American Water Works Association, and Water Environment Federation. 1998. *Standard Methods for the Examination of Water and Wastewater*. 20th Edition. (<http://www.standardmethods.org/>)

5.2.9 Standard Methods 9260 H: *Vibrio cholerae*

This method should be used for the contaminant identified below and listed in Appendix B:

Analyte(s)	Agent Category
<i>Vibrio cholerae</i> (cholera)	Bacteria

Samples are enriched in alkaline peptone broth and incubated for up to 8 hours. Thiosulfate-citrate-bile salts-sucrose TCBS agar plates are inoculated with the incubated broth and incubated for 24 hours. *Vibrio cholerae* isolates are plated on tryptic soy agar with 0.5% NaCl. Biochemical confirmation is done using multiple tests including but not limited to ONPG, Indole, and Voges-Proskauer. Slide agglutination assays are used for serological identification.

Please note: This procedure has not been fully validated. At a minimum, the following sample processing quality control checks should be performed and evaluated before using this protocol: positive control,

negative control, and blank. This method was originally developed for water matrices, further research will be conducted to develop and standardize sample processing protocols for other matrices.

Source: American Public Health Association, American Water Works Association, and Water Environment Federation. 1998. *Standard Methods for the Examination of Water and Wastewater*. 20th Edition. (<http://www.standardmethods.org/>)

5.2.10 Literature Reference for Caliciviruses

This method should be used for the contaminant identified below and listed in Appendix B:

Analyte(s)	Agent Category
Caliciviruses	Viruses

This molecular detection method is used for the concentration, extraction, and RT-PCR analysis of human *Noroviruses* in water and clinical (stool) samples. Water samples are collected by filtration (1MDS filter) and viruses are eluted using a beef extract solution (1.5%, pH 9.0). Viruses are concentrated using celite adsorption (pH 4.0), filtration, and elution with sodium phosphate (0.15 M, pH 9.0), followed by further concentration and processing to remove inhibitors (ultracentrifugation, solvent extraction, and MW-exclusion filtration). Concentrated viruses are used either directly or following RNA extraction (GITC) for two-step (RT followed by PCR) RT-PCR analysis using gene-specific (norovirus pol gene) primer sets. Detection of amplicons is by agarose gel electrophoresis and subsequent confirmation (genotyping) by sequence analysis of cloned amplicons.

Please note: This procedure has not been fully validated. At a minimum, the following sample processing quality control checks should be performed and evaluated before using this protocol: positive control, negative control, and blank. PCR quality control checks should be performed according to the current version of the *EPA Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples*. Please call the point of contact identified in Section 3 for additional information. This method was originally developed for water matrices; further research will be conducted to develop and standardize sample processing protocols for other matrices.

Source: Parshionikar, S. U., Willian-True, S., Fout., G. S., Robbins, D. E., Seys, S. A., Cassady, J. D., and Harris, R. 2003. *Waterborne Outbreak of Gastroenteritis Associated with a Norovirus*. Applied and Environmental Microbiology 69(9): 5263-5268. <http://aem.asm.org/cgi/reprint/69/9/5263>

5.2.11 Literature Reference for Enteroviruses

This method should be used for the contaminant identified below and listed in Appendix B:

Analyte(s)	Agent Category
Enteroviruses	Viruses

This molecular detection method is used for the simultaneous detection of several human enteric viruses (enteroviruses, reoviruses, and rotaviruses) in groundwater using a multiplex RT-PCR approach. Water samples are collected by filtration (1MDS filters); and viruses are eluted using a beef extract solution (1.5%, pH 9.5). Viruses are concentrated using celite adsorption (pH 4.0), filtration, and elution with sodium phosphate (0.15 M, pH 9.0), followed by further concentration and processing to remove

inhibitors (ultracentrifugation, solvent extraction, and MW-exclusion filtration). Concentrated samples are analyzed by a two-step multiplex RT-PCR (RT followed by PCR) using virus-specific primer sets. Detection of amplicons is by gel electrophoresis and subsequent confirmation by hybridization (dot-blot) using digoxigenin-labeled internal (nested) probes.

Please note: This procedure has not been fully validated. At a minimum, the following sample processing quality control checks should be performed and evaluated before using this protocol: positive control, negative control, and blank. PCR quality control checks should be performed according to *EPA Draft Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* document or call the point of contact identified in Section 3. This method was originally developed for water matrices, further research will be conducted to develop and standardize sample processing protocols for other matrices.

Source: Fout, G. S., Martinson, B. C., Moyer, M. W. N., and Dahling, D. R. 2003. *A Multiplex Reverse Transcription-PCR Method for Detection of Human Enteric Viruses in Groundwater*. Applied and Environmental Microbiology Vol. 69 No. 6: 3158-3164. (<http://aem.asm.org/cgi/reprint/69/6/3158.pdf>)

5.2.12 Literature Reference for Hepatitis A Viruses

This method should be used for the contaminant identified below and listed in Appendix B:

Analyte(s)	Agent Category
Hepatitis A Viruses	Viruses

This method is used to detect Hepatitis A virus in groundwater samples. It is a multiplex RT-PCR procedure optimized for hepatitis A viruses. Water samples are collected by filtration (1MDS filters) and viruses are eluted using a beef extract solution (1.5%, pH 9.5). Viruses are concentrated using celite adsorption (pH 4.0), filtration, and elution with sodium phosphate (0.15 M, pH 9.0), followed by further concentration and processing to remove inhibitors (ultracentrifugation, solvent extraction, and MW-exclusion filtration). Concentrated samples are analyzed by a two-step multiplex RT-PCR (RT followed by PCR) using two hepatitis A-specific primer sets. Detection of amplicons is by gel electrophoresis with subsequent confirmation by hybridization (dot-blot) using digoxigenin-labeled internal (nested) probes.

Please note: This procedure has not been fully validated. At a minimum, the following sample processing quality control checks should be performed and evaluated before using this protocol: positive control, negative control, and blank. PCR quality control checks should be performed according to *EPA Draft Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* document or call the point of contact identified in Section 3. This method was originally developed for water matrices; further research will be conducted to develop and standardize sample processing protocols for other matrices.

Source: Fout, G. S., Martinson, B. C., Moyer, M. W. N., and Dahling, D. R. 2003. *A Multiplex Reverse Transcription-PCR Method for Detection of Human Enteric Viruses in Groundwater*. Applied and Environmental Microbiology. 69(6): 3158-3164. (<http://aem.asm.org/cgi/reprint/69/6/3158.pdf>)

5.2.13 Literature Reference for Venezuelan Equine Encephalitis (VEE) Virus

This method should be used for the contaminant identified below and listed in Appendix B:

Analyte(s)	Agent Category
Venezuelan Equine Encephalitis (VEE) Virus	Viruses

The VEE-specific RT-PCR assay is applied to human sera viruses that are isolated in either Vero cells, C6/36 cells, or newborn mice. VEE is identified using an indirect immunofluorescence assay. QIAmp viral RNA kit (Qiagen) is used to extract RNA without the use of TaqExtender and amplification is done using gene-specific RT-PCR–seminested PCR. Annealing temperature is 55°C in the thermocycler.

Please note: This procedure has not been fully validated for matrices other than human sera. At a minimum, the following sample processing quality control checks should be performed and evaluated before using this protocol: positive control, negative control, and blank. PCR quality control checks should be performed according to *EPA Draft Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* document or call the point of contact identified in Section 3. This method was originally developed for clinical matrices, further research will be conducted to develop and standardize sample processing protocols for other matrices.

Source: Linszen, B., Kinney, R. M., Aguilar, P., Russell, K. L., Watts, D. M., Kaaden, O., and Pfeffer M. 2000. *Development of Reverse Transcription-PCR Assays Specific for Detection of Equine Encephalitis Viruses*. *Journal of Clinical Microbiology*. 38(4):1527-1535. (<http://jcm.asm.org/cgi/reprint/38/4/1527.pdf>)

5.2.14 Literature Reference for *Toxoplasma gondii*

This method should be used for the contaminant identified below and listed in Appendix B:

Analyte(s)	Agent Category
<i>Toxoplasma gondii</i> (Toxoplasmosis)	Protozoa

This method uses PCR amplification of *T. gondii* oocyst DNA using gene-specific (B1 gene) primers (one primer end - labeled with biotin) and subsequent detection and confirmation by a sandwich hybridization capture assay and Enzyme Immunoassay (EIA). Amplicons are generated by PCR and are then denatured and hybridized with FITC-labeled internal (nested) oligoprobes. Following hybridization, amplicons are bound to avidin-coated (96-well) plates and reacted with anti-FITC monoclonal antibody conjugated to horseradish peroxidase. Following substrate reaction, hybridization products are detected colorimetrically (OD 450).

Please note: This procedure has not been fully validated. At a minimum, the following sample processing quality control checks should be performed and evaluated before using this protocol: positive control, negative control, and blank. PCR quality control checks should be performed according to *EPA Draft Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* document or call the point of contact identified in Section 3.

Source: Schwab, K. J. and McDevitt, J. J. 2003. *Development of a PCR-Enzyme Immunoassay Oligoprobe Detection Method for Toxoplasma gondii Oocysts, Incorporating PCR Controls*. *Applied and Environmental Microbiology*. 69(10):5819-5825.

5.2.15 *Entamoeba histolytica*: Filtration/IMS/PCR

This method should be used for the contaminant identified below and listed in Appendix B:

Analyte(s)	Agent Category
<i>Entamoeba histolytica</i>	Protozoa

A standardized method/protocol for the analysis of *Entamoeba histolytica* has not been identified. Further research will be conducted prior to the next revision of this document to identify an appropriate method/protocol. In the interim, PCR test kits may be obtained for analysis of this organism. Please contact the appropriate point of contact identified in Section 3 if the need to analyze for *Entamoeba histolytica* arises.

Section 6.0: Conclusions

Methods listed in Appendix A (chemical methods) and Appendix B (biological methods) should be used to assess the extent of contamination and the effectiveness of decontamination in response to a homeland security event.

As stated in the introduction, the primary objective of this effort was not necessarily to identify the “best” method, but rather to have a balanced approach between leveraging existing determinative techniques and methodologies and providing consistent analytical results. The method selected for each analyte matrix pair was the most general, appropriate, and broadly applicable of available methods. This is a living document and recommended methods are subject to change based on advances in technology.

Any questions concerning the information in this document should be directed to the appropriate point(s) of contact identified in Section 3.

Abbreviations and Acronyms

1 MDS	#1 Mark D. Sobsey filter
AEM	<i>Applied and Environmental Microbiology</i>
AGI sampler	All Glass Impinger Sampler
AOAC	AOAC International
ASTM	ASTM International
BSL	Biosafety Level
°C	Degrees Celsius
Campy-BAC	<i>Campylobacter-Brucella</i> agar base with sheep blood and antibiotics
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CLP	Contract Laboratory Program
CVAA	Cold Vapor Atomic Absorption
CVAFS	Cold Vapor Atomic Fluorescence Spectrometry
DAPI	4',6-diamidino-2-phenylindole
DHS	Department of Homeland Security
DIC	Differential Interference Contrast
DNA	Deoxyribonucleic Acid
DNPH	2,4-dinitrophenylhydrazine
DOD	Department of Defense
ED	Electron Diffraction
EDTA	Ethylenediaminetetraacetic acid
EDXA	Energy Dispersive X-ray Analysis

EEB	EHEC Enrichment Broth
EHEC	Enterohemorrhagic <i>Escherichia coli</i>
EIA	Enzyme Immunoassay
ELISA	Enzyme-Linked Immunosorbent Assay
EMMI	Environmental Monitoring Methods Index
EPA	U.S. Environmental Protection Agency
EQL	Estimated Quantitation Limit
FA	Fluorescence Assay
FBI	Forensic Bureau of Investigation
FDA	Food and Drug Administration
FID	Flame Ionization Detector
FITC	Fluorescein isothiocyanate
FSIS	Food Safety and Inspection Service
GC	Gas Chromatograph or Gas Chromatography
GC/MS	Gas Chromatograph/Mass Spectrometer or Gas Chromatography/Mass Spectrometry
GFAA	Graphite Furnace Atomic Absorption Spectrophotometer or Graphite Furnace Atomic Absorption Spectrophotometry
GITC	Guanidinium iso-thiocyanate
HAV	Hepatitis A Virus
HPLC	High Performance Liquid Chromatograph or High Performance Liquid Chromatography
IC	Ion Chromatograph or Ion Chromatography
ICP	Inductively Coupled Plasma
ICP-AES	Inductively Coupled Plasma - Atomic Emission Spectrometry
ICR	Information Collection Rule
IMS	Immunomagnetic Separation
IO	Inorganic

ISO	International Organization for Standardization
ISE	Ion Specific Electrode
K-D	Kuderna-Danish
LIA	Lysine Iron Agar
LRN	Laboratory Response Network
LSE	Liquid/Solid Extraction
MCP	Membrane <i>Clostridium perfringens</i>
MS	Mass Spectrometer or Mass Spectrometry
MSD	Matrix Spike Duplicate
MW	Molecular Weight
NA	Not Applicable
NEMI	National Environmental Methods Index
NERL-CI	National Exposure Risk Laboratory-Cincinnati
NHSRC	National Homeland Security Research Center
NIOSH	National Institute for Occupational Safety and Health
NOS	Not Otherwise Specified
NTIS	National Technical Information Service
ONPG	Ortho-nitrophenyl- β -D-galactopyranoside
OSHA	Occupational Safety and Health Administration
OW	Office of Water
PAHs	Polycyclic Aromatic Hydrocarbons
PCBs	Polychlorinated biphenyls
PCDDs	Polychlorinated dibenzo-p-dioxins
PCDFs	Polychlorinated dibenzofurans
PCR	Polymerase Chain Reaction

PFE	Pressurized Fluid Extraction
QC	Quality Control
RNA	Ribonucleic Acid
RP/HPLC	Reversed-Phase High Performance Liquid Chromatography
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
SAED	Selected Area Electron Diffraction
SM	<i>Standard Methods for the Examination of Water and Wastewater</i>
SPE	Solid-Phase Extraction
SW	Solid Waste
TCBS	Thiosulfate Citrate Bile Salts Sucrose
TC SMAC	Tellurite Cefixime Sorbitol MaConkey agar
TCLP	Toxicity Characteristic Leaching Procedure
TEM	Transmission Electron Microscope or Microscopy
TOXNET	National Library of Medicine, Toxicological Database
TS	Thermospray
TSI	Triple Sugar Iron
USDA	U.S. Department of Agriculture
USGS	U.S. Geological Survey
UV	Ultraviolet
VEE	Venezuelan Equine Encephalitis
VOCs	Volatile Organic Compounds
VOA	Volatile Organic Analysis
XLD	Xylose lysine desoxycholate

Appendix A: Chemical Methods

Page Intentionally Blank

Appendix A: Chemical Methods

Analyte(s)	CAS No.	Determinative Technique	Determinative Method Identifier	Solid Phase Sample Prep Procedure	Oily Solid Sample Prep Procedure	Aqueous and Liquid Phase Sample Prep Procedure	Gas Phase Sample Prep Procedure
Aflatoxin	1402-68-2	HPLC-FL	994.08 (AOAC)	994.08 (AOAC)	994.08 (AOAC)	994.08 (AOAC)	Not of concern in this matrix
Aldicarb (Temik)	116-06-3	HPLC-FL	8318A (SW-846)	8318A (SW-846)	8318A (SW-846)	8318A (SW-846)	TO-13A
Alpha amanitin	NA	Immunoassay	LRN	LRN	LRN	LRN	LRN
Ammonia	7664-41-7	Spectrophotometry	4500-NH3 G (SM)	Not of concern in this matrix	Not of concern in this matrix	4500-NH3 B (SM)	6015 (NIOSH) / ID-188 (OSHA)
Arsenic III compound, Cadmium and other metals	22569-72-8	ICP-MS/ICP-AES	6020A/6010C (SW-846)	3050B (SW-846)	3031/3050B (SW-846)	200.2 (OW)	IO-3.5 / IO-3.4 / IO-3.1 (ORD)
Arsenic trichloride (analyze for Arsenic)	7784-34-1	ICP-MS/ICP-AES	6020A/6010C (SW-846)	3050B (SW-846)	3031/3050B (SW-846)	200.2 (OW)	IO-3.5 / IO-3.4/IO-3.1 (ORD)
Arsine	7784-42-1	GFAA	7010 (SW-846)	3050B (SW-846)	Not of concern in this matrix	200.2 (OW)	6001 (NIOSH)
Asbestos	1332-21-4	TEM	ASTM(dust) / ISO-10312 (air)	ASTM D5755-03 (soft surfaces-microvac) or D6480-99 (hard surfaces-wipes)	Not of concern in this matrix	Not of concern in this matrix	ISO-10312 (filter)
Boron trichloride	10294-34-5	ICP-AES	Journal Article: J. Anal. At. Spectrom., 2000, 15, 277-279	Not of concern in this matrix	Not of concern in this matrix	Not of concern in this matrix	Journal Article: J. Anal. At. Spectrom., 2000, 15, 277-279
Boron trifluoride	7637-07-2	ISE	ID-216SG (OSHA)	Not of concern in this matrix	Not of concern in this matrix	Not of concern in this matrix	ID - 216SG (OSHA)
Botulinum toxin	NA	Immunoassay	LRN	LRN	LRN	LRN	LRN

Appendix A: Chemical Methods

Analyte(s)	CAS No.	Determinative Technique	Determinative Method Identifier	Solid Phase Sample Prep Procedure	Oily Solid Sample Prep Procedure	Aqueous and Liquid Phase Sample Prep Procedure	Gas Phase Sample Prep Procedure
Brevetoxins	NA	HPLC-MS	994.08 (AOAC)	994.08 (AOAC)	994.08 (AOAC)	994.08 (AOAC)	Not of concern in this matrix
Bromadiolone	28772-56-7	HPLC-UV	8321B (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Cadmium	7440-43-9	ICP-MS/ICP-AES	6020A/6010C (SW-846)	3050B (SW-846)	3031/3050B (SW-846)	200.2 (OW)	IO-3.5 / IO-3.4/IO-3.1 (ORD)
Carbofuran (Furadan)	1563-66-2	HPLC	8318A (SW-846)	8318A (SW-846)	8318A (SW-846)	8318A (SW-846)	TO-13A / Method 74 (OSHA)
Carbon disulfide	75-15-0	GC/MS	8260B (SW-846)	5035A (SW-846)	3585 (SW-846)	5030C (SW-846)	TO-15
Chlorine	7782-50-5	IC	6011 (NIOSH)	Not of concern in this matrix	Not of concern in this matrix	4500-CI G	6011 (NIOSH)
Chloropicrin	76-06-2	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Chlorosarin	1445-76-7	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Chlorosoman	7040-57-5	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Cyanide	57-12-5	Spectrophotometry	CLP ILM05.3 CN	CLP ILM05.3 CN	Not of concern in this matrix	CLP ILM05.3 CN	7904 (NIOSH)
Cyanogen chloride	506-77-4	GC/MS	8260B (SW-846)	5035A (SW-846)	3585 (SW-846)	5030C (SW-846)	TO-15

Appendix A: Chemical Methods

Analyte(s)	CAS No.	Determinative Technique	Determinative Method Identifier	Solid Phase Sample Prep Procedure	Oily Solid Sample Prep Procedure	Aqueous and Liquid Phase Sample Prep Procedure	Gas Phase Sample Prep Procedure
Cyclohexyl Sarin (GF)	329-99-7	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Dichlorvos	62-73-7	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Dicrotophos	141-66-2	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Diesel Range Organics	NA	GC/FID	8015C (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	Not of concern in this matrix
Dimethylphosphite	868-85-9	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Distilled Mustard (HD) / Mustard Gas (H)	505-60-2	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Ethylchloroarsine (ED)	598-14-1	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846) Note: For liquid matrices use 3520C (SW-846)	TO-13A
Ethylene oxide	75-21-8	GC/MS	8260B (SW-846)	5035A (SW-846)	3585 (SW-846)	5030C (SW-846)	TO-15
Fenamiphos	22224-92-6	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Fluoroacetate salts	NA	Ion Chromatography/ GC/ECD	300.1 (OW)	Journal Article: Analytical Letters, 1994, 27 (14), 2703-2718	Journal Article: Analytical Letters, 1994, 27 (14), 2703-2718	300.1 (OW)	S301-1 (NIOSH)

Appendix A: Chemical Methods

Analyte(s)	CAS No.	Determinative Technique	Determinative Method Identifier	Solid Phase Sample Prep Procedure	Oily Solid Sample Prep Procedure	Aqueous and Liquid Phase Sample Prep Procedure	Gas Phase Sample Prep Procedure
Formaldehyde	50-00-0	HPLC	8315A (SW-846)	8315A (SW-846)	Not of concern in this matrix	8315A (SW-846)	TO-15
Gasoline Range Organics	NA	GC/FID	8015C (SW-846)	5035A (SW-846)	3585 (SW-846)	5030C (SW-846)	Not of concern in this matrix
1-methylethyl ester ethyl-phosphonofluoridic acid (GE)	1189-87-3	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Hydrogen bromide	10035-10-6	IC	4110B (SM) / 7903 (NIOSH)	Not of concern in this matrix	Not of concern in this matrix	4110B (SM)	7903 (NIOSH)
Hydrogen chloride	7647-01-0	IC	4110B (SM) / 7903 (NIOSH)	Not of concern in this matrix	Not of concern in this matrix	4110B (SM)	7903 (NIOSH)
Hydrogen cyanide	74-90-8	Spectrophotometry / ISE	6010 (NIOSH)	Not of concern in this matrix	Not of concern in this matrix	Not of concern in this matrix	6010 (NIOSH)
Hydrogen fluoride	7664-39-3	IC	7906 or 7903 (NIOSH)	Not of concern in this matrix	Not of concern in this matrix	Not of concern in this matrix	7906 / 7903 (NIOSH)
Hydrogen sulfide	7783-06-4	IC	6013 (NIOSH)	Not of concern in this matrix	Not of concern in this matrix	Not of concern in this matrix	6013 (NIOSH)
Kerosene	64742-81-0	GC/FID	8015C (SW-846)	5035A (SW-846)	3545A/3541/3580A (SW-846)	5030C (SW-846) Note: For liquid matrices use 3520C/3535A (SW-846)	Not of concern in this matrix
Lewisite 1 (L-1)	541-25-3	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A

Appendix A: Chemical Methods

Analyte(s)	CAS No.	Determinative Technique	Determinative Method Identifier	Solid Phase Sample Prep Procedure	Oily Solid Sample Prep Procedure	Aqueous and Liquid Phase Sample Prep Procedure	Gas Phase Sample Prep Procedure
Lewisite 2 (L-2)	40334-69-8	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Lewisite 3 (L-3)	40334-70-1	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Mercury	7439-97-6	CVAA	7471B (s) / 7470A (aq)	7471B	Not of concern in this matrix	7470A	IO-5
Metals, NOS	NA	ICP-MS/ICP-AES	6020A/6010C (SW-846)	3050B (SW-846)	3031/3050B (SW-846)	200.2 (OW)	See specific metals methods
Methyl parathion	298-00-0	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Mevinphos	7786-34-7	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Microcystin	NA	Immunoassay	LRN	LRN	LRN	LRN	LRN
Nicotine	54-11-5	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Nitrogen Mustard (HN-2) [unstable compound]	51-75-2	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
O-ethyl-S-(2-diisopropylaminoethyl)methyl phosphorite (VX)	50782-69-9	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Osmium tetroxide (analyze for Osmium)	20816-12-0	ICP-AES	6010C (SW846)	5050 (SW-846)	Not of concern in this matrix	5050 (SW-846)	IO-3.4 (ORD)

Appendix A: Chemical Methods

Analyte(s)	CAS No.	Determinative Technique	Determinative Method Identifier	Solid Phase Sample Prep Procedure	Oily Solid Sample Prep Procedure	Aqueous and Liquid Phase Sample Prep Procedure	Gas Phase Sample Prep Procedure
Oxamyl	23135-22-0	HPLC	8318A (SW-846)	8318A (SW-846)	8318A (SW-846)	8318A (SW-846)	TO-13A
Paraquat	4685-14-7	HPLC-UV	549.2 (OW)	Problematic	Problematic	549.2 (OW)	Not of concern in this matrix
Perfluoroisobutylene (PFIB)	382-21-8	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Phencyclidine	60124-79-0	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Phorate	298-02-2	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Phosgene	75-44-5	GC/MS	8260B (SW-846)	5035A (SW-846)	3585 (SW-846)	Not of concern in this matrix	TO-6
Phosphine	7803-51-2	UV-VIS	6002 (NIOSH)	Not of concern in this matrix	Not of concern in this matrix	Not of concern in this matrix	6002 (NIOSH)
Phosphorus trichloride	7719-12-2	Spectrophotometry	6402 (NIOSH)	Not of concern in this matrix	Not of concern in this matrix	Not of concern in this matrix	6402 (NIOSH)
Picrotoxin	124-87-8	HPLC-MS	994.08 (AOAC)	994.08 (AOAC)	994.08 (AOAC)	994.08 (AOAC)	Not of concern in this matrix
Red Phosphorous (RP) (analyze for total phosphorous)	7723-14-0	Spectrophotometry (colorimetric)	365.1 (NERL)	Not of concern in this matrix	Not of concern in this matrix	365.1 (NERL)	Not of concern in this matrix
Ricin	9009-86-3	Immunoassay	LRN	LRN	LRN	LRN	LRN

Appendix A: Chemical Methods

Analyte(s)	CAS No.	Determinative Technique	Determinative Method Identifier	Solid Phase Sample Prep Procedure	Oily Solid Sample Prep Procedure	Aqueous and Liquid Phase Sample Prep Procedure	Gas Phase Sample Prep Procedure
Sarin (GB)	107-44-8	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Saxitoxin	35523-89-8	HPLC-MS	994.08 (AOAC)	994.08 (AOAC)	994.08 (AOAC)	994.08 (AOAC)	Not of concern in this matrix
Semivolatile Organic Compounds, NOS	NA	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Soman (GD)	96-64-0	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Strychnine	57-24-9	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Sulfur Dioxide	7446-09-5	IC	6004 (NIOSH)	Not of concern in this matrix	Not of concern in this matrix	Not of concern in this matrix	6004 (NIOSH)
T-2 Mycotoxins	NA	HPLC-MS	994.08 (AOAC)	994.08 (AOAC)	994.08 (AOAC)	994.08 (AOAC)	Not of concern in this matrix
Tabun (GA)	77-81-6	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Tear gas (CS) chlorobenzylidene malonitrile	2698-41-1	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Tetanus toxin	NA	Immunoassay	LRN	LRN	LRN	LRN	LRN
Tetraethyl pyrophosphate	107-49-3	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A

Appendix A: Chemical Methods

Analyte(s)	CAS No.	Determinative Technique	Determinative Method Identifier	Solid Phase Sample Prep Procedure	Oily Solid Sample Prep Procedure	Aqueous and Liquid Phase Sample Prep Procedure	Gas Phase Sample Prep Procedure
Tetramethylene-disulfotetramine	80-12-6	HPLC-UV	8321B (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Titanium tetrachloride (analyze for total titanium)	7550-45-0	ICP-MS/ICP-AES	6020A/6010C (SW-846)	3050B (SW-846)	Not of concern in this matrix	Not of concern in this matrix	Not of concern in this matrix
Trimethyl phosphite	121-45-9	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846) Note: For liquid matrices use 3520C (SW-846)	TO-13A
VE	21738-25-0	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
VG	78-53-5	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
VM	21770-86-5	GC/MS	8270D (SW-846)	3545A/3541 (SW-846)	3545A/3541/3580A (SW-846)	3520C/3535A (SW-846)	TO-13A
Volatile Organic Compounds, NOS	NA	GC/MS	8260B (SW-846)	5035A (SW-846)	3585 (SW-846)	5030C (SW-846)	TO-15

Appendix B: Biological Methods

Page Intentionally Blank

Appendix B: Biological Methods

Agent Category	Analyte(s)	Waterborne			Dustborne			Aerosol		
		Determinative technique	Determinative Method Identifier	Sample preparation procedure and/or sampling method	Determinative technique	Determinative Method Identifier	Sample preparation procedure and/or sampling method	Determinative technique	Determinative Method Identifier	Sample preparation procedure and/or sampling method
Bacteria	<i>Bacillus anthracis</i> (Anthrax)	Culture / PCR	LRN	As specified by LRN protocol	Culture / PCR	OSS3 (OSHA)	Sampling with wet swabs	Culture / PCR	OSA-7 (OSHA)	XMZ / Anderson Button Sampler / DFU
Bacteria	<i>Brucella</i> spp. (Brucellosis)	Culture	LRN	As specified by LRN protocol	Culture	LRN	Swabs, socks, swipes CDC/NIOSH Sampling techniques	Culture	LRN	Wetted-wall cyclones, CDC/NIOSH Sampling techniques
Bacteria	<i>Burkholderia mallei</i> (glanders)	Culture	LRN	As specified by LRN protocol	Culture	LRN	Swabs, socks, swipes CDC/NIOSH Sampling techniques	Culture	LRN	Wetted-wall cyclones, CDC/NIOSH Sampling techniques
Bacteria	<i>Burkholderia pseudomallei</i> (melioidosis)	Culture	LRN	As specified by LRN protocol	Culture	LRN	Swabs, socks, swipes CDC/NIOSH Sampling techniques	Culture	LRN	Wetted-wall cyclones, CDC/NIOSH Sampling techniques
Bacteria	<i>Clostridium perfringens</i>	Culture	ICR method	ICR- As specified in ICR protocol	Culture	ICR method	Swabs, socks, swipes CDC/NIOSH Sampling techniques	Culture	ICR method	Wetted-wall cyclones, CDC/NIOSH Sampling techniques
Bacteria	<i>Francisella tularensis</i> (Tularemia)	Culture	LRN	As specified by LRN protocol	Culture	LRN	Swabs, socks, swipes CDC/NIOSH Sampling techniques	Culture	LRN	Wetted-wall cyclones, CDC/NIOSH Sampling techniques
Bacteria	<i>Coxiella burnetii</i> (Q-fever)	Culture	LRN	As specified by LRN protocol	Culture	LRN	Swabs, socks, swipes CDC/NIOSH Sampling techniques	Culture	LRN	Wetted-wall cyclones, CDC/NIOSH Sampling techniques
Bacteria	<i>Salmonella typhi</i> (Typhoid fever)	Culture/ELISA	USDA/FSIS 4	As specified by FSIS protocol	Culture/ELISA	USDA/FSIS 4	Swabs, socks, swipes CDC/NIOSH Sampling techniques	Culture/ELISA	USDA/FSIS 4	Wetted-wall cyclones, CDC/NIOSH Sampling techniques
Bacteria	<i>Vibrio cholerae</i> (Cholera)	Culture	SM 9260H	Concentration by placing Moore swabs in flowing wastewater for 1 week	Culture	SM 9260H	Swabs, socks, swipes CDC/NIOSH Sampling techniques	Culture	SM 9260H	Wetted-wall cyclones, CDC/NIOSH Sampling techniques
Bacteria	<i>Yersinia pestis</i> (Plague)	Culture	LRN	As specified by LRN protocol	Culture	LRN	Swabs, socks, swipes CDC/NIOSH Sampling techniques	Culture	LRN	Wetted-wall cyclones, CDC/NIOSH Sampling techniques
Bacteria	<i>Campylobacter jejuni</i>	Culture	SM 9260G	Filtration of large volumes of water using 0.45 or 0.22 micron filter	Culture	SM 9260G	Swabs, socks, swipes CDC/NIOSH Sampling techniques	Culture	SM 9260G	Wetted-wall cyclones, CDC/NIOSH Sampling techniques
Bacteria	<i>E. coli</i> O157:H7	Culture	SM 9260F	Collect 100 mL sample in sterile container	Culture	SM 9260F	Swabs, socks, swipes CDC/NIOSH Sampling techniques	Culture	SM 9260F	Wetted-wall cyclones, CDC/NIOSH Sampling techniques
Bacteria	<i>Shigella</i> spp.	Culture	SM 9260E	Filtration of large volumes of water using 0.45 or 0.22 micron filter	Culture	SM 9260E	Swabs, socks, swipes CDC/NIOSH Sampling techniques	Culture	SM 9260E	Wetted-wall cyclones, CDC/NIOSH Sampling techniques
Viruses	Arenaviruses	Biosafety Level 4 - Ship directly to CDC laboratory	Method not identified	As specified by CDC	Biosafety Level 4 - Ship directly to CDC laboratory	Method not identified	As specified by CDC	Biosafety Level 4 - Ship directly to CDC laboratory	Method not identified	As specified by CDC
Viruses	Caliciviruses	RT-PCR	AEM Vol. 69 No. 9; 5263-5268	As specified in AEM Vol. 69 No. 9; 5263-5268	RT-PCR	As specified in AEM Vol. 69 No. 9; 5263-5268 (method was developed for water but may be adaptable to other matrices)	Swabs, socks, swipes	RT-PCR	As specified in AEM Vol. 69 No. 9; 5263-5268 (method was developed for water but may be adaptable to other matrices)	AGI sampler (modify for viruses)
Viruses	Nairovirus	Biosafety Level 4 - Ship directly to CDC laboratory	Method not identified	As specified by CDC	Biosafety Level 4 - Ship directly to CDC laboratory	Method not identified	As specified by CDC	Biosafety Level 4 - Ship directly to CDC laboratory	Method not identified	As specified by CDC
Viruses	Hemorrhagic fever	Biosafety Level 4 - Ship directly to CDC laboratory	Method not identified	As specified by CDC	Biosafety Level 4 - Ship directly to CDC laboratory	Method not identified	As specified by CDC	Biosafety Level 4 - Ship directly to CDC laboratory	Method not identified	As specified by CDC
Viruses	Rift valley fever	Biosafety Level 4 - Ship directly to CDC laboratory	Method not identified	As specified by CDC	Biosafety Level 4 - Ship directly to CDC laboratory	Method not identified	As specified by CDC	Biosafety Level 4 - Ship directly to CDC laboratory	Method not identified	As specified by CDC
Viruses	Encephalomyelitis	Antibody / Integrated cell culture PCR	Method not identified	Method not identified	Antibody / Integrated cell culture PCR	Method not identified	Swabs, socks, swipes	Antibody / Integrated cell culture PCR	Method not identified	AGI sampler (modify for viruses)

Appendix B: Biological Methods

Agent Category	Analyte(s)	Waterborne			Dustborne			Aerosol		
		Determinative technique	Determinative Method Identifier	Sample preparation procedure and/or sampling method	Determinative technique	Determinative Method Identifier	Sample preparation procedure and/or sampling method	Determinative technique	Determinative Method Identifier	Sample preparation procedure and/or sampling method
Viruses	Variola (Smallpox)	Biosafety Level 4 - Ship directly to CDC laboratory	Method not identified	As specified by CDC	Biosafety Level 4 - Ship directly to CDC laboratory	Method not identified	As specified by CDC	Biosafety Level 4 - Ship directly to CDC laboratory	Method not identified	As specified by CDC
Viruses	Yellow fever	Integrated cell culture PCR	Method not identified	Method not identified	Integrated cell culture PCR	Method not identified	Swabs, socks, swipes	Integrated cell culture PCR	Method not identified	AGI sampler (modify for viruses)
Viruses	Enteroviruses	RT-PCR	AEM Vol. 69 No. 6; 3158-3164	As specified in AEM Vol. 69 No. 6; 3158-3164	RT-PCR	As specified in AEM Vol. 69 No. 6; 3158-3164 (method was developed for water but may be adaptable to other matrices)	Swabs, socks, swipes	RT-PCR	As specified in AEM Vol. 69 No. 6; 3158-3164 (method was developed for water but may be adaptable to other matrices)	AGI sampler (modify for viruses)
Viruses	Hepatitis A viruses	RT-PCR (HAV)	AEM Vol. 69 No. 6; 3158-3164	As specified in AEM Vol. 69 No. 6; 3158-3164	RT-PCR (HAV)	As specified in AEM Vol. 69 No. 6; 3158-3164 (method was developed for water but may be adaptable to other matrices)	Swabs, socks, swipes	RT-PCR (HAV)	As specified in AEM Vol. 69 No. 6; 3158-3164 (method was developed for water but may be adaptable to other matrices)	AGI sampler (modify for viruses)
Viruses	VEE virus	RT-PCR	J. Clin. Microbiol. Vol. 38 No. 4; 1527-1535	As specified in J. Clin. Microbiol. Vol. 38 No. 4; 1527-1535	RT-PCR	Method not identified	Swabs, socks, swipes	RT-PCR	Method not identified	AGI sampler (modify for viruses)
Protozoa	<i>Cryptosporidium parvum</i> (Cryptosporidiosis)	FA	Method 1622, EPA OW April 2001	Filtration	FA	Method 1622, EPA OW April 2001	Swabs, socks, swipes	FA	Method 1622, EPA OW April 2001	Wetted-wall cyclone
Protozoa	<i>Toxoplasma gondii</i> (Toxoplasmosis)	RT-PCR	Applied and Environmental Microbiology, 69 (10):5819-5825	As specified in Applied and Environmental Microbiology, 69 (10):5819-5825	RT-PCR	Applied and Environmental Microbiology, 69 (10):5819-5825	Swabs, socks, swipes	RT-PCR	Applied and Environmental Microbiology, 69 (10):5819-5825	Wetted-wall cyclone
Protozoa	<i>Entamoeba histolytica</i>	RT-PCR	PCR test kits: Mo Bio, Qiagen	Filtration	RT-PCR	PCR test kits: Mo Bio, Qiagen	Swabs, socks, swipes	RT-PCR	PCR test kits: Mo Bio, Qiagen	Wetted-wall cyclone