Response Protocol Toolbox:
Planning for and Responding to
Drinking Water Contamination Threats and Incidents

Module 4: Analytical Guide

Interm Final - December 2003

Diagram:

1. Threat Warning
2. Initial Threat Evaluation
   - Is Threat Possible?
     - Immediate Operational Response Actions
       - Site Characterization and Sampling
         - Is Threat Credible?
           - Public Health Response Actions
             - Sample Analysis
               - Is Incident Confirmed?
                 - Remediation and Recovery

THREAT EVALUATION PROCESS

EXPANDED RESPONSE ACTIONS
OTHER RESPONSE PROTOCOL TOOLBOX MODULES

Module 1: Water Utility Planning Guide *(December 2003)*
Module 1 provides a brief discussion of the nature of the contamination threat to the public water supply. The module also describes the planning activities that a utility may undertake to prepare for response to contamination threats and incidents.

Module 2: Contamination Threat Management Guide *(December 2003)*
Module 2 presents the overarching framework for management of contamination threats to the drinking water supply. The threat management process involves two parallel and interrelated activities: 1) evaluating the threat, and 2) making decisions regarding appropriate actions to take in response to the threat.

Module 3: Site Characterization and Sampling Guide *(December 2003)*
Module 3 describes the site characterization process in which information is gathered from the site of a suspected contamination incident at a drinking water system. Site characterization activities include the site investigation, field safety screening, rapid field testing of the water, and sample collection.

Module 4: Analytical Guide *(December 2003)*
Module 4 presents an approach to the analysis of samples collected from the site of a suspected contamination incident. The purpose of the Analytical Guide is not to provide a detailed protocol. Rather, it describes a framework for developing an approach for the analysis of water samples that may contain an unknown contaminant. The framework is flexible and will allow the approach to be crafted based on the requirements of the specific situation. The framework is also designed to promote the effective and defensible performance of laboratory analysis.

Module 5: Public Health Response Guide *(available March 2004)*
Module 5 deals with the public health response measures that would potentially be used to minimize public exposure to potentially contaminated water. It discusses the important issue of who is responsible for making the decision to initiate public health response actions, and considers the role of the water utility in this decision process. Specifically, it examines the role of the utility during a public health response action, as well as the interactions between the utility, the drinking water primacy agency, the public health community, and other parties with a public health mission.

Module 6: Remediation and Recovery Guide *(available March 2004)*
Module 6 describes the planning and implementation of remediation and recovery activities that would be necessary following a confirmed contamination incident. The remediation process involves a sequence of activities, including: system characterization; selection of remedy options; provision of an alternate drinking water supply during remediation activities; and monitoring to demonstrate that the system has been remediated. Module 6 describes the types of organizations that would likely be involved in this stage of a response, and the utility’s role during remediation and recovery.
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DISCLAIMER

The mention of trade names or commercial products does not constitute endorsement or recommendation for use.
ACRONYMS

AA Atomic absorption
AMDIS Automated mass spectral deconvolution and identification system
AOAC Association of Analytical Communities (formerly Association of Official Analytical Chemists)
APCI Atmospheric pressure chemical ionization
APHL Association of Public Health Laboratories
API Atmospheric pressure ionization
ASTM American Society for Testing and Materials
BSL Biosafety level
BT Biological terrorism
CDC Centers for Disease Control
CLIA Clinical Laboratory Improvement Amendments
CVAA Cold-vapor atomic absorption
CW Chemical warfare
CWA Clean Water Act
CWC Chemical Weapons Convention
DNA Deoxyribonucleic acid
DOD Department of Defense
DOE Department of Energy
DOJ Department of Justice
DOT Department of Transportation
ELCD Electrolytic conductivity detector
EPA Environmental Protection Agency
ESI Electrospray ionization
ETV Environmental Technology Verification
FA Immunofluorescence assay microscopy
FBI Federal Bureau of Investigation
FDA Food and Drug Administration
FEMA Federal Emergency Management Agency
FERN Food Emergency Response Network
FRMAC Federal Radiological Management Center
GC Gas chromatography
GFAA Graphite furnace atomic absorption
HASP Health and safety plan
HazMat Hazardous materials
HHS Health and Human Services
HPLC High performance liquid chromatography
HRMS High resolution mass spectrometry
IC Ion chromatography
ICAP Inductively coupled argon plasma
ICP-AES Inductively coupled plasma atomic emission spectroscopy
ICP-MS Inductively coupled plasma mass spectrometry
ICP-OES Inductively coupled plasma optical emission spectroscopy
ICR Information Collection Rule
IFA Immunofluorescence assay microscopy
IMS Immunomagnetic separation
IOC Intergovernmental Oceanographic Commission
ISE Ion-selective electrode
ISO International Organization for Standardization
LC  Liquid chromatography
LLE  Liquid-liquid extraction
LRN  Laboratory Response Network
MARLAP Multi-Agency Radiological Laboratory Analytical Protocols
MCL  Maximum Contaminant Level
MeV  Megaelectron volts
MS  Mass spectrometry
MWCO Molecular weight cut-off
NEMI National Environmental Methods Index
NIST National Institute of Standards and Technology
NRC National Research Council
ORD Office of Research and Development
OSHA Occupational Safety and Health Administration
OSW Office of Solid Waste
OW Office of Water
PCR Polymerase chain reaction
PDA Photodiode array detector
PID Photoionization detector
PPE Personal protective equipment
PSI Pounds per square inch
QA Quality assurance
QC Quality control
RF Response factor
RIFA Radioimmunofocus assay
RNA Ribonucleic acid
RT-PCR Reverse Transcriptase-PCR
SDWA Safe Drinking Water Act
SM Standard Methods for the Analysis of Water and Wastewater
SOP Standard operating procedure
SPE Solid-phase extraction
SPME Solid-phase microextraction
SQM Semi-quantitative mode
TBD To be determined
UCMR Unregulated Contaminant Monitoring Rule
URL Uniform Resource Locator
USAMRIID United States Army Medical Research Institute of Infectious Diseases
USDA United States Department of Agriculture
UV Ultraviolet
VEE Venezuelan equine encephalitis virus
VHF Viral hemorrhagic fever
GLOSSARY

Definitions in this glossary are specific to the Response Protocol Tool Box but conform to common usage as much as possible.

**Analyte** – the name assigned to a substance or feature that describes it in terms of its molecular composition, taxonomic nomenclature, or other characteristic.

**Analytical Approach** – a plan describing the specific analyses that are performed on the samples collected in the event of a water contamination threat. The analytical approach is based on the specific information available about a contamination threat.

**Analytical Confirmation** – the process of determining an analyte in a defensible manner.

**Analytically Confirmed** – in the context of the *analytical approach*, a contaminant is considered to be analytically confirmed if it has undergone analytical confirmation, as defined herein.

**Basic Screen** - utilizes standardized methods based on established analytical techniques for the analysis of samples collected in response to a contamination threat.

**Biosafety Level 1** – suitable for work involving well-characterized biological agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. Work is generally conducted on open bench tops using standard microbiological practices.

**Biosafety Level 2** – suitable for work involving biological agents of moderate potential hazard to personnel and the environment. Laboratory personnel should have specific training in handling pathogenic agents and be directed by competent scientists. Access to the laboratory should be limited when work is being conducted, extreme precautions should be taken with contaminated sharp items, and certain procedures should be conducted in biological safety cabinets or other physical containment equipment if there is a risk of creating infectious aerosols or splashes.

**Biosafety Level 3** – suitable for work done with indigenous or exotic biological agents that may cause serious or potentially lethal disease as a result of exposure by inhalation. Laboratory personnel must have specific training in handling pathogenic and potentially lethal agents and be supervised by competent scientists who are experienced in working with these agents. All procedures involving the manipulation of infectious materials are conducted within biological safety cabinets or other physical containment devices, or by personnel wearing appropriate personal protective clothing and equipment. The laboratory must have special engineering and design features.

**Biosafety Level 4** – suitable for work with the most infectious biological agents. Access to the two Biosafety Level 4 laboratories in the U.S. is highly restricted.

**Chain of Custody** – the tracking and documentation of physical control of evidence.

**Chemical Weapons** – the chemicals that the CWC has placed on its Schedule 1 list.

**CLIA Laboratory** – a laboratory regulated under the CLIA program.
**Clinical Laboratory Improvement Amendments (CLIA)** – regulation that covers all laboratory testing (except research) performed on humans in the U.S. Any laboratory participating in the CLIA program that does diagnostic testing, verification, or proficiency testing is exempt from select agent regulation, but the laboratory must follow specific procedures.

‘Confirmed’ – in the context of the *threat evaluation* process, a water contamination incident is ‘confirmed’ if the information collected over the course of the threat evaluation provides definitive evidence that the water has been contaminated.

**Core Field Testing** – analysis performed at the investigation site for radiation, cyanide, residual chlorine, and pH. Core field testing is performed as part of *site characterization* and is composed of two elements, a *field safety screen* and *rapid field testing*.

‘Credible’ – in the context of the *threat evaluation* process, a water contamination threat is characterized as ‘credible’ if information collected during the threat evaluation process corroborates information from the *threat warning*.

**Environmental Chemistry Laboratory** – any laboratory that is set up to perform analysis of water samples for compliance with the Safe Drinking Water, Clean Water Acts, or other applicable environmental regulation, as well as other chemical parameters that are important to system operation and overall water quality. These laboratories have the instrumentation necessary to implement methods for chemical analysis of a water sample.

**Established Analytical Techniques** – these techniques are commonly employed in a large number of laboratories and form the basis of many standardized analytical methods.

**Expanded Field Testing** – analysis of water at the site of a suspected contamination incident for parameters beyond those covered under core field testing (e.g., VOCs, chemical weapons, biotoxins, etc.).

**Expanded screen** – application of exploratory techniques for chemical contaminants that do not have standardized methods. Additionally, the expanded screen provides laboratories with additional options regarding the instrumentation used to implement both established and exploratory techniques; however, the results may not be considered definitive and thus may require confirmation.

**Exploratory Techniques** – techniques capable of detecting chemicals that are not included in existing standardized methods. They may employ less common instrumentation than those in the standardized methods, or they may simply not yet be specifically used in standardized drinking water methods.

**Field Safety Screening** – screening performed to detect any environmental hazards (i.e., in the air and on surfaces) that might pose a threat to the *site characterization* team. Monitoring for radioactivity as the team approaches the site is an example of field safety screening.

**Field Sample Concentrate** – the term used for the retentate from the ultrafiltration device used for the sampling/concentration of unknown microbial contaminants.

**Filtrate** – in ultrafiltration, the water that passes through the filtration membrane.
**Food Emergency Response Network (FERN)** – a laboratory network for the analysis of contaminants in food developed through integration with the existing LRN for pathogen analysis and established forensic chemistry laboratories that serve as reference laboratories for other FDA laboratories.

**Grab Sample** - a sample collected at random from the source.

**Hazard Assessment** – the process of evaluating available information about the site to identify potential hazards that might pose a risk to the site characterization team. The hazard assessment results in assigning one of four levels to risk: low hazard, radiological hazard, high chemical hazard, or high biological hazard.

**Incident Command System** – a standardized on-scene emergency management concept specifically designed to allow its user(s) to adopt an integrated organizational structure equal to the complexity and demands of single or multiple incidents, without being hindered by jurisdictional boundaries.

**Incident Commander** – the individual responsible for the management of all incident operations.

**Infectious** – capable of causing infection. In the context of waterborne pathogens, infection is caused by exposure to water.

**Laboratory Analytical Screening** – application of multiple analytical techniques in a laboratory setting to screen for a wide range of analytes and to confirm tentative results. (Laboratory analytical screening is different and separate from laboratory safety screening, field safety screening, and rapid field testing.)

**Laboratory Compendium** – a comprehensive, web-based, searchable database of laboratory capability for environmental analysis in water, air, soil, sediments, and other media.

**Laboratory Guide** – a plan prepared by a specific laboratory detailing their approach and capabilities for the 24/7 processing of emergency water samples.

**Laboratory Response Network (LRN)** – a network of laboratories developed by the CDC, APHL, and FBI for the express purpose of dealing with bioterrorism threats, including pathogens and some biotoxins.

**Laboratory Safety Screening** – screening for various hazards that is conducted when samples are received at the laboratory, and which is designed to reduce the risks to laboratory personnel that may handle the samples.

**Non-standardized method** – a method that does not meet the definition of a standardized method.

**Opportunity Contaminant** – contaminants that might be readily available in a particular area, even though they may not be highly toxic or infectious or easily dispersed and stable in treated drinking water.

**Pathogen** - an infectious microorganism that is capable of causing disease.
**Personal protective equipment (PPE)** – equipment and supplies designed to protect employees from serious injuries or diseasees resulting from contact with chemical, radiological, biological, or other hazards. PPE includes face shields, safety glasses, goggles, laboratory coats, gloves, and respirators.

‘Possible’ – in the context of the *threat evaluation* process, a water contamination threat is characterized as ‘possible’ if the circumstances of the *threat warning* appear to have provided an opportunity for contamination.

**Presumptive results** – results of chemical and/or biological field testing that need to be confirmed by further laboratory analysis. Typically used in reference to the analysis of pathogens.

**Priority Contaminants** – list of chemicals, biotoxins, and radionuclides that were ranked highly due to their availability, properties and potential to harm public health if introduced into the drinking water supply.

**Rapid field testing** - analyzing the water at the site of contamination using rapid field water testing technology to tentatively identify any chemicals or pathogens. Rapid field testing is performed as part of site characterization.

**Reference laboratory** – the core, advanced technology, LRN laboratories that can provide analytical confirmatory testing of contaminants.

**Retentate** - in ultrafiltration, the retentate contains the particles that do not pass through the filtration membrane.

**Select Agent** – biological contaminants, including some pathogens and biotoxins, that are regulated by HHS. Only certain laboratories, registered with the CDC in accordance with the Select Agent Act, are permitted to confirm the presence of, and maintain cultures of, select agents.

**Sentinel Laboratory** – an LRN laboratory that reports unusual results that might indicate a possible outbreak, and refers specimens that may contain select biological agents to *Reference laboratories* within the LRN.

**Site Characterization** – the process of collecting information from an *investigation site* in order to support the evaluation of a drinking water contamination threat. Site characterization activities include the site investigation, *field safety screening*, *rapid field testing* of the water, and sample collection.

**Standardized Method** – a method that has been produced as a standard by a recognized method development body (EPA, ASTM, AOAC, ISO, etc.) or applicable regulatory authority. A standardized method has been subjected to review and validation and is capable of generating data of sufficient quality for its intended use. Standardized methods often contain steps to defensibly confirm the presence and/or quantity of specific contaminants.

**Tentative Identification** – the contaminant identity is hypothesized based on available information from the site characterization report. Examples of situations in which tentative identification might occur include: a specific contaminant named in a threat; tentatively positive results for a specific
contaminant during field safety screening or rapid field testing; physical evidence at the site pointing to a specific contaminant; and clinical evidence of the identity of the disease-causing agent.

**Threat** – an indication that a harmful *incident*, such as contamination of the drinking water supply, may have occurred. The threat may be direct, such as a verbal or written threat, or circumstantial, such as a security breach or unusual water quality.

**Threat Evaluation** – part of the threat management process in which all available and relevant information about the threat is evaluated to determine if the threat is ‘possible’ or ‘credible’, or if a contamination *incident* has been ‘confirmed.’ This is an iterative process in which the threat evaluation is revised as additional information becomes available. The conclusions from the threat evaluation are considered when making *response decisions*.

**Threat Management** – the process of evaluating a contamination threat and making decisions about appropriate response actions. The threat management process includes the parallel activities of the threat evaluation and making response decisions. The threat management process is considered in three stages: ‘possible’, ‘credible,’ and ‘confirmatory.’ The severity of the threat and the magnitude of the response decisions escalate as a threat progresses through these stages.

**Ultrafiltration** – a filtration process for water that uses membranes to preferentially separate very small particles that are larger than the membrane’s molecular weight cut-off, typically greater than 10,000 daltons.

**Water Contamination Incident** – a situation in which a contaminant has been successfully introduced into the system. A water contamination incident may or may not be preceded by a water contamination threat.

**Water Contamination Threat** – a situation in which the introduction of a contaminant into the water system is threatened, claimed, or suggested by evidence. Compare *water contamination threat* with *water contamination incident*. Note that even a threat against a water system is a crime under the Safe Drinking Water Act as amended by the Bioterrorism Act.

**Water Utility Emergency Response Manager (WUERM)** – the individual(s) within the drinking water utility management structure that has the responsibility and authority for managing certain aspects of the utility’s response to an emergency (e.g., a contamination threat) particularly during the initial stages of the response. The responsibilities and authority of the WUERM are defined by utility management and will likely vary based on the circumstances of a specific utility.
1 Introduction

1.1 Objectives and Organization of This Module
The primary intended users of this module include laboratory personnel and planners who would provide analytical support to a water utility in the case of a contamination threat to the water supply. This module is intended to be a planning tool for laboratories that may need to provide an analytical response in the case of a contamination threat, not a “how-to” manual for use during the actual incident. As part of planning for such an incident, laboratories may want to prepare such a manual specific to their needs and capabilities. They should also exercise and improve the manual from lessons learned via conducting drills, which may encourage laboratories to “think outside the box” in responding to contamination threats.

While this module is not based expressly on regulatory requirements, it should be recognized that failure to plan for an emergency contamination incident might lead to tragic public health consequences. Accordingly, the objectives of this document are to:

- Describe special laboratory considerations for handling and processing emergency water samples suspected of contamination with a harmful substance.
- Present model approaches and procedures for analysis of water samples suspected of contamination with a known or unknown substance. These approaches and procedures are developed to take advantage of existing methodologies and infrastructure.
- Encourage planners to develop a site-specific analytical approach and laboratory guide that conform to the spirit and general principles of the model approaches. Sometimes these models may represent the best and/or only way of dealing with the analytical issues involved. Frequently, they provide an example of the most comprehensive approach.

Planners and laboratory analysts are encouraged to review this module in its entirety, as well as the other modules in the Response Protocol Toolbox, to obtain a more comprehensive understanding of the analytical response approach for water contamination threats. As suggested in the Overview and Application to the Response Protocol Toolbox, the modules that perhaps are most relevant to laboratories are Modules 3 and 4, but depending on the nature of the laboratory, the other modules also may prove helpful. Because of the varied audience for Module 4, particularly certain sections in Module 4, an attempt has been made to provide explanations of the subject matter in the various sections that are directed toward certain audiences. For instance, the bulk of the technical material for the analytical approach is found in Sections 6 and 8. Section 5 describes the framework for the development of an analytical approach, so planners may find Section 5 more useful, whereas laboratory analysts may find the technical material in Sections 6 and 8 more relevant. Furthermore, different laboratories may also require different levels of sophistication in the technical material.

It is hoped that the presentation of the material represents an effective compromise among the needs and capabilities of the wide audience of utilities and laboratories that wish to develop analytical capability to support the evaluation of water contamination threats. This module is organized into ten sections as described below.

Section 1: Introduction: describes the overall organization of the document and discusses the concept of due diligence as applied to laboratories in terms of meeting two main goals: the safety of laboratory personnel and provision of quality analytical data.
Section 2: Description of Laboratory Infrastructure in the U.S.: describes existing laboratory resources that might be involved in the implementation of the analytical approaches presented in this module.

Section 3: Considerations for Laboratory Analysis of Emergency Samples: briefly discusses infrastructure, staffing, personnel safety, and sample capacity considerations for laboratory analysis of water samples suspected to be contaminated with an unknown substance.

Section 4: Field Screening and Sample Collection: provides an overview of the field screening and sampling procedures presented in Module 3 and describes how these activities are linked to laboratory analysis.

Section 5: Analytical Approach for Unidentified Contaminants in Water: presents a framework for developing an analytical approach for emergency water samples in response to a specific contamination threat.

Section 6: Analytical Approach for Chemical Contaminants: presents a tiered approach for the analysis of unknown chemical contaminants, including biotoxins and radionuclides, in water samples.

Section 7: Examples of the Analytical Approach to Site-Specific Situations for Chemical Contaminants: presents examples of how several, hypothetical utility laboratories with differing capabilities have chosen to plan their analytical approach, based on the discussion in Section 6. Laboratories that choose to perform analysis of emergency water samples and serve a broader client base, such as Federal, State, and commercial labs, may need to adopt a more comprehensive approach, such that they maintain flexibility to provide analytical support in a variety of situations.

Section 8: Analytical Approach for Microbiological Contaminants: presents a tiered approach for the analysis of unknown microbiological agents in water samples.

Section 9: References and Resources: lists the references used in the development of this module as well as additional information resources.

Section 10: Appendices: provides additional information and materials that may be of value to the reader.

1.2 Laboratory Goals for Responding to Contamination Threats to Water Systems in the Context of the Response Protocol Toolbox

The analytical approach discussed in this module is directed at any laboratory potentially involved in analysis of emergency water samples. Water utilities are most familiar with their own systems, and they are often interested in dealing with incidents of all sorts at the local level (i.e., through the use of their own personnel and their own laboratories). In fact, it is not practical for HazMat responders and hazard materials laboratories to become involved in every incident that occurs at the thousands of
water systems across the country. The responsibility falls on water utilities, who routinely investigate water quality complaints, whether they originate from intentional contamination or not. Accordingly, during intentional water contamination events, it should be the general goal of non-utility laboratories to support the utilities.

For any laboratory to exercise due diligence in responding to contamination threats to water systems, it must meet two specific, essential goals. The first goal is ensuring the safety of laboratory personnel. The second goal is the timely generation of comprehensive, quality analytical results to support difficult decisions that must be made during an emergency situation.

1.2.1 Safety

Specific safety considerations for laboratories wishing to receive and analyze emergency water samples are discussed in more depth in Section 3. It is important to realize that details important for laboratory safety are integrated into the threat evaluation (Module 2) and the site characterization (Module 3) processes, even though they occur outside of the laboratory setting. Information in these other modules impact laboratory safety in several ways. First, the site characterization and threat evaluation processes help define the hazard conditions at the site of sample collection, which govern who should collect the samples and which laboratories should analyze them. For highly hazardous samples, samples should be collected and analyzed by specifically trained personnel, namely HazMat responders and laboratories that specialize in the handling of specific hazardous substances. This mitigates potential exposure to unprepared laboratory personnel. As an example, if the site characterization results indicate a potential radiological hazard, samples would be delivered only to laboratories that specialize in the analysis and handling of radiological materials.

Second, in the threat management process, water contamination incidents range from ‘possible’ to ‘confirmed’. The principle set forth in Module 2 is that, to date, there are many, perhaps thousands, of ‘possible’ incidents (ones that are not really intentional contamination incidents) for every ‘confirmed’ incident (one in which contamination has occurred). Those incidents falling into the ‘confirmed’ category are those that generate samples containing a harmful contaminant. Even among those samples, it is expected that the vast majority will not contain highly hazardous materials (e.g., chemical or biological warfare agents or radionuclides). Naturally, there is a continuum, described in Module 2 as ‘credible’, between the ‘possible’ and ‘confirmed’ categories. A contamination threat is deemed ‘credible’ if additional information collected during the evaluation supports the likelihood that contamination has occurred. However, the number of ‘credible’ cases is expected to be just a few more than the ‘confirmed’ ones, which is still vastly less than the number of ‘possible’ ones.

In summary, it is likely that most “emergency” water samples will be sent on the basis of a ‘probable’ contamination threat. Samples sent to a laboratory as a result of a ‘probable’ contamination threat should be treated as if they contain a potentially harmful substance. However, the site characterization, along with the threat evaluation process, should result in most highly hazardous samples being screened out before they reach the laboratory. From a safety standpoint, it is important for a laboratory to realize that it will **not be expected to determine every potential contaminant**. For instance, utility laboratories typically may expect to receive samples from ‘possible’ incidents. The utility laboratories will need additional laboratory support for ‘credible’ incidents, and specialty laboratories would be called into service for ‘confirmed’ incidents.
1.2.2 Analytical Goals

Utilities and laboratories familiar with regulatory compliance monitoring are accustomed to thinking about water analysis in terms of contaminant concentrations that may cause chronic (long-term) toxicity. In responding to intentional contamination incidents, an important paradigm shift is that one goal of the analysis may be to help rule in or rule out the presence of significantly elevated levels of certain types/classes of contaminants. Other goals may exist, and their significant features are discussed below.

Analytical goals for the three levels of the threat evaluation process

The threat evaluation process is important to a laboratory’s analytical goals, because part of providing timely, accurate results is the proper allocation of analytical resources. The allocation of laboratory resources to a threat will be determined by the analytical goals of the laboratory and the incident’s credibility category. Regardless of category, it is important to remember that even if one contaminant is identified during the analysis, the presence of additional contaminants should also be investigated.

- ‘Possible’ — For the vast majority of cases, because it is unlikely that there will be an actual contaminant, it is very important to report accurate results and to not misidentify an instrumental response. These results need to be rapid, but not instantaneous. Keep in mind that many of the decisions about water system operations will have been made before the analytical results are back from the laboratory. While speed and accuracy are necessary analytical goals for any scenario, they take on a special meaning during the evaluation of ‘possible’ incidents.

- ‘Credible’ — In the few ‘credible’ cases, laboratories may receive water samples containing potentially harmful contaminants; however, the activities performed during the threat evaluation and site characterization processes should reduce the likelihood that samples containing high hazard materials (as defined in Module 3, Section 4.1.2) reach the laboratory. Thus, laboratories should exercise due diligence to meet the goals of protecting their personnel and providing timely, accurate, analytical results. The laboratory investigation should be focused on those types of contaminants discussed in Sections 6 (toxic industrial chemicals, biotoxins, and radionuclides) and Section 8 (some waterborne pathogens, etc.).

- ‘Confirmed’ — For the rare ‘confirmed’ incidents, the laboratories receiving these materials should be ones with specific capabilities for the contaminants (chemical, biological, or radiological), which will be suspected or known as a result of the site characterization. Environmental laboratories will be capable of many analyses, but are prohibited from handling materials such as Schedule 1 chemical warfare agents. This Module should not be construed to suggest that laboratories intentionally handle materials for which they are not licensed.

Data Interpretation

Another goal of analysis is to interpret data in an appropriate manner. Often, part of the interpretation involves understanding baseline concentrations. The importance of knowing baseline levels of contaminants at a location cannot be overemphasized. Not only does this affect the site characterization (Module 3) and threat management process (Module 2) in terms of the proper use of analytical data, but it may serve the larger goal of creating greater public acceptance of water from a distribution system that was once contaminated. Most laboratories currently do not retain this information because they do not necessarily know the sampling location. However, the laboratory may be aware of typical background levels through analysis of routine samples. Laboratories should be aware of issues regarding background data, particularly if they are asked to render an opinion on the presence of an unusual contaminant.
1.3 Role of Laboratories in Response to Contamination Threats

While this Toolbox is aimed primarily at utilities, very few utility labs will be able to independently implement Module 4 in its entirety. However, they may find the information in Module 4 useful in understanding their analytical options, planning their analytical approach, and developing their laboratory guide.

Federal, State, and commercial laboratories will play a critical support role in the response to contamination threats – specifically, some of these laboratories will be responsible for implementing the analytical approaches presented in this module. These laboratories may already be familiar with some of the material in Module 4. However, they should work with their utility clients to ensure that the utilities are familiar with the capabilities of the laboratory. In working with the utility, the laboratory should be aware that response to a contamination threat is led by the incident commander, who may be from the water utility. Please see Module 1, Section 4.4 for more details on the Incident Command System and the role of the laboratory within it.

Utilities need to have confidence that laboratories that agree to process emergency water samples operate according to the following guidelines:

- Apply the analytical approach presented in this module according to the circumstances of a particular incident and the needs of the client.
- Maintain facilities and implement procedures for ensuring the security and integrity of samples and analytical results that may be considered as evidence for use in prosecution.
- Receive and process emergency samples 24/7. The laboratory should develop an appropriate plan for staffing, sample receipt, and internal chain of custody.
- Provide results to the client in a time frame stipulated by the client. The laboratory should be prepared to provide the client with an estimate of the time frame in which results may be available. As discussed in Module 2, the utility may need to take certain response measures before analytical results are available. Accordingly, the time frame may be dictated by site-specific factors, such as the hydraulic residency time within a segment of a distribution system.
- Implement appropriate quality assurance and quality control (QA/QC) procedures, and report the QC data along with the analytical results. See Section 3 for additional discussion.
- Use proper channels for reporting results.
- Provide support in the analysis and interpretation of analytical results.
- Have a back-up plan for processing samples should the laboratory’s facility become unusable or unavailable.

Laboratories may wish to develop their laboratory guide in accordance with these guidelines, and to share these plans with their clients. Timely and accurate results from the laboratory may provide valuable input for making decisions about how to proceed with a response to a contamination threat. Identification of a harmful contaminant in a water sample would likely trigger additional public health measures, including additional sampling to characterize the spread of the contaminant, and possibly some initial remediation efforts. Likewise, if laboratory results reveal nothing out of the ordinary, the response would likely be terminated, and any precautionary public health measures could be cancelled or scaled down.
2 Description of Laboratory Infrastructure in U.S.

The analytical approach described in the module was developed under the assumption that it would be implemented using existing laboratory infrastructure. If an environmental laboratory response network, designed specifically to analyze environmental samples, is developed in the future, the approaches presented in this document may be revised to take advantage of the new resource. Figure 4-1 summarizes laboratory infrastructure, as it currently exists, for the analysis of environmental samples. Discussion of both access to and roles of these laboratories during the response to an intentional contamination threat or incident follows.

![Figure 4-1. Summary of Types of Laboratories by Contaminant Class](image)

The following two sections provide a general description of the laboratory infrastructure for the analysis of chemical and microbiological contaminants in a water matrix. A comprehensive, web-based, searchable database of laboratory capability for environmental analysis in water, air, soil, sediments, and other media will be provided in the Laboratory Compendium when available. It is not a listing of laboratories approved, certified, or recommended to analyze samples from intentional contamination incidents. Rather, the Compendium is designed to be a tool for searching for laboratories and determining their ability to perform various analytical techniques, such as those presented in this module.

### 2.1 Chemistry Laboratories

In addition to laboratories within water utilities, standard and specialized chemistry laboratories within Federal, State, local, city, and municipal government agencies, as well as commercial laboratories, may support analysis of chemicals in water samples. Some laboratory resources may also be available from the academic and industrial sectors. For example, a major chemical manufacturer in an area might want to bring their laboratory operations to bear during the evaluation of an incident in which their products are suspected in a water contamination threat. Many academic and industrial laboratories, however, may not necessarily be set up to rapidly respond to water contamination threats without extensive planning. Regardless of their origin, it is anticipated that four broad categories of analytical chemistry laboratories would play a role in implementing the chemistry procedures in the analytical approach: environmental chemistry, radiochemistry, biotoxins, and chemical weapons.

#### 2.1.1 Environmental Chemistry Laboratories

This group forms the largest sector of the laboratory infrastructure for analysis of chemicals in water, and includes many EPA, State, and commercial water analysis laboratories. Environmental chemistry laboratories are typically set up to perform analysis of water samples for compliance with the Safe
Drinking Water or Clean Water Acts, as well as other chemical parameters that are important to system operation and overall water quality. It is important to realize that these laboratories are typically involved in the analysis of contaminants at concentrations associated with chronic (long-term) toxicity, not the acutely (short-term) toxic levels potentially associated with an intentional contamination incident. While it may seem intuitive that laboratories capable of determining contaminants at low concentrations should not experience difficulties at high concentrations, this is not necessarily the case for a number of technical and practical reasons.

These laboratories typically have the instrumentation necessary to implement standardized methods for chemical analysis in a water matrix. Because many of these laboratories are involved in regulatory compliance, the laboratory and staff may already be accredited and certified to implement these methods. However, unless the laboratory tests for the particular chemical analyte on a routine basis, the laboratory will not necessarily be able to run the associated method without advanced notice. This includes maintaining an inventory of standards and reagents, setting up the instrument for a particular method, and having staff trained to run the method. This may be particularly relevant in the context of chemical analysis in the case of a suspected intentional contamination incident since many of the chemicals of greatest concern are not routinely analyzed for in water, even though standardized methods are available.

Some environmental chemistry laboratories may have unique capabilities for analysis of select radionuclides or biotoxins, but this is not the expected norm. Analysis for these chemicals may need to be performed by a specialty laboratory as discussed below. Further, there are a number of research laboratories within the government and academic sectors that may be available on a limited basis. These laboratories are typically involved in method development, and thus are equipped with advanced equipment and highly trained analysts that provide capability for implementation of exploratory techniques that are currently beyond the means of other environmental chemistry laboratories.

2.1.2 Radiochemistry Laboratories

If a radioactive contaminant is suspected, analyses should be performed by a laboratory specifically equipped to handle such material and analyze for a range of radionuclides. EPA, DOE, States, and commercial firms have laboratories dedicated to the analysis of radioactive and/or nuclear material. For further information about EPA’s laboratory services and radiological emergency response programs, see http://www.epa.gov/radiation/programs.htm.

Another source of support in the case of an attack utilizing a radiological contaminant is the Federal Radiological Management Center (FRMAC) operated by the Federal Emergency Management Agency (FEMA). FRMAC is set up to provide rapid response teams and emergency management services related to incidents involving radioactive materials. This center works with a number of Federal and State agencies and draws on a variety of resources including fixed and mobile laboratories. FRMAC also maintains databases of national laboratory resources and radiological capabilities. Information regarding FEMA’s Radiological Emergency Preparedness Program can be found at http://www.fema.gov/rrr/rep/index.shtm.

2.1.3 Biotoxin Laboratories

Currently, few laboratories are set up specifically for the analysis of biotoxins. Those in existence primarily focus on the analysis of marine biotoxins in coastal waters and seafood products. Some
Laboratory Response Network (LRN) laboratories (see Section 2.2.1) may have the capability to analyze for select biotoxins in water samples, assuming proper sample preparation. In addition, there are a number of laboratories in government and academia that perform biotoxin analysis, usually for other matrices than water (e.g., seafood or agricultural products). It is possible that some biotoxin analyses could be performed in qualified environmental chemistry laboratories using techniques such as GC/MS, HPLC, immunoassay, and possibly LC/MS; however, such capability may not be currently widespread.

2.1.4 Chemical Weapon Laboratories

For the purposes of the Response Protocol Toolbox, a “chemical weapon” refers to those chemicals that the Chemical Weapons Convention (CWC) has placed on its Schedule 1 (http://www.cwc.gov/Regulations/cfr-15/part-712-s1.html). This list includes toxic chemicals with few or no legitimate other purposes, that were developed or used primarily for military purposes (http://www.cwc.gov/Industry_Outreach/Publications/002/cwc-b0001.html). CWC also monitors chemicals on two other Schedules and certain “unscheduled discrete organic chemicals.” A list of chemicals in the CWC Schedules appears in the appendix in Table 4-18. Some of these other chemicals (not on Schedule 1) may be present in water through a number of routes other than intentional contamination. For instance, there are some relatively non-toxic chlorinated hydrocarbons listed on Schedule 2 because they can be precursors to Schedule 1 chemicals. Coincidentally, these chemicals may also be disinfection byproducts, a term which refers to the hundreds of substances formed in very small amounts by reactions between drinking water disinfectants and substances naturally present in the water.

Only a few laboratories are qualified and permitted to work with concentrated Schedule 1 chemical weapons surety material. In fact, the only two chemical weapons surety laboratories in the U.S. are the U.S. Army’s Edgewood laboratory and Lawrence Livermore National Laboratories. These laboratories can only be accessed through specific channels (e.g., through certain Federal agencies such as FBI). A broader group of laboratories can work with dilute chemical weapons materials, such as might be encountered in a water contamination incident; however, the ability to access these laboratories through normal channels is uncertain, and even if such capability can be accessed, analysis may not be widely available. In contrast, many environmental laboratories work routinely with Schedule 2, Schedule 3, and unscheduled chemicals, and analytical standards for many of these are readily available.

2.2 Microbiological Laboratories

The analysis of waterborne pathogens will likely be performed either by an environmental microbiology laboratory or a laboratory that is part of the Laboratory Response Network (LRN). This may include hospital laboratories, medical laboratories, public health laboratories, and environmental microbiology laboratories. However, the missions and capabilities of these two distinct sets of laboratories are significantly different, and neither may be particularly well prepared for the analysis of all biological terrorism (BT) contaminants of concern in a water matrix. The potential role of each of these types of laboratories in responding to a water contamination threat involving pathogens is discussed in the following two sections.
2.2.1 Laboratory Response Network

The Laboratory Response Network (LRN) was developed by the Centers for Disease Control (CDC), the Association of Public Health Laboratories (APHL), and the FBI for the express purpose of dealing with bioterrorism (BT) threats, including pathogens and some biotoxins. Various laboratories within each State participate in the LRN (see for contact information). Laboratories that are part of the LRN can analyze the select agents (subject to legislative requirements set forth in the Select Agent Regulation (42 CFR 72, ). The legislation requires that, subject to certain exemptions, entities possessing biological agents that are listed as select agents must register with CDC and/or USDA’s Animal and Plant Inspection Service (for veterinary purposes, and demonstrate compliance with specific safety and security standards for handling these agents.

Two details of select agent regulation that might be of immediate interest are: 1) The USA Patriot Act places restrictions on persons who possess select agents and provides criminal penalties for possession of such agents that cannot be justified for specified peaceful purposes. 2) Any diagnostic or Clinical Laboratory Improvement Amendments (CLIA) laboratory that does diagnostic testing, verification, or proficiency testing is exempt from the regulation. However, the director of such a laboratory must notify the Department of Health and Human Services (HHS, see contact info above) immediately upon identifying specific select agents, and it must transfer the agents to a registered facility or destroy them (unless directed otherwise by law enforcement or HHS) within 7 calendar days of identification of the select agent, subject to certain exemptions. Retention of any select agent as a positive control or reference sample is not permitted in this case.

Thus, by legislation, confirmatory analysis of samples containing select pathogens, such as Bacillus anthracis, Brucella spp., Yersinia pestis, Francisella tularensis, and C. botulinum toxins, among others, must be performed through the LRN. It should be noted that the LRN also performs analysis of biological samples other than the “select” agents, and many of the waterborne pathogens of concern are not select agents. Since the LRN will be involved in an analytical response to any bioterrorism incident, it is important to understand the structure and organization of this network. The LRN, composed of city, county, State, and Federal public health laboratories, is in a perpetual state of evolution as capabilities are matched with current needs. Figure 4-2 provides a schematic overview of the current LRN structure.
The LRN membership is organized into “Sentinel Labs,” which recognize an agent, rule it out, and/or refer the sample to the next level for confirmatory testing, performed in “Reference Labs”. At the top of the pyramid are “National Labs” (namely CDC and USAMRIID), which are capable of definitive characterization of even the most hazardous biological agents.

Although the LRN is set up to identify and characterize dangerous pathogens through an upward referral system, most LRN laboratories are not equipped to process water samples that may contain these pathogens or their toxic byproducts. Specifically, they do not have appropriate protocols to process the relatively large sample volumes needed for analysis of pathogens at low concentrations that are still of public health concern. For example, some Reference laboratories have received standardized environmental protocols for handling dry samples that might contain Bacillus anthracis, Brucella spp., Yersinia pestis, Francisella tularensis, C. botulinum toxins, and Staphylococcus enterotoxins; however, protocols are not yet in place for handling water samples containing these agents.

The LRN can accept specimens and samples from hospitals, clinics, the FBI and other law enforcement groups, emergency medical services, the military, and other agencies. Thus, it may be reasonable to expect that water utilities could work through their local or State health departments to deliver samples to LRN laboratories. However, this is complicated by regulations governing the transport of samples that are known or suspected of containing select pathogens. Specifically, such samples can be shipped only in an approved and appropriately marked container (see Section 6 in Module 3 for details, including the use of technical escort services). While these containers are adequately sized to ship clinical specimens and culture tubes, none are of sufficient size or integrity to ship the large volumes of water (a minimum of 10 liters) that are necessary to achieve the desired detection limit. These issues need to be resolved before LRN laboratories can be engaged in the response to a BT incident at a water system. The protocols presented in this module are one proposed solution.
Another challenge arises from the fact that public health and environmental protection are legislated and managed differently by each state. Only four states have a single state agency that is responsible for both public and environmental health. All other states have one agency responsible for public health issues and another for environmental issues. In addition, some states also separate their state public health (clinical) laboratories from the state environmental laboratories. Only 14 state public health laboratories also serve as the state environmental laboratories (Arkansas, Colorado, Connecticut, Iowa, Kansas, Louisiana, Maine, New Mexico, North Carolina, South Carolina, South Dakota, Tennessee, Virginia, and Wisconsin). Personnel at these 14 laboratories, however, rarely handle both clinical and environmental samples. Some states even have strict rules that prohibit cross-training of public health (clinical) microbiologists and environmental microbiologists. To further complicate interactions and communications among agencies, the individual responsible for drinking water is located in the health agency in half of the states and in the environmental agency in the other half of the states.

2.2.2 Environmental Microbiological Laboratories

Environmental microbiological laboratories, including those of EPA, state environmental agencies, and the commercial sector, typically perform analyses for waterborne pathogens. Most of these laboratories have the equipment and staff necessary to perform classical microbiological methods, and routinely analyze for indicators of fecal contamination such as fecal and total coliforms and *E. coli*. Culture techniques are available for many of the more common waterborne pathogens such as *Vibrio cholerae*, *Salmonella enteriditis* Typhi, and *Shigella* spp.; however, analyses for these pathogens are not routinely performed in most environmental microbiological laboratories. While some environmental microbiological laboratories have expanded capabilities to analyze for parasites such as *Cryptosporidium* and *Giardia* or to perform molecular assays for some organisms, these capabilities are not widespread.

While many environmental microbiological laboratories are well equipped to analyze for microbiological contaminants in a water matrix, they generally lack the infrastructure, training, and methods to analyze for many pathogens of concern. Furthermore, as discussed in Section 2.2.1, only laboratories registered for the analysis of select agents are legally permitted to analyze for those agents, and currently most registered labs reside in the LRN. Thus, even if environmental microbiological laboratories develop additional capabilities for pathogen analysis, they could not perform such analyses without registering for select agents.

2.3 Integration of Laboratory Resources

Sections 2.1 and 2.2 presented a brief overview of the laboratory infrastructure that will likely be called upon to implement the procedures presented in this module. While the core infrastructure may exist for both chemical and microbiological analysis, no mechanism currently exists to provide coordination in a manner conducive to optimal analytical response. At a minimum, this will create a greater logistical burden on the organization coordinating sampling and shipment to qualified laboratories. In the worst case, these inefficiencies may result in an incomplete analysis of an unknown, shipment to the wrong laboratory, or delays in receiving time sensitive information.

Formation of environmental laboratory response networks would help to address these coordination issues. Such networks are in existence for the analysis of clinical samples (CDC’s LRN) and food samples (FDA’s *Food Emergency Response Network* [FERN]). The FERN was developed through integration with the existing LRN for pathogen analysis and establishment of regional forensic
chemistry laboratories that serve as reference laboratories for other FDA laboratories. In the absence of a formal network, the analytical response to water contamination threats may be supported by the laboratory infrastructure as it currently exists.

Accordingly, the approaches described in this module were developed for implementation by the existing laboratory infrastructure. Some states have established/will establish network-like entities to coordinate laboratory efforts. The following steps may help states better integrate laboratory resources and provide a more coordinated response to water contamination threats:

- Establish environmental chemistry laboratories that are capable of implementing both basic and expanded screens (see Sections 6.3 and 6.4) for unknown chemicals in water samples.
- Establish environmental microbiology laboratories within the LRN that are capable of performing Sentinel testing for pathogens of concern in a water matrix.
- Determine those biotoxins that will likely be analyzed for in environmental chemistry laboratories and those that will be analyzed for in LRN laboratories. Considering the range of techniques used to measure biotoxins, there may be some overlap in biotoxin capability.
- Establish a clear sample referral system for the analytical confirmation of tentatively identified contaminants, in cases where the environmental chemistry laboratory cannot perform it. This concept is integrated into the LRN for microbiological analysis but is not formally defined for chemical analyses.
3 Considerations for Laboratory Analysis of Emergency Samples

Laboratories participating in the analysis of emergency water samples that may contain an unknown and potentially dangerous substance have additional responsibilities beyond those associated with routine analyses. This section briefly discusses some of the special issues related to safety, infrastructure, responsiveness, data reporting, quality assurance, and legal admissibility of scientific evidence that laboratories should consider before engaging in the analysis of emergency samples. This discussion is not intended to comprehensively address all issues that laboratories may face, but focuses on issues specifically dealing with the analysis of emergency water samples. This discussion applies largely to fixed laboratories, not mobile laboratories. Mobile laboratories often are designed to meet specific needs, so it is anticipated that most analyses will be performed by fixed laboratories, which are likely capable of a wider range of analytical methodologies.

This section primarily deals with operations within the laboratory during the analysis of emergency water samples. It is also important, however, for the laboratory to keep in mind its role within the incident command structure, which, as discussed in Module 1, is through the “laboratory point of contact.” The incident command structure, which must be developed at the local level, establishes and clarifies the roles of each of the various participants (water utility emergency response manager, first responders, state drinking water program personnel, laboratory personnel, etc.) and how they should interact with each other.

3.1 Safety

This section seeks to promote the safety of laboratory personnel during the analysis of samples arising from the suspected contamination of the water supply, which is subject to regulations with which most laboratories should be familiar. In addition, the entire Toolbox, including the analytical approach presented in Sections 6 and 8 of this module, is intended to enhance the safety of laboratory personnel. For instance, it is important to realize that during the site characterization process, field safety screening and rapid field testing have occurred. This may identify potentially hazardous samples before a decision is made regarding the laboratory that will receive the samples. In fact, part of site characterization is the site hazard assessment, in which the site is categorized as low hazard, radiological hazard, high chemical hazard, or high biological hazard. The results of this site hazard assessment should dictate the laboratory that will be used for analysis, and they should also help assure that only laboratories equipped to deal with highly hazardous materials will receive samples potentially containing such materials.

Considering that the vast majority of evaluated threats that generate laboratory samples will likely not prove to be intentional contamination incidents (see discussion in Section 1.2 of this module), additional risks may be manageable. Due to site characterization procedures, environmental chemistry laboratories may have increased confidence that they will not be processing hazardous biological agents. In the unlikely case that such a sample does reach the laboratory, the measures described below and the analytical approach in Sections 6 and 8 are designed to reduce the risk to laboratory personnel. Accordingly, many more laboratories may be willing and able to help respond to potential intentional contamination incidents.
3.1.1 Health and Safety Plan (HASP)
Under current regulations, laboratories are required to have a plan in place to ensure worker safety. Some laboratories may wish to treat certain emergency water samples as hazardous material as defined in Module 3, whether it be chemical, biological or radiological in nature, and to develop a specific health and safety plan (HASP) to address this potential risk, although there is currently no requirement to do so in most cases. Information on HASPs is available at http://www.ertresponse.com/health_safety/index.htm, along with an electronic expert system jointly developed by EPA and OSHA (http://www.osha.gov/dts/osta/oshasoft/ehasp/) to help determine the appropriate controls of health and safety hazards for a specific situation.

Laboratory personnel involved in the handling and analysis of water samples should have appropriate, current safety training that will allow them to conform to applicable regulations. Laboratories, although not required, may wish to explore some of the measures contained in regulations for the handling of hazardous materials, such as OSHA 1910.120 (http://www.osha.gov). If planners and laboratories do not conclude that these regulations are applicable to them, they may still wish to adopt some of the principles in these regulations. For instance, laboratory personnel may work in cooperation with a designated “buddy” and maintain visual and/or vocal contact with the buddy at all times during the analysis. This system may provide an additional level of protection compared to regulations applicable for most environmental chemistry laboratories, which do not necessarily rely on the “buddy” system.

3.1.2 Personal Protective Equipment (PPE)
Analysis of potentially hazardous samples during an emergency situation may require additional personal protective equipment (PPE) above that normally used in the laboratory. Such PPE requirements should be determined during the creation of the site-specific HASP described in Section 3.1.1. The requirements should be contained in the HASP, and PPE should be freely available to laboratory personnel. Personnel should be trained, competent, and medically certified (particularly for respiratory PPE) to use all necessary levels of PPE, in accordance with applicable regulations (i.e., OSHA 1910.120 or OSHA 1910.132 (http://www.osha.gov), along with local or State requirements).

Conventional PPE may be used in conjunction with hand-held “sniffer-type” instruments, which can monitor for a variety of preselected volatile organic and inorganic compounds. References such as http://www.chrismanual.com, http://www.osha.gov/SLTC/personalprotectiveequipment/index.html, and http://www.cdc.gov/niosh/npptl/default.html, should be consulted for more detail on special PPE. For instance, butyl gloves and full-face shields should be considered for optimal protection, particularly during pouring and splitting of non-volatile samples when maximum risk of accidental exposure could occur.

Although not necessarily a personal protection issue, care should be used when using PPE to preserve the forensic integrity of the samples. For example, dirty laboratory coats and gloves could result in cross-contamination of samples. Hair bonnets, aside from some safety benefit, may prevent loose or attached hair from contacting samples, which could potentially introduce a variety of contaminants into the sample.
3.1.3 Communication to Increase Information Quality about Hazardous Samples

Information about the collection site may be an **invaluable** component in personnel safety. In general, laboratories may be asked to analyze samples that originate from two types of sources: 1) those with rigorous sample collection procedures and 2) those using less-than-perfect sample collection procedures. In either case, and particularly the latter, it is recommended that the laboratory be fully informed about the sample collection and site investigation procedures, including any **field safety screening** and **rapid field testing** results, to ensure not only personnel safety but also analytical integrity. The laboratory may wish to take several steps to become more fully informed. One such step is to inquire if the field personnel followed the procedures and performed the tests suggested in Module 3 for site characterization. Another possible step is to establish a system to review procedures used to collect the samples and assess the reliability of the source of the samples. A third step might be for laboratories to enhance their communication with the field sampling teams.

With respect to enhancing communications, representatives from the laboratory ideally would be present during site characterization and sampling to provide as much information as possible to the laboratory analysts regarding sample collection, field screening, sample transport, and eventual analysis. Representatives from the laboratory might then accompany samples to the laboratory and interact with the laboratory personnel while maintaining contact with the field personnel. A less ideal case might be that the laboratory can readily communicate interactively in real time with the field sampling team so that they can provide details of sampling strategies, environmental conditions, and other pertinent information to the laboratory. In any case, the written site characterization report (see Module 3) should be provided to the laboratory at the time of sample delivery or faxed separately. Direct verbal communication, such as a conference call to discuss the site characterization findings, should improve the quality and clarity of the information in such a report.

It is important to note that different organizations may use different terminology when discussing issues related to potential contamination events, ranging from a description of the contaminants, to what is meant by field testing, to descriptions of laboratory results (i.e., is a “negative” result good or bad?). The glossary to this module attempts to standardize these terms, but it is imperative that laboratories ensure that necessary and effective communication is **not** impeded by inconsistent terminology.

3.1.4 Special Procedures for Safe Handling of Hazardous Water Contaminants

**Examination of incoming packages**

Infrastructure requirements for the safe receipt of packages are discussed in the next section. In addition, the staff in the receiving area should be trained in the identification of safety hazards associated with packages. Sometimes these hazards are indicated by warning labels required by DOT regulations (http://hazmat.dot.gov/) and/or information contained on the shipping papers. Other hazards may result from improper packaging and shipment. They include, but are not limited to: obvious leaks, breaks in packaging tape or custody seals, damage to packaging, unusual stains on shipping container, strange odors, etc. (Note: It is not advisable to smell or taste the package).

**Laboratory safety screening upon receipt**

During the site characterization process, samples from a site are subjected to field safety screening and/or rapid field testing, as described in Section 4.1 of this module. To reduce risks associated with potential, undetected hazards, laboratories may wish to screen the sample for various hazards upon receipt at the laboratory, regardless of the reported field safety screening results. Specifically, some of the safety screening techniques employed in the field (see Module 3) may also be used in the
Avoiding aerosols

The water solubility of potential contaminants sometimes contributes to their safe handling. Namely, as long as the contaminant remains in the bulk aqueous solution, the principal risk involves ingestion. Therefore, steps should be taken to avoid volatilizing or aerosolizing water samples, which would then increase the inhalation risk. For instance, the pressurized portion of the *ultrafiltration* device used for concentrating microbial samples (Section 8) is a closed system which prevents the formation of aerosols, assuming there are no leaks. Analytical methods should be appropriately selected to reduce volatilization and the formation of aerosols (e.g., performing extractions in sealed vials). Accordingly, separatory funnel liquid-liquid extraction, which may release aerosols when vented, is not recommended unless laboratories can utilize appropriate hoods or other precautions.

Some aerosol generation is unavoidable. Samples, particularly biological ones, may produce aerosols when shaken vigorously for homogenization, and these aerosols should be contained. Purge-and-trap technology for analysis of volatile organic compounds produces aerosols, which are contained within the instrument, provided that the purge vessel is not accidentally broken during analysis. Also, inductively coupled plasma and some atomic absorption techniques for trace metal analysis must produce aerosols to function properly. For optimal protection, these instruments have to be properly vented to minimize inhalation risk. Manufacturers of modern instruments have designed their instruments to minimize aerosol release (many of these instruments are used in industrial clean rooms), but laboratories should ensure that the venting on their instruments is adequate. If this is not possible, because of the short instrument run times for metal analysis, laboratories may consider performing these analyses after other procedures have been used to screen for the presence of other volatilizable or aerosolizable contaminants.

Dilution

As a general principle, dilution of a hazardous water sample with laboratory-grade water helps reduce risks associated with handling of the sample and its analysis for chemical contaminants. Dilution, however, may have undesirable effects on the ability to detect and quantify the contaminants. Accordingly, it should be used carefully only as part of an overall handling (and/or analysis) strategy that balances contaminant effects (e.g., health and safety concerns) with instrument response and timeliness of the results. Contaminant effects may be important primarily for extremely hazardous chemicals, such as Schedule 1 chemical warfare agents and biological warfare agents. The need to dilute may be reduced by safety screening performed both during site characterization (Module 3) and also upon receipt by the laboratory.

If dilution is desired, “log dilutions” may be attempted. For instance, first, a 1/1,000 dilution may be analyzed, followed by a 1/100 dilution if nothing is detected in the highest dilution, followed by a 1/10 dilution, and finally the undiluted sample. In deciding on a dilution scheme, it is important to keep in mind that most drinking water methods are designed to detect contaminants in the low parts-per-billion
range. Concentrations that are hundreds of times this level may be present in samples from the site of an intentional contamination incident. Appropriate QC measures should be implemented, particularly with regard to the purity of the dilution water. Also, before diluting samples in the laboratory, it should be verified that samples were not diluted in the field prior to shipment to the laboratory.

Although not directly related to safety, dilution may also protect the analytical instrument from becoming saturated with a contaminant. Procedures for reducing the saturation are usually possible, but they take time, limiting the availability of the instrument. Working out the correct dilution requires time, which could be an important consideration. On the other hand, the presence of sufficient analyte to saturate the instrumentation may indicate a significant contamination incident. The decision to implement dilution should accordingly be made during planning.

Reduction of sample volumes used
Like dilution, reducing the volumes of sample handled may help minimize exposure for both chemical and biological contaminants. Some techniques (like those discussed in Section 6) may involve using smaller volumes of sample. For example, direct aqueous injection typically uses a few microliters of sample. Micro-liquid extraction uses about 40 milliliters (mL), and large volume extractions may use 1 liter (L) or more. A sufficient volume of sample is required to achieve the desired detection limits, but it may be helpful to plan the analytical approach based on the desired goals. For example, if the goal is to quantify a high concentration of contaminant (high micrograms per liter), then less sample may be required than for a low concentration (low to mid-micrograms per liter).

Irradiation and other means to reduce pathogenic infectivity, particularly of samples contaminated with both chemicals and pathogens
Some laboratories may consider irradiating (UV or gamma) the samples prior to chemical analysis to reduce the risk of possible exposure to pathogens. There may be other ways of achieving the goal of reducing pathogenic infectivity, such as pasteurization, other heat sterilization, and/or direct filtration. However, all of these approaches to reduce pathogen infectivity may alter the identity and/or quantity of some chemicals by thermal, photochemical, or physical means. Also, in the case of filtration, significant leaching of interfering substances from the filter material can occur. Therefore, when using techniques to reduce pathogenic infectivity, it is important to keep in mind the goals of the analysis, such as analysis of chemicals, detection of viable organisms, etc.

Currently there is no general consensus on proper use of irradiation and/or other techniques to reduce risks associated with sample handling and analysis while maintaining the integrity of the sample and the analysis. Accordingly, these techniques for reducing pathogenic infectivity are not generally recommended (Module 3, Section 4.4.1). The laboratory may wish to explore these techniques, however, if the authorities in charge of the threat evaluation process (Module 2) believe there is very good reason to suspect that the sample is contaminated with both biological and chemical contaminants. In this case, one way to proceed would be to split the sample prior to irradiation. One portion could be irradiated and analyzed for chemicals, and a non-irradiated portion could be analyzed for pathogens, following confirmation that there are no highly hazardous volatile chemicals in the sample. (Note that an irradiated sample could be used for the pathogens, provided the analysis did not require the presence of viable organisms, as is the case with many molecular assays.) Informed decisions could then be made about how to safely proceed with other portions of the original, non-irradiated sample. Appropriate hoods and other physical control measures should be used when handling such samples.
3.2 Laboratory Infrastructure

Infrastructure must be adequate not only to support the analysis of the target analyte in a water matrix, but also must be sufficient to ensure the safety of laboratory staff and the security of samples and data. The following subsections describe infrastructure considerations applicable to all laboratories, followed by those applicable to specific analytical classes. Details of laboratory infrastructure are discussed in more detail in this section, particularly as they relate to safety, security, and analytical quality. This section is not intended to be a laboratory design manual, but rather to highlight areas in which infrastructure considerations may play an important role when handling emergency samples.

General considerations for laboratory capability

The infrastructure must be consistent with the goals of emergency analysis. Ample instrumentation and laboratory equipment should be available to meet analysis goals. This infrastructure should be compatible with relevant laboratory accreditation requirements, quality assurance plans, and requirements of auditing groups. This may help ensure that laboratories function well during an emergency and do not forego sound laboratory practice due to emergency conditions.

Safety equipment

Infrastructure includes any safety equipment such as eyewashes, safety showers, spill containment devices/supplies, etc. It also includes first aid kits, which should include inexpensive antidote kits for various poisons, such as cyanide and organophosphate compounds. Personnel should be trained on the availability, indications, and use of these antidotes. Safety supplies and training materials are readily available from numerous companies servicing industrial safety needs.

Chemical/biological hoods and physical control measures

Appropriate chemical hoods, biosafety cabinets, containment glove boxes, and/or other physical control measures should be incorporated into laboratory infrastructure and may be required by the HASP. Exhaust and pollution control systems must be evaluated to determine proper operation prior to every analysis (i.e., through the use of appropriate monitors for hood face velocity, etc). The chemical hood, biosafety cabinet, and/or containment glove box should be cleared of all other equipment, samples, reagents, supplies, etc., except those required to open and process the sample itself. Some laboratories may wish to deactivate any contaminants that exist on the outside of the sample container or the sample opening equipment. In this case, the deactivating solution (10% bleach, chemical neutralizers, etc) may be kept in the hood or glove box. Note that deactivating solutions, including bleach, have finite shelf-lives and must be refreshed accordingly.

Ventilation and physical control requirements for chemical samples vary with hazard classification. Most laboratories should currently have sufficient controls for low hazard samples. In general, occupational exposure to hazardous chemicals in laboratories is described in OSHA standard 1910.1450 (http://www.osha.gov). This standard also forms the basis for laboratory safety guidelines for laboratories possessing one milliliter or less of a pure Schedule 1 chemical warfare agent (http://www.usapa.army.mil/pdffiles/p385_61.pdf). This link is provided for reference, and should not imply that a laboratory with a liter of water that contains only 10 microliters of pure agent and that has the appropriate physical controls should consider itself an approved laboratory capable of analyzing these substances.

For biological samples, appropriate equipment is required based on the desired biosafety level (BSL). For BSL-2, biosafety hoods may be required, while BSL-3 requires a specially designed facility. See http://www.cdc.gov/od/ohs/pdfiles/4th BMBL_pdf for design considerations for biosafety hoods and for more information on BSL requirements for particular biological agents in various forms, e.g. dried...
or in solution. For example, although *B. anthracis* is considered an organism that may be safely contained using Biosafety Level 2 (BSL-2, [http://www.bt.cdc.gov/documents/PPTResponse/table3abiosafety.pdf](http://www.bt.cdc.gov/documents/PPTResponse/table3abiosafety.pdf)) conditions, if the procedure is likely to generate aerosols, it should be handled using BSL-3 conditions.

**Sample receipt, handling, and security**
Adequate infrastructure is required to achieve the goals of safety for laboratory personnel charged with the receipt, handling, and analysis of potentially hazardous forensic samples. In some cases, the same infrastructure used for receipt of routine samples may be sufficient, but depending on the existing facilities, some upgrades may be necessary to safely handle hazardous samples.

Infrastructure-related safety issues begin with initial sample receipt. The sample receipt area should be isolated from the analysis area and include features that allow effective handling and security of samples. Furthermore, samples should be handled and secured in a manner that will support the admissibility of the results into a court of law. This may include electronic sample tracking and impenetrable storage areas.

Samples should be opened in a manner to maximize personnel safety and minimize laboratory contamination. For example, samples may be opened in a dedicated glove box or hood which is physically and geographically separate from the hoods used in the analytical laboratory. Laboratories may wish to take pictures of samples and/or install automatic video surveillance equipment in order to document sample receipt, handling, and security. Likewise, laboratories may wish to conduct physical measurements (e.g., determine the mass and/or volume of the water sample, upon receipt and at various stages of analysis). This may be important for safety as well as forensic reasons.

**Dual-use facilities for routine and emergency samples**
For routine analysis, laboratories often can anticipate the concentration range of certain chemicals in water samples and plan accordingly. However, in the case of an unknown, there may not be any information regarding the potential concentration of a particular chemical, and some samples could contain very high concentrations. This can lead to significant problems, such as a laboratory becoming contaminated when unusually high concentrations of a contaminant are present. To minimize the potential for laboratory contamination when working with unknown samples, precautions should be taken, such as working in a dedicated sample prep area, carrying samples in buckets with gas-tight, friction-fit lids containing absorbent material in the bottom, and other measures to physically prevent the accidental introduction of contaminants (including vapors) from water samples into the laboratory.

Aside from safety issues, accurate analytical determinations are related to good laboratory practices, particularly in the area of cross-contamination of samples. (Note that radioactive contamination may be preventable by identifying the presence of radioactivity during initial sample screening procedures.) In addition to degrading the validity of analytical results, cross-contamination can affect aspects of the quality assurance features of a method. For example, contamination of the reagent blank can occur. The reagent blank is an aliquot of reagent water or other blank matrix that is treated exactly as a sample. The reagent blank is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus. If a chemical from an unknown sample contaminates the reagent blank, the accuracy of all subsequent measurements to identify and quantify that chemical in a sample may be compromised. In principle, concerns related to the reagent blank and other related quality assurance issues are procedurally dealt with by the quality control section of the chemical or biological analysis method. However, it is still possible for difficulties to arise, especially
with volatile material that may easily pass through the air from one sample to another or to the reagent blank.

In the case of microbiological contaminants, the high sensitivity of molecular techniques makes them susceptible to contamination by trace amounts of biological materials. For example, molecular methods utilizing the polymerase chain reaction (PCR) are extremely sensitive to the presence of any target DNA. It is imperative that PCR product analysis be carried out in an area physically separated, with separate airflow, from the areas where the samples are prepared for analysis. To avoid contaminating reagents, it is desirable to have a separate reagent preparation facility. These considerations warrant a specialized laboratory facility, specifically designed to deal with the demands of molecular methods. Frequently, a core facility strategy is used to minimize the impact of this space requirement. In this case, there should be separate preparation areas for suspected biological contaminants, to prevent possible cross contamination with routine environmental samples, and to minimize the opportunity for contamination of a laboratory with biological contaminants.

Sample capacity and turn-around time
Laboratory infrastructure involves elements that are desirable for ensuring sufficient analytical capacity and rapid turn-around time in the case of an emergency incident, while maintaining quality standards. This includes elements of laboratory staffing, planning of work schedules, and creating provisions for the availability of staff during an emergency. In addition, the facility should allow for 24/7 access and control of safety measures. For instance, laboratory personnel should be able to override systems that reduce automatic hood ventilation at preset times to save energy. Laboratories may choose to perform frequent exercises to meet the combined goals of analysis speed and accuracy.

Sample disposal
Laboratories should have waste disposal plans in place, subject to applicable regulations. As discussed previously, most samples will likely contain no or little contamination, and hence existing laboratory waste disposal practices should suffice. In the relatively rare instance that the sample does contain a hazard, then it is likely that law enforcement will want the sample retained as evidence and provide guidance as to its storage and ultimate disposition when no longer needed. In any case, all regulations governing the disposal of hazardous substances should be adhered to when disposing of samples.

3.3 Data Analysis and Reporting
The responsibility of the laboratory during an emergency does not end with sample analysis. At a minimum, the laboratory should report the results in a timely manner to the designated recipients. Furthermore, the laboratory may be called upon to assist in analysis and interpretation of the data. While specific arrangements for the reporting and evaluation of analytical results need to be made between the laboratory and the client, the following general guidelines will apply in most situations:

1. The laboratory and the client (e.g., the water utility emergency response manager or the designated incident commander) should agree on the format and content of the report as part of their planning. In this manner, incident command will know what to expect and can practice interpreting the report. There are efforts in the forensic community to provide standardized guidance on report writing (i.e., the minimum types of information that should be contained in such a report). When it is available, this guidance may be included in this module.

An important issue in the content of the report arises when the laboratory is asked to provide an analysis of a water sample for which the contaminant is unknown (e.g., applying a laboratory
analytical screening procedure to a water sample, as described in Section 6.3). Environmental laboratories are often familiar with determining specific analytes and providing reports regarding those analytes. For example, the laboratory may apply a standardized method and report on the analytes listed in the method. However, if the laboratory observes non-method analytes during the analysis, several questions arise about how these should be reported, such as:

- What is a significant result in terms of the concentration of the non-target analytes?
- Should all non-detects be reported?
- How much detail is appropriate in identifying and/or quantifying the analytes?

In principle, the report should be thorough enough so that one does not miss anything important, but if too much information is reported, the laboratory may confuse the client. These are complex issues that are likely to be situation-specific and may also be dependent on local policy. Also, reporting priorities may change during the course of the evaluation of a water contamination threat/incident. For example, during the initial phases of the evaluation, the water utility emergency response manager may set a high priority on identifying any unexpected substances in the water, but only above certain concentrations (e.g., normal background levels). Later on, as critical decisions may need to be made, management may be more concerned about analytical confirmation of identification and accurate quantification. Planning and table-top exercises may help the water utility and laboratory decide on what report content is best for them, particularly at various stages of the evaluation.

2. During a suspected contamination incident, it is important that all relevant information be managed through the incident command. This is especially true for analytical results that could be subject to misinterpretation if not placed into the context of the situation. Thus, analytical results should be reported only to those individuals designated by incident command, and it will be their responsibility to inform other stakeholders. The water utility emergency response manager (or the designated incident commander) and laboratory contact should be aware of the various applicable Federal, State and local legal requirements, especially for reporting infectious diseases. If they are not, the laboratory should inform incident command of these requirements when reporting the results to incident command, provided such reporting is permitted by regulatory requirements.

3. In a crisis situation, the laboratory may be asked to provide tentative results (sometimes called a “rolling report”) prior to complete review and confirmation, especially in cases where more than a couple of hours is required for confirmation. The laboratory may need to provide appropriate caveats regarding the validity of the data at this stage of the analysis. Depending on the analytical methods used, such caveats, perhaps in the form of a clearly presented narrative summary, might include: methods and techniques used, the probability of false negative/false positive results, limit of detection, method accuracy/precision, quantitative versus qualitative results, and the time necessary to confirm tentative results. It is the responsibility of incident command to weigh all of this information and make decisions about appropriate response actions.

4. The laboratory should be available to assist in the analysis and interpretation of the results. Not only will the laboratory staff have a unique perspective regarding the reliability of the method and interpretation of results, but they also may have substantial experience with the application of the methodology to other samples. Thus, the analyst may have the ability to discriminate
between results that fall within the normal range of occurrence and those likely to be indicative of an actual contamination incident. As an example, chloropicrin and cyanogen chloride are potentially hazardous chemicals at high concentrations in water. However, these same compounds can occur at very low levels in disinfected drinking water as disinfection by-products, resulting from the reaction of the disinfectant with materials naturally present in the water. An analyst with experience determining these compounds would likely recognize elevated levels outside of the range typically encountered in treated drinking waters. The issue of baseline (background) levels is critical to the interpretation of analytical results and thus to the threat evaluation (Module 2). A more difficult interpretation of the results would occur if low levels of such “naturally occurring yet potentially hazardous contaminants” are detected, since these levels could represent typical background or the tail of a transient contaminant slug, in which case additional sampling and analysis may be necessary.

3.4 Quality Assurance and Quality Control

Standardized methods available in fixed laboratories are designed and verified to provide accurate and defensible results. However, methods for analysis are only as good as the people who perform them, assuming suitable analytical technology is at their disposal. It is common experience that some individuals obtain better results from analytical methods compared to others due to the skill level of the analyst, and this may be particularly true during the analysis of an unidentified contaminant using non-standardized methods, as discussed in Section 6. To address these types of concerns, EPA has developed extensive guidelines regarding the quality of data generated internally and for non-EPA organizations. For more details, see http://www.epa.gov/quality1/exmural.html#genreqts.

There are currently specific QA plans/guidance being developed for forensic analysis of unknowns for both biological and chemical samples. This QA guidance may be included in a future version of this module. Many laboratories may already have quality assurance plans that meet these requirements. Until (and after) this QA guidance is formalized, laboratories should be aware of legal issues involving admissibility of scientific evidence, as discussed in the following section.

One consideration with respect to QA/QC for analytical methods used during the evaluation of suspected water contamination incidents is the objective of the analysis. For instance, one goal may be to qualitatively confirm the presence of a particular contaminant. Another goal would be to quantitatively determine the concentration of a contaminant. Selecting the objectives of a particular analysis is part of planning for a site-specific response to a contamination threat. In many cases, the threat evaluation process discussed in Module 2 will drive the purpose of the analysis toward quantifying chemicals at concentrations from milligrams per liter to low micrograms per liter of chemicals, and pathogens at their infective doses. The analytical approach presented in Sections 6 and 8 reflects this goal. However, depending on the specific plan, other goals may be applicable.

3.5 Admissibility of Scientific Evidence

If criminal or terrorist activity is the suspected cause of a contamination incident, any samples collected and analyses performed may be considered evidence by law enforcement agencies. Many environmental chemistry laboratories are currently performing water analyses to support regulatory compliance, and they are familiar with the need to treat samples with precautions necessary for legal defensibility. Analysis for regulatory compliance purposes, however, may operate somewhat differently than analysis of emergency samples in response to a contamination threat, which has the
dual role of both providing an emergency response to protect public health and also supporting a criminal investigation.

The objective of the emergency response is to provide the water utility emergency response manager (or the designated incident commander) with timely and accurate scientific results (Module 1). Supporting a criminal investigation may involve additional time and effort. Improper decisions made during emergency response may also result in public outcry and/or legal action, whether criminal or civil, so the quality of data required for a response action may rival that of the criminal investigation.

Meeting these dual goals requires careful planning and analysis of the public health needs and legal requirements in place for a specific location. Nevertheless, utility planners should be aware that most of the groundwork is already in place at laboratories, and may be readily built upon through appropriate selection of the analytical approach, as well as training exercises. Some of this groundwork is described below.

To maintain the credibility of evidence throughout the sampling and analysis process, the laboratory must demonstrate an unbroken chain of custody and ensure that basic principles of admissibility of scientific evidence are met. Chain of custody begins with sample collection and shipment to the laboratory. Sample custody typically becomes the responsibility of the laboratory upon sample receipt. Documentation should clearly track the sample and analytical results from the point of sample receipt through reporting of results. Furthermore, when designated laboratory staff are not in possession of the sample or related information, it should be secured. If there are gaps in the chain of custody or periods during which the sample or results could have been compromised, the analytical results could be dismissed by the court. In cases in which the analysis leads to prosecution of a perpetrator, chain of custody should continue until law enforcement has determined that any remaining sample is not needed as evidence.

Certain principles govern the admissibility of scientific evidence in a court of law, and laboratories processing samples considered as evidence should adhere to these principles to improve the legal defensibility of their results. There are no universally accepted criteria for the admissibility of scientific evidence, although State and Federal courts typically use one of three standards: Federal (or State) Rules of Evidence, the Frye standard, or the Daubert standard. A more detailed discussion of the admissibility of scientific evidence can be found in the Reference Manual on Scientific Evidence, 2nd ed., which can be downloaded from the Federal Justice Center’s web site at http://www.fjc.gov/public/pdf.nsf/lookup/sciman00.pdf/$file/sciman00.pdf.

Note that in the case of scientific evidence, states may develop their own criteria, other than Frye or Daubert, which are more stringent than the relevancy issue considered in the rule of evidence. In considering the principles that govern the admissibility of scientific evidence in the context of the analysis of water samples for known or unknown contaminants, it is most useful to consider the most stringent standard – Daubert. It is clear that a laboratory would play a crucial role in the selection and implementation of an analytical approach that would result in data that would pass the Daubert tests. There are currently efforts underway in the forensic community to prepare guidance for proper implementation, particularly as it refers to the validity of the analytical results. While this guidance is not available at this time, it may be included in future versions of this module. In the meantime, a few example areas that should be addressed that would aid in meeting the Daubert standard include:

- Quantification of Type I (false positive) and Type II (false negative) error rates through appropriate quality assurance and quality control (QA/QC).
• Use of standardized methods, or methods that have been subject to rigorous peer review if standardized methods are unavailable.
• Participation in a certification or accreditation program for the relevant methodologies.
• Participation in interlaboratory studies (or third-party performance evaluation studies).
• Supporting documentation for methods, SOPs, QA plans, etc.
• Training and competency testing for analysts.
• Special training for scientists and analysts required to give expert testimony.
4 Site Characterization and Sampling

While this module focuses on the analytical approach, laboratory personnel need to have a basic understanding of the processes implemented prior to transfer of sample custody to the analytical laboratory. This understanding may be of value to the laboratory for the purpose of developing procedures for sample receipt that will ensure the safety of laboratory personnel, a meaningful analytical approach, the timeliness and quality of the analysis, and the legal defensibility of the results.

The processes leading up to sample analysis, namely site characterization and sampling, are covered in detail in Module 3, “Site Characterization and Sampling Guide,” but it is worthwhile to briefly discuss a few points here. Readers are encouraged to refer to Module 3 for more complete explanations and extensive description of site characterization activities, including a general discussion of who will perform the activities under various hazard conditions. Figure 4-3 illustrates the steps leading up to sample shipment. The glossary provides definitions of terms used.

![Diagram of Site Characterization and Sampling Process]

Figure 4-3: Site Characterization and Sampling Process
4.1 Site Characterization

The left side of Figure 4-3 shows the threat evaluation process conducted in parallel with the site characterization and sampling procedures. Throughout the entire evaluation of the incident, the credibility of the threat is continually reevaluated as new information becomes available. If at any point the threat is determined to be not credible, the process is discontinued. Thus, samples might only be sent to the laboratory if the threat is still deemed credible following sample collection, as indicated in Figure 4-3. The threat evaluation protocol is discussed in more detail in Module 2.

The site characterization process begins with an evaluation of the site hazards. Based on the available data and initial threat evaluation, a determination will be made regarding the need to implement special hazardous material handling techniques. This may dictate the organization or team that is sent to the site to perform field screening and sampling. While it may be necessary to consider the details of a specific threat/incident when determining the level of protection required for the response team, planning for a response to various scenarios should occur well in advance of an actual threat. Specifically, response plans should document who would be called to respond to contamination threats under different hazard conditions.

Upon site entry, field safety screening protocols may be implemented. The purpose of field safety screening is to detect any immediate threats to the response team from contaminants in the atmosphere or on surfaces. Core safety screening focuses on threats from radiation, but the safety screening may be expanded to include volatile chemicals, chemical weapons, and biological weapons as appropriate for the situation. If any of these threats are detected, it would likely be necessary to immediately evacuate the site and send in teams properly equipped to deal with the hazard tentatively identified during the safety screening. The site characterization team should have training in the uses of safety screening equipment (see section 4.2.2 of Module 3) and be familiar with its capabilities and limitations.

After the field safety screen has been completed, the appropriate team may continue the site characterization, including rapid field testing of the water. Recommended core field testing consists of radiation monitoring (in the water), cyanide, chlorine residual, and pH. Section 4.3.2 of Module 3 discusses options for expanded field testing. Note that these core tests are based on historically reliable or verified technology. Few of these technologies for rapid field water testing beyond the core tests, however, have undergone a thorough and independent performance evaluation. Without reliable performance data, any field testing technology for chemicals, pathogens and/or radionuclides should be used with caution. The same is true for technology for safety screening, particularly for biological contaminants. This document is somewhat forward looking, and it assumes that some of the existing kits will provide some useful data; however, these kits have not yet been verified. Verification of several of these technologies is being carried out through EPA’s Environmental Technology Verification program in 2003, and the verification reports can be found, when available, at http://www.epa.gov/etv/.

There are three objectives of field testing the water: 1) provide additional information to assess the credibility of the incident; 2) provide tentative identification of contaminants that would need to be analytically confirmed in the laboratory; and 3) determine if hazards tentatively identified in the water require special precautions during sampling. Note that these goals are distinct from the analytical testing that occurs at the laboratory, even if the same technology is employed at the laboratory as part of their safety screen upon sample receipt (Section 3.1.4).
4.2 Sampling
After field testing is completed, samples may be collected using those precautions appropriate for the hazards identified. A sampling kit that contains bottles and supplies for the proper collection and identification of each sample should be available. In Section 4.2.2 of Module 3, Table 3-1 describes such a sampling kit. Note also that Table 3-1 does not contain any personal protective equipment, except that required to add the chemicals used to preserve the samples. If any personal protective equipment is required, it should be included as part of the health and safety plan (HASP) included in the sample kit (Table 3-1).

In general, samples for chemical analyses may be collected in clean glass or plastic containers according to sampling procedures appropriate for the analysis. The specific sample containers suggested for chemical analytes are listed in Table 3-2 of Module 3, and correspond to the particular analysis in the analytical approach set forth in Sections 6 and 8 of this module. The table lists the number, type, and volume of each sample container, as well as any preservatives or dechlorinating agents appropriate for the sample. Laboratories may find the information in these tables useful to increase their familiarity with incoming samples.

4.3 Sample Transport and/or Storage
The process flow chart in Figure 4-3 indicates that samples would typically be collected after field testing regardless of threat credibility; however, a decision must be made whether or not to send samples to the laboratory immediately following sample collection. This decision should largely be based on the outcome of the threat evaluation. If the threat is deemed credible, the samples may be sent to the appropriate laboratory for immediate analysis. Packaging and transport of the sample to the laboratory is discussed in Section 6 of Module 3. If the threat is not deemed credible, the process is halted; however, some analysis may still be performed on the samples at the discretion of the utility or other parties involved in the response.

If samples are not sent to the laboratory for analysis, they should be held in cold storage (protected from freezing at 4°C) under safe and secure conditions for at least one week following collections, and possibly longer if dictated by other parties that could be involved in the response (FBI, CDC, EPA, etc.). If the samples are stored, then the samples must be securely stored and appropriately preserved (Section 4.2). Proper chain-of-custody procedures should be maintained, and holding times must be within acceptable bounds to meet the analytical goals for the contaminant of interest.

4.4 Site Characterization Report and Chain of Custody
Chain of custody is initiated at the time of sampling, along with other sampling documentation. If samples are sent to the laboratory, all relevant documentation should be submitted at the same time including:

- Contact information for the sampling organization.
- Sample documentation, including chain-of-custody forms.
- Details about any pretreatment performed on the samples in the field, such as dilution, preservation, dechlorination, etc., if not already included elsewhere.
- Site characterization report, including site investigation forms, field safety screening results, and results from rapid field testing conducted on the water.
- The current stage of the threat evaluation. Note: the laboratory should be cautioned against reducing handling precautions for lower threat evaluation stages without good reason.
• Instructions on data reporting (i.e., to whom, what format, special reporting requirements, etc.).

This information may be of tremendous value in the development of an analytical approach for a water sample that may be contaminated with a harmful substance, as discussed in greater detail in Section 5. The laboratory should be aware that the site characterization report may be incomplete and/or inaccurate. If the laboratory has any questions, communication (see section 4.5 below) becomes extremely important. The laboratory may also wish to verify the preservation and/or dechlorination of some arriving samples (e.g., through the use of test strips for free chlorine and pH). Deviations from the preservation and/or dechlorination described on the sample documentation should be noted by the laboratory, and the laboratory should adjust their sample processing accordingly.

In addition, chain-of-custody procedures should be followed carefully to support future criminal and/or civil legal action regarding the incident. Chain-of-custody should not stop with sample receipt but should also include an internal chain-of-custody procedure. Internal chain-of-custody should be established for all samples regardless of any questions about chain-of-custody maintained prior to receipt by the laboratory.

4.5 Communication
Multiple laboratories may be involved, depending on the nature of the analytes. All relevant laboratories should be included in the communication process. Communication between the laboratory(ies) and sampling team should begin no later than the time of sample collection, especially in the case of a highly credible threat. In some cases, a laboratory may choose to send personnel to the site to ensure that proper sampling techniques are followed, assist in the interpretation of field test results, and facilitate chain of custody. This interaction may help laboratories identify any potential hazards associated with the samples and consider site-specific information in developing an analytical approach for the specific incident. If laboratory staff cannot be present during sample collection, a conference call might be scheduled to discuss the information outlined above, as it relates to laboratory safety and development of an analytical approach, which is discussed in more detail in the following section.

Communication should be appropriately documented for future reference. Written notes may be taken, and electronic recording devices may be used, but only to the extent allowed by privacy laws, particularly for telephone conversations.
5 General Considerations for an Analytical Approach for Unidentified Contaminants in Water

In the case of a water contamination threat, it will likely be necessary to make rapid decisions, based on limited information, about the contaminants that will be evaluated by the laboratory. These decisions may impact the samples that are collected, selection of the laboratory, and the specific analyses that would ultimately be performed (defined as the analytical approach). In some cases, evidence from the site or results from field screening may provide some indication about the identity of the contaminant, and the analytical approach can be adjusted accordingly. However, it is generally assumed that the presence and identity of a contaminant in the water sample would need to be analytically confirmed with limited evidence to guide the analysis. Furthermore, even if a tentative identification of the contaminant has been made in the field, circumstances may warrant analysis for other contaminants in addition to those tentatively identified.

A number of officials from various organizations, including the laboratory, may need to be involved in the development of an analytical approach in response to a specific contamination threat. During the response to a contamination threat, the laboratory would need to establish clear lines of communication with incident command, the affected utility, sampling teams, and any other stakeholders that are not coordinated directly through incident command. This collective group of decision officials would need to evaluate available information and work together to develop an analytical approach that is appropriate for the circumstances of the specific threat.

The analytical approach may need to be developed rapidly – in a time frame of minutes to hours. Planning, preparation, and communication are key to making such decisions in a rapid and effective manner. After careful planning, laboratories may wish to formalize their analytical and management approach in a laboratory guide that is analogous to the response guide (Module 1) prepared by utilities that summarizes the actions the utilities plan to take during an emergency.

This section lays out a framework for developing an analytical approach in response to a specific contamination threat. The technical aspects of the analytical approach for chemicals and pathogens are described in Sections 6 and 8, respectively. Hypothetical examples of specific approaches developed to meet site-specific objectives are given in Section 7. While this framework may be used in real-time during a response, it should also be used for preparation and planning for such a response. Laboratories and their customers (utilities, States, Federal agencies, etc.) should work though this process under various test scenarios to become familiar with the process, and to understand the capabilities and limitations of a laboratory in implementing the approaches described in Sections 6 and 8. Laboratories may find they need to expand their capability in a particular area to provide adequate coverage for target analytes, or it may become apparent that limitations may require use of other laboratories for some specialty analyses.

This section is intended not necessarily for laboratories, which may be more interested in Sections 6 and 8, but for planners and managers, who may be more interested in the concepts involved. Section 5 is divided into three subsections. The first subsection presents a decision tool that may serve as a model for the development of an analytical approach to a specific contamination threat, based on different levels of tentative identification. The next subsection, “Initial Assessment of Available Information” describes potentially useful information from a specific contamination threat to support the development of an analytical approach. The final subsection describes the general approach used for the analysis of unknown contaminants.
5.1 **Framework for Development of an Analytical Approach**

Once the decision to send samples to a laboratory for analysis has been made (see Section 4), it will be necessary to develop an analytical approach that is appropriate for a specific contamination threat or incident. Figure 4-4 presents a decision tool that is intended to aid in the development of the analytical approach. Figure 4-4 is geared towards the analysis of water samples potentially containing an unknown chemical or biological contaminant that was collected from a site characterized as having low hazard conditions. It is assumed that radiation screening has been performed prior to the start of Figure 4-4. In particular, if the field testing results for radiation were positive, one would go down an entirely different, and fairly well-defined, analytical path (see Section 6.4.8).

![Decision Process Diagram](image)

**Figure 4-4. Decision Process for the Development of an Analytical Approach for Potentially Contaminated Water Samples**

It is important to emphasize that the decision process depicted in Figure 4-4 should be used as a planning tool, not just during an incident. Laboratories, in conjunction with their clients, should work through this process prior to an emergency situation to develop and refine their own analytical plans and procedures. The decision process begins with an evaluation of available information about the contamination threat that might be of value in developing an analytical approach. The information used to support this initial assessment is described further in Section 5.2 and in greater detail in Module 2.
The first decision point in the process is an assessment of whether or not there is sufficient information to make a tentative identification of the contaminant as chemical and/or biological. If this is possible, then an entire class of contaminants is eliminated from consideration, allowing focus on the tentatively identified contaminant class. If the information is not sufficient to make a determination between chemical and biological contaminants, then the sample may be treated as a complete unknown. The recommended analytical approach for a complete unknown is described in Section 5.3.

The second decision point involves tentative identification of the specific contaminant. At this point, the contaminant identity is hypothesized based on available information from the site characterization report. Examples of situations in which tentative identification might occur include: a specific contaminant named in a threat; tentatively positive results for a specific contaminant during field safety screening or rapid field testing; physical evidence at the site pointing to a specific contaminant; and clinical evidence of the identity of the disease causing contaminant. It is important to note, however, that each of these situations has a different level of credibility for the purpose of tentative identification. In general, tentative identification may focus the analytical approach on the specific contaminant subclass, leading to confirmatory analyses for the particular contaminant. For example, tentative identification of a class of pesticides (e.g., organophosphates) may be based on results from a test kit, and this information might be used to focus the analytical approach on specific pesticides within that class. Depending on the strength of the tentative identification, as determined with due diligence by the water utility emergency response manager, it may be wise to consider heading down both paths simultaneously (Module 2). If the evidence for tentative identification is sufficiently strong, then screening may be delayed. Otherwise, screening for a broad array of potential contaminants should begin simultaneously with the confirmatory analysis for the tentatively identified contaminant.

The third decision point is based on the results of the confirmatory analysis used for the tentatively identified contaminant. If the presence of the contaminant was analytically confirmed, then the results are reported to incident command. In the case of potential multiple contaminants, multiple analyses may be required before incident command decides if the analysis is complete. (Note: For the purpose of this document, analytical confirmation refers to the legally defensible identification of an analyte. There are efforts in the analytical forensic community to rigorously define what constitutes confirmation, and results of these efforts may be included in later versions of this module. In the interim, laboratories should pay careful attention to principles that govern the admissibility of scientific evidence [Section 3.5].)

The fourth decision point involves the possibility of additional screening for chemicals or biologicals if the presence of the tentatively identified contaminant was not confirmed or additional contaminants are suspected. At this point, communication between the laboratory and incident command will likely be necessary, because the decision to perform additional screening may be based on recently available information regarding the threat. For example, new evidence may come to light indicating that, despite initial indications, the threat is no longer credible. However, in case the threat is still deemed credible, it may be necessary to revise and/or expand the analytical approach, possibly in conjunction with a fresh review of the site characterization report.

In any situation involving tentative identification of a contaminant, the reliability of the information should be carefully evaluated. In some cases, it may be determined that the source of information is too unreliable to provide the basis for a tentative identification. For example, if a specific contaminant is named in a threat made over the phone, the reliability of the information might be considered suspect. In this case, the analytical approach might start with the named contaminant, but additional
screening might be warranted depending on other considerations such as the credibility of the incident. For this reason, development of the analytical approach may be iterative, as indicated by the decision point “Is additional screening necessary?” If the answer to this question is “yes,” available information would be reevaluated to refine or broaden the analytical approach.

5.2 Initial Assessment of Available Information

While previous water contamination threats may indicate that the most likely scenario is an unknown contaminant, it is possible that there may be some information from the incident that may inform the development of the analytical approach. For example, negative results from reliable field tests may eliminate some contaminant classes from further consideration. Likewise, information from the site characterization report may be useful in tailoring the screening to the specific situation. Even if the available information is insufficient to make a tentative identification at the grossest level (i.e., chemical or microbiological), it still may be of value during application of the screening.

The first step of the process shown in Figure 4-4 is an evaluation of available information from the current threat that might provide clues about the identity of the suspected contaminant. Information that should be considered in developing an analytical approach for a specific contamination threat includes the following:

- **Public health information** may be available if exposed individuals seek medical attention, and the clinical data for these patients may provide insight regarding the identity of the contaminant. Such information may be used to make a tentative identification of the contaminant or contaminant class, and in some cases might provide definitive identification. It is important to note that even though exposure has occurred, it does not necessarily imply that drinking water is the source of the contamination; other routes of exposure, such as food, air, or surfaces might be considered depending on the situation.

- **Tentatively positive results** from field testing may be used to tentatively identify a specific contaminant or contaminant class. Negative results may be used to exclude certain contaminants from further consideration. The credibility of the field test results must be evaluated in the context of the reliability of the field test equipment and procedures.

- **Physical evidence** at the site may provide insight regarding the identity of the contaminant. For example, empty containers might have markings indicating the nature of the contaminant, and analysis of the residual material in the container may confirm the identity of the substance. Other physical evidence from the site, such as dead animals or plants, might be used to formulate a hypothesis about the identity of the substance, and thus inform the analytical approach.

- **Information about a specific contaminant** from a database or fact sheets may be used to make a tentative identification of the suspect contaminant. For example, field screening results and/or physical evidence might be used in conjunction with detailed information about potential contaminants to make a tentative identification of the contaminant or contaminant class.

- **Location-specific contamination threats** should also be considered during development of an analytical approach for a specific contamination threat. For example, utilities may have
identified potential contaminant sources (e.g., chemical warehouses) during their vulnerability assessment, and such opportunity contaminants might be explicitly included in the analytical approach.

- **Background concentrations of the specific contaminant at the site** may be extremely important in determining if a contamination incident has occurred. In some cases and for some contaminants, background levels may be at detectable concentrations. **If unrecognized, background concentration of a contaminant may be confused with an actual contamination incident.**

- **Threat credibility** may provide an indication of the severity of the incident, and thus may indirectly impact the analytical approach. For example, if a threat is deemed highly credible, then complete screening for both chemical and biological contaminants might serve as the analytical approach regardless of other information collected from the site.

- **Consequences** of failing to properly identify an unknown contaminant (or not confirming the absence of harmful contaminants) should also be considered in developing an analytical approach. The higher the potential consequences of a misdiagnosis of the incident, the more rigorous the analytical approach should be.

### 5.3 Overview of Analytical Approach For Unknowns

In the case of a complete unknown, the problem of confirming the presence/absence of a contaminant or identifying and quantifying a specific contaminant presents a significant challenge. The difficulty arises from the large number of potential contaminants of concern, and the impracticality of screening for them all. To address this issue, the analytical approach for unknowns was based on contaminant classes derived from a prioritization of chemicals and pathogens of public health concern if present in the drinking water supply. The approach used to prioritize potential contaminants is briefly discussed in Section 2.1 of Module 1.

The analytical approach for unknown contaminants in water presented in this module is comprehensive for selected, *priority contaminants* and provides coverage for hundreds of additional contaminants not on this list. The following assumptions and principles were used in the development of these approaches:

- Selection of target analytes was based on an assessment of contaminants likely to pose a threat to **public health** if introduced into the drinking water supply.
- Existing laboratory infrastructure and analytical methodologies were utilized when possible.
- Both chemical and microbiological procedures are tiered, with a progression through field safety screening and rapid field testing to laboratory testing to confirmatory analysis.
- Samples analyzed in a laboratory that cannot be confirmed by the laboratory performing the test are referred to laboratories that can perform a confirmatory analysis. This upward referral system is formalized for microbiological analysis, through the Laboratory Response Network, while it is not as well defined for chemical analysis.
- The entire approach relies on the systematic elimination of potential contaminants, both to ensure the safety of sampling and laboratory personnel, and to aid in identification of the unknown contaminant.
Considering the principles and assumptions upon which the comprehensive analytical approach is founded, it is likely that the approach may evolve as new methodologies are developed and refined. Furthermore, laboratories with advanced capabilities may wish to implement alternative procedures that either expand coverage or streamline the analysis. Such modifications would be acceptable assuming that they provide at least equivalent coverage of target analytes compared to the standard approach and meet the data quality objectives of the analysis, such as legal defensibility of data. The comprehensive analytical approach in this module should serve as a baseline against which any alternate approach should be compared.

It is also important to realize that identification of unknown contaminants in water samples is not an exact science. There should be no expectation than any combination of technology and analytical personnel will guarantee successful identification of unknown contaminants. However, application of appropriate technologies and training of laboratory personnel will likely increase the probability of success. The successful identification of an unknown is dependent upon the skill of the laboratory personnel and the laboratory having appropriate analytical technology. With the many types of analytical techniques and methods in existence, it is likely that no one analyst, no matter how skilled, can use all these techniques and methods. A more thorough identification of unknown contaminants may involve a team of chemists and microbiologists to provide consultation and/or perform various analyses.

Since the procedures and laboratories used for the analysis of chemical and microbiological contaminants are significantly different, discrete analytical screening procedures have been developed for each. The screening for chemical contaminants is presented in Section 6, while the screening for microbiological contaminants is presented in Section 8. These procedures are briefly discussed in the following subsections.

Not only may different laboratories be involved in the analysis of chemical and microbiological contaminants, but some select analyses or extreme hazards should be handled only by a limited number of specialized laboratories. Since different laboratories may be involved in implementation of the analytical approach for a specific water contamination threat, communication and coordination among the various laboratory personnel involved in the analysis of an unknown sample is critical. For example, other laboratories should be made aware of potentially harmful contaminants revealed by other laboratories. This may be vital to the safety of the laboratory personnel as well as the expeditious identification of unknowns.

5.3.1 Screening for Chemicals, Including “Unknowns”

Screening for chemicals, including “unknowns,” is described in detail in Section 6. Because the target audience for Section 6 is laboratories, the following overview is presented for the benefit of planners and managers. In summary, the chemical screen integrates several analytical techniques to cover a broad range of chemical classes. These analytical techniques include not only wet chemistry and instrumental analysis, with which laboratories are typically familiar, but also various hand-held equipment and commercially available test kits, such as those based on immunoassays.

The overall screen is broken up into two parts, one referred to as a basic screen and the other as an expanded screen. The suite of techniques utilized in the expanded chemical screen is comprehensive for all prioritized contaminants of concern discussed in Section 6.1.1. Furthermore, the screen may be capable of detecting hundreds of additional chemicals that were not identified as high priority, but
could still pose some problem if used in an intentional contamination threat or incident. It is important to note that the screens are not prescriptive and labs have a great deal of flexibility in building an analytical approach that is consistent with their existing capabilities and experience while meeting the needs of their clients. However, it is strongly recommended that screening for chemicals encompass a wide range of possible contaminants given the large number of chemicals potentially available. See Section 6.1 for more details.

The basic screen utilizes established analytical techniques in conjunction with legally defensible, standardized methods for the analysis of contaminants in water. However, these established techniques do not provide complete coverage for all target analytes. For instance, currently there are no standardized methods for analysis of the biotoxins of concern in water. To address these gaps, exploratory techniques, which do not have standardized methods, are used in the expanded screen. For instance, immunoassays or liquid chromatography-mass spectrometry may be incorporated into the expanded screen to cover biotoxins. Of the many analytical techniques available, the ones utilized in the expanded screen are those that may show the most promise for water analysis. Particularly, those that have established applications in media other than water provide a basis for inclusion in the expanded screen.

In the screening procedure, analysis of contaminants is divided into chemical classes, such as organic, inorganic, and radionuclides:

- Organic analyses utilized in this approach are comprised of some combination of the following three steps: 1) extraction or recovery of the contaminant from the aqueous matrix, 2) separation of the compounds through gas chromatography or liquid chromatography; and/or 3) detection and identification of the analyte. Preparatory and extraction techniques for organic constituents should be broad enough to recover a variety of compound classes (e.g., a range of hydrophilic properties and molecular weights). A variety of techniques are used for detection of organic constituents. When mass spectrometry is used for detection, qualitative identification may be realized through electron ionization mass spectral library comparisons.

- The inorganic analyses include several analytical techniques: classical wet chemistry and instrumental techniques such as inductively coupled plasma mass spectrometry, inductively coupled atomic emission spectrometry, atomic absorption spectrometry for trace metals, and ion chromatography for anionic and cationic contaminants.

- The analysis of radionuclides during an emergency incident relies on conventional radiological techniques, but falls into a separate paradigm than other chemicals because radionuclides may be characterized by both the type of radioactivity they emit as well as specific radioisotopes.

Figure 4-4 illustrates an important issue in the screening for unknown chemicals, namely that confirmatory analysis may be required in the case of a tentatively identified chemical. In general, a positive result from a rapid field test or safety screening (performed in the field or laboratory) would be considered tentative identification and require independent confirmation. By contrast, chemicals identified through the application of standardized methods typically do not require independent analytical confirmation because recommended confirmatory steps are often incorporated into the methods themselves. In some cases, another laboratory with specialized capability may need to perform the confirmatory analysis. For instance, confirmatory analyses for chemical weapons would be performed only in few established laboratories designed and permitted to work with these substances.
When possible, confirmatory analyses should be performed using existing standardized methods accepted for analysis of the target analyte in a water matrix. When a standardized method is unavailable, confirmatory analysis may need to be performed through application of methods that rely upon different separation and/or detection techniques as a means of independently verifying the identity of a chemical contaminant.

5.3.2 Screening for Microbiologicals, Including “Unknowns”

The microbiological screen is designed to recover several classes of organisms, including parasites, bacteria, and viruses. Furthermore, the approach may recover some high molecular weight biotoxins, such as botulinum toxins. The four basic steps of the microbiological screen include: 1) concentration and recovery in the field; 2) rapid field testing; 3) testing at a Sentinel laboratory; and, if needed, 4) testing at a Reference laboratory.

To obtain the detection limits necessary to provide results from microbiological analysis that are meaningful from a public health perspective, it is often necessary to concentrate the water sample by several orders of magnitude (e.g., 100 liters concentrated to 100 milliliters). In the microbiological screen (Section 8), concentration is performed by ultrafiltration, a physical separation process capable of complete retention of parasites, bacteria, viruses, and potentially some large molecular weight biotoxins. Ultrafiltration is capable of processing large volumes of finished water in a short period of time, while continuously concentrating pathogens and other suspended solids in the retentate. The accumulated material is then removed from the membrane through mechanical or chemical means and collected as a retentate. This low volume retentate may be then subjected to microbiological analysis, so it should be handled with appropriate caution to ensure the safety of the sampler and the viability of any organisms collected.

The rationale for performing this concentration step in the field as an integral part of sample collection is based on two considerations. First, containers approved for the shipment of large volumes of liquid suspected of containing select pathogens do not exist; thus there may be no effective means of quickly transferring a large water sample to another facility for processing. Second, concentration of microbiological contaminants in the field allows for the useful application of field screening devices (e.g., pathogen field test kits), the next step of the microbiological screen.

After concentration, the concentrated sample is divided into three aliquots. Two of these aliquots are packaged for shipment to the laboratory, while the third may be used for field testing. The method for field testing will depend on the technology utilized, but in the case of pathogen field test kits, a small volume of the concentrate may be directly applied to the pathogen field test kit, followed by a short reaction period and evaluation of the test result. A positive result should be considered tentative, and the information used accordingly in the context of the overall threat evaluation.

The remaining two aliquots are sent to a laboratory, such as an LRN Sentinel Lab, capable of performing tests for tentative identification of target pathogens. One aliquot may be used to perform culture techniques for the target pathogens. If the initial results of the culture method are analyzed, and colonies of a particular morphology are considered tentatively positive, they are forwarded to an LRN Reference laboratory. The second aliquot may be used for PCR and subsequent molecular analysis for target pathogens, which will provide more rapid, yet still tentative, results compared to the culture technique. However, molecular assays do not necessarily produce specimens that can be forwarded on to LRN Reference laboratories for confirmatory analysis. It is important to note that
LRN PCR techniques for select agents may not be performed by Sentinel labs, although Sentinel labs can perform certain non-LRN PCR techniques.

Upon tentative identification of a target pathogen, the suspect cultures are isolated and processed for referral to higher level LRN laboratories capable of confirming the presence of select pathogens through a combination of culture and PCR techniques. Once the sample enters this level of the LRN, the existing network, and methodologies will be utilized to further characterize the pathogen.
6 Analytical Approach for Chemical Contaminants

6.1 Overview of Contaminant Issues

6.1.1 Types of Chemical Contaminants Covered by the Analytical Approach

Module 1 of this Toolbox provides an overview of the nature of intentional contamination threats that drinking water facilities might face. Water from the drinking water distribution systems is considered the most significant vulnerability, as opposed to raw source water or wastewater. The focus of the analytical approach in this section is on approximately 35 specific contaminants, selected from a list of chemicals, biotoxins, and radionuclides, prioritized based on the contaminant’s potential threat to public health from a national perspective (see Module 1). General water quality parameters from distribution systems (pH, turbidity, chlorine residual, total organic carbon, etc.) are discussed in terms of site characterization (Module 3, Table 3-2). These general water quality parameters are potential indicators of chemical contamination at best, and may not cover all of the contaminants of concern.

The approximately 35 specific contaminants are not necessarily listed in this module or in Table 1-1 of Module 1. There are several reasons for this. First, it may not be advisable to specifically list these, since such information could be used with malicious intent. Second, a particular contaminant may be very important in a particular locality, but not have been included on the national priority list. Third, as part of fulfilling the goal of due diligence in providing a comprehensive screen (Section 1.2.2), it is necessary to include other chemicals in the analytical approach that encompass a broad range of effects, not just those that may have the highest public health impact.

The approach taken in this module is to divide potential contaminants into classes based on their analytical grouping (i.e., how contaminants with similar properties are analytically determined). This helps to limit the number of methods to those that encompass the largest number of analytes. The chemical types, resulting analytical groups, and examples of specific contaminants, are shown in Table 4-1. Suitable analytical methods are discussed further in Sections 6.4 and 6.5.

Table 4-1. Summary of Types of Chemical Contaminants, their Analytical Group, and the Class of Contaminants Determined by the Analysis

<table>
<thead>
<tr>
<th>Chemical types</th>
<th>Analytical Group</th>
<th>Example Contaminants*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic</td>
<td>Volatiles</td>
<td>Acetone, acrylonitrile, chloroform, methyl t-butyl ether,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>tetrachloroethylene, toluene,</td>
</tr>
<tr>
<td></td>
<td>Semivolatiles</td>
<td>Organophosphates (e.g., malathion, mevinphos, dichlorvos,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>etc.), cyanazine, chlorinated insecticides, chlordane,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pentachlorophenol</td>
</tr>
<tr>
<td></td>
<td>Non-volatiles</td>
<td>Sodium trifluoroacetates, surfactants</td>
</tr>
<tr>
<td></td>
<td>Carbamate compounds</td>
<td>Aldicarb, carbofuran, oxamyl</td>
</tr>
<tr>
<td></td>
<td>Quaternary nitrogen compounds</td>
<td>Diquat, paraquat</td>
</tr>
<tr>
<td></td>
<td>Pharmaceuticals</td>
<td>Nicotine, illicit drugs</td>
</tr>
<tr>
<td>Inorganic</td>
<td>Trace metals</td>
<td>Mercury, lead, cobalt</td>
</tr>
<tr>
<td></td>
<td>Nonmetals</td>
<td>Arsenic salts</td>
</tr>
<tr>
<td></td>
<td>Organometallics</td>
<td>Organomercury compounds</td>
</tr>
<tr>
<td>Cyanides</td>
<td>Cyanides</td>
<td>Cyanide salts, cyanogen chloride</td>
</tr>
<tr>
<td>Radionuclides</td>
<td>Radiologicals</td>
<td>Cesium-137, Cobalt-60, Strontium-92</td>
</tr>
<tr>
<td>CW Agents</td>
<td>Schedule 1 only**</td>
<td>(e.g., VX, sarin, nitrogen and sulfur mustards, Lewsites)</td>
</tr>
</tbody>
</table>

* Not every contaminant in a particular analytical group is listed in this column.
**See Section 2.1.4 for a discussion of Schedule 1 agents.
6.1.2 Selection of Appropriate Methods for Contaminant Analysis

As described in Section 5 of this module, there are two general approaches to the chemical screen. One approach is referred to as the “basic” screen, while the other is referred to as the “expanded” screen. Together, they are a comprehensive screen for the 35 priority contaminants and also provide coverage for hundreds of other potential water contaminants, although it is not feasible (or necessary) to determine all of the hundreds of thousands of chemicals in existence. Fewer laboratories may be capable of implementing the expanded screen (Section 6.5).

It must be emphasized that this module along with Sections 6 and 8 are not intended to represent a prescriptive “how-to” laboratory manual. Rather, the model screening procedure (Sections 6.3-6.5, 8.1) is intended to be a planning tool for laboratories to formulate a laboratory guide specific to their needs and capabilities. For instance, the basic screen presented in Section 6.4 is composed of relatively reliable and accessible techniques, which hopefully will encourage more laboratories to develop some screening capability for potential harmful chemical contaminants (i.e., beyond regulatory requirements). However, planners may not wish to employ any elements of the basic screen, but rather rely only on techniques used in the expanded screen (Section 6.5), backed up by suitable analytical confirmation to ensure defensibility of the results. Planners must exercise due diligence in planning their analytical approach, as well as all other response activities, to protect public health and safeguard the water supply.

For the selection of appropriate methods to include in the screen, it is important to realize that planning is a vital part of site-specific response to a contamination incident. In many cases, planning for the site characterization (Module 3) will drive the selection of methods. In some cases, it may be desired only to identify the contaminant, but accurate quantification may be necessary in others. Depending on the specific plan, various goals and analytical methods may be applicable.

An intentional contamination incident may produce contaminant concentrations ranging from extremely high (milligrams per liter), when much contaminant is added to a relatively small amount of water, to very low (low to mid microgram per liter), as in the tail of a transient contaminant slug in a drinking water distribution pipe. Accordingly, the analytical methods described in this approach were selected to be conservative and reflect the goal of defensibly determining lower concentrations of chemicals. This great variability in analytical needs that may arise in each threat/incident is another reason that this section is not a prescriptive “how-to” manual.

6.1.3 Initial Processing of Contaminants prior to Laboratory Analysis

Regardless of the type of screening that the laboratory uses, it is likely that laboratories will receive samples that have been characterized to some extent, and perhaps tentatively identified, at the site. Figure 4-5 indicates the types of laboratories that might analyze emergency water samples. The site hazard assessment performed during site characterization will help the water utility emergency response manager (or the designated incident commander) decide which laboratories to use for a particular sample set. If a chemical contaminant has been tentatively identified in the field, it may aid in the selection of an appropriate laboratory. It will be necessary to then apply specific analytical techniques to confirm its identity and/or quantify the concentration. Otherwise, the sample may be subjected to a screening procedure, which is described in more detail below. Note that the screening procedure box does not explicitly list radionuclides, since radionuclide analysis involves a different paradigm, as discussed in Section 6.4.8, below. Also, some radionuclide screening is performed upon receipt by the laboratory as discussed in Section 3, in addition to the field screening in Section 4.
Figure 4-5. Initial Analytical Approach to Processing Samples with Chemical Contaminants

*Specialty labs include the LRN and those only accessible through law enforcement (e.g., CW labs).

At the starting point for Figure 4-5, the contaminant identity is hypothesized based on available information from the site characterization report. Examples of situations in which tentative identification might occur include: a specific contaminant named in a threat; tentatively positive results for a specific contaminant during field safety screening or rapid field testing; physical evidence at the site pointing to a specific contaminant; and clinical evidence of the identity of the disease-causing contaminant.

It is important to note, however, that each of these situations has a different level of credibility for the purpose of tentative identification (Module 2) and that additional contaminants may be present. In general, tentative identification may focus the analytical approach on the specific contaminant subclass, leading to confirmatory analysis for the particular contaminant. For example, tentative identification of a class of pesticides (e.g., organophosphates) may be based on results from a test kit, and this information might be used to focus the analytical approach on specific pesticides within that class.

In most cases, it is anticipated that a specific chemical contaminant will not be identified with sufficient reliability to be considered tentatively identified, and it will be necessary to conduct screening for potential chemical contaminants. Depending on the strength of the tentative identification, it may be wise to consider heading down both paths of Figure 4-5 simultaneously. If the evidence for tentative identification is sufficiently strong, and if there are no other contaminants suspected, then screening may be delayed. Otherwise, screening should begin simultaneously with the confirmatory analysis, as described in the Section below. At the other extreme, during a ‘confirmed’ incident, the contaminant will likely be known, and the point of analysis in this case is to further characterize the contaminated area to support the planning stage of remediation and recovery.
6.2 Confirmatory analysis

It is necessary to confirm the identity and/or concentration of a contaminant identified either in the field or in the laboratory. In the case of a tentatively identified chemical contaminant, Figure 4-5 indicates that one of several types of distinct, specialized laboratories might be involved in conducting confirmatory analysis. The functions of these laboratories are described in more detail in Section 2.1 as part of the infrastructure discussion. Some confirmatory analysis may only take place in specialty laboratories, such as those for Schedule 1 CW agents. A discussion of these techniques and the associated equipment is beyond the scope of this document since these laboratories presumably have established procedures for these specialty analytes, and an overview of these techniques would not be particularly helpful to the target audience for this document. In addition, access to these laboratories is restricted and must be made by the appropriate authorities.

For radionuclides, analytical confirmation may be viewed differently than most chemicals because radionuclide analysis can take the form of screens for gross alpha, beta, or gamma radiation, and/or it can take the form of analysis for the specific radionuclides themselves, such as strontium-90. Thus, analytical confirmation may involve the process of confirming quantity of gross radiation or identifying the radionuclide. The type of analysis that is performed will be based on the analytical goals. For example, radionuclides in drinking water regulation are often characterized by their gross alpha and gross beta emissions. For the purpose of emergency analysis of water contaminants, a tiered approach is suggested, with screening for gross radiation in the field or upon receipt by the laboratory followed by identification of the radionuclide, which may be important for a more complete understanding of risks to public health as well as potential criminal actions against perpetrators.

On the other hand, many environmental chemistry laboratories can confirm a significant number of chemical contaminants through the use of a variety of standardized methods. In the context of this module, standardized methods are produced as a standard by a recognized method development organization (EPA, ASTM, AOAC, ISO, etc.) and contain valid steps to defensibly confirm the presence and/or quantity of specific contaminants. For the analysis of emergency water samples, a standardized method need not be promulgated for compliance monitoring of drinking water samples.

Environmental chemistry laboratories are often familiar with promulgated, standardized methods and may have the equipment and expertise to perform confirmatory analysis using such standardized methods. A number of standardized methods are published by various organizations, and may be selected from an appropriate method database, such as the National Environmental Methods Index (NEMI, http://www.nemi.gov). NEMI contains methods reviewed and selected by the National Methods and Data Comparability Board (http://wi.water.usgs.gov/pmethods/), a partnership of water-quality experts from Federal agencies, States, Tribes, municipalities, industry, and private organizations. The Board provides a framework and forum for comparing, evaluating, and promoting monitoring approaches for water quality. Methods are available in NEMI to support the analytical approach for the priority chemical contaminants, and a laboratory can use the NEMI database to select a confirmatory method for a particular chemical contaminant based on its particular expertise and equipment. NEMI is searchable by analyte, and a search will often return several methods for the same analyte, developed to meet a variety of needs. For example, some methods are EPA drinking water methods, some are EPA SW-846 methods, and some were developed by USGS or DOE for their environmental monitoring programs, etc.

Although consulting NEMI first is recommended, there are other sources of standardized methods, some of which are shown in Table 4-2. There are other comparable standardized methods available from standards setting organizations like ASTM (http://www.astm.org), AOAC (http://www.aoac.org),
and ISO (http://www.iso.org/). In choosing a method, keep in mind that some of these standardized methods may be prescribed for a specific intended purpose, such as drinking water compliance monitoring. However, in the context of this module, the methods are not prescribed, because there is no regulatory driver for doing so. Rather, all of these methods resources are to be used as planning tools to assist laboratories in selecting methods for use in implementing the analytical approach presented in this module prior to analyzing actual emergency water samples. For many laboratories, planning may be simplified through the use of methods they are already familiar with, such as EPA drinking water and SW-846 methods.

Table 4-2. Sources of Standardized Methods

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Publisher</th>
<th>How to obtain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>US EPA Office of Research and Development (ORD)</td>
<td><a href="http://www.epa.gov/nerlcwww/ordmeth.htm">http://www.epa.gov/nerlcwww/ordmeth.htm</a></td>
</tr>
<tr>
<td>EPA SW-846 methods</td>
<td>Mainly for solid waste, but many are applicable for drinking water, using appropriate preparation techniques.</td>
<td>US EPA Office of Solid Waste</td>
<td><a href="http://www.epa.gov/epaanswer/hazwaste/test/main.htm">http://www.epa.gov/epaanswer/hazwaste/test/main.htm</a></td>
</tr>
<tr>
<td>EPA drinking water manuals</td>
<td>Printed versions of drinking water methods</td>
<td>US EPA ORD</td>
<td>For ordering details, see <a href="http://www.epa.gov/nerlcwww/methmans.html">http://www.epa.gov/nerlcwww/methmans.html</a></td>
</tr>
<tr>
<td>40 CFR Parts 136 and 141</td>
<td>Promulgated list of defensible methods widely accepted in the analytical community for water and wastewater.</td>
<td></td>
<td><a href="http://www.epa.gov/docs/epacfr40/chapt-1.info/subch-D.htm">http://www.epa.gov/docs/epacfr40/chapt-1.info/subch-D.htm</a></td>
</tr>
</tbody>
</table>

If standardized methods are not available for the contaminant of interest, then analytical confirmation may involve the techniques described below for basic and/or expanded screening (Sections 6.3 and 6.4). In this situation, the requirements for analytical confirmation are generally not well defined and are further complicated by the issue of legal defensibility of scientific data. Section 3.5 of this module provides an overview of rules that govern the admissibility of scientific evidence into a court of law and points out the pitfalls associated with using methods that have not been standardized. There are currently efforts underway in the forensic community to resolve these issues and develop standard approaches for the analysis (including confirmation) of samples contaminated with an unknown substance. While guidance from the forensic community is not available at this time, it may be included in future versions of this module. Ultimately, it may be the responsibility of laboratories to take appropriate steps to support the admissibility of data produced using exploratory techniques that have not yet been widely accepted in the scientific community (see Section 3.5).
6.3 **Laboratory Analytical Screening for Chemical Contaminants, Including “Unknowns”**

The laboratory analytical screening described here is **different and separate** from the laboratory safety screening (Section 3.1.4 of this Module) and the field safety screening and rapid field testing (briefly described in Section 4 of this Module and more completely in Module 3). Briefly, the field safety screening and rapid field testing were performed during characterization of the contamination site, before any laboratory work is performed. The entire site characterization process is designed to reduce risk to the site characterization team and any individuals coming into contact with samples collected from the site. Site characterization activities are not limited to only field safety screening and rapid water testing, which may only capture a few contaminants, but also include a site investigation that may identify other signs of hazards. These activities aid in the site hazard assessment and help ensure that samples are sent to a laboratory prepared to deal with both the analysis and any safety issues. For example, if pathogens are suspected, the samples should not be sent to an environmental chemistry lab.

The overall concept of laboratory analytical screening for an unknown chemical in a water sample is presented in Figure 4-6. This chemical screen consists of two core elements: 1) application of multiple analytical techniques to screen for a wide range of analytes; and 2) analytical confirmation of tentative results. In the first core element, the combination of analytical techniques used to achieve broad coverage of the contaminant classes of concern includes both standardized methods and exploratory techniques. The standardized methods are derived from those same sources as listed in Section 6.2. Exploratory techniques are those that do not necessarily form the basis of standardized methods for water analysis, but are capable of detecting chemicals that are not included in existing standardized methods. Both of these elements are implemented in the laboratory.

Two different chemical screens were constructed from the established and exploratory techniques. The first, indicated in Figure 4-6 as the “Basic Screen,” (Section 6.4) uses only standardized methods, and the method analytes include a significant percentage of the priority chemical contaminants as well as many other chemicals. The second, referred to as the “Expanded Screen,” (Section 6.5) combines established techniques and exploratory techniques to achieve the broadest coverage, including all priority chemical contaminants. Additionally, the Expanded Screen provides laboratories with additional options regarding the instrumentation used to implement both established and exploratory techniques; however, the results may not be as definitive as those for established techniques.

The second core element in Figure 4-6 is analytical confirmation of the results. As shown in the figure, some methods utilized in the “Basic” screen contain required steps for defensible confirmation. Some do not, and other confirmatory analysis should be performed, as described in Section 6.2. As shown in Figure 4-6, it also would be necessary to confirm the results of most exploratory techniques, unless the technique is applied within a methodology that utilizes suitable validation procedures. The strategy of using the variety of techniques in the “Expanded Screen” may lend itself to fulfilling the requirements discussed in Section 6.2 for confirming an analyte, particularly one for which no standard method exists. For example, analytical confirmation may sometimes be accomplished through determination of the contaminant by two or more analytical techniques that operate on independent principles (e.g., LC/UV and GC/MS). In comparison, analytical techniques that operate on the same principles (e.g., different immunoassays) may be unreliable for confirmation purposes and should be used with caution.
Figure 4-6. Overview of Screening and Confirmatory Analyses for Chemical Contaminants
6.4 Basic Screening using Established Techniques with Standardized Methods for Target Analytes

The basic screen is designed to capture many of the chemical contaminants of concern using a relatively small number of well-defined analytical techniques. The techniques chosen for this basic screening analysis are summarized in Table 4-3. This is to say, if the methods in Table 4-3 are performed, then the screen may cover a large percentage of the priority chemical contaminants (Table 4-1). Furthermore, many other contaminants of concern, but of lower priority, may be screened for as well. To increase confidence in the results, only standardized methods (e.g., EPA drinking water, SW-846, or comparable methods) are used for the basic screen. Comparability involves analytes determined, detection limits, QA/QC, etc. See Section 6.2 for information about comparable methods.

As discussed previously, EPA standardized methods are suggested mainly because many labs may be familiar with some or all of these methods, and many laboratories are certified and/or accredited to determine water contaminants using these methods. This increases the defensibility of the results, since the laboratories would have experience in performing all of the required QC within acceptable ranges. Also, in most cases, the use of EPA methods also means that if a method analyte is determined by these methods, no further analytical confirmation would be necessary since many of these methods contain confirmatory steps. In choosing a method, keep in mind that some of these standardized methods may be prescribed for a specific intended purpose, such as drinking water compliance monitoring. However, in the context of this module, the methods are not prescribed, because there is no regulatory driver for doing so.

The basic screen may serve as a springboard for more complete characterization of the sample, in an “outside-the-box” manner. Namely, a significant advantage to using the techniques in the Table 4-2, or comparable ones, is that many rely on chromatography and/or mass spectrometry, so the data should be capable of being evaluated for the presence of not only target analytes, but also other compounds. If a contaminant is discovered that is not in the analyte list of the method used, the identification should be considered tentative until confirmed (Section 6.2). In the absence of confirmation, expanded screening of the sample should be applied (Section 6.5).

Information from the basic screen may also be used to inform the selection of exploratory techniques. For instance, if a target analyte or unknown peak is not found in a Method 524.2 analysis, the compound is likely not a volatile organic; if no peak is present in a Method 8270D analysis, the unknown may be inorganic in nature or thermally unstable.
Table 4-3. Suggested Analytical Techniques for Performing the Basic Chemical Screen, broken out by the classes given in Table 4-1.

<table>
<thead>
<tr>
<th>Chemical (general class)</th>
<th>Analytical Technique</th>
<th>EPA Method</th>
<th>Analyte List</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volatiles (organic)</td>
<td>Purge-and-trap PID/ELCD</td>
<td>502.2, 8021B</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Purge-and-trap GC/MS</td>
<td>524.2, 8260B</td>
<td></td>
</tr>
<tr>
<td>Semivolatiles (organic, includes many pesticides)</td>
<td>Solid-phase extraction GC/MS</td>
<td>525.2, 8270D/3535A</td>
<td>B</td>
</tr>
<tr>
<td>Carbamate pesticides (organic)</td>
<td>HPLC – fluorescence detection</td>
<td>531.1, 531.2</td>
<td>C</td>
</tr>
<tr>
<td>Quaternary nitrogen compounds (organic)</td>
<td>HPLC – UV</td>
<td>549.2</td>
<td>D</td>
</tr>
<tr>
<td>Trace metals (inorganic)</td>
<td>ICP-AES, ICP-MS, graphite furnace AA</td>
<td>200.7, 200.8; 200.9</td>
<td>E</td>
</tr>
<tr>
<td>Total mercury (inorganic, includes organomercury compounds)</td>
<td>Cold vapor AA</td>
<td>245.1, 245.2 with persulfate</td>
<td></td>
</tr>
<tr>
<td>Cyanides</td>
<td>Wet chemistry</td>
<td>335.2, 335.3, 335.4</td>
<td>G</td>
</tr>
<tr>
<td>Radionuclides</td>
<td>Gross alpha, gross beta, Gross gamma</td>
<td>900, or technique preferred by lab¹</td>
<td>H</td>
</tr>
</tbody>
</table>

1. Method titles are listed in Table 4-19 in the appendix (Section 10)
2. Method 200.8 may be applied in the time-saving “direct analysis” mode that does not require digestion, if the NTU of the water sample is <1. Note that the NTU of the water may be quickly determined in-house and/or may be available in the site characterization report supplied with the sample. The use of the scanning mode in Method 200.8 is also suggested.
3. Many radionuclide laboratories have developed in-house methods for rapid screening of radionuclides. These may be similar to EPA 900-Series methods, which were developed for the measurement of very low, environmental levels of radioactivity in drinking water but may require several days to obtain a result. See Section 6.4.8 for further discussion. Analytical confirmation may be required via EPA Method or other defensible methods.
4. Standard may contain these. See Table 4-20 in the appendix (Section 10).

As mentioned in Section 1, this module is primarily intended for the purpose of planning an analytical response. The users may wish to develop their own basic screen after evaluation of their particular capabilities and experience as well as the needs and concerns of their clients. (Some hypothetical examples of basic screens are given in Section 7 of this module.) For a utility laboratory, the basic screen would be based on the utility’s perception and knowledge about threats in their area and the capabilities of their laboratories. Because there is no regulatory requirement to apply any basic screen, a laboratory may, in principle, choose not to employ any of the standardized methods in Table 4-3. In this case, however, it would be prudent from a public health perspective to employ an expanded screen based on exploratory techniques capable of determining the analytes of interest, such as those listed in Table 4-20.

There are several important notes about Table 4-3:

- Screening of radionuclides involves a different paradigm than conventional chemicals. See Section 6.4.8 for details.
- The preparation method for 8270D listed is 3535A, based on solid-phase extraction. Separatory funnel preparation for 8270D, or any other separatory funnel method, is not included because separatory funnels may release aerosols when vented, potentially increasing the risk of exposure for laboratory personnel, unless appropriate hoods and/or other control measures are employed. Granted, separatory funnel techniques are safely used every day but, considering that other sample preparation methods (such as 3535A)
sorbent tube is heated and backflushed with helium to desorb the trapped sample components into a capillary gas chromatography (GC) column interfaced to a mass spectrometer (MS). The column is temperature-programmed to facilitate the separation of the method analytes, which are then detected with the MS. Compounds eluting from the GC column are identified by comparing their measured mass spectra and retention times to reference spectra and retention times in a database. Reference spectra and retention times for analytes are obtained by the measurement of calibration standards under the same conditions used for samples. The instrument should be calibrated for the analytes of interest, using analytes similar to those found in Analyte List A (Table 4-20).

Some laboratories possess purge-and-trap instrumentation with a PID detector in series with an ELCD detector. This combination provides the ability to determine most of the analytes in list A. Because of the nature of the PID and ELCD detectors, however, they cannot necessarily provide the additional level of information and analytical confidence as a mass spectrometer (see Section 6.5.1 below). Accordingly, Methods 502.2 and 8021B are included primarily to increase the number of laboratories capable of this analysis.

6.4.2 Semivolatiles (Organic)
Solid-phase extraction involves the removal of analytes from water onto a solid sorbent material (based on EPA Method 525.2). Analytes, internal standards, and surrogates are extracted from a water sample by passing 1 liter of sample water through a cartridge or disk containing a solid matrix with a chemically bonded C18 organic phase (solid-phase extraction, SPE). The organic compounds are eluted from the SPE cartridge or disk with small quantities of ethyl acetate followed by methylene chloride, and this extract is concentrated further by evaporation of some of the solvent. The sample components are separated, identified, and measured by injecting an aliquot of the concentrated extract into a high resolution fused silica capillary column of a gas chromatography/mass spectrometry (GC/MS) system.

Compounds eluting from the GC column are identified by comparing their measured mass spectra and retention times to reference spectra and retention times in a database. Reference spectra and retention times for analytes are obtained by the measurement of calibration standards under the same conditions used for samples. The instrument should be calibrated for the analytes of interest, using analytes similar to those found in list B (Table 4-20).

6.4.3 Carbamate Pesticides (Organic)
EPA Methods 531.1 and 531.2 can be utilized to detect carbamate pesticides and degradation products, namely the N-methylcarbamolyoximes and N-methylcarbamates. (Note that Method 531.1 requires a different preservative.) Compared to the other EPA methods in the basic screening analysis, EPA Methods 531.1 and 531.2 do not encompass many analytes. It may be worth noting that at least one chemical (aldicarb) in this class has an immunoassay test available for screening purposes.

This immunoassay is not recognized for compliance monitoring, so it is not included in the basic screening analysis, but might be useful as a field test. In Methods 531.1 and 531.2, the water
are available, it may be prudent for laboratories to consider not using the separatory funnel preparation method.

- The methods encompass many analytes. In terms of obtaining standards to use in conjunction with the respective methods, it may be helpful to focus on certain analytes. The analytes that may be included in the standard are identified in the appendix (Table 4-20) by the list number included in Table 4-3. Laboratories may wish to add analytes to those listed in the appendix, and may already be doing so (e.g., to meet local regulation). These analytes must meet the same QC acceptance criteria and be part of the overall quality systems in the laboratory, so that the added analytes are as defensible as the method analytes.

- The methods listed in Table 4-3 determine a number of analytes that probably would not be involved in a contamination incident (in addition to those that are priority contaminants). However, for the purposes of a screen, it is valuable to include some of the additional analytes that are picked up by the method, especially since there is no way to predict which contaminants might be used in a threat or incident.

- The standardized methods listed in Table 4-3 do not include some analytes of concern, such as the biotoxins, chemical warfare agents, and pharmaceuticals. This may be due to the fact that since these are not regulated compounds in drinking water, there has been little impetus to develop methods for these analytes. This may change, due to the inclusion of some algal biotoxins, namely microcystins, in the Contaminant Candidate List (http://www.epa.gov/safewater/ccl/cclfs.html) and Unregulated Contaminant Monitoring Rule (http://www.epa.gov/safewater/standard/ucmr/main.html).

- The methods in Table 4-3 are not always the same ones that are required for compliance with the Safe Drinking Water Act as amended. For instance, Method 551.1, which is used for compliance monitoring of some disinfection by-products, does not encompass a sufficient number of chemicals considered as priority contaminants from an acute health effect standpoint. After all, disinfection by-products largely produce a long-term health risk, not the acute risk to human health that would be the most pressing concern during an intentional contamination threat/incident.

The following is a brief discussion of the analytical methods utilized in the Basic Screen, broken down by the chemical classes listed in Table 4-1. Many laboratories may be familiar with the individual methods listed, as these are typically the EPA drinking water compliance methods. However, the purpose of the module is not to insist that only EPA drinking water compliance methods may be used. As mentioned above, there are other fine method sources, including, but not limited to, those in Table 4-3. If these other methods are employed, they should be capable of determining the analytes of interest (i.e., Table 4-20), and they should conform to the spirit and intent of the methods described briefly below.

6.4.1 Volatiles (Organic)
Purge-and-trap concentration and analyses by GC/MS (based on EPA Method 524.2 or 8260B) may be utilized to identify organic compounds that are volatile in nature. To summarize the method, volatile organic compounds with low water solubility are extracted (purged) from the sample matrix by bubbling an inert gas through the aqueous sample. Purged sample components are trapped in a tube containing suitable sorbent materials. When purging is complete, the
sample is filtered and a 400-µL aliquot is injected into a reversed-phase HPLC column. Separation of analytes is achieved using gradient elution chromatography. After elution from the HPLC column, the analytes are hydrolyzed to form a methyl amine, which is further reacted to form a highly fluorescent derivative that is detected by a fluorescence detector. The derivatization procedure imparts specificity to the analysis, and a sample component is identified by comparison of its retention time to the retention time of a reference chromatogram. If the retention time of an unknown compound corresponds, within limits, to the retention time of a standard compound, then identification is considered positive. Because of specificity limitations of fluorescent detectors, identification requires expert judgment when sample components are not resolved chromatographically.

When peaks obviously represent more than one sample component, or any time doubt exists over the identification of a peak on a chromatogram, appropriate alternate techniques should be applied to identify the peak, such as the use of a second chromatography column, several of which are contained in the method. SW-846 Method 8321B also employs HPLC instrumentation for the analysis of carbamates, and it also describes the use of thermospray mass spectrometric detection, possibly providing additional information about the target compound. However, for technical reasons revolving around data interpretation, the thermospray interface is considered obsolete, and is in the process of being replaced (http://www.epa.gov/epaoswer/hazwaste/test/new-meth.htm).

6.4.4 Quaternary Nitrogen Compounds (Organic)

EPA Method 549.2 is a high performance liquid chromatography (HPLC) method for the determination of diquat and paraquat in drinking water sources and finished drinking water. In the method, a volume of water sample is subjected to solid-phase extraction. The eluate from the extraction device is separated through the use of liquid chromatography with UV detection. A photodiode array detector may be utilized to provide simultaneous detection and analytical confirmation of the method analytes.

6.4.5 Trace Metals (Inorganic)

a) **Inductively Coupled Plasma Mass Spectrometry (ICP-MS).** EPA Method 200.8 provides procedures for the determination of 21 elements in a single run and provides mass spectral qualitative identification with significantly better detection limits than most standard instrumental elemental analysis techniques. The method involves a sample preparation procedure with a digestion step using nitric and hydrochloric acids, although there is an alternative “direct analysis” for samples with turbidity less than 1 NTU, which is applicable to many treated drinking water samples as they typically have turbidity less than 0.3 NTU. The method describes the multi-element determination of trace elements by inductively coupled plasma mass spectrometry (ICP-MS). Sample material in solution is introduced by pneumatic nebulization into a radiofrequency plasma where energy transfer processes cause desolvation, atomization, and ionization. The ions are extracted from the plasma through a vacuum interface and detected on the basis of their mass-to-charge ratio by a mass spectrometer. The mass spectrometer may be operated in the “scanning” or the “single ion monitoring” mode. While detection limits are slightly less in the scanning mode, the use of the scanning mode offers
benefits in terms of identifying non-method analytes. Details are discussed below in Section 6.5.2a, as part of the expanded screen.

It should be noted that Method 200.8 is designed for compliance monitoring, and if acidification of the sample is not performed in the field, the method recommends that the sample be held 16 hours after acidification, prior to analysis. This may not be practical in responding to contamination incidents. (See Module 3, Section 3.2, for more details on preservation issues.) Acid addition in the field may result in the release of toxic gases, such as hydrogen cyanide, depending on the nature of the contamination. As a safeguard, sample preservation in the field should occur only after the potential presence of cyanide has been eliminated through field screening using devices such as portable colorimeters or probes. According to the procedures in Module 3, sample collection should always follow field testing (including cyanide), so this check should be automatic. If cyanide is found, the speed of reporting of Method 200.8 analytes may not be an issue since discovery of elevated cyanide levels would elevate the credibility of the threat and prompt immediate response actions, which would provide additional time for careful forensic analysis.

Because of the specificity and detection limits of ICP-MS for all the elements in Analyte List E (Table 4-20), it may be considered a superior technique for trace metal analysis. However, in the interest of more laboratories being capable of performing trace metal screening, it should be recognized that inductively coupled plasma atomic emission spectrometry and graphite furnace atomic absorption spectrometry are widely used for trace metal determination. Practitioners should be familiar with the comparative advantages and disadvantages of the techniques for trace metal determination listed in Table 4-3, particularly regarding detection limits and interferences for some of the analytes in Analyte List E (Table 4-20).

b) **Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES).** While ICP-MS is a trace elemental technique with excellent specificity and detection limits for some of the contaminants of concern, other trace elemental techniques are available in many laboratories and may be used as part of the expanded screening procedure. EPA Method 200.7 provides procedures for the determination of 32 elements in a single run through the use of ICP-AES. ICP-AES is sometimes referred to as ICAP (for inductively coupled argon plasma) or ICP-OES (for optical emission spectrometry). The instruments measure the atomic line emission spectra by optical spectrometry. The aqueous sample is acidified prior to instrument analysis. The analysis begins with a nebulization and the aerosol stream is transported to the plasma torch, which may be viewed by an optical spectrometer either head on (radially) or from the side (axially) depending on the instrument. Each orientation provides different performance characteristics, particularly in terms of interferences and detection limits, but each of these is dependent on the matrix as well as the instrument design. The speed of the analysis is also influenced by instrument design. For example, the optical system may allow either sequential or simultaneous determination of the analytes, with the simultaneous determination allowing faster analysis; it also may allow tentative identification of non-method analytes, similar to ICP-MS (above).

c) **Atomic Absorption (AA) Spectrometry.** Graphite furnace atomic absorption spectrometry (EPA Method 200.9) can also be used for a select number of metals. The acid-digested sample is
placed in the instrument and is pretreated prior to atomization. The atomization occurs after a
purging with an inert gas and drying. The atomization is performed by rapid heating and
produces an atomic cloud. The atomic cloud then absorbs the element-specific atomic emission
from a hollow cathode lamp or electrodeless discharge lamp. Instrument design influences
performance characteristics, like detection limits and number of elements that may be analyzed
at one time. The atomic absorption technique may be used with other introduction techniques
than graphite furnace, as shown in the next section for total mercury.

6.4.6 Total Mercury (Including Organomercury Compounds)
EPA Methods 245.1 and 245.2 allow the determination of total mercury, which includes
inorganic mercury and many organomercury compounds, following digestion with potassium
persulfate, which oxidizes the organomercury compound to mercuric ion before measurement.
The total mercury is determined through the use of cold vapor atomic absorption. This technique
greatly enhances the detection limit for mercury determination and offers other benefits as well.
Total mercury may be determined as part of the trace metal analysis using methods in Table 4-3
(Section 6.4.5), except GF AA, Method 200.9. However, Methods 245.1 and 245.2, which form
the basis for the dedicated mercury analysis instruments currently found in many laboratories,
were included here.

6.4.7 Cyanides
Ideally, the core field testing recommended in Module 3 will detect the presence of cyanide, so
laboratory analysis of cyanide would most likely serve a confirmatory role. Depending on the
nature of the field test, it may use similar chemistry to the EPA Methods 335.2, 335.3, and 335.4,
as described below, but typically the field test will not employ distillation. Thus, the field tests
primarily detect free cyanide ion (not cyanide complexes).

It should be noted that most cyanide field tests are designed for quantitative analysis of cyanide
at low levels (near the MCL), and they typically provide only a qualitative indication of the
presence of high cyanide concentrations. Accordingly, it may not be necessary to perform
redundant tests in the laboratory, although it may be deemed prudent to do so. Such decisions
may be based on the concentration of cyanide in the water or the form of cyanide known or
suspected. For instance, a titration procedure for cyanides is used when the concentrations
exceed 1 mg/L (EPA Method 335.2). A reflux distillation is used to release the cyanides, which
are then absorbed in a scrubber solution. The titration uses a silver nitrate solution to titrate
cyanide in the presence of a silver sensitive indicator. For lesser concentrations, cyanide in
water is determined through the use of colorimetry (EPA Method 335.3 and 335.4). UV
digestion and distillation are used to convert the cyanides to hydrogen cyanide. Cyanides are
then converted to cyanogen chloride and then subsequent reactions with pyridine and barbituric
acid give a red colored complex.

The form of cyanide added to water may be important in deciding on which cyanide test is
appropriate for use. Free cyanide in water may result from the addition of soluble cyanide salts
or water-soluble organic cyanogenic compounds. Methods 335.2, 335.3, and 335.4 measure total
cyanide, which could be a mix of free cyanide, complexed cyanide, and some organic
cyanogenic compounds. Because free cyanide is the most toxic, without knowing the concentrations of the other forms, which have widely varying toxicity, the use of the total cyanide concentration would be conservative with respect to public health implications.

6.4.8 Radionuclides

Basic screening for radionuclides represents a somewhat different paradigm than conventional chemicals because radionuclides can be characterized by both the type of radiation they emit as well as their exact chemical identity. Accordingly, initial screening for radionuclides may involve an examination of gross radioactivity. However, any initial screening that indicates the presence of a radionuclide should be followed by analytical confirmation of the chemical identity. A schematic for radionuclide screening is shown in Figure 4-7. Note that in Section 3, screening for radioactivity upon sample receipt is recommended. Accordingly, the decision point in Figure 4-7 that asks “Does lab policy require screening for radioactivity?” provides flexibility for local planning decisions.

![Figure 4-7. Schematic for Basic Radionuclide Screening](http://www.epa.gov/radiation/docs/readytorespond/ready3.htm)

The results of field testing for radioactivity (Figure 4-7) must be compared to background levels to determine whether the site may have been contaminated with radioactive material. Although discussed in Module 1, it is worth noting here that EPA has developed protective action guidelines for radiation levels in water (http://www.epa.gov/radiation/docs/readytorespond/ready3.htm).
The analysis for gross alpha and beta radiation (EPA Method 900.0) may be conducted as a screening method for alpha and beta particle activities in water and used to determine if specific radiological analyses are needed. Preliminary analysis can first be conducted in the field using appropriate field portable or hand-held devices, but may be verified in the laboratory. EPA Method 900.0 can measure alpha particles with energies above 3.9 mega electronvolts (MeV) and beta particles with energies above 0.1 MeV. In this method, the water sample is evaporated and dried, and the dried residue is counted for alpha and beta by a gas flow proportional counting system or scintillation detector system. Note that as part of their safety plan, laboratories may wish to screen samples upon arrival for gamma radiation using appropriate technologies.

If the presence of radioactive material is indicated by the initial screening, specific radioisotopes may be determined by radiochemical specific procedures, using EPA 900-Series methods or other legally defensible techniques. These procedures often involve separation of the radionuclide from the sample by precipitation procedures and subsequent determination by gas flow proportional counting system or scintillation detector system for alpha and beta emitters and an appropriate gamma detector for gamma emitters. For example, strontium-89 and strontium-90 can be measured by EPA Method 905.0. Strontium-89 and strontium-90, are precipitated as carbonates from the sample. Additional precipitation steps allow separation from other radionuclides and interferences. The daughter product of strontium-90, yttrium-90 is separated by a hydroxide precipitation step and is beta counted by a low background beta counting system. The combined strontium-89 and strontium-90 residue is also beta counted. The results for each radionuclide are determined.

Due to the unique nature of radionuclide analysis, some laboratories have developed in-house procedures for radionuclide analysis that make use of their special skills and capabilities to enhance the speed of analysis, especially since some EPA 900-Series methods are not rapid methods. For example, EPA 905.0 for radioactive strontium in drinking water recommends a two-week in-growth period for obtaining the yttrium isotope from the purified strontium. Modification of the method results in much faster results. Reduction in analysis time could be accomplished by measuring the total amount of an element’s radionuclide, not the isotopic distribution. Also, for some isotopes, faster results may be obtained by simply reducing the volume of water sample processed.

In summary, many laboratories may choose to utilize a specialized method or technique for initial sample screening that may not be necessarily identical to those described in EPA 900-Series methods, although these methods must be used when reporting drinking water compliance monitoring results.

### 6.5 Expanded Screening using Established and/or Exploratory Techniques with Standardized or Non-standardized Methods

The purpose of the expanded screen is to capture chemical contaminants not picked up by the basic screen, namely those analytes listed in Table 4-20. The expanded screen may also detect those analytes covered by the basic screen more rapidly. It should be a sufficiently broad screen to “go fishing” for many possible contaminants. The expanded screen can be used with a combination of standardized or non-standardized methods. Note that in the case of standardized methods, no additional analytical confirmation may be required because the method used may
itself be considered definitive if applied correctly. In other cases, please see Section 6.2 for a further discussion of confirmation of analytical results.

In practice, the expanded screen can be used in addition to the basic screen, because the results of the basic screen may provide a “springboard” to guide the selection of techniques for the expanded screen. Alternatively, some laboratories may choose to utilize only the expanded screen, comprised of potentially sensitive techniques, including those summarized in Table 4-4. In the latter case, preliminary results can be cautiously used to make response decisions, but must be followed up with confirmatory analysis since screening techniques are not necessarily definitive or legally defensible.

<table>
<thead>
<tr>
<th>Contaminant Type</th>
<th>Expanded Screening Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic</td>
<td>GC, GC/MS, HPLC, LC/MS, Immunoassay test kits*</td>
</tr>
<tr>
<td>Inorganic</td>
<td>IC, AA, ICP, ICP-MS</td>
</tr>
<tr>
<td>Cyanides</td>
<td>Wet chemistry*</td>
</tr>
<tr>
<td>Biotoxin</td>
<td>Immunoassay test kits, GC/MS, HPLC, and LC/MS</td>
</tr>
<tr>
<td>Radiological</td>
<td>Handheld equipment*</td>
</tr>
<tr>
<td>Chemical Warfare Agents</td>
<td>GC/MS with direct injection, purge &amp; trap, and SPE/SPME, test kits,<em>handheld equipment</em></td>
</tr>
</tbody>
</table>

*See Section 4.1 for important disclaimers and information about the use of these technologies. Note that when these technologies are being used in the expanded screen, they are being applied in the laboratory, not the field.

Given the variety of choices presented in Table 4-4, the question naturally arises about how to select suitable methods and equipment for the expanded screen. This section attempts to address this in a number of ways:

- Although noted in Section 6.4, it is worth repeating here that information gained during the basic screen may help guide the selection of techniques and methods for use in an expanded screen. For example, many of the techniques in the basic screen rely on chromatography and/or mass spectrometer, so the data should be capable of being evaluated for the presence of not only target analytes, but also other compounds. In addition, clues about the identity of the contaminant may be provided by the mass spectra or chromatographic retention behavior (e.g., early eluting compounds may indicate a lower boiling point, poor peak shape on a non-polar chromatography column may suggest certain (polar) functional groups, etc).

- Combining observations from multiple basic screening techniques may be helpful. For instance, if no target analyte or unknown peak is found in a Method 524.2 analysis, the unknown compound is likely not a volatile organic; if no peak is present in a Method 8270D analysis, the unknown may be inorganic in nature or thermally unstable. This reduces the possibilities, although requires knowledge of the properties of potential contaminants. Some of these many properties may be obvious to skilled analysts. One resource to help with this is the Water Contaminant Information Tool (see Module 2), currently under development.
Many EPA standardized drinking water compliance methods are not included in Table 4-4, but may be used to implement the analysis summarized in Table 4-4 for expanded screening. This increases the number of analytes covered and, depending on the method, may decrease analysis time. Some standardized methods (see Section 6.2), including drinking water and SW-846 methods, may be useful to apply directly in the expanded screen, even though they are not necessarily intended for Table 4-20 analytes. In fact, by their design, SW-846 methods may allow for some "quick and dirty" approaches to screening that might be significant time savers when faced with the short turnaround times associated with the initial phases of an incident.

A discussion of the expanded screening techniques listed in Table 4-4 is provided below, along with some appropriate precautionary notes. Many readers will recognize some of these precautionary notes as being components of standardized methods. The discussion below should not be considered an exhaustive technical discussion on the subject of analysis of unknowns, but is intended to be an overview of those that are more widely used, or highly promoted. Thus, this information is primarily intended for planners and some laboratories, in terms of investing in resources and techniques that may be very useful for expanding the screening capability to look for unknown chemicals. Skilled analysts may wish to pursue other sources, such as technical books, scientific journals, and peer-interaction, for detailed insight and application of these techniques to specific technical challenges.

### 6.5.1 Expanded Screening for Organic Chemicals

Contaminants are often extracted from water samples to facilitate the analysis (e.g., to obtain a suitable amount of contaminant for analysis). Regardless of the detector system employed, there are a few widely used sample preparation techniques. Following sample preparation, a variety of detection techniques are used to analyze the sample. Figure 4-6 illustrates which preparation techniques are commonly used with which detection techniques.

#### Sample Preparation

**NOTE:** Large Volume Liquid/Liquid Extraction (LLE) generally may not be recommended for aerosolizable samples. This technique, although utilized in some EPA methods, requires operation of separatory funnels that may release aerosols when vented. The generation of these aerosols may represent a larger health hazard than other techniques, unless laboratories take precautions, such as appropriate hoods and sampling handling techniques. Many of the same goals of large volume liquid/liquid extraction may be accomplished through the use of the techniques described below. The techniques below may also have other benefits, such as reducing the consumption of sample and solvent.

**a) Direct Aqueous Injection.** Although a potentially powerful analytical technique, the use of direct aqueous injection with gas chromatography may present technical difficulties in chromatographic separation and may reduce the lifetime of the GC column and the detector. However, some modern GC capillary columns are designed to survive the rigors of direct aqueous injection, provided appropriate QC is applied to instrument operations and required maintenance is performed. For the high concentrations of contaminants that may be present
during an emergency incident, the use of direct injection of aqueous samples with GC/MS library matching may prove valuable, particularly for initial and rapid screening of analytes for which laboratories do not possess the equipment utilized in a standardized method. For all but a few analytes, analytical confirmation procedures may be necessary, and laboratories should plan carefully, in terms of developing skills to applying this technique, verifying their GC columns are suitable, implementing QC requirements, and preparing for additional maintenance requirements of both column and detector.

b) **Micro Liquid-Liquid Extraction (micro-LLE).** Liquid micro extraction involves the use of small volumes of solvent (~ 2 mL) to extract analytes from a small volume (~ 40 mL) of water (for an example, see EPA Method 551.1). This approach is usually much quicker than the typical LLE because of the ease of the microextraction and because no concentration/solvent exchange is required. If the analyte is known, then a calibration standard should be analyzed along with other requisite quality control samples to quantify the analyte. If the analyte is unknown, then a screening approach should be taken. The extraction may be immediately followed by GC/MS analysis, which can provide qualitative identification from the mass fragmentation library search. Analytical confirmation procedures may be necessary, and laboratories should plan carefully in terms of QC requirements and additional maintenance requirements of both column and detector. For the high, perhaps acutely toxic, concentrations of contaminants that may be present during an emergency incident, the use of liquid-liquid microextraction of aqueous samples with a suitable solvent, such as methylene chloride (dichloromethane) may prove particularly valuable, particularly for initial and rapid screening of analytes for which laboratories do not possess the equipment utilized in a standardized method. Micro-LLE may not provide adequate detection limits for lower concentrations, which may occur if the water sample represents the tail of a transient contaminant slug.

c) **Continuous Liquid-Liquid Extraction (Cont LLE).** This technique, as described in EPA Method 3520C, is applicable to the isolation and concentration of water-insoluble and slightly soluble organics. Its use can result in excellent detection limits, like solid-phase extraction described below, although detection limits for specific analytes may be different.

d) **Solid-Phase Extraction (SPE).** Solid-phase extraction (sometimes referred to as liquid-solid extraction) is described in Section 6.4.2 for EPA Method 525.2 as one of the techniques for basic screening analysis. Like micro liquid-liquid extraction, SPE extracts many contaminants, but can achieve larger concentration factors compared with the former technique, which may be necessary for some analytical goals (Section 1.3). However, many variants of the solid-phase extraction technique are possible. For instance, instead of using a C18 adsorbent, there are many other adsorbents available. The adsorbents have markedly different properties and may be used to extract contaminants that are not amenable to C18 adsorbents. Different elution solvents can also be used as an experimental variable. As a safety note, SPE should produce few aerosols.

e) **Solid-Phase Microextraction (SPME).** A relatively recent variation of solid-phase adsorption is solid-phase micro extraction (SPME). Like SPE, SPME should produce few aerosols. SPME involves the use of a fiber coated with sorbent material, and the sorbent coated fiber is exposed to either the aqueous sample or the headspace from the sample. The analytes then adsorb to the coating on the fiber. After exposure to the sample, the fiber can be introduced
into the detection system, although the technical details depend on the detector (i.e., GC or HPLC). For instance, after exposure to the sample, the SPME fiber is inserted into the injector of a GC, and contaminants are released to the column by thermal desorption. Fibers are available with several types of coating to enable extraction of analytes of different properties, such as volatiles or semivolatiles. Accordingly, SPME could be used for a quick screen. As with micro-LLE, another quick screen, the detection limits may only be useful in the case of elevated, perhaps acutely toxic, contaminant concentrations. Lower concentrations like those found in the tail of a transient contaminant slug, might not be detected using SPME.

f) **Headspace Collection.** The headspace above an aqueous sample may be injected in a GC. Commercial equipment, interfaced with the GC, is designed to facilitate this analysis, although manual injections may be made.

g) **Flow Injection.** In the flow injection analysis experiment, an aqueous sample or sample extract is injected directly into an LC/MS in such a manner that it bypasses the LC column. Thus, the analytes are not chromatographically separated, but the technique can prove useful if high concentrations of a single analyte are present, or if sample preparation is employed that is selective of particular analytes.

**Detection Techniques**

a) **Multidetector GC in Screening Mode.** In Table 4-3, a multidetector GC is utilized for specific analytes as an alternative to a mass spectrometer. However, the intent of using multidetector GC in the analysis of unknowns is primarily as a screening tool, not necessarily to quantify or identify, but rather to determine if the sample contains a component not normally found in the background. Multidetector (sometimes called multispectral) GC refers to the use of GC with a variety of detectors (there are over a dozen on the market) and then comparing the data from all the detectors. Examples of detectors include atomic emission, electron capture, infrared, flame ionization, nitrogen-phosphorus specific, thermal conductivity, etc.

Various GC detectors respond to contaminants in different ways, and the evaluation of all the data from the various detectors increases the selectivity of the analysis. For example, detectors like flame ionization detectors tend to respond to most contaminants (but not with high sensitivity), while electron capture detectors respond with great sensitivity (but mostly to halogenated compounds and are less sensitive to certain other compounds). The detectors may be used in series with one GC, or in parallel through the use of multiple GCs. In the latter case, the peaks are correlated through the use of relative retention times, i.e., the retention time of the peak of interest compared to a standard. Multidetector GC should be applied carefully because of differing response of different detectors to coeluting contaminants, and confirmatory/supplemental analysis may be necessary. For the expanded screen, because methods are used other than the standardized EPA methods for drinking water compliance monitoring, it may be possible to utilize other detectors than specified in the standardized methods.

b) **Gas Chromatography with Electron Impact Ionization Mass Spectrometry.** The subsequent analysis of contaminants extracted from water may be conducted by the use of gas chromatography/mass spectrometry. When the mass spectrometry is performed using electron
impact ionization, eluting peaks may show distinctive fragmentation patterns, which may be used in identification, particularly through the use of a variety of computerized tools for library matching to ionization patterns of known compounds. Thus, the use of electron ionization in full scan mode (i.e., \( m/z \) 35 to the upper scan limit of the particular instrument) may be particularly useful when performing the analysis for unknowns using the GC/MS for analyte separation and detection. Note that for the analysis of small molecules, it may be necessary to start the scan below \( m/z \) 35.

It is desirable to review peaks that do not match the retention time of analytes listed in the method. Water samples may contain hundreds or thousands of compounds, so each laboratory must decide a threshold for examining unidentified peaks. For instance, as a starting point, the laboratory may attempt to identify an unknown peak if it is present at >10% (height threshold) of the internal standard using the automated programs that are available with many instrument software packages. The threshold may need to be reduced (e.g., to 1%) or increased (e.g., to 20%) depending on the sampling location, as water samples vary in the number and intensity of their normal background peaks.

Usually, the program performs a spectral search using a user-defined library (such as NIST, EPA, Wiley, etc), and will report the compound with the best spectral match as the tentatively identified compound with an estimated concentration (based on height, assuming a response factor of 1.0). Some of the guidelines in evaluating the spectra are:

- The major ions in the reference spectrum (ions greater than 10% of the most abundant ion) should be present in the sample spectrum.
- The relative intensities of the major ions should agree within +/- 20%.
- The base mass ion present in the reference spectrum should be present in the sample spectrum.
- Ions present in the sample spectrum but not in the reference spectrum should be reviewed for possible coelution or background contamination.

Identification of unknown compounds relies on more than the efficiency of the library search program. The expertise of the analyst in evaluating the mass spectra and library matches is critical for assigning a tentative identification to the unknown. Electron impact mass spectrometers may vary slightly by manufacturer, vintage, and mass analyzer design. An analyst’s familiarity with the nuances of a particular instrument may greatly enhance a laboratory’s ability to confidently identify an unknown. In addition, there are a number of options within library search software, such as fitting algorithm, threshold parameters, result constraints, etc., and a particular analyst may be more proficient with one of these options for performing library-enhanced identification. For example, one approach may be that if the mass spectral library “reverse fit” is greater than 800 (of a possible 1,000), the peak is reported as identified in the report. If the fit is 600-800, the analyst should review the library search to determine if the identification was skewed by a coelution or high background. If the analyst is not able to improve upon the initial identification (fit between 600-800), the unknown should be listed by the class of compound assigned to that peak (e.g., “alkylated aromatic”, “halogenated aliphatic”, etc.) and an approximate molecular weight. If the report finds an unknown with a fit below 600, and the analyst cannot improve on the identification, the peak should simply be listed
as “unknown.” The library search report should be scrutinized by an experienced GC/MS analyst to determine how the “unknowns” should be reported.

The above procedures are based on the library match and represent only a tentative identification. To increase confidence in the identification, a reference standard can be purchased, when available, to help confirm tentatively identified compounds. Primary dilution standards may be prepared and analyzed using the same procedure as for samples. Comparison of the retention time and mass spectrum of the known reference standard with the data from the unknown may verify or negate the tentative identification.

c) **High Performance Liquid Chromatography-UV Detector.** Analogous to multidetector GC, HPLC with UV detection can be used to determine if organic compounds not amenable to gas chromatographic procedures (e.g., nonvolatile or thermally unstable compounds) are present in amounts greater than background. Calibration and quality control samples should be included to provide an accurate analysis. Analytical confirmation may be necessary using established techniques, such as GC/MS. Due to technical issues with different HPLC methods, some laboratories currently dedicate HPLC instruments to standardized methods currently being run in the laboratory. Accordingly, it may be desirable, albeit not always practical, to dedicate an HPLC instrument to screening activities.

Analysis for specific pesticides and other chemicals are possible, and various HPLC methods for specific chemicals appear in the sources of methods described in Section 6.2. Screening procedures using HPLC may be more successful if targets are selected. For instance, a HPLC analysis for pharmaceutical compounds of concern in water might be developed to supplement existing methods. This method would probably involve some type of extraction (micro-LEE, SPE, SPME, etc.) and analysis by HPLC with UV detection. Contaminants in the extracts can be separated with HPLC and detected through the use of a UV photodiode array detector (PDA), which can record a UV spectrum that can be used for qualitative identification, although not always definitely. If peaks cannot be identified from the UV spectrum, LC/MS, described below, may be attempted under the exact same conditions (e.g., chromatography column and elution solvent program), provided such conditions are consistent with LC/MS operation.

d) **High Performance Liquid Chromatography-Mass Spectrometry (LC/MS).** Many polar hydrophilic compounds cannot be easily extracted from an aqueous sample. In addition, there are contaminants of large molecular weight (e.g., biotoxins) or thermally unstable compounds that are not amenable to GC analysis but can sometimes be analyzed by LC/MS. Direct aqueous injection HPLC allows analysis of a sample without extraction or concentration. SPME and SPE (and other extraction procedures) may be utilized for compounds that can be extracted. Identification of unknowns can be performed but there are no standardized (EPA, NIST, Wiley, etc.) mass spectral libraries, as in GC/MS. Analyst interpretation can help identify possible compound fragments and structure. There are several ionization techniques available. Two of the more popular ones that fall into the category of atmospheric pressure ionization (API) are atmospheric pressure chemical ionization (APCI), which allows the analysis of neutral analytes, and electrospray ionization (ESI), which allows the analysis of ionic analytes.
Analysis of samples by LC/MS is often not a straightforward or conclusive endeavor, due to complexities of the ionization process. For instance, depending on the composition of the sample, when ESI-MS is operated in the positive ion mode, it can produce $[M+H]^+$, $[M+Na]^+$, and other adduct ions, which sometimes can be useful to the analyst and sometimes confounding. For this and other reasons inherent to ESI-MS, quantification may not be always as straightforward as with other LC detectors. Qualitative analysis of unknowns by LC/MS may be hampered because the technique is not readily amenable to the development of libraries, such as those available with electron impact ionization GC/MS. For qualitative analysis, LC/MS may be considered best suited to providing an estimate of the molecular weight of the compound.

In summary, more than a decade after its commercialization, LC/MS is not standardized for drinking water analysis, although it has proved extremely useful for analysis of target analytes in other industries. Nonetheless, LC/MS can be an added tool in an expanded screen for unknown chemicals in specific cases, and may be useful for certain classes of pesticides, such as carbamates. For the analysis of specific target compounds, a skilled analyst may be able to establish methods to analyze for pesticides and pharmaceuticals in a particular water matrix, thereby eliminating the need for a combination of HPLC detectors and derivatization schemes. However, these methods may not be as robust as standardized methods in terms of the analysis of waters from various sources. In addition, there may be many opportunities for misinterpretation and misapplication of LC/MS data, so additional analytical confirmation steps may be necessary to ensure confidence in and legal defensibility of the analytical results.

e) **Tandem Mass Spectrometry (MS/MS).** Both GC and HPLC may be utilized in conjunction with tandem mass spectrometry, also known as MS/MS. Different MS/MS instruments operate under different principles to achieve similar results, but in essence can be considered to be like two mass spectrometers connected by a collision cell. The first mass spectrometer separates ionized molecules, which are broken apart in the collision cell, and the resulting fragments are separated in the second mass spectrometer. This produces a great deal of information that can be used to identify the original molecules, but even when used with electron impact ionization, this technique does not necessarily produce searchable libraries. Like other advanced mass spectrometric techniques, MS/MS is not as widely available as MS and requires a high degree of skill. However, it can produce unique and powerful results in the right hands.

f) **High Resolution Mass Spectrometry (HR/MS).** GC or HPLC, combined with a high-resolution mass spectrometer, may provide exact mass data of an eluting compound, allowing for calculation of elemental composition of both molecular and fragmentation ions. This information is useful in the identification of unknown organic compounds, especially when the result of mass spectral library research is not conclusive or when the standard of a tentatively identified compound is not available. Careful quality control procedures are required, and the technique is not always definite, especially for unknown compounds, because many compounds may produce fragments with the same exact masses. Due to high capital cost and complexity of use, HR/MS is not widely available.

g) **Immunoassays.** There are a large number of immunoassay tests kits for organic chemicals, such as pesticides and biotoxins. These may be useful for screening a sample for specific unknowns either in the field or in the laboratory. These kits may be used for speed or if
instrumental methods are not available in a laboratory. However, use of these kits requires that
the goals of the analysis be planned because some kits are slower than the instruments, especially
if analytical confirmation time is considered. Also, appropriate training is necessary in the use of
these tests. Not all are the same, or easy to use. Laboratories should be aware of the kits’
reliability and levels of detection before using.

A large collection of test kits for chemicals, such as carbamate and quaternary ammonium
pesticides, and many other contaminants is summarized at
http://www.aoac.org/testkits/TKDATA2.HTM. This is by no means a complete list, and there
are a number of vendors with products not in this listing. It is important to note that most of
these test kits are not recognized by any standard setting organization. Not all of these products
have been studied in detail as to their efficacy for drinking water, which may contain interfering
and/or cross-reacting substances. Efforts are underway to confirm the efficacy of some of these
kits for particular purposes, and as results become available, they may be reported in the Module
3, Section 3.2. In the meantime, it may be important to keep in mind the trade-offs these kits
offer in terms of sample throughput and false negatives/positive results. In general, a positive
result from one of these test kits should be considered tentative and confirmed through a more
rigorous laboratory analysis.

Immunoassays have been developed for the analysis of urine for chemicals that may be classed
as illicit pharmaceuticals (e.g., narcotics and psychedelics). While it seems unlikely that these
substances would be used as water contaminants, there is some historical precedence for
concern. Immunoassays represent a very sensitive approach to screening of these compounds,
and they are available from a variety of sources. Because many of these tests were designed for
urine analysis, the performance of these tests for detecting the parent compound in water should
be verified, due to the potential for cross reactivity with substances in water, which may result in
a false positive detection. Because immunoassays may be interfered with by chlorine-based
disinfectants, the residual should be quenched before use. Established procedures also exist for
the analytical confirmation of many of these substances from urine using techniques such as
GC/MS.

6.5.2 Expanded Screening of Inorganic Chemicals

Like the determination of organic chemicals, there are often a number of similar preparation
steps that are used in the analysis of inorganic chemicals. These are numerous and vary with the
methodology being employed. As a starting place to select a sample preparation approach, it
may be useful to refer to relevant standardized methods. For instance, if the goal is to look for
trace metals not listed in a particular method, it may be useful to refer to a method in which the
sample is prepared for metal analysis. This is not necessarily an exact process, and some metals
have certain characteristics that may cause them to not be amenable to a preparation technique
applicable to another. For instance, a digestion method for nickel may not be applicable for
mercury analysis. Following preparation, the samples may be analyzed by a number of

a) **ICP-AES or ICP-MS in Semiquantitative Mode.** Analogous to multi-detector GC and
HPLC with UV detection, the ICP-AES and ICP-MS methods (EPA Methods 200.7 and
200.8, respectively) can also be expanded to provide a broad screen approach to identifying unknown trace metals. Under the semiquantitative mode (SQM, referred to by various names depending on instrument manufacturer), the ICP-MS instrument, operated in scanning mode, may be capable of providing semiquantitative results for more than 60 elements including major atomic cations, metals, semi-metals, rare earth elements and selected radionuclides (uranium and thorium) without actually including all of the elements being analyzed in the calibration standards. If an element is suspected, then standards may be obtained to perform quantitative analysis. (Note: radioactive materials should be handled by a specialized laboratory.) Some ICP-AES instruments can provide semiquantitative results for many, albeit fewer, elements than ICP-MS instruments, most of which are capable of SQM through the use of the correct software. The following paragraph discusses ICP-MS operated in scanning mode, and SQM for appropriate ICP-AES instruments functions analogously.

The original calculation algorithm of SQM may be applicable to all naturally occurring elements. Additional information may be obtained for elements that are not considered in the default algorithm, such as man-made radioisotopes or stable fission products with long half-lives, especially for isotopes in mass range between 210 and 238. Under the SQM mode, the concentrations of the naturally occurring elements are calculated based upon instrument response factors (RF) for each element. No internal standards are necessarily added when using the SQM method. Following the external calibration procedure, a blank and a single calibration standard of 20-30 elements are typically used to update the RF before each analytical run. Increasing the number of elements in the standard generally may improve the accuracy of the analysis. For elements not included in the calibration standard, the RFs are updated indirectly by software interpolation from the calibrated elements. SQM identifies and assigns signal intensity based upon the natural abundance distributions for each isotope, corrects the molecular interferences by subtracting the signal of the assigned polyatomic species from the total signal, further corrects for polyatomic and isobaric interferences given in the elemental equation, and calculates the final concentration.

b) **Ion Chromatography (IC).** Ion chromatography forms the basis of several EPA methods to determine ions of regulatory interest, such as EPA Method 300.1 for several common drinking water ions and disinfection byproducts. By the correct choice of operating conditions and ion chromatography columns, determination of many different types of ions have appeared in the literature, including those for some potential, albeit not high priority, drinking water contaminants of concern from a drinking water security perspective.

c) **Wet Chemistry.** Wet chemistry forms the basis of many types of chemical test kits. The chemistry and detectors for test kits approved for compliance monitoring are traceable to EPA methods. Wet chemistry techniques, through the use of so-called “autoanalyzers,” form the basis of many types of chemical analysis, for environmental and clinical applications. Manufacturers of these devices often provide full detailed methodology for defensible application of wet chemistry to a variety of analytes. Titrimetric methods are also available and can be used to analyze background water quality parameters such as alkalinity.
d) **Ion Selective Electrodes.** Ion selective electrodes (ISE, also known as electrochemical probes) can be utilized to analyze for some background water quality parameters. A simple example of an ISE is the familiar pH probe for the hydrogen ion. Other ISEs for a variety of ions (such as ammonia, calcium, chloride, fluoride, nitrate, potassium, silver, sodium, and sulfide) also may be available from a variety of manufacturers and may be considered. Some parameters that can be monitored by ISE may be useful in characterizing the extent of contamination or verifying credibility of a contamination threat (see the discussion on field testing in Module 3).

### 6.5.3 Expanded Screening of Cyanides

Expanded screening of cyanides typically involves the use of sample preparation techniques and wet chemical detection chemistries that are not used in standard EPA methods. For instance, the distillation step may be omitted, as it is for cyanide methods based on the Conway diffusion technique. Note that distillation is required for determination of total cyanide concentration, which, as discussed in Section 6.4.7, may be the most conservative approach with respect to public health implications. However, free cyanide concentration measured without distillation, may still be useful, particularly in detecting high, acutely toxic concentrations.

Distillation also is not used in the rapid field tests for cyanide (Section 4.1), but may be applicable for expanded cyanide screening. As discussed in Section 3, some laboratories may consider using these rapid field tests as safety screening tests upon receipt of the sample by the laboratory. Note that several rapid field tests for cyanide have been evaluated as part of EPA’s Environmental Technology Verification program, including some ion selective electrodes. The reports, available at [http://www.epa.gov/etv/verifications/vcenter1-23.html](http://www.epa.gov/etv/verifications/vcenter1-23.html) indicate that some of these rapid field test kits, although acceptable for field use, may perform better when used under controlled laboratory conditions than in the field, especially in cold weather.

### 6.5.4 Expanded Screening of Biotoxins

Some biotoxins have been monitored routinely for quite a while, particularly in conjunction with naturally occurring outbreaks of biotoxins in marine environments. There are hundreds of biotoxins from dozens of different plant and animal species. The broad screening for microbiological contaminants (Section 8) may capture some of the higher molecular weight biotoxins, and analysis of some biotoxins may be supported by LRN laboratories, as indicated in Table 4-1. In addition, the LRN currently may utilize immunoassays for biotoxin analysis of botulism, ricin, and other biotoxins where applicable. The results of the LRN analysis should be further communicated to any other laboratories to which chemical samples are sent.

**Immunnoassays.** Many/all of the biotoxins listed in Table 1-1 have commercially available immunoassay kits. A large collection of test kits is summarized at [http://www.aoac.org/testkits/TKDATA2.HTM](http://www.aoac.org/testkits/TKDATA2.HTM). This is by no means a complete list, and there are a number of vendors with products not in this listing. It is important to note that most of these test kits are not recognized by any standard setting organization, and the applicability of these products to disinfected drinking water, which may contain interfering and/or cross-reacting substances, is not well studied. Efforts are underway to confirm the efficacy of some of these
kits for particular purposes, and as results become available, they may be reported in the Appendix to Module 3 that lists field test equipment. In the meantime, it may be important to keep in mind the trade-offs these kits offer in terms of sample throughput and false negative/false positive results. In general, a positive result from one of these test kits should be considered tentative and confirmed through a more rigorous laboratory analysis.

A key part of laboratory analytical screening for a biotoxin is the ability to confirm its tentative identification (e.g., via an immunoassay test kit). Confirmatory analyses are discussed in more detail below, and usually involve GC/MS, LC, or LC/MS. The IOC Manual on Harmful Marine Microalgae, available at http://unesdoc.unesco.org/images/0012/001220/122021eo.pdf, contains many examples of these types of analyses, chiefly intended for analysis of algal biotoxins. One type of assay that has been widely used for the determination of biotoxins in seafoods over the past 60 years is the mouse bioassay. The applicability of this technique to water samples is not well investigated, and most laboratories do not have facilities for the care of laboratory animals. In addition, immunoassay techniques are less sensitive than instrumental analysis techniques.

The biotoxins may be considered as organic chemicals, and the same types of sample preparation and instrumental analysis techniques may be applicable, depending on the molecular weight of the biotoxin. Low molecular weight toxins may be treated much like any organic chemical described in Section 6.5, and may be analyzed by the same type of analytical techniques (e.g., GC/MS) as described therein. Because biotoxins tend to be very water soluble, liquid chromatography techniques have been used to determine biotoxins in water. When LC/MS is used, the same precautions may be necessary as those for other organic chemicals (see Section 6.5.1). The analysis of biotoxins is one area where LC/MS has proved particularly valuable, especially if the molecular weight of the biotoxin precludes its analysis by GC/MS. Again, the skill of the analyst is critical in obtaining meaningful results for LC/MS analysis of biotoxins.

6.5.5 Expanded Screening of Radionuclides

Radiological analysis, as noted above, should be performed only by licensed, specialty laboratories, and the need for such analysis should be indicated by the field screening equipment for alpha, beta, and gamma emitters. As discussed in Module 3, the site characterization will determine the appropriate laboratory to receive radioactive samples (e.g., high levels of radiation would indicate a radiation hazard, and only the appropriate radiation laboratory would receive the sample). If the laboratory should wish to screen samples before accepting them or analyzing them, then inspection of samples for alpha, beta, and gamma radioactivity using probes for these emitters may be prudent (e.g., the devices used by the first responder/sample collector as described in section 3.1.4). Again, a positive test from the screening devices should be sent to specialty laboratories for confirmatory analysis.

The basic screen discussed in Section 6.4.8 is rather comprehensive because it requires identification of the specific radionuclide if indicated by the screens for gross alpha, beta, and gamma radiation. Therefore, the expanded screen is designed to capture radionuclides that do not fall into the energy range of the gross radionuclide screen for gross alpha and beta. Fortunately, these radionuclides have specific EPA 900-Series methods designed for their
Two other techniques that may be particularly useful for radionuclide analysis are gamma spectroscopy, which can directly identify the gamma emitting radionuclide and inductively coupled plasma mass spectrometry (ICP-MS). Principal considerations in the use of both of these techniques include detection limits and availability of instrumentation. Often, these considerations are based on the particular radionuclide involved. To better understand and deal with the needs and roles of the wide variety of analytical radiochemistry techniques at the disposal of radionuclide laboratories, the Multi-Agency Radiological Laboratory Analytical Protocols (MARLAP) Manual has been developed by the Environmental Protection Agency, the Department of Energy, the Nuclear Regulatory Commission, the Department of Defense, the National Institute of Standards and Technology, along with some states (http://www.epa.gov/radiation/marlap/). This effort is but one component in the EPA’s Radiological Emergency Response Plan (http://www.epa.gov/radiation/rert/docs/rerp-1-00.pdf) in support of EPA’s responsibilities for protecting the environment and in support of the Federal Radiological Emergency Response Plan (http://www.nrt.org/production/nrt/home.nsf/0/5c23c5d580746e48525660c005b56b5?OpenDocument).

6.5.6 Expanded Screening of Chemical Weapons

As discussed in Section 2.1.4, the term chemical weapons refers to the substances, such as those shown in Table 1.1, that appear on Schedule 1 of the Chemical Weapons Convention (CWC, http://www.cwc.gov/Regulations/cfr-15/part-712-s1_html). There are other substances, such as chemical weapon precursors, that are monitored by the CWC under Schedules 2 or 3, which also may be present in water at very low concentrations (typically less than 10 µg/L) due to industrial pollution or the formation of disinfection byproducts. Table 4-18 in the Appendix (Section 10) lists the Scheduled chemicals. Chemicals on Schedules 2 or 3 have fewer restrictions compared with Schedule 1, since many have legitimate industrial applications. Thus, environmental laboratories could potentially analyze for many Schedule 2 or 3 chemicals. This section focuses on the analysis of Schedule 1 chemicals, which are most restricted.

The Schedule 1 agents used as chemical weapons are extremely hazardous to handle and most environmental chemistry laboratories do not have the facilities or the procedures in place to handle these agents. In addition, most of the agents are not available commercially to prepare analytical standards for quantification. The chemical weapons agents will need to be analyzed by special chemical weapons laboratories for confirmatory analysis. However, in the unlikely case that an environmental chemistry laboratory receives a sample containing a chemical weapon, screening techniques can be used by environmental chemistry laboratories to identify the presence of the agents in water.

The best analytical approach may be to utilize the preparatory procedures (direct injection, micro-LLE, SPE, and/or SPME) as discussed above, followed by GC/MS for identification. This may only be able to determine the presence, not concentration, of the agent because an analytical standard would not be available. However, the standard electron impact mass spectral libraries...
frequently contain mass spectra of these compounds and can be used for tentative identification. As an aid to increasing confidence in chemical warfare agents’ GC/MS library matches, the National Institute of Standards and Technology has developed the Automated Mass Spectral Deconvolution and Identification System (AMDIS), [http://chemdata.nist.gov/mass-spc/amdis/](http://chemdata.nist.gov/mass-spc/amdis/).

In the unlikely case CW agents are present, the expanded screen for organic chemicals may be procedurally designed to reduce risk, namely through reduction of aerosols. As with any organic chemical, an additional way to reduce risk would be through sample dilution. The laboratory may first start with the most dilute sample (1/1,000) and if nothing is detected may proceed to analyze the next dilution (1/100), followed by the 1/10 dilution, and lastly the undiluted sample. If the laboratory proceeds through the undiluted sample and nothing is detected, the sample may be deemed non-detect for the chemical weapons that would be captured by the screen. If the chemical weapons agents are identified in the screen, proper notifications should be made to law enforcement to gain access to laboratory resources that can confirm the presence of the chemical weapons agent.
7 Examples of the Analytical Approach to Site-Specific Situations for Chemical Contaminants

Section 6 describes an analytical approach based on chemical contaminants considered important from a national perspective. As noted above, local authorities may wish to develop their own analytical approach based on their perceived needs and concerns. Here are some brief examples of analytical approaches based on the concept of basic and expanded screening. The first five (Section 7.1) are derived from the needs of specific, hypothetical utilities, and the final three (Section 7.2) are geared toward laboratories that serve a wider base of clients.

The overriding principle in these examples is the flexibility that laboratories have in developing and updating an analytical plan for the analysis of unknowns in water. Utility laboratories may have the greatest level of flexibility with respect to the comprehensiveness of their screen if they have made arrangements with other labs to perform more comprehensive screening as necessary. For example, utility laboratories may choose to perform some level of basic screening that may be applied in the case of a 'possible' threat; however, analysis may need to be performed at another laboratory in response to a threat deemed 'credible'. On the other hand, laboratories that serve a wider customer base should have a more comprehensive and robust screening procedure to cover the range of potential scenarios that might be encountered in working with various utilities.

7.1 Examples for Utility Laboratories

These five examples are targeted at utility laboratories that chose to develop an analytical approach to support analysis of their own emergency water samples, and make arrangements to engage other laboratories with broader capabilities when necessary. This is to say, it is part of upfront planning for a utility to decide when they will handle samples in-house and when they will send them to external laboratories. In many of these examples, the utility laboratories often take a “springboard” approach to screening, using the results of the basic screen to make decisions in the expanded screen.

Example #1: Based on the results of a vulnerability assessment, a local water utility has concluded that their consumers are at only a very small risk from water contamination incidents, and so do not want to invest much of their limited resources in overly detailed analytical approach. They possess a purge-and-trap-GC instrument with conventional photoionization and electrolytic conductivity detectors in series, and are certified to perform analysis of Method 502.2 analytes. They possess a GC/MS for another purpose, so they choose to expand their laboratories capabilities by making a small purchase of a solid-phase extraction manifold and supplies suitable for Method 525.2 analytes. They allow their laboratory staff the time to become proficient with Method 525.2 to ultimately obtain certification by their state. In the meantime, they plan on using the technique in the expanded screen. They decide to exclude carbamate pesticides and quaternary ammonium compounds from their analytical approach because they are not available anywhere nearby, and the utility does not believe that they will be important because there are opportunity contaminants available locally. One of these opportunity contaminants is glyphosate, which, although not very toxic to humans, is widely used in the area. They search NEMI, or another source such as those listed in Table 4-2, and find...
a suitable method. They do not possess any capability for trace metal analysis, so they contract this out to a third party who performs Method 200.7. In looking at the analyte list for Method 200.7, the laboratory sees that mercury can be analyzed by Method 200.7 and seeks data from the contracted third party that their instrument provides sufficient detection limits for arsenic and mercury. The utility laboratory realizes Method 200.7 most likely will determine many organomercury compounds (as total mercury), so they decide that the benefit of enhancing their mercury screening capabilities through the addition of Method 245.2 to their basic screen is not worth the expense. In their locality, the first responders to contamination incidents possess, and are trained in, the use of portable cyanide detection systems and radiation detectors. They buy a low-cost gamma detector to screen samples in the laboratory as a safety precaution. Thus, instead of Table 4-3, their basic analysis may be summarized in Table 4-5. Clearly, this is not as rigorous as Table 4-3, but does meet many of the same needs.

During an incident, if the threat evaluation and site characterization indicated that greater analytical capability is required for expanded screening or confirmatory analysis, they plan to rely on state resources, although there is a four-hour delay in getting the samples to the state laboratories. If the water utility emergency response manager (or the designated incident commander) must have results faster, they plan to perform direct aqueous injection into their GC/MS with the hope of getting a successful GC/MS library match. They also consider performing a micro-liquid liquid extraction using methylene chloride for the same purpose. However, due to the resulting additional instrument maintenance requirements, which may make the instrument unavailable for its intended purpose, they do not want to do this routinely.

Table 4-5. Basic Screen for Chemical Analysis for Example #1

<table>
<thead>
<tr>
<th>Chemical (general class)</th>
<th>Analytical Technique</th>
<th>EPA Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volatiles (organic)</td>
<td>Purge-and-trap PID/ELCD</td>
<td>502.2</td>
</tr>
<tr>
<td>Semivolatiles (organic)</td>
<td>Solid-phase extraction GC/MS</td>
<td>525.2</td>
</tr>
<tr>
<td>Trace metals (inorganic)</td>
<td>Contracted out</td>
<td>200.7</td>
</tr>
<tr>
<td>Cyanides</td>
<td>By first responders</td>
<td>N/A</td>
</tr>
<tr>
<td>Radionuclides</td>
<td>By first responders</td>
<td>N/A</td>
</tr>
<tr>
<td>Local contaminant (Glyphosate)</td>
<td>HPLC-UV</td>
<td>547</td>
</tr>
</tbody>
</table>

Example #2: A utility laboratory has a great number of legally defensible protocols (some of which are EPA methods) already in place, except for radionuclides, which are handled by a separate department. They examine the analytes in the methods in Table 4-3, and determine to the best of their ability and interest that their protocols already cover all the contaminants about which they are concerned. Thus, their version of an analytical approach for chemicals may appear as shown in Table 4-6.
Table 4-6. Basic Screen for Chemical Analysis for Example #2

<table>
<thead>
<tr>
<th>Chemical (general class)</th>
<th>Analytical Technique</th>
<th>EPA Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volatiles (organic)</td>
<td>Purge-and-trap GC/MS</td>
<td>In-house protocol</td>
</tr>
<tr>
<td>Semivolatile (organic)</td>
<td>Solid-phase extraction GC/MS</td>
<td>In-house protocol</td>
</tr>
<tr>
<td>Carbamate pesticides (organic)</td>
<td>Derivatization GC/MS</td>
<td>In-house protocol</td>
</tr>
<tr>
<td>Quaternary nitrogen compounds (organic)</td>
<td>HPLC – UV</td>
<td>In-house protocol</td>
</tr>
<tr>
<td>Trace metals (inorganic)</td>
<td>ICP-MS</td>
<td>In-house protocol</td>
</tr>
<tr>
<td>Total mercury (including organomercury compounds)</td>
<td>Cold vapor AA</td>
<td>In-house protocol</td>
</tr>
<tr>
<td>Cyanides</td>
<td>Wet chemistry</td>
<td>In-house protocol</td>
</tr>
<tr>
<td>Radionuclides</td>
<td>First responders</td>
<td>In-house protocol</td>
</tr>
</tbody>
</table>

In addition, the laboratory possesses a significant capability in terms of instrumentation and analytical expertise to perform the expanded screening analysis, such as interpreting electron impact mass spectra and performing immunoassays. Thus, Table 4-6 is consistent with their needs and may comprise their analytical approach.

Example #3: A water utility decides its water security concerns are reflected in the approach presented in Section 6. The utility has access to laboratories that are capable of performing the methods in Table 4-3, except radionuclide analysis, which is handled by emergency responders in accordance with state policy. However, they do not have access to expanded screening techniques, and choose not to include in-house expanded screening, since their local emergency operations policy does not allow time for detailed expanded screening. Thus, decisions would typically be based on the results from the basic analytical screen. However, the utility has made arrangements with a commercial laboratory to perform some expanded screening if deemed necessary based on the circumstances of the incident.

Table 4-7. Basic Screen for Chemical Analysis for Example #3

<table>
<thead>
<tr>
<th>Chemical (general class)</th>
<th>Analytical Technique</th>
<th>EPA Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volatiles (organic)</td>
<td>Purge-and-trap GC/MS</td>
<td>524.2</td>
</tr>
<tr>
<td>Semivolatile (organic)</td>
<td>Solid-phase extraction GC/MS</td>
<td>525.2</td>
</tr>
<tr>
<td>Carbamate pesticides (organic)</td>
<td>HPLC – fluorescence detection</td>
<td>531.1</td>
</tr>
<tr>
<td>Quaternary nitrogen compounds (organic)</td>
<td>HPLC – UV</td>
<td>549.2</td>
</tr>
<tr>
<td>Trace metals (inorganic)</td>
<td>Graphite furnace AA</td>
<td>200.9</td>
</tr>
<tr>
<td>Total mercury (including organomercury compounds)</td>
<td>Cold vapor AA</td>
<td>245.1 with persulfate</td>
</tr>
<tr>
<td>Cyanides</td>
<td>Wet chemistry</td>
<td>335.3</td>
</tr>
<tr>
<td>Radionuclides</td>
<td>Emergency responders</td>
<td></td>
</tr>
</tbody>
</table>

Example #4: A water utility only has capability for basic water quality parameters, such as pH, turbidity, and chlorine residual. The utility has always sent samples to the state laboratory for routine analysis and does not have resources to do differently, even in the case of an emergency. They do, however, make plans for how to most efficiently deliver the samples to the state laboratory, and recruit employees from other city and county offices to help implement the plan if the regular staff of two persons is not available. Note that the techniques used by the state...
laboratory (shown in Table 4-8) are the same as in Table 4-3. However, from the utility’s perspective, the state laboratory governs the selection of the techniques.

<table>
<thead>
<tr>
<th>Chemical (general class)</th>
<th>Analytical Technique</th>
<th>EPA Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volatiles (organic)</td>
<td>Purge-and-trap GC/MS</td>
<td>524.2</td>
</tr>
<tr>
<td>Semivolatile (organic)</td>
<td>Solid-phase extraction GC/MS</td>
<td>2525A/8270D</td>
</tr>
<tr>
<td>Carbamates pesticides (organic)</td>
<td>HPLC – fluorescence detection</td>
<td>531.1</td>
</tr>
<tr>
<td>Quaternary nitrogen compounds (organic)</td>
<td>HPLC – UV</td>
<td>549.2</td>
</tr>
<tr>
<td>Trace metals (inorganic)</td>
<td>ICP-MS</td>
<td>200.8</td>
</tr>
<tr>
<td>Cyanides</td>
<td>Wet chemistry</td>
<td>335.3</td>
</tr>
<tr>
<td>Radionuclides</td>
<td>Gross alpha, gross beta, gross gamma radiation</td>
<td>technique referred by state lab</td>
</tr>
</tbody>
</table>

**Example #5:** A utility laboratory has a purge-and-trap GC/MS, a regular GC/MS, and an ICP-MS. Their basic screen is represented in Table 4-9.

<table>
<thead>
<tr>
<th>Chemical (general class)</th>
<th>Analytical Technique</th>
<th>EPA Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volatiles (organic)</td>
<td>Purge-and-trap GC/MS</td>
<td>524.2</td>
</tr>
<tr>
<td>Semivolatile (organic)</td>
<td>Solid-phase extraction GC/MS</td>
<td>525.2</td>
</tr>
<tr>
<td>Trace metals (inorganic)</td>
<td>ICP-MS</td>
<td>200.8</td>
</tr>
</tbody>
</table>

While this leaves some gaps in their analytical coverage, they plan to address these deficiencies through an expanded screen. They acquired immunoassay test kits that have undergone some level of validation, such as ETV (http://www.epa.gov/etv). For these and other kits, the laboratory understands the limitations of these test kits (see Section 6.5.1 and 6.5.4) and has arranged analytical confirmation of the analytes with state laboratories should the need arise. The laboratory also has an agreement with a local university that has a mass spectrometry facility including an LC/MS. The utility laboratory takes steps to ensure that the university laboratory/staff will be available in the case of an emergency and all chain-of-custody rules and legal defensibility issues have been met. The utility also has made arrangements to contract out the confirmatory analysis, if necessary. Although the utility recognizes there are potential problems with this arrangement, it believes this plan meets its needs.

### 7.2 Examples for Laboratories with a Wide Client Base

The concept of basic screening takes on a different dimension for laboratories that serve a wide client base because utility laboratories are most likely faced with ‘possible’ incidents. Accordingly, they may feel reasonably confident in the comprehensiveness of the screening procedures, illustrated in examples #1–#5. On the other hand, laboratories that serve a wide client base have a larger opportunity to receive a sample from a ‘credible’ incident. There are two reasons for this. First, these laboratories serve more clients, so on a strictly mathematical basis, they have a greater chance. Second, utility laboratories in ‘credible’ situations may be
more likely to refer samples to another laboratory, which may have greater capability to apply a more focused analytical approach in response to a specific incident (as discussed in Section 5).

Accordingly, laboratories that choose to perform analysis of emergency water samples and serve a broader client base, such as Federal, State, and commercial labs, may need to adopt a more comprehensive approach such that they maintain flexibility to provide analytical support in a variety of situations. However, as the following three examples illustrate, this goal can be met in a variety of ways.

The first two examples represent extreme cases of laboratories preparing to respond to a ‘possible’ and/or ‘credible’ incident. The first example represents a laboratory that does not define a basic screen and will perform analyses as part of a comprehensive expanded screening program. The second example depicts a laboratory that attempts to be comprehensive by including a very large number of standard methods in their basic screen. The third example blends the first two examples and adopts a screening approach that aligns closely with the complete “Basic Screen” (Table 4-3) and has some capability for the “Expanded Screen” (Table 4-4), as well.

**Example #6.** Utilities in a county are all serviced by the same central laboratory. However, the needs of the utilities are somewhat different, so after a meeting between all utility and laboratory representatives, it is agreed that the plan is to perform no basic screens. The decision was based on response times for utilities, mandated by the locality, being too short for the basic screen. The laboratory will instead perform the expanded screen using exploratory techniques that have short response time, such as those discussed in Section 6.5 (Table 4-4). The laboratory has skilled analysts and modern equipment and is confident of its capability. The laboratory also is capable of performing confirmatory techniques for some contaminants, if necessary. The planners realize that this approach may not meet all desired QC goals for every contaminant, but are prepared to live with the consequences of basing decisions on this kind of data.

**Table 4-10. Basic Screen for Chemical Analysis for Example #6**

<table>
<thead>
<tr>
<th>Chemical (general class)</th>
<th>Analytical Technique</th>
<th>EPA Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

**Example #7.** A state laboratory that supports a large number of utilities. The laboratory performs many EPA drinking water and SW-846 methods routinely, and it wants to include as many techniques as possible in the basic screen. It chooses to do this since the needs of water systems it serves vary greatly, and most are small and understaffed to the point that the utilities generally rely on the state for all analyses and analytical decisions. The laboratory also realizes that only a broad screen will be sufficient to cover the majority of potential emergency scenarios that its utility clients may face. Also, the laboratory wants to exercise “due diligence” up to the level of a ‘credible’ threat, as defined by the threat evaluation, and it realizes that more analytes are captured by these methods than are in the basic screen in Table 4-3, and that some of the methods are more sensitive for particular analytes. In all, the laboratory feels that the inclusion of all these methods would be valuable for their clients.
Note that the laboratory chooses preparation methods for the SW-846 methods to make them compatible with the drinking water methods, so that it does not have to prepare the sample twice in many cases. The laboratory feels that its basic screen best meets the needs of its clients. It does perform some expanded screening to try to identify unknown chromatographic peaks in its analysis and through combining all the analytical results from the various techniques.

### Table 4-11. Basic Screen for Chemical Analysis for Example #7

<table>
<thead>
<tr>
<th>Chemical (general class)</th>
<th>Analytical Technique</th>
<th>EPA Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volatiles (organic)</td>
<td>Purge-and-trap GC/MS</td>
<td>8260B</td>
</tr>
<tr>
<td>Semivolatiles (organic)</td>
<td>Solid-phase extraction GC/MS</td>
<td>3535A/8270D</td>
</tr>
<tr>
<td>Nitrogen and phosphorus-containing pesticides (organic)</td>
<td>GC/NPD</td>
<td>507</td>
</tr>
<tr>
<td>Chlorinated pesticides, herbicides, and organohalides (organic)</td>
<td>GC/ECD</td>
<td>508.1</td>
</tr>
<tr>
<td>Chlorinated acids (organic)</td>
<td>GC/ECD</td>
<td>515.1</td>
</tr>
<tr>
<td>Phenols (organic)</td>
<td>solid-phase extraction GC/MS</td>
<td>528</td>
</tr>
<tr>
<td>Carbamate pesticides (organic)</td>
<td>HPLC – fluorescence detection</td>
<td>531.1</td>
</tr>
<tr>
<td>Glyphosate (organic)</td>
<td>HPLC-UV</td>
<td>547</td>
</tr>
<tr>
<td>Quaternary nitrogen compounds (organic)</td>
<td>HPLC – UV</td>
<td>549.2</td>
</tr>
<tr>
<td>Chlorination disinfection byproducts, chlorinated solvents, and halogenated pesticides/herbicides (organic)</td>
<td>GC/ECD</td>
<td>551.1</td>
</tr>
<tr>
<td>Water soluble alcohols/non-chlorinated organics (organic)</td>
<td>GC/FID with direct aqueous injection</td>
<td>8015B/5031</td>
</tr>
<tr>
<td>Organochlorine pesticides (organic)</td>
<td>GC/MS</td>
<td>8081A</td>
</tr>
<tr>
<td>Organophosphorus compounds (organic)</td>
<td>GC/MS</td>
<td>8141A</td>
</tr>
<tr>
<td>Chlorinated herbicides (organic)</td>
<td>GC/MS after methylation or pentafluorobenzylation</td>
<td>8151A</td>
</tr>
<tr>
<td>Trace metals (inorganic)</td>
<td>ICP-MS in scanning mode</td>
<td>200.8</td>
</tr>
<tr>
<td>Total mercury (including organomercury compounds)</td>
<td>Cold vapor AA</td>
<td>245.2 with persulfate</td>
</tr>
<tr>
<td>Cyanides</td>
<td>Wet chemistry</td>
<td>335.3</td>
</tr>
<tr>
<td>Biotoxins</td>
<td>Immunoassays with confirmatory techniques</td>
<td></td>
</tr>
<tr>
<td>Radionuclides</td>
<td>Gross alpha, gross beta, gross gamma radiation</td>
<td>technique referred by lab</td>
</tr>
</tbody>
</table>

### Example #8: A laboratory serves an area that has several drinking water treatment plants located downstream from an agricultural area, an industrial area, and/or a nuclear power plant. In addition, there are geological formations that contribute to high inorganic contaminant levels. Fortunately, the laboratory is very well equipped and staffed, with strong capabilities and experience in monitoring all the classes of contaminants in Table 4-3. In addition, the laboratory expands the analyte list (see Table 4-20) for these methods to include as many analytes as possible.
Table 4-12. Basic Screen for Chemical Analysis for Example #8

<table>
<thead>
<tr>
<th>Chemical (general class)</th>
<th>Analytical Technique</th>
<th>EPA Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volatiles (organic)</td>
<td>Purge-and-trap GC/MS</td>
<td>524.2</td>
</tr>
<tr>
<td>Semivolatiles (organic)</td>
<td>Solid-phase extraction GC/MS</td>
<td>3535A/8270D</td>
</tr>
<tr>
<td>Carbamate pesticides (organic)</td>
<td>HPLC – fluorescence detection</td>
<td>531.1</td>
</tr>
<tr>
<td>Quaternary nitrogen compounds (organic)</td>
<td>HPLC – UV</td>
<td>549.2</td>
</tr>
<tr>
<td>Trace metals (inorganic)</td>
<td>ICP-MS</td>
<td>200.8</td>
</tr>
<tr>
<td>Total mercury (including organomercury compounds)</td>
<td>Cold vapor AA</td>
<td>245.2 with persulfate</td>
</tr>
<tr>
<td>Cyanides</td>
<td>Wet chemistry</td>
<td>335.3</td>
</tr>
<tr>
<td>Radionuclides</td>
<td>Gross alpha, gross beta, gross gamma</td>
<td>technique preferred by lab</td>
</tr>
</tbody>
</table>

Due to sources of potential contaminants other than intentional contamination, the laboratory also maintains careful records of ranges of contaminants it normally encounters, so that during an emergency incident, it does not confuse background levels of contaminants with intentionally introduced ones.

For expanded screening, the laboratory utilizes its existing instrumentation with the techniques discussed in Section 6.5. The laboratory is particularly interested in developing an HPLC screen for pharmaceuticals (which it was already interested in due to potential regulatory issues), and would like to explore micro-LLE and SPME for this purpose. Therefore, the laboratory acquires the required equipment and supplies, and encourages its staff to investigate these techniques.
8 Analytical Approach for Biological Contaminants

8.1 Overview of Contaminant Issues

8.1.1 Types of Biological Contaminants Covered by the Analytical Approach

Two broad classes of pathogens may be encountered in a contaminated water sample: 1) traditional waterborne pathogens such as enteric bacteria, viruses and protozoa; and 2) exotic and select agents that may be suitable for a water route of transmission. Table 4-13 lists some of these pathogens. This section is intended as a guide for selecting analytical approaches for samples suspected of containing known and unknown microbial contaminants. Specific methodologies will be addressed in a laboratory analysis guide for biological contaminants (under development).

<table>
<thead>
<tr>
<th>Pathogen general class</th>
<th>Organism</th>
<th>Select Agent*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria (non-spore forming)</td>
<td><em>Brucella spp.</em></td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td><em>Burkholderia pseudomallei</em></td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td><em>Campylobacter spp.</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>E. coli 0157:H7</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Francisella tularensis</em></td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td><em>Salmonella spp.</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Shigella spp.</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Vibrio cholerae</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Yersinia pestis</em></td>
<td>Yes</td>
</tr>
<tr>
<td>Bacteria (spore forming)</td>
<td><em>Bacillus anthracis</em></td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td><em>Clostridium botulinum</em> A</td>
<td>Yes</td>
</tr>
<tr>
<td>Bacteria (Rickettsia)</td>
<td><em>Coxiella burnetti</em></td>
<td>Yes</td>
</tr>
<tr>
<td>Protozoa</td>
<td><em>Cryptosporidium parvum</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Entamoeba histolytica</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Giardia intestinalis</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Toxoplasma gondii</em></td>
<td></td>
</tr>
<tr>
<td>Viruses</td>
<td><em>Enteroviruses</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Hepatitis A</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Hepatitis E</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Noroviruses</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Rotavirus</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Variola</em></td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td><em>VEE</em></td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td><em>VHF</em></td>
<td>Yes</td>
</tr>
</tbody>
</table>

* See [http://www.cdc.gov/od/sap/docs/salist.pdf](http://www.cdc.gov/od/sap/docs/salist.pdf)

8.1.2 Selection of Appropriate Analytical Approach for Contaminant Analysis

The analytical approach for these biological contaminants can be divided into two distinct steps, sample collection and sample analysis. All information prior to and during the threat evaluation (Module 2) needs to be carefully considered to assess the microbial contaminant threat and to
apply a relevant sample collection and sample analysis procedure. Different sample collection and analysis approaches are described below.

The overall approach for sample collection and analysis for microbial contaminants is based on the target contaminant. When the target contaminant is known, then established and validated technologies for sample collection and methods for analysis are utilized. When the target contaminant is unknown or when the contaminant is known but established and validated procedures for collection are not available, then an ultrafiltration device is used for sample collection and exploratory methods of analysis are utilized.

8.1.3 Microbial Sample Collection Considerations

Figure 4-8 illustrates the overall approach for sample collection for microbial contaminants. This approach utilizes established and validated sample collection techniques when the target contaminant has been tentatively identified based on available information from the site characterization report. Examples of situations in which tentative identification might occur include: a specific contaminant named in a threat; physical evidence at the site pointing to a specific contaminant; and clinical evidence of the identity of the disease-causing contaminant. In the case where the contaminant is unknown, sample collection is performed through the use of an ultrafiltration sampler (see Section 8.3).

---

Figure 4-8. Analytical Approach for the Collection of Microbial Contaminants
The ultrafiltration approach may also be desirable for a number of reasons. First, the sample may contain a mixture of microbial contaminants. Second, due to the low infectious dose of most waterborne pathogens, a sample of 100 liters of finished water may need to be concentrated to obtain suitable detection limits. Third, sample concentration technology for viruses and protozoa require separate field equipment and procedures and are practical only if the microbial contaminant is suspected or identified as one amenable for concentration by each of these specific technologies. Thus, in a contamination threat or incident, it may be necessary to utilize a more general sample concentration procedure based on ultrafiltration that targets a wider range of microorganisms.

### 8.1.4 Microbial Processing and Analysis Considerations

Making a determination as to whether the microbial contaminant has been tentatively identified is important since it will determine the type of laboratory that can do the processing and analysis. Non-select agents or BSL levels 1 and 2 contaminants that are regarded as waterborne pathogens, including the enteric bacteria, viruses and protozoa, can be sent to an environmental laboratory. If the contaminant is unknown or is a select agent, the sample is to be shipped to a specialty laboratory (Figure 4-9).

![Figure 4-9. Approach for the Processing and Analysis of Microbial Contaminant Groups](image)

*The specialty laboratory includes the Laboratory Response Network Laboratories among other specialty labs.

### 8.2 Established Techniques for Sample Collection of Known Waterborne Contaminants

If a microbial contaminant has been tentatively identified, the available and established techniques may be the more direct approach for sample collection and analysis. The advantage of these techniques is that a broader base of expertise and more analytical laboratories are available to water utilities than is available for select agents and for exploratory techniques.
Established and validated methods for sample collection and analysis of microbial contaminants in water are limited to a few microorganisms, mainly some enteric bacteria, viruses and protozoa, and a few other organisms with a known waterborne transmission route, listed in Table 4-14. If the contaminant is not known or the available site investigation and intelligence information is not considered sufficiently reliable for the use of an established method, the procedure for microbial unknowns (Section 8.4) is to be used.

Table 4-14. Known Waterborne Pathogens with Established Techniques for Sample Collection and Analysis

<table>
<thead>
<tr>
<th>Pathogen (general class)</th>
<th>Organism</th>
<th>Analytical Technique</th>
<th>Method¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protozoa</td>
<td>Cryptosporidium parvum Giardia intestinalis</td>
<td>IMS/IFA²</td>
<td>EPA 1622/23 EPA 1623</td>
</tr>
<tr>
<td>Viruses</td>
<td>Enteroviruses Hepatitis A</td>
<td>Mammalian cell culture</td>
<td>EPA/600/4-84/013 EPA ICR manual</td>
</tr>
</tbody>
</table>

1. SM refers to Standard Methods for the Examination of Water and Wastewater (Clesceri et. al., 1999). The EPA methods may be found at [http://www.epa.gov/nerlcwww/](http://www.epa.gov/nerlcwww/).
2. Immunomagnetic separation (IMS) and immunofluorescence assay (FA) microscopy.

8.2.1 Bacteria Collection with Established Techniques

The established sample collection procedures for bacteria are essentially grab samples that are later processed by membrane filtration of typically 100 milliliters to two liters. A four liter grab sample will allow for analysis for different bacteria by plating multiple membrane filters on separate selective or enrichment media. Special shipping regulations apply to samples known to contain pathogens (see Module 3, Section 6 for requirements for shipment of infectious materials, [http://www.bt.cdc.gov/labissues/PackagingInfo.pdf](http://www.bt.cdc.gov/labissues/PackagingInfo.pdf)). If select agents are not known or are not suspected to be in the water, then the sample is considered an environmental sample, and volumes of four liters or less can be collected and shipped using standard sampling containers.

8.2.2 Virus Collection with Established Techniques

The established sample collection procedures for viruses involve concentration on positively charged filters (ICR Microbial Laboratory Manual, EPA/600/R-95/178, April 1996, [http://www.epa.gov/nerlcwww/](http://www.epa.gov/nerlcwww/)). This method is reasonably effective for concentration of enteric viruses from large volumes of water. Samples in excess of 100 liters can be easily concentrated by this method. The ICR method has been used to concentrate 1,200 liters of finished water. The processed filters can be shipped to the laboratory, or viruses adsorbed to the filter can be eluted in the field and shipped as a one-liter concentrate to a laboratory for further processing by conventional reconcentration procedures.
8.2.3 Protozoa Collection with Established Techniques

The established procedure for protozoa sampling in water consists of obtaining a 10-liter grab sample or field concentration by filtration (Method 1623: Cryptosporidium and Giardia in Water by Filtration by IMS/FA, EPA-821-R-99-006, April 1999, http://www.epa.gov/nerlcwww/). The ten-liter grab sample or the filter is shipped to the laboratory for further processing.

8.3 Established Techniques for Analysis of Known Waterborne Contaminants

Established analytical techniques are based on culture assays in selective media for bacteria, selected cell lines for viruses and IFA with antibodies for protozoa identification (Table 4-14). Culture based techniques are not available for some contaminants and these techniques have long turn-around times for analysis. Thus, it may be necessary to utilize exploratory methods (Section 8.4.3) due to time restraints or the absence of an established culture method for a particular pathogen. Exploratory methods are primarily molecular-based, and involve PCR and probe hybridization as a presumptive test, followed by sequence analysis to confirm. If necessary, samples could be simultaneously analyzed by both rapid exploratory methods and established methods.

8.3.1 Bacteriological Analysis with Established Techniques

Sample collection for laboratory analysis of pathogenic bacteria in water samples consists of processing a grab sample by membrane filtration and transferring the membrane onto selective or enrichment media for presumptive identification (Figure 4-10). The enteric bacteria and other non-select pathogenic bacteria can be confirmed by conventional methods based on biochemical profiles, serological identification, or other culture based methods. The vast majority of enteric bacteria can be analyzed with BSL-2 containment level; thus an environmental laboratory with that containment level could proceed with confirmatory identification of non-select organisms.

If the presumptive tests indicate the possible presence of a select agent, the sample and the isolated culture must be referred to a specialty laboratory (Figure 4-10). Federal regulations prohibit the handling and maintenance of select agents unless the laboratory has registered with the CDC under the requirements of the Select Agent Rule (see Section 2.2).
Figure 4-10. Bacteriological Screening with Established Techniques

8.3.2 Virological Analysis with Established Techniques

Established techniques for virological analysis consist of plating the sample concentrate on cell lines receptive to the target viruses. The target viruses anticipated in a water sample are the enteric viruses. There are cell lines receptive to a number of them (see Table 4-13). It may be necessary to inoculate a sample on multiple cell lines to cover the range of possible virus targets. A presumptive positive result of a cell culture test is the production of cytopathic effects on the cell monolayer as observed by light microscopy. Established virus confirmation and identification techniques consist of serological neutralization with specific antibodies against the various viruses or by means of pools of antisera with respective identification tables. If a presumptive identification suggests the presence of a select virus agent, the sample, culture and other processed sample materials must be referred to a specialty laboratory (Figure 4-11).

If an established technique is not available for a specific virus or if the virus is known not to grow in cell culture, exploratory PCR-based techniques may be the more appropriate approach for presumptive identification. Presumptive identification is based on PCR amplification and probing. Analytical confirmation is conducted by sequence analysis of the amplified material.
8.3.3 Parasitological Analysis with Established Techniques

Established sample collection techniques for protozoa in water consist of obtaining a grab sample of 10 liters or alternatively, performing filtration in the field (EPA Method 1623, http://www.epa.gov/nerlcwww/). The grab sample or the filter is sent to an environmental laboratory for processing. In the laboratory, protozoa retained by the filter are eluted, concentrated by centrifugation and purified by immunomagnetic separation (IMS). *Giardia* cysts and *Cryptosporidium* oocysts are identified and counted by the indirect fluorescent assay with an epifluorescence (or UV) microscope (Figure 4-12).

Exploratory techniques such as PCR can be readily applied to cysts and oocysts purified by the immunomagnetic separation step of Method 1623. In addition, the infectivity of *Cryptosporidium* oocysts can also be determined by cell culture infectivity after IMS purification.

---

**Figure 4-11. Virological Analysis with Established Techniques**

**Collection**
- On-site Filtration
  - 100-1000 Liters

**Process**
- Elute and Reconcenrate

**Assay**
- Specific Cell Lines

- Tentative Identification as Select Agent?
  - Yes
    - Refer Presumptive Select Agents to Specialty Lab
  - No
    - Perform Established Confirmatory Analysis on Non-select Agents
8.4 Method for Sample Collection, Field Processing, and Laboratory Analysis for Unknown Microbial Contaminants

Sample collection, processing, and analysis of an unknown microbial contaminant are inherently linked because the procedure relies on the use of an ultrafiltration device in the field (see Module 3). Together, the collection, processing, and analysis may be considered to constitute a method for unknown microbial contaminants, and the individual parts are discussed below. This method, including a suitable ultrafiltration device, is currently under development.

8.4.1 Sample Collection and Concentration for Unknown Microbials

Ultrafiltration is a size exclusion process, and particles larger than the molecular weight cut-off (MWCO) of the membrane, are concentrated in the *retentate* and separated from the *filtrate*. Ultrafiltration can concentrate viruses, bacteria, spores or parasites as long as the organisms are larger than the MWCO of the membrane selected. Thus, the method is suitable for sampling water with an unknown biological contaminant. Also, ultrafiltration of water samples can be used to concentrate some biotoxins if the MWCO of the selected membrane is sufficiently small.

The overall procedure, illustrated in Figure 4-13, consists of concentrating large (100 liters or more) volumes of water by cross-flow disposable hollow fiber ultrafiltration cartridges, and subsequently recovering pathogens and other concentrated microorganism and particles in the filter retentate. The recovered retentate is referred to as the *field sample concentrate*. The field sample concentrate will be separated into three aliquots. Ultrafiltration of 100 liters or more of finished (e.g., tap) water will result in a retentate volume of approximately 250 ml. A small aliquot (1/20th of the field sample concentrate) can be used for field screening (Section 8.4.2). Approximately one half of the field sample concentrate can be used for presumptive PCR testing (Section 8.4.3). The remainder can be used for confirmatory culture techniques (Section 8.4.4). The reason for dividing the field sample concentrate is that some of the reagents for molecular analysis that may be added in the field will essentially inactivate any microbes present in the sample.
The basic elements of an ultrafiltration field concentration apparatus (Figure 4-14) consist of an input reservoir for sample collection, a peristaltic pump, a cross-flow hollow fiber membrane cartridge, a retentate reservoir and a filtrate reservoir. Operating the unit in cross-flow mode is preferable because “caking” or fouling of membranes is reduced. Non-reactive membranes are preferable, such as polysulfone or low protein binding membranes.

Sample filtration is conducted by recirculating water (necessary to maintain cross-flow conditions) with a 5-10 PSI differential between the input and retentate pressure. The pressure differential forces water through the hollow fiber membrane which retains particles (including microbes) larger than the membrane pores in an increasingly concentrated liquid retentate volume. The water containing particles that passes through the filter is known as filtrate. The volume of the retentate is reduced to approximately 250 ml by recirculating the concentrated sample through the smaller retentate loop for capture in the retentate reservoir. The retentate reservoir vessel can be used for sample shipping if necessary. The sample retentate can also be recovered by backflushing (reversing the flow through of the hollow fiber membrane cartridge).

Figure 4-13. Sample Collection and Processing for Unknown Microbial Contaminants
8.4.2 Field Screening and Processing of Sample

Field processing of the small aliquot of the field sample concentrate from the ultrafiltration concentrator consists of field screening for microbial contaminants (Figure 4-15), which also is a component of site characterization (see Module 3). This field screening, in turn, may result in rapid actions to prevent further adverse consequences to safety and health of the samplers, laboratory personnel, and the general public. It is important to remember that both field screening by equipment and field test assays have only limited use, and any results need to be confirmed by additional laboratory testing.

Figure 4-14. Field Concentrator Schematic

Figure 4-15. Field Processing for Microbials
Field screening equipment encompasses a wide range of technologies including lateral flow chromatography, antibody identification, hand-held or field deployable PCR based assays, and others (Table 4-15). The specific technology chosen will affect the interpretation of field results. The Office of Justice Programs of the Department of Justice (DOJ) has provided a list of available technologies for field screening (http://www.ojp.usdoj.gov/nij/pubs-sum/190747.htm). (The DOJ document contains neither an endorsement of any specific technology nor an evaluation of their performance.)

Many of the available field screening assay tests (Table 4-15) are less sensitive than laboratory-based assays by perhaps several orders of magnitude, and thus there is a potential for false negative results. Due to possible cross-reactivity, these technologies may also lead to false positive reactions. The results of field screening should be used as part of a threat characterization process and not as part of a conclusive analytical procedure.

Table 4-15. Exploratory Field Screening Techniques for Waterborne Contaminants and Select Agents

<table>
<thead>
<tr>
<th>Pathogen (general class)</th>
<th>Organism</th>
<th>Screening Technique*</th>
<th>Commercial Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Waterborne Contaminants</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Bacteria | Campylobacter spp.  
E. coli 0157:H7  
Salmonella spp. | PCR  
PCR  
PCR | Yes |
| Protozoa | TBD | TBD |
| Viruses | TBD | TBD |
| **Select Agents** | | | |
| Spores | Bacillus anthracis  
Clostridium botulinum A | PCR and Smart tickets  
PCR | Yes |
| Bacteria | Brucella spp.  
Francisella tularensis  
Yersinia pestis | PCR | Yes |
| Protozoa | TBD | TBD |
| Viruses | TBD | TBD |

* TBD = to be determined

8.4.3 Presumptive Testing for Microbials with Exploratory PCR Techniques

For presumptive testing of the field sample concentrate from the ultrafiltration concentrator, exploratory techniques such as PCR testing may be utilized as a means to obtain faster presumptive results for critical samples. These presumptive results may be used during the threat evaluation (Module 2) and site characterization (Module 3) procedures. Although PCR techniques are widely used in the clinical and medical fields, these techniques are still regarded as exploratory in the field of environmental microbiology. PCR has three main benefits in a presumptive test: 1) results can be obtained faster; 2) multiple targets can be assayed in a short period of time; and 3) cultural methods are not available yet for some microbial contaminants of interest. Another advantage of PCR testing is that samples could be analyzed concurrently with the conventional established methods. Table 4-16 lists PCR techniques for the biological contaminants of concern.
**Table 4-16. Exploratory Analytical Techniques for Presumptive Testing of Waterborne Contaminants and Select Agents**

<table>
<thead>
<tr>
<th>Pathogen general class</th>
<th>Organism</th>
<th>Presumptive Testing Technique</th>
<th>Confirmation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td><em>Campylobacter</em> spp.</td>
<td>PCR</td>
<td>Probe or sequencing</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em> 0157:H7</td>
<td>PCR</td>
<td>Probe or sequencing</td>
</tr>
<tr>
<td></td>
<td><em>Salmonella</em> spp.</td>
<td>PCR</td>
<td>Probe or sequencing</td>
</tr>
<tr>
<td></td>
<td><em>Shigella</em> spp.</td>
<td>PCR</td>
<td>Probe or sequencing</td>
</tr>
<tr>
<td></td>
<td><em>Vibrio cholerae</em></td>
<td>PCR</td>
<td>Probe or sequencing</td>
</tr>
<tr>
<td><strong>Protozoa</strong></td>
<td><em>Cryptosporidium parvum</em></td>
<td>PCR</td>
<td>Probe or sequencing</td>
</tr>
<tr>
<td></td>
<td><em>Giardia intestinalis</em></td>
<td>PCR</td>
<td>Probe or sequencing</td>
</tr>
<tr>
<td></td>
<td><em>Entamoeba histolytica</em></td>
<td>PCR</td>
<td>Probe or sequencing</td>
</tr>
<tr>
<td></td>
<td><em>Toxoplasma gondii</em></td>
<td>PCR</td>
<td>Probe or sequencing</td>
</tr>
<tr>
<td><strong>Viruses</strong></td>
<td><em>Enteroviruses</em></td>
<td>RT-PCR</td>
<td>Probe or sequencing</td>
</tr>
<tr>
<td></td>
<td><em>Hepatitis A</em></td>
<td>RT-PCR</td>
<td>Probe or sequencing</td>
</tr>
<tr>
<td></td>
<td><em>Hepatitis E</em></td>
<td>RT-PCR</td>
<td>Probe or sequencing</td>
</tr>
<tr>
<td></td>
<td><em>Noroviruses</em></td>
<td>RT-PCR</td>
<td>Probe or sequencing</td>
</tr>
<tr>
<td></td>
<td><em>Rotavirus</em></td>
<td>RT-PCR</td>
<td>Probe or sequencing</td>
</tr>
<tr>
<td><strong>Spores</strong></td>
<td><em>Bacillus anthracis</em></td>
<td>PCR</td>
<td>Probe or sequencing</td>
</tr>
<tr>
<td></td>
<td><em>Clostridium botulinum</em> A</td>
<td>PCR</td>
<td>Probe or sequencing</td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td><em>Brucella</em> spp.</td>
<td>PCR</td>
<td>Probe or sequencing</td>
</tr>
<tr>
<td></td>
<td><em>Burkholderia pseudomallerei</em></td>
<td>PCR</td>
<td>Probe or sequencing</td>
</tr>
<tr>
<td></td>
<td><em>Francisella tularensis</em></td>
<td>PCR</td>
<td>Probe or sequencing</td>
</tr>
<tr>
<td></td>
<td><em>Yersinia pestis</em></td>
<td>PCR</td>
<td>Probe or sequencing</td>
</tr>
<tr>
<td><strong>Rickettsia</strong></td>
<td><em>Coxiella burnetti</em></td>
<td>PCR</td>
<td>Probe or sequencing</td>
</tr>
<tr>
<td><strong>Viruses</strong></td>
<td><em>Variola</em></td>
<td>PCR</td>
<td>Probe or sequencing</td>
</tr>
<tr>
<td></td>
<td><em>VEE</em></td>
<td>RT-PCR</td>
<td>Probe or sequencing</td>
</tr>
<tr>
<td></td>
<td><em>VHF</em></td>
<td>RT-PCR</td>
<td>Probe or sequencing</td>
</tr>
</tbody>
</table>

*These viruses are not considered to pose a major threat in water

Protozoa and bacteria in the field sample concentrate are further concentrated by centrifugation of the sample in the laboratory (Figure 4-16). Possible spores, bacteria or protozoa in the sample are recovered in the pellet whereas viruses will be retained in the supernatant. DNA from spores, vegetative bacteria or protozoa is extracted from the pellet. DNA extraction kits designed to remove co-contaminants from environmental samples are more desirable since ultrafiltration will tend to concentrate soil-derived PCR inhibitors (e.g., humic and fulvic acids). The supernatant is further concentrated by a second phase of ultrafiltration. RNA-containing viruses are the anticipated target in a water sample; thus, a procedure for RNA extraction and purification is to be followed. The purified RNA is used for reverse transcriptase-PCR (RT-PCR) to desired viral RNA targets.

Primers and probes for PCR presumptive testing for waterborne and non-select agents may be obtained from the scientific literature. Some commercial vendors provide primers and probes for select agents, although these are not necessarily utilized by the LRN for its analysis. The LRN system utilizes a restricted set of primers and probes for detection of select agents. (If a DNA virus, such as variola, is strongly suspected, a different technique will be necessary for extraction and purification of its DNA, although variola is not considered a priority threat in water).
8.4.4 Confirmatory Testing for Microbials by Culture and Conventional Assays

Processing of the field sample concentrate for culture assays (Figure 4-17) consists of an initial centrifugation step to further concentrate bacteria (vegetative cells and spores) and protozoa in the sample. Bacteria and protozoa will be concentrated in the pellet, and viruses will remain in the supernatant. Protozoa are separated from the bacteria by IMS. Bacteria will remain in the IMS supernatant (liquid phase) and the protozoa will be concentrated with the beads (solid phase). Viruses are further concentrated for cell culture analysis. Table 4-17 lists established confirmatory analytical techniques for waterborne and select agents.
Figure 4-17. Sample Processing for Confirmatory Microbiological Assays

Table 4-17. Established Confirmatory Analytical Techniques for Waterborne Contaminants and Select Agents

<table>
<thead>
<tr>
<th>Pathogen (general class)</th>
<th>Organism</th>
<th>Confirmatory Techniques</th>
<th>Additional Exploratory Techniques</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Waterborne Contaminants</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td>Campylobacter spp. E. coli 0157:H7 Salmonella spp. Shigella spp. Vibrio cholerae</td>
<td>Biochemical and Serological Tests</td>
<td>PCR and Sequencing</td>
</tr>
<tr>
<td>Protozoa</td>
<td>Cryptosporidium parvum Giardia intestinalis Entamoeba histolytica Toxoplasma gondii</td>
<td>IFA and Microscopy</td>
<td>PCR and Sequencing</td>
</tr>
<tr>
<td>Viruses</td>
<td>Enteroviruses Hepatitis A Hepatitis E Noroviruses Rotavirus</td>
<td>Plaque neutralization N/A N/A N/A IFA</td>
<td>RT-PCR and Sequencing IFA, RIFA, PCR RT-PCR and Sequencing RT-PCR and Sequencing RT-PCR and Sequencing</td>
</tr>
<tr>
<td><strong>Select Agents</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spores</td>
<td>Bacillus anthracis Clostridium botulinum A</td>
<td>Biochemical and Serological Tests</td>
<td>PCR and Sequencing</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Brucella spp. Burkholderia pseudomallei Francisella tularensis Yersinia pestis</td>
<td>Biochemical and Serological Tests</td>
<td>PCR and Sequencing</td>
</tr>
<tr>
<td>Rickettsia</td>
<td>Coxiella burnetti</td>
<td>Biochemical and Serological Tests</td>
<td>PCR and Sequencing</td>
</tr>
<tr>
<td>Viruses</td>
<td>Variola VEE, VHF</td>
<td>TBD</td>
<td>PCR and Sequencing PCR and Sequencing</td>
</tr>
</tbody>
</table>
The procedures outlined in this section are intended for use in water samples where select agents are suspected to be present, thus these procedures should be performed in microbiology laboratories that use appropriate Biological Safety Level practices. For all suspected pathogens, refer to the CDC publication “Biosafety in Microbiological and Biomedical Laboratories,” 4th edition (HHS Publication No. 93-8395, http://www.cdc.gov/od/ohs/pdffiles/4th%20BMBL.pdf). Some BSL requirements are summarized below, broken out by pathogen class.

**Bacterial assays**
Pathogenic enteric bacteria can be handled in an environmental BSL-2 laboratory. Nonetheless, select agents should only be analyzed for presumptive identification by laboratories approved for presumptive identification of select agents. *Brucella* spp. (BSL-3), *Burkholderia mallei* (BSL-2), *B. pseudomallei* (BSL-2), *Yersinia pestis* (BSL-2), and *Francisella tularensis* (BSL-3) are highly infectious and have caused laboratory-acquired infections. Because of the highly infectious nature of these pathogens, consultation with a state public health laboratory is recommended if any of these are suspected in a sample.

**Protozoa**
The solid phase of the IMS procedure is further processed by EPA Method 1623 for IFA identification of *Cryptosporidium* and *Giardia*. The protozoa *Cryptosporidium parvum*, *Giardia intestinalis*, *Entamoeba histolytica*, and *Toxoplasma gondii* can be handled in an environmental BSL-2 laboratory.

**Viruses**
Viruses in the supernatant from the centrifugation step above are further concentrated by a second ultrafiltration to reduce the assay volume for cell culture analysis. Pathogenic enteric viruses can be handled in an environmental BSL-2 laboratory. Nonetheless, select agents should only be analyzed for presumptive identification by laboratories approved for presumptive identification of select agents. *Variola* (BSL-3), *VEE* (BSL-3) and *VHF* (BSL-4) are highly infectious, and suspected samples should be referred to an LRN laboratory. Because multiple virus targets are pursued, the sample is likely to be inoculated onto more than one cell line.

Public health laboratories primarily use tube cultures of mammalian cells which are amenable only for small inoculation volumes. However, tube culture is a well established method and fairly efficient for virus detection. Infectious viruses are amplified in the receptive cells and are used as the source of infectious material for subsequent identification and confirmation assays. Identification and analytical confirmation consists of neutralization assays with specific antisera. PCR and sequencing of positive samples is also an exploratory technique that could be used to further identify and characterize the isolated virus. DNA sequencing is used for fingerprinting studies and for definitive identification of pathogens.
9 References and Resources

References and information cited or used to develop this module are listed below. The URLs of several sources are cited throughout the text. These URLs were correct at the time of the preparation of this document. If the document is no longer available at the URL provided, please search the sponsoring organization’s Web site or the World Wide Web for alternate sources. A copy of referenced documents may also be provided on the CD version of this module, although readers should consult the referenced URL for the latest version.


OSHA. 2002. “Safety and Health Topics Personal Protective Equipment”


OSHA. 2003b. “Occupational Safety and Health Administration Homepage”


US Coast Guard. 2001. “Chemical Hazards Response Information System”


USGS. 2003. “Methods and Data Comparability Board Home Page”
## 10 Appendices

### 10.1 Chemicals that appear in CWC Schedules

Table 4-18. List of chemicals that appear in CWC Schedules 1, 2, and 3

<table>
<thead>
<tr>
<th>Schedule 1 Chemical Agents</th>
<th>Schedule 2 Chemical Agents</th>
<th>Schedule 3 Chemical Agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-Alkyl (≤C10, incl. cycloalkyl) alkyl (Me, Et, n-Pr or i-Pr)-phosphonofluoridates (e.g. Sarin: O-Isopropyl methylphosphonofluoridate Soman: O-Pinacolyl methylphosphonofluoridate)</td>
<td>Amiton: O,O-Diethyl S-[2-(diethylamino)ethyl] phosphorothiolate and corresponding alkylated or protonated salts</td>
<td>Phosgene: carbonyl dichloride</td>
</tr>
<tr>
<td>O-Alkyl (≤C10, incl. cycloalkyl)N,N-dialkyl (Me, Et, n-Pr or I-Pr)-phosphoramidocyanidates (e.g. Tabun: O-Ethyl N,N-dimethyl phosphoramidocyanidate)</td>
<td>PFIB: 1,1,3,3,3-Pentafluoro-2-(trifluoromethyl)-1-propene</td>
<td>Cyanogen chloride</td>
</tr>
<tr>
<td>O-alkyl (H or ≤C10, incl. cycloalkyl)S-2-dialkyl (Me, Et, n-Pr or I-Pr)-aminoethyl alkyl (Me, Et, n-Pr or I-Pr) phosphonothiolates and corresponding alkylated or protonated salts (e.g. VX: O-Ethyl S-2-disopropylaminoethyl methyl phosphonothiolate)</td>
<td>BZ: 3-Quinuclidinyl benzilate</td>
<td>Hydrogen cyanide</td>
</tr>
<tr>
<td>Sulfur mustards: 2-Chloroethylchloromethylsulfide Mustard gas: Bis(2-chloroethyl)sulfide Bis(2-chloroethylthio)methane Sesquimustard: 1,2-Bis(2-chloroethylthio)ethane 1,3-Bis(2-chloroethylthio)-n-propane 1,4-Bis(2-chloroethylthio)-n-butane 1,5-Bis(2-chloroethylthio)-n-pentane Bis(2-chloroethylthiomethyl)ether O-Mustard: Bis(2-chloroethylthioethyl)ether</td>
<td>Chemicals, except for those listed in Schedule 1, containing a phosphorus atom to which is bonded one methyl, ethyl or propyl (normal or iso) group but not further carbon atoms, e.g., Methylphosphonyl dichloride Dimethyl methylyphosphonate Exemption: Fonofos: O-Ethyl S-phenyl ethylphosphonothiolothionate</td>
<td>Chloropicrin: trichloronitromethane</td>
</tr>
<tr>
<td>Lewisites: Lewisite 1:2-Chlorovinyldichloroarsine Lewisite 2:Bis(2-chlorovinyl)chloroarsine Lewisite 3:Tris (2-chlorovinyl)arsine</td>
<td>N,N-Dialkyl (Me, Et, n-Pr or i-Pr) phosphoramidic dihalides</td>
<td>Phosphorus oxychloride</td>
</tr>
<tr>
<td>Nitrogen mustards:</td>
<td>Dialkyl (Me, Et, n-Pr or i-Pr) N,N-</td>
<td>Phosphorus trichloride</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Schedule 1 Chemical Agents</th>
<th>Schedule 2 Chemical Agents</th>
<th>Schedule 3 Chemical Agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>HN1: Bis(2-chloroethyl)ethyamine</td>
<td>dialkyl (Me, Et, n-Pr or i-Pr)-phosphoramidates</td>
<td></td>
</tr>
<tr>
<td>HN2: Bis(2-chloroethyl)methylamine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HN3: Tris(2-chloroethyl)amine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saxitoxin</td>
<td>Arsenic trichloride</td>
<td>Phosphorus pentachloride</td>
</tr>
<tr>
<td>Ricin</td>
<td>2,2-Diphenyl-2-hydroxyacetic acid</td>
<td>Trimethyl phosphate</td>
</tr>
<tr>
<td>Alkyl (Me, Et, n-Pr or I-Pr)phosphonyldifluorides e.g., DF:Methylphosphonyldifluoride</td>
<td>Quinuclidine-3-ol</td>
<td>Triethyl phosphate</td>
</tr>
<tr>
<td>O-Alkyl(incl. Cycloalkyl)O-2-dialkyl (Me, Et, n-Pr or I-Pr)-aminoethyl alkyl (Me, Et, n-Pr or I-Pr) phosphonites and corresponding alkylated or protonated salts e.g., QL: O-Ethyl O-2-disopropylaminoethyl methylphosphonite</td>
<td>N,N-Dialkyl (Me, Et, n-Pr or I-Pr) aminoethyl-2-chlorides and corresponding protonated salts</td>
<td>Dimethyl phosphate</td>
</tr>
<tr>
<td>Chlorosarin: O-Isopropyl methylphosphonochloridate</td>
<td>-Dialkyl (Me, Et, n-Pr or I-Pr) oethane-2-ols and corresponding protonated salts</td>
<td>Diethyl phosphate</td>
</tr>
<tr>
<td>Chlorosoman: O-Pinacolyl methylphosphonochloridate</td>
<td>N,N-Dialkyl (Me, Et, n-Pr or I-Pr) aminoethane-2-thiols and corresponding protonated salts</td>
<td>Sulfur monochloride</td>
</tr>
<tr>
<td></td>
<td>Thiodiglycol: Bis(2-hydroxyethyl) sulfide</td>
<td>Sulfur dichloride</td>
</tr>
<tr>
<td></td>
<td>Pinacolyl alcohol: 3,3-Dimethylbutane-2-ol</td>
<td>Thionyl chloride</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethylidithanolamine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methylidithanolamine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Triethanolamine</td>
</tr>
</tbody>
</table>
### 10.2 Methods Used for the Basic Screen

Table 4-19. Titles of methods used in Table 4-3 for the basic screen. (See Table 4-2 for information on how to obtain these methods.)

<table>
<thead>
<tr>
<th>EPA Method number</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Drinking water methods</strong></td>
<td></td>
</tr>
<tr>
<td>200.8, May 1994, Revision 5.4*</td>
<td>Determination Of Trace Elements In Waters And Wastes By Inductively Coupled Plasma Mass Spectrometry</td>
</tr>
<tr>
<td>200.9, May 1994, Revision 2.2*</td>
<td>Trace Elements in Water, Solids, and Biosolids by Stabilized Temperature Graphite Furnace Atomic Absorption Spectrometry</td>
</tr>
<tr>
<td>245.1, May 1994, Revision 3.0*</td>
<td>Mercury (Manual Cold Vapor Technique)</td>
</tr>
<tr>
<td>245.2, March 1983*</td>
<td>Mercury (Automated Cold Vapor Technique)</td>
</tr>
<tr>
<td>335.2, March 1983</td>
<td>Cyanide, Total (Titrimetric; Spectrophotometric)</td>
</tr>
<tr>
<td>335.3, March 1983</td>
<td>Cyanide, Total (Colorimetric, Automated UV)</td>
</tr>
<tr>
<td>335.4, March 1983</td>
<td>Cyanide, Total (Colorimetric, Semi-automated UV)</td>
</tr>
<tr>
<td>525.2, 1995, Revision 2.0*</td>
<td>Determination of Organic Compounds in Drinking Water By Liquid-solid Extraction and Capillary Column Gas Chromatography/Mass Spectrometry</td>
</tr>
<tr>
<td>531.1, 1995, Revision 3.1* 531.2, September 2001, Revision 1.0</td>
<td>Measurement of N-methylcarbamoyloximes and N-methyl-carbamates in Water by Direct Aqueous Injection HPLC with Post-Column Derivatization</td>
</tr>
<tr>
<td>549.2, 1997, Revision 1.0*</td>
<td>Determination of Diquat and Paraquat in Drinking Water by Liquid-Solid Extraction and High Performance Liquid Chromatography with Ultraviolet Detection</td>
</tr>
<tr>
<td>900, August 1980*</td>
<td>Proscribed Procedures for the Determination of Radioactivity in Water</td>
</tr>
<tr>
<td><strong>SW-846 methods</strong></td>
<td></td>
</tr>
<tr>
<td>3535A, January 1998, Revision 1</td>
<td>Solid-Phase Extraction (SPE) [preparation technique for 8270D]</td>
</tr>
<tr>
<td>5030B, December 1996, Revision 2</td>
<td>Purge-and-Trap for Aqueous Samples</td>
</tr>
<tr>
<td>8021B, December 1996, Revision 2</td>
<td>Aromatic and Halogenated Volatiles by Gas Chromatography Using Photoionization and/or Electrolytic Conductivity Detectors</td>
</tr>
<tr>
<td>8260B, December 1996, Revision 2</td>
<td>Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)</td>
</tr>
<tr>
<td>8270D, January 1998, Revision 4</td>
<td>Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)</td>
</tr>
</tbody>
</table>

* These methods are approved at 40 CFR Part 141 for regulatory compliance monitoring of drinking water.
### 10.3 Analyte Lists for Basic Screen

**Table 4-20. Analyte Lists Corresponding to Table 4-3.**

*Note:* Laboratories may recognize these as commercially available standard mixes or combinations thereof. This does not imply endorsement of any company, product, or service. The standard mixes do not necessarily include all analytes that the method is capable of determining. For example, in list B, only 90 analytes are included, although commercially available mixes could be selected to encompass more than 150 compounds. Laboratories should be encouraged to include as many analytes as possible in the standards mix to increase the breadth of the screen. Because the analytic lists are intended for the basic screen, which is designed to produce defensible results, laboratories may wish to modify the analytic lists to reflect their accreditations and certifications. However, additional analytes may become part of the expanded screen, so laboratories may wish to include those anyway. Analytes added to those listed must meet the same QC acceptance criteria and be part of the overall quality system in the laboratory, so that the added analytes will be as defensible as those listed.

The exclusion or inclusion of an analyte in the lists in this table does not reflect its potential hazard as a water contaminant. Rather, these analytes were chosen for the convenience of commercial availability of standard mixes. In practice, site-specific plans and situations may be used (and are encouraged) to modify these lists.

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,1,1,2-Tetrachloroethane</td>
<td>2,2',3,3’,4,4’,6'-</td>
<td>Aldicarb</td>
<td>diquat</td>
<td>arsenic</td>
<td>mercury</td>
<td>free cyanide</td>
<td>cesium-137</td>
</tr>
<tr>
<td>1,1,1-Trichloroethane</td>
<td>Octachlorobiphenyl</td>
<td>aldicarb sulfone</td>
<td>paraquat</td>
<td>cadmium</td>
<td></td>
<td></td>
<td>iridium-192</td>
</tr>
<tr>
<td>1,1,2,2-Tetrachloroethane</td>
<td>2,2’,3’,4,6-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cobalt-60</td>
</tr>
<tr>
<td>1,1,2-Trichloroethane</td>
<td>Hexachlorobiphenyl</td>
<td>carbofuran</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>strontium-90</td>
</tr>
<tr>
<td>1,1-Dichloroethane</td>
<td>Tetrachlorobiphenyl</td>
<td>Hydroxycarbofuran</td>
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<td></td>
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</tr>
<tr>
<td>1,1-Dichloroethene</td>
<td>2,3-Dichlorobiphenyl</td>
<td>methiocarb</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>1,1-Dichloropropene</td>
<td>2,4,5-Trichlorobiphenyl</td>
<td>methomyl</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1,2,3-Trichlorobenzene</td>
<td>2,4-Dinitrotoluene</td>
<td>Oxamyl</td>
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<td></td>
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</tr>
<tr>
<td>1,2,3-Trichloropropane</td>
<td>2,6-Dinitrotoluene</td>
<td>Propoxur</td>
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<tr>
<td>1,2,4-Trichlorobenzene</td>
<td>2-Chlorobiphenyl</td>
<td>Sevin (carbaryl)</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>1,2,4-Trimethylbenzene</td>
<td>a-BHC</td>
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<td></td>
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<tr>
<td>1,2-Dibromo-3-Chloropropane</td>
<td>Acenaphthylene</td>
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</tr>
<tr>
<td>1,2-Dibromoethane</td>
<td>a-Chlordane</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1,2-Dichlorobenzene</td>
<td>Alachlor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,2-Dichloroethane</td>
<td>Aldrin</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1,2-Dichloropropane</td>
<td>alpha-Chlordane</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,3,5-Trimethylbenzene</td>
<td>Anthracene</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
## Analyte Lists Corresponding to Table 4-3

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
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</thead>
<tbody>
<tr>
<td>1,3-Dichlorobenzene</td>
<td>Atrazine</td>
<td></td>
<td></td>
<td></td>
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</tr>
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<td>1,3-Dichloropropane</td>
<td>Azinphos methyl</td>
<td></td>
<td></td>
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<td>1,4-Dichlorobenzene</td>
<td>b-BHC</td>
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</tr>
<tr>
<td>2,2-Dichloropropane</td>
<td>Benz(a)anthracene</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2-Chlorotoluene</td>
<td>Benzo(a)pyrene</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>2-Nitropropane</td>
<td>Benzo(a)pyrene</td>
<td></td>
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</tr>
<tr>
<td>4-Chlorotoluene</td>
<td>Benzo(b)fluoranthene</td>
<td></td>
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<tr>
<td>acrylonitrile</td>
<td>Benzo(g,h,i)perylene</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>allyl chloride</td>
<td>Benzo(k)fluoranthene</td>
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<td></td>
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<tr>
<td>Benzene</td>
<td>Ethylhexyl adipate</td>
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<td>Ethylhexyl phthalate</td>
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<td>Bromochloromethane</td>
<td>Bolstar</td>
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<td>Bromodichloromethane</td>
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<td>Bromoform</td>
<td>Butylbenzyl phthalate</td>
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<td>Bromomethane</td>
<td>Chlorobenzilate</td>
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<td>Butyl chloride</td>
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## Analyte Lists Corresponding to Table 4-3

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