EPA
United States
Environmental Protection
Agency

Wastewater Response Protocol Toolbox:

Planning For and Responding To Wastewater Contamination Threats and Incidents

December 2011

Module 4:
Analytical Guide

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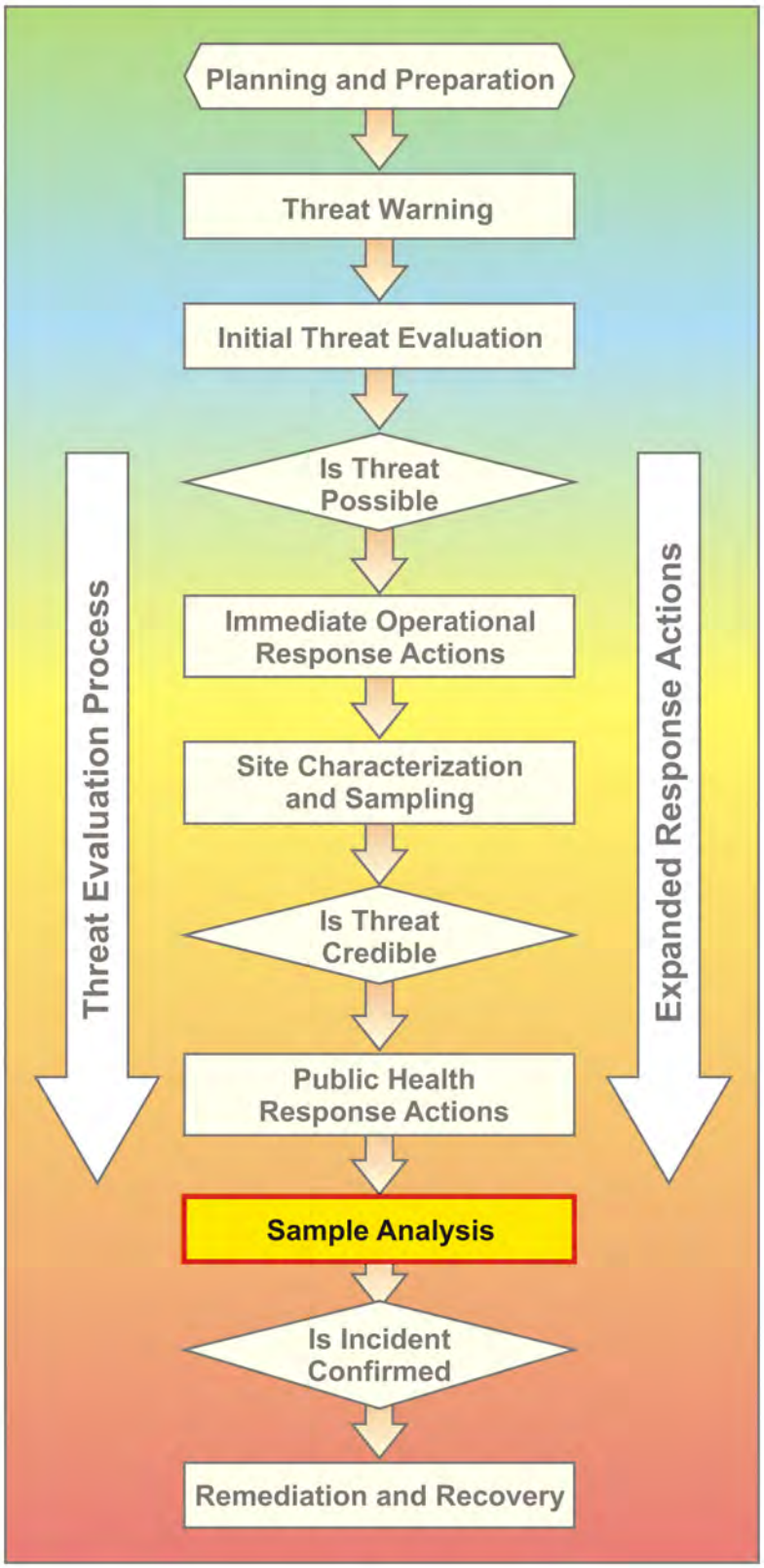
Table of Contents - Module 4


Planning and Preparation
Threat Warning
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

1 Introduction

1.1 Objectives of this Module

The primary intended users of this module include laboratory personnel and planners who would provide analytical support to a wastewater utility in the event of a contamination threat. This module is intended to be a planning tool for labs rather than a how-to manual for use during an actual incident. As part of planning for such an incident, laboratories may want to prepare a detailed 'Laboratory Guide' specific to their needs and capabilities. Also, laboratories may want to consider how they coordinate with networks of other laboratories so as to provide added capability and capacity.

The objectives of this module include:

1. Describing how laboratories can respond to contamination events.
2. Describing special laboratory considerations for handling and processing emergency wastewater samples suspected of contamination with a harmful substance.
3. Presenting model approaches and procedures for analysis of wastewater samples suspected of contamination with a known or unknown substance. These analytical approaches are intended to take advantage of existing methodologies and infrastructures.
4. Encouraging planners to develop a site-specific analytical approach and Laboratory Guide that conforms to the general principles of the model approaches presented in this module.

Roles of Laboratories in Response to Contamination Threats

While utility labs, especially at larger utilities, may become quite involved with preliminary screening and preliminary analysis of samples from suspected contamination events, most will not be able to implement all of the analytical protocols described in Module 4. Federal, state, and commercial labs may be called upon to provide more sophisticated, in-depth analyses.

2 Current Laboratory Infrastructure in U.S.

The analytical approach described in this module was developed under the assumption that it would be implemented using the existing laboratory infrastructure in this country. EPA established the Environmental Response Laboratory Network (ERLN) to assist in addressing chemical, biological, and radiological threats during nationally significant incidents. The Water Laboratory Alliance (WLA), which launched in October 2009, is the water component of the ERLN and provides the Water Sector (drinking water and wastewater systems) with an integrated nationwide network of laboratories. The WLA provides additional analytical capability and capacity to an event involving intentional and unintentional water

contamination involving chemical, biological and radiochemical contaminants. For more information, visit http://www.epa.gov/erln/water.html.

Also, the WLA has a *Water Laboratory Alliance – Response Plan* (WLA-RP) (EPA 817-R-10-002, November 2010) that outlines the processes and procedures for a coordinated laboratory response to water contamination incidents that may require more analytical laboratory capability and capacity than a typical laboratory can provide. It addresses analytical demand during the emergency response, remediation, and recovery phases of a natural disaster, accident, or terrorist incident affecting the water sector. (http://water.epa.gov/infrastructure/watersecurity/wla/upload/WLAResponsPlan_November2010.pdf)

EPA has constructed a Laboratory Compendium to assist utilities and other responders in locating appropriate labs for analysis of contaminants during a contamination incident. The Laboratory Compendium is a database of laboratory capabilities for environmental analysis in water, air, soil, sediment, and other media. Instructions on acquiring access to the Laboratory Compendium are available at the following website: http://www.epa.gov/compendium.

The ERLN is also part of a larger federal network of laboratories called the lntegrated Consortium of Laboratory Networks (ICLN). The Department of Homeland Security established the ICLN to coordinate laboratory networks to respond to acts of terrorism and other major incidents. ICLN is composed of networks of Federal laboratories from U.S. Department of Agriculture, Department of Health and Human Services (Centers for Disease Control and Prevention, Food and Drug Administration), Department of Defense, and the Environmental Protection Agency.

Analytical Goals

In responding to contamination incidents (intentional or unintentional), keep in mind the following analytical goals or points:

- Protect laboratory personnel and provide timely, accurate results.
- Confirm or rule out the presence of significantly elevated levels of certain types or classes of contaminants.
- Check for the presence of additional contaminants, not just one.
- Report accurate results and not misidentify an instrumental response, which could lead to a false positive result.
- Focus on harmful contaminants including radionuclides, biotoxins, pathogens, and high concentrations of industrial chemicals.
- Consider background concentrations of a contaminant in a specific location when analyzing the data from wastewater samples.

The networks of laboratories analyze clinical and environmental samples for chemical, biological, and radiological analytes associated with terrorist as well as natural events.

It is likely that most emergency wastewater samples will be sent for analysis 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 process, along with the threat evaluation process, should result in most highly hazardous samples being screened before they reach the laboratory. Some organizations have an "All Hazards Receipt Facility" (AHRF) which is activated to screen unknown samples before those samples are sent to a 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 labs may need additional laboratory support for 'credible' incidents, and specialty laboratories likely would be called into service for 'confirmed' incidents.

Figure 4-1 and the narrative below summarize the typical laboratory infrastructure, as it currently exists, for the analysis of environmental samples.

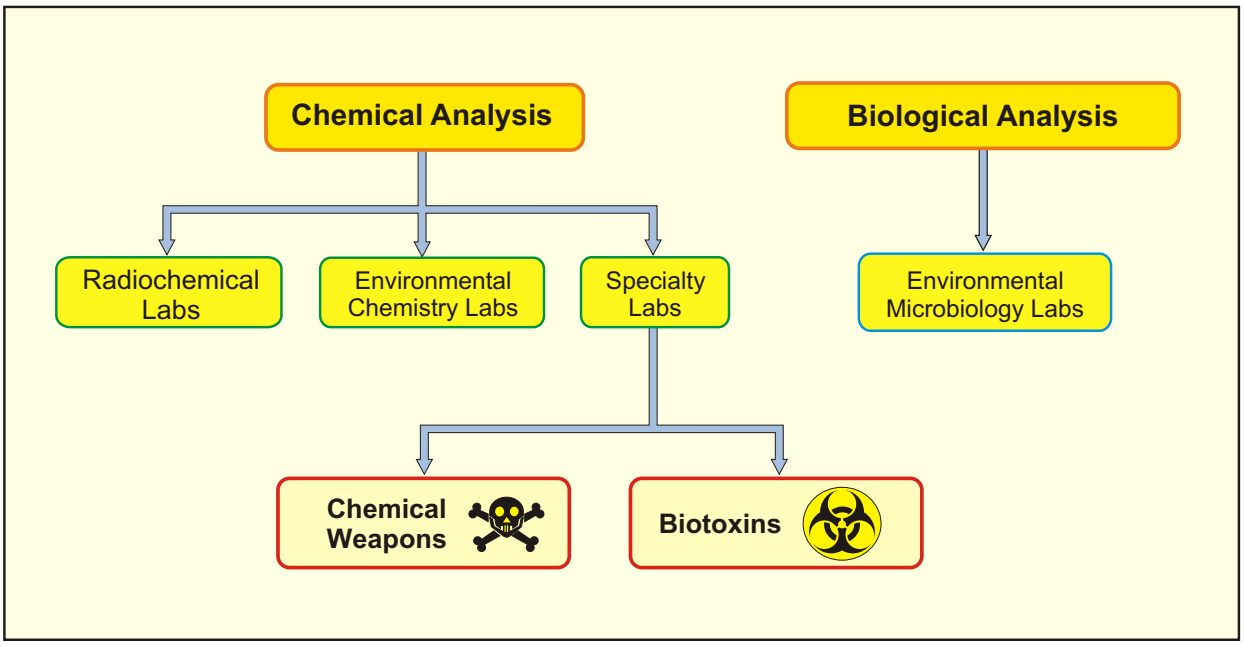


Figure 4-1. Types of Laboratories for Analysis of Environmental Samples.

2.1 Environmental Chemistry Labs

This group includes many EPA, state, utility, and commercial water analysis labs. Most environmental chemistry labs are set up to perform analysis of wastewater samples for compliance with the Clean Water Act and/or the Resource Conservation and Recovery Act, as well as some state and local regulations. Because these laboratories are typically certified to utilize regulatory compliance methods, unless the lab tests for a particular analyte on a routine basis, they may not necessarily be able to utilize a method for a specific contaminant without advance notice.

There are also a number of research laboratories within the government and academic sectors that may be available on a limited basis. These labs may be equipped with advanced instrumentation and highly trained analysts who can implement exploratory techniques.

2.2 Radiochemistry Labs

If a radioactive contaminant is suspected, analysis should be performed by a laboratory specifically equipped to handle such material and analyze for a range of radionuclides. EPA, Department of Energy (DOE), states, and some commercial firms have labs specifically dedicated to the analysis of radioactive material. Information concerning EPA's radiological emergency response and laboratory services is available at http://www.epa.gov/radiation/emergency-response-overview.html. Another source of support is the Federal Radiological Monitoring and Assessment Center (FRMAC) operated by the Department of Energy: http://www.nv.doe.gov/nationalsecurity/homelandsecurity/frmac/.

2.3 Biotoxin Labs

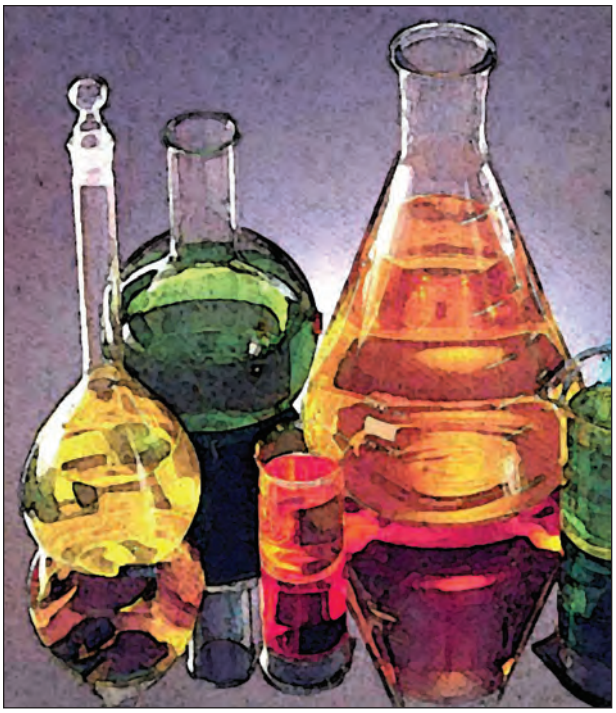
Currently, few laboratories are set up specifically for the analysis of biotoxins. There are a number of laboratories in government and academia that perform biotoxin analysis, usually for matrices other than wastewater (e.g., seafood and agricultural products). It is possible that some biotoxin analyses could be performed in qualified environmental chemistry labs using techniques such as gas chromatography-mass spectrometry (GC/MS), high performance liquid chromatography (HPLC), immunoassay, and possibly liquid chromatography-mass spectrometry (LC/MS). However, this capability is not currently widespread.

2.4 Chemical Warfare Labs

Chemical Weapons are those weapons that the Chemical Weapons Convention (CWC) has placed on a list known as Schedule 1. These are toxic chemicals with few or no legitimate uses other than for military purposes. There are only a handful of laboratories in the U.S. that are qualified and permitted to perform analysis for Schedule 1 chemical weapons material. Among other qualifications, these labs possess appropriate analytical instrumentation, are supplied with analytical standards of Schedule 1 chemical weapons material, and have implemented necessary safety measures. Some of these labs can only be accessed via certain federal agencies such as the FBI and include the U.S. Army Edgewood Laboratory and the Lawrence Livermore National Laboratories. EPA is developing capability and capacity to analyze environmental samples potentially contaminated with chemical warfare agents and degradents at seven fixed laboratories and two mobile laboratories.

2.5 Microbiological Laboratories

The analysis of waterborne pathogens will likely be performed by an environmental microbiology lab. Environmental microbiology laboratories (including those of EPA, state environmental agencies, utilities, and the commercial sector) routinely analyze water samples for indicators of fecal contamination (e.g., fecal coliform bacteria, total coliform



bacteria, and *E. coli*). An analytical limitation is that specific culture analyses for waterborne pathogens such as *Salmonella* spp. and *Shigella* spp. are not routinely performed in most environmental microbiology laboratories. In the event that a contamination threat or event involves select agents such as *Bacillus anthracis*, *Brucella* spp., *Yersinia pestis*, *Francisella tularensis*, and *C. botulinum* toxins, among others, samples would probably be transported by federal authorities to a lab within the Centers for Disease Control and Prevention Laboratory Response Network.

As discussed later in this module, the presence of microbiological pathogens in wastewater typically does not constitute the same health risk as when these pathogens are found in drinking water. Therefore, there may not be the same need to analyze potentially contaminated wastewaters for harmful microbes as there is for chemical contaminants.

3 Health and Safety

It is important to realize that details important for laboratory safety are integrated into the Threat Evaluation (Module 2) and Site Characterization (Module 3) processes even though they occur outside of the laboratory setting. The threat evaluation and site characterization processes help to define the hazard conditions at the site of sample collection, identify who should collect the samples and determine which laboratories should analyze them.

The following are some important considerations for the safety of personnel who will be processing laboratory samples that may contain unknown, possibly dangerous substances.

Currently, laboratories should have a plan in place to ensure worker safety. Some laboratories may wish to treat certain emergency wastewater samples as hazardous material, whether they be chemical, biological, or radiochemical in nature. They may also decide 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.

Laboratory personnel involved in the handling and analysis of wastewater samples should have appropriate current safety training that will allow them to adhere to applicable regulations. Laboratories 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/pls/oshaweb/owadisp.show_document?p_table=standards&p_id=9707).

Additionally, there is health and safety suggestions contained in various government publications including *Biosafety in*

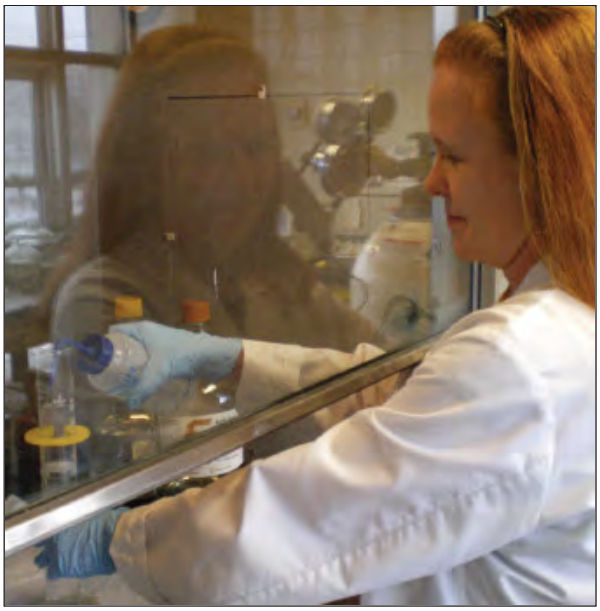


Figure 4-2. Lab Personnel Using a Protective Lab Hood.

Microbiological and Biomedical Laboratories, 5th Edition. National Center for Infectious Diseases, Centers for Disease Control and Prevention, Office of Health and Safety, 2009. http://www.cdc.gov/biosafety/publications/bmbl5.

Analysis of potentially hazardous samples during an emergency situation may require additional personal protective equipment (PPE) above that normally used in the laboratory. These PPE requirements should be determined during the creation of the site-specific HASP. These may include, among others, the use of butyl gloves and full face shields especially during pouring and splitting of non-volatile samples.

Appropriate hoods (Figure 4-2) and other physical control measures should always be utilized when handling samples containing potentially hazardous unknown contaminants. The laboratory should also be outfitted with safety equipment such as eyewashes, safety showers, spill containment devices, and first aid kits. The laboratory should be fully informed about the sample collection and site investigation procedures, including any field safety screening and rapid field testing results. However, 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.
The water solubility of potential contaminants sometimes contributes to their safe handling. Steps should be taken to avoid volatilizing or aerosolizing wastewater samples, which would then increase the inhalation risk. Accordingly, separatory funnel liquid-liquid extractions, which may release aerosols when vented, are not recommended unless laboratories utilize appropriate hoods or other precautions.

Dilution of a hazardous wastewater sample with laboratory-grade water helps reduce risks associated with handling of the sample and its analysis for chemical contaminants. Dilution, however, may interfere with the ability to detect and quantify contaminants. If dilution is desired, 'log dilutions' may be utilized. For instance, a 1/1000 dilution may be analyzed first, followed by a 1/100 dilution if nothing is detected in the highest dilution. These can be followed by a 1/10 dilution, and finally the undiluted sample.

Like dilution, reducing the volumes of sample handled may help minimize exposure for both chemical and biological contaminants. Certain

analytical techniques involve using smaller sample volumes. For example, micro-liquid extraction utilizes only about 40 ml compared with large volume extractions which utilize 1L or more. Selecting analytical approaches requiring smaller volumes of sample may help to limit risk to lab personnel dealing with suspect samples.

Approaches to limiting the potential exposure to unknown pathogens prior to chemical analysis may be to irradiate (UV or gamma), or pasteurize, the samples. Currently there is no general consensus on proper use of irradiation to reduce risk associated with sample handling and analysis while maintaining the integrity of the sample and analysis. Therefore, these techniques for reducing pathogen exposure are not validated methods and are experimental at best. However, they could be utilized by the laboratory, on portions of the sample, as an exploratory technique. It should be noted that UV sterilization or heat sterilization may also alter the identity or quantity of some chemicals.

4 Analytical Approach for Unidentified Contaminants in Wastewater

In the case of a complete unknown, the problem of 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 all of them. To address this issue, EPA recommends using an analytical approach for unknowns that is based on contaminant classes derived from a prioritization of chemicals and pathogens of concern if present in a wastewater system.

The recommended analytical approach for unknown contaminants in wastewater presented in this module is comprehensive for selected priority contaminants and provides coverage for hundreds of additional contaminants. The following assumptions and principles were used in the development of this approach:

- Selection of target analytes was based on an assessment of contaminants likely to pose a threat to public health, public safety, utility employee health and safety, property, utility operations/infrastructure, and the environment.
- Existing laboratory infrastructure and analytical methods were utilized.
- Analytical procedures are tiered, with a progression from field safety screening and rapid field testing, through laboratory screening, to confirmatory analysis.
- Samples that cannot receive confirmatory analysis in the lab performing the initial testing are subsequently referred to laboratories that can perform a confirmatory 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.

It is also important to realize that identification of unknown contaminants in wastewater samples is not an exact science. This is especially true given the difficult analytical matrix presented by wastewater. There is no guarantee that any combination of technology will always yield successful identification of unknown contaminants.

It should be emphasized that Module 4 is not intended to represent a prescriptive how-to laboratory manual. Rather, this model screening procedure is intended to be a recommended planning tool for laboratories to formulate a Laboratory Guide specific to their own needs and capabilities. The Laboratory Guide for the lab dealing with emergency samples is similar to the Emergency Response Plan prepared by the utility in that both can be based extensively on information presented in the EPA Wastewater Response Protocol Toolbox, but both should still be customized to local needs and resources.

Also, the *Water Laboratory Alliance – Response Plan* (WLA-RP) provides a structure to coordinate laboratory capability and capacity to prevent duplication of effort, maximize efficiencies and effectiveness, improve communication, and increase analytical support. Laboratories are encouraged to increase awareness of the WLA-RP through notification and discussion with the state drinking water programs and emergency management agencies.

Additionally, EPA has recently published additional guidance on sample collection entitled *Sampling Guidance for Unknown Contaminants in Drinking Water* (EPA 817-R-08-003, November 2008) (see www.epa.gov/watersecurity; search under Water Laboratory Alliance). The guidance integrates recommendations for pathogen, toxin, chemical, and radiochemical sample collection, preservation, and transport procedures to support multiple analytical approaches for the detection and identification of potential contaminants in drinking water.

5 Basic Screening for Organic and Inorganic Chemicals Using Standard Methods

The recommended chemical screen integrates a number of analytical techniques to cover a broad range of chemical classes. These techniques include not only wet chemistry and instrumental analysis, with which laboratories are typically familiar, but also hand-held equipment and commercially available test kits, such as those based on immunoassays.

The overall screening approach for unknown chemicals is broken into two parts, the basic screen (Section 5) and the expanded screen (Section 6). The basic screen utilizes established (standardized) analytical methods for the analysis of contaminants in wastewater. The WLA-RP also has a section on Basic Field/Safety Screening to assist laboratories in procedures for dealing with unidentified contaminants. Typically, these methods are produced as a standard by a recognized method development organization and contain steps to defensibly confirm the presence and/or quantity of specific contaminants. Table 4-1 lists several sources of standard methods.

Standardized methods may be selected from an appropriate method database, such as the Water Contaminant Information Tool

Table 4-1: Sources of Standardized Methods

Name	Description	Publisher	How to obtain
Water Contaminant Information Tool (WCIT)	Contains methods compiled from a number of sources. **May be consulted first.**	US EPA Office of Water	http://www.epa.gov/wcit
EPA SW-846 methods	Compendium of analytical and sampling methods that have been evaluated and approved for use in complying with RCRA regulations.	US EPA Office of Solid Waste	http://www.epa.gov/epaoswer/hazwaste/test/main.htm
40 CFR Parts 136 and 141	Promulgated list of defensible methods widely accepted in the analytical community for water and wastewater.	US EPA Office of Resource Conservation and Recovery and US EPA Office of Water	http://ecfr.gpoaccess.gov
National Environmental Method Index (NEMI)	On-line database containing chemical, microbiological, biological, toxicity, and physical methods for comparison.	US Geological Survey and US EPA	www.nemi.gov

(WCIT) (http://www.epa.gov/wcit/). The National Environmental Methods – Index (NEMI) contains methods compiled from many sources. These methods are reviewed and selected by the National Methods and Data Comparability Board (http://acwi.gov/methods/). Some of these methods are EPA wastewater methods, some are EPA SW-846 methods (Test Methods for Evaluating Solid Waste, Physical/Chemical Methods), and others were developed by USGS or DOE for their environmental monitoring programs.

Also, EPA's National Homeland Security Research Center's *Standardized Analytical Methods for Environmental Restoration Following Homeland Security Events* (SAM) (EPA 600-R-10-122, October 2010) (www.epa.gov/sam/) identifies analytical methods to be used by laboratories tasked with performing analyses of environmental samples following a homeland security event.

The basic screen is designed to capture many of the chemical contaminants of concern using a relatively small number of well-defined, standardized analytical techniques (Figure 4-3). The techniques chosen for basic screening analysis are summarized in Table 4-2.

If the methods in this table are performed, then the basic screen may cover a large percentage of the priority chemical contaminants. Furthermore, many other contaminants of concern, but of lower priority, may be screened

Table 4-2: Suggested Analytical Techniques for Performing the Basic Screen, Arranged by Chemical Class

Chemical (general class)	Analytical Technique	EPA Method (SW 846)	Clean Water Act Method 40 CFR Part 136	Analyte List
Volatiles (organic)	Purge-and-trap PID/ELCD Purge-and-trap GC/MS	8021B 8260B	601 602 624	A
Semivolatiles (organic, includes many pesticides)	Solid-phase extraction GC/MS	8270D 3535A	625	B
Trace metals (inorganic)	ICP-AES, ICP-MS, graphite furnace AA	6010 6020A 7010	200.7 200.8 200.9	C
Total mercury (inorganic, includes organomercury compounds)	Cold vapor AA	7471B	245.1 245.2	D
Cyanides	Wet chemistry	9012A	335.4	E
Radionuclides	Gross alpha, gross beta, gross gamma	7110B	900.0	F

for as well. To increase confidence in the results, only validated methods should be used for the basic screen (e.g., SW-846 or comparable methods). Table 4-3 below lists contaminants that may be detected by the basic screen standardized methods listed in Table 4-2.

Figure 4-3. Lab Personnel Using an Analytical Approach

Table 4-3: Analyte Lists Corresponding to Table 4-2

A	B	C	D	E	F
1,1,1,2-Tetrachloroethane	2,2',3,3',4,4',6-Heptachlorobiphenyl	Arsenic	Mercury	Free cyanide (see method)	Cesium-137
1,1,1-Trichloroethane	2,2',3,3',4,5',6,6'-Octachlorobiphenyl	Cadmium			Iridium-192
1,1,2,2-Tetrachloroethane	2,2',3',4,6-Pentachlorobiphenyl	Chromium			Cobalt-60
1,1,2-Trichloroethane	2,2',4,4',5,6'-Hexachlorobiphenyl	Cobalt			Strontium-90
1,1-Dichloroethane	2,2',4,4'-Tetrachlorobiphenyl	Copper			
1,1-Dichloroethene	2,3-Dichlorobiphenyl	Lead			
1,1-Dichloropropene	2,4,5-Trichlorobiphenyl	Mercury			
1,2,3-Trichlorobenzene	2,4-Dinitrotoluene				
1,2,3-Trichloropropane	2,6-Dinitrotoluene				
1,2,4-Trichlorobenzene	2-Chlorobiphenyl				
1,2,4-Trimethylbenzene	a-BHC				
1,2-Dibromo-3-chloropropane	Acenaphthylene				
1,2-Dibromoethane	a-Chlordane				
1,2-Dichlorobenzene	Alachlor				
1,2-Dichloroethane	Aldrin				
1,2-Dichloropropane	Anthracene				
1,3,5-Trimethylbenzene	Atrazine				
1,3-Dichlorobenzene	Azinphos methyl				
1,3-Dichloropropane	b-BHC				
1,4-Dichlorobenzene	Benz(a)anthracene				
2,2-Dichloropropane	Benzo(a)pyrene				
2-Chlorotoluene	Benzo(b)fluoranthene				
2-Nitropropane	Benzo(g,h,i)perylene				
4-Chlorotoluene	Benzo(k)fluoranthene				
Acrylonitrile	bis(2-Ethylhexyl)adipate				
Allyl chloride	bis(2-Ethylhexyl)phthalate				
Benzene	Bolstar				
Bromobenzene					
Bromochloromethane					

Table 4-3 (cont.): Analyte Lists Corresponding to Table 4-2

A	B
Bromodichloromethane	Butachlor
Bromoform	Butylbenzylphthalate
Bromomethane	Chlorobenzilate
Butyl chloride	Chloroneb
Carbon disulfide	Chlorothalonil
Carbon tetrachloride	Chlorpyrifos
Chloroacetonitrile	Chrysene
Chlorobenzene	cis-Permethrin
Chloroethane	Coumaphos
Chloroform	Cyanazine
Chloromethane	Dacthal
Cis-1,2-Dichloroethene	d-BHC
Cis-1,3-Dichloropropene	Demeton (mixed isomers)
Dibromochloromethane	Diazinon
Dibromomethane	Dibenz(a,h)anthracene
Dichlorodifluoromethane	Dichlorvos
Diethyl ether	Dieldrin
Ethyl methacrylate	Diethyl phthalate
Ethylbenzene	Dimethyl phthalate
Hexachlorobutadiene	Di-n-butyl phthalate
Hexachloroethane	Disulfoton
Isopropylbenzene	Endosulfan I
Methacrylonitrile	Endosulfan II
Methanol (solvent)	Endosulfan sulfate
Methyl acrylate	Endrin
Methyl methacrylate	Endrin aldehyde
Methyl tert-butyl ether	Ethoprop
Methylene chloride	Etridiazole
m-Xylene	Fensulfothion
Naphthalene	Fenthion
n-Butylbenzene	Fluorene
Nitrobenzene	g-BHC
n-Propylbenzene	g-Chlordane

Table 4-3 (cont.): Analyte Lists Corresponding to Table 4-2

A	B
o-Xylene	Heptachlor
Pentachloroethane	Heptachlor epoxide (Isomer B)
p-Isopropyltoluene	Hexachlorobenzene
Propionitrile	Hexachlorocyclopentadiene
p-Xylene	Indeno(1,2,3-cd)pyrene
sec-Butylbenzene	Lindane
Styrene	Merphos
tert-Butylbenzene	Methoxychlor
Tetrachloroethene	Methyl parathion
Tetrahydrofuran	Metolachlor
Toluene	Metribuzin
trans-1,2-Dichloroethene	Mevinphos
trans-1,3-Dichloropropene	Naled
trans-1,4-Dichloro-2-butene	p,p'-DDD
Trichloroethene	p,p'-DDE
Trichlorofluoromethane	p,p'-DDT
Vinyl chloride	Pentachlorophenol
	Phenanthrene
	Phorate
	Propachlor
	Pyrene
	Ronnel
	Simazine
	Stirophos
	Tokuthion
	trans-Nonachlor
	Trichloronate

6 Expanded Screening for Chemicals

The purpose of the expanded screen is to capture chemical contaminants not picked up by the basic screen. The expanded screen may also more rapidly detect some analytes covered by the basic screen. The expanded screen should be sufficiently broad to permit the analyst to screen for many possible contaminants.

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. For example, many of the techniques in the basic screen 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. Combining observations from multiple basic screening techniques may also be helpful.

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 should be followed up with confirmatory analysis because screening techniques, including some listed in Table 4-4, are not necessarily definitive. Some details regarding utilization of the expanded screening techniques are included below to help guide the reader in the selection of appropriate techniques relative to wastewater analysis.

Table 4-4: Expanded Screening for Contaminants (Arranged by Class of Contaminant)

Contaminant Type	Expanded Screening Technique
Organic	GC, GC/MS, HPLC, LC/MS, Immunoassay test kits
Inorganic	IC, AA, ICP, ICP-MS
Cyanides	Wet chemistry
Biotoxin	Immunoassay test kits, GC/MS, HPLC, and LC/MS
Radiological	Handheld equipment
Chemical Warfare Agents	GC/MS with direct injection, purge & trap, and SPE/SPME, test kits, handheld equipment

6.1 Expanded Screening for Organic Compounds - Sample Preparation Techniques

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 wastewater 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.

Regardless of the detector system employed, there are a number of widely used sample preparation techniques. These include the following:

Large Volume Liquid/Liquid Extraction (LLE)

This technique (SW846-Method 3510C) is not advisable for aerosolizable samples because it requires the use of separatory funnels that may release aerosols when vented. The generation of these aerosols may represent a larger health hazard than other techniques, unless labs take precautions such as appropriate hoods.

Direct Aqueous Injection

Although a powerful analytical technique, the use of direct aqueous injection of wastewater samples into a GC may present technical difficulties in chromatographic separation and could reduce the lifetime of the GC column and the detector (Figure 4-4). While the high concentrations of contaminants that might be present during an emergency incident may cause the use of direct injection of wastewater samples to prove valuable, particularly for initial and rapid screening of analytes, the analytical system should be carefully monitored for loss of performance. For all but a few analytes, confirmatory analyses may be required.

Figure 4-4. Lab Personnel Using Syringe to Inject GC.

Micro Liquid-Liquid Extraction (micro-LLE)

Liquid micro extraction involves the use of small volumes of solvent (e.g., 2 ml) to extract analytes from a small volume (e.g., 40 ml) of water. For the high concentrations of contaminants that may be present during an emergency incident, the use of micro-LLE of aqueous samples with a suitable solvent, such as methylene chloride, could prove particularly valuable for initial and rapid screening of analytes. The extraction could be immediately followed by GC/MS analysis which can provide qualitative identification. However, micro-LLE may not provide adequate detection limits for lower concentrations which may occur at the tailing edge of a contaminant slug.

Continuous Liquid-Liquid Extraction (Cont LLE)

This technique, as described in SW846-Method 3520C, may be used for the isolation and concentration of water insoluble and slightly soluble organics. Its use can result in excellent detection limits, although analysis times can be long.

Solid-Phase Extraction (SPE)

Solid-phase extraction, sometimes referred to as liquid-solid extraction (SW846-Method 3535A), is one of the techniques for basic screening analysis. Like micro-LLE, SPE extracts many contaminants, but can achieve larger concentration factors compared with the former technique. C18 adsorbents are commonly used. Many other adsorbents can also be employed to extract contaminants not amenable to C18 adsorbents. Different elution solvents can be used. A safety advantage associated with SPE is that it produces few aerosols.

Solid-Phase Microextraction (SPME)

SPME involves the use of a fiber coated with sorbent material. The sorbent coated fiber is exposed to either the aqueous sample or the headspace from the sample, and the analytes then adsorb to the coating on the fiber. After exposure to the sample, the fiber is introduced into the detection system (i.e., GC or HPLC). For example, 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. As with micro-LLE, another quick screen, the detection limits achievable via the use of SPME may only be useful in the case of elevated contaminant concentrations. Like SPE, SPME should produce few aerosols.

Headspace Collection

The headspace above an aqueous sample may be injected into a GC (SW846-Method 3810). Commercially available equipment, interfaced with the GC, is designed to facilitate this analysis.

Flow Injection

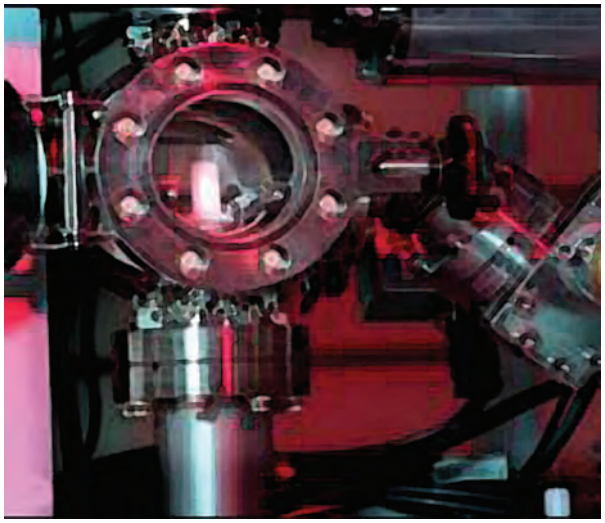
In flow injection, an aqueous sample or sample extract is injected directly into an LC/MS in such a manner that it bypasses the LC column. Thus the analytes are not chromatographically separated, but the technique can prove useful if high concentrations of a single analyte are present, or if sample preparation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

6.2 Expanded Screening for Organic Compounds - Detection Methods

In addition to the sample preparation techniques described above, there are a number of detection methods available for organic chemical contaminants:

Gas Chromatography with Electron Impact Ionization Mass Spectrometry

The subsequent analysis of contaminants extracted from wastewater may be conducted by the use of GC/MS. When the mass spectrometry is performed using electron impact ionization, eluting peaks 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. Usually, the program performs a spectral search using a user-defined library (such as National Institute of Standards and Technology - NIST, EPA, Wiley, etc.) and will report the compound with the best spectral match as the tentatively identified compound with an estimated concentration.



It is desirable to examine the peaks for more than just the analytes for which the instrument is calibrated. The analyst may utilize a threshold for examining unidentified peaks that exceed 10% (height threshold) of the internal standard.

Multidetector GC in Screening Mode

A multidetector GC is utilized for specific analytes as an alternative, and sometimes complement, to a mass spectrometer. The intent of using multidetector GC in the analysis of unknowns is primarily as a screening tool. There are more than a dozen detectors available including electron capture, infrared, flame ionization, nitrogen-phosphorous 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, and sometimes the sensitivity, of the analysis. For example, flame ionization detectors respond to a wide variety of contaminants, but typically with low sensitivity. On the other hand, electron capture detectors are more sensitive and react more specifically to halogenated compounds. The detectors may be used in series with one GC, or in parallel through the use of multiple GCs.

High Performance Liquid Chromatography-Ultraviolet (UV) Detector

Analogous to multidetector GC, HPLC with UV detection can be used to determine if organic compounds not amenable to GC procedures (e.g., non-volatiles or thermally unstable compounds) are present in amounts greater than background. Calibration and quality control samples should be included to provide accurate analysis. Analytical confirmation may be necessary using established techniques such as GC/MS, although derivatization of the compounds may be necessary to make them amenable to GC/MS analysis.

High Performance Liquid Chromatography-Mass Spectrometry (LC/MS)

Many polar hydrophilic compounds cannot be easily extracted from an aqueous sample. Additionally, 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 mass spectral libraries, as in GC/MS. Analyst interpretation can help identify possible compound fragments and structure.

More than a decade after its commercialization, LC/MS is not commonly used for 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.

Tandem Mass Spectrometry (MS/MS)

Both GC and HPLC may be used in conjunction with tandem mass spectrometry, also known as MS/MS. Different MS/MS instruments operate under different principles to achieve similar results, but essentially 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 does not necessarily produce searchable libraries. MS/MS is not as widely available as MS and requires a high degree of skill.

High Resolution Mass Spectrometry (HRMS)

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 definitive, especially for unknown compounds, because many compounds produce fragments with the same exact masses.

Immunoassays

There are a number of immunoassay test kits available for organic chemicals, such as pesticides and biotoxins. These may be useful for screening a sample for specific unknowns in the field or in the laboratory. These kits may be used for speed or if instrumental methods are not available in the lab. 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. Laboratories should be aware of the kits' reliability and levels of detection before using them. 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 wastewater, which may contain interfering and/or cross reacting substances. These problems can lead to false positive and false negative results. In general, a positive or negative result from one of these test kits should be considered tentative and be confirmed through more rigorous laboratory analysis.

6.3 Expanded Screening for Inorganic Chemicals

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

Like the determination of organic chemicals, there are a number of preparation steps that are required for the analysis of inorganic chemicals. These vary with the methodology being employed. 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 a wastewater sample of similar composition to the one in question is prepared for metal analysis. This is not 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 example, a digestion method for nickel may not be suitable for mercury analysis. Following preparation, the samples can be analyzed by a number of techniques, described below:

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 (CWA Methods 200.7 and 200.8) can also be expanded to provide a broad screening approach to identifying unknown trace metals. Under the semiquantitative mode, 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). (Note: radioactive materials should be handled by a specialized laboratory).

Ion Chromatography

Ion chromatography forms the basis of several EPA methods to determine ions of regulatory interest (e.g., CWA Method 300.1). By the correct choice of operating conditions and ion chromatography columns, determination of many different types of ions have appeared in the literature.

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 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 to analyze background water quality parameters such as alkalinity.

Ion Selective Electrodes (ISE)

Ion selective electrodes (ISE, also known as electrochemical probes) can be utilized to analyze for some background wastewater quality parameters. A simple example of an ISE is the familiar pH probe for the hydrogen ion. Other ISEs are available for a variety of ions and may be considered (e.g., ammonia, calcium, chloride, fluoride, nitrate, potassium, silver, sodium, and sulfide). Some parameters that can be monitored by ISEs

may be useful in characterizing the extent of contamination or verifying the credibility of a contamination threat as part of the rapid field testing of wastewater procedure during site characterization.

6.4 Expanded Screening for Cyanides

Free cyanide concentration, measured without distillation, is useful in detecting acutely toxic cyanide. Therefore, distillation is not used in the rapid field tests for cyanide or for safety screening upon the receipt of samples in the laboratory. Distillation is required for determination of total cyanide concentration and is the most conservative approach with respect to public health concerns. Distillation may be applicable for expanded cyanide screening.

6.5 Expanded Screening for 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. Analysis of some biotoxins may be supported by the CDC Laboratory Response Network (LRN) laboratories. The LRN may utilize immunoassays for screening for botulinum toxin, ricin, and some other biotoxins.

Immunoassay kits are commercially available for a number of biotoxins. It is important to note that most of these kits are not recognized by any standard setting organization, and potential interferences and/or cross reacting substances in wastewater are not well studied. Because these tests are susceptible to false positive and negative results, a positive or negative result should be considered tentative and should be confirmed through a more rigorous laboratory analysis. Confirmatory analyses usually involve GC/MS, LC, or LC/MS. Because biotoxins tend to be very water soluble, LC/MS may be particularly useful for biotoxin analysis, although specialized sample preparation techniques may be required. The skill of the analyst is critical for this technique to be used effectively.

6.6 Expanded Screening for Chemical Weapons

The term chemical weapons refers to the substances that appear on Schedule 1 of the Chemical Weapons Convention. The Schedule 1 agents 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 laboratories for confirmatory analysis.

In the unlikely event that an environmental chemistry laboratory receives a sample containing a chemical weapon, screening techniques can be used to detect the presence of the agents in wastewater. In addition, the laboratory should notify appropriate ICS personnel. The best analytical approach may be to utilize the preparatory procedures for organic chemical analysis described above (direct injection, micro-LLE, SPE, SPME) followed by GC/MS for identification. This approach may only be able to determine the presence, not concentration, of the agent because an analytical standard would not be available. 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 NIST has developed the Automated Mass Spectral Deconvolution and Identification System (AMDIS) (http://chemdata.nist.gov/mass-spc/amdis/).

In the unlikely event that chemical weapons agents are present, the expanded screen for organic chemicals is procedurally designed to reduce risk to personnel handling the sample, 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, it may be that the sample is a non-detect for the chemical weapon that would be captured by the screen. If chemical weapons agents are identified in the screen, proper notifications should be made to the Incident Commander or appropriate official within the ICS structure. Also notify law enforcement who may be able to gain access to laboratory resources that can confirm the presence of the chemical weapons agent. EPA is developing the capability and capacity at seven fixed laboratories and two mobile laboratories to analyze environmental samples potentially contaminated with chemical warfare agents and degradents. Other notifications may be required by applicable laws and regulations.

6.7 Basic and Expanded Screening for Radionuclides

Screening for radionuclides is somewhat different than screening for other chemical contaminants since 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 measurement 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 4-5. The results of field testing for radioactivity should be compared to background levels to determine whether the site may have been contaminated with radioactive material.

The analysis for gross alpha and beta radiation may be conducted as a screening method for alpha and beta particle activities in wastewater and used to determine if specific radiological analyses are needed. Preliminary analysis can

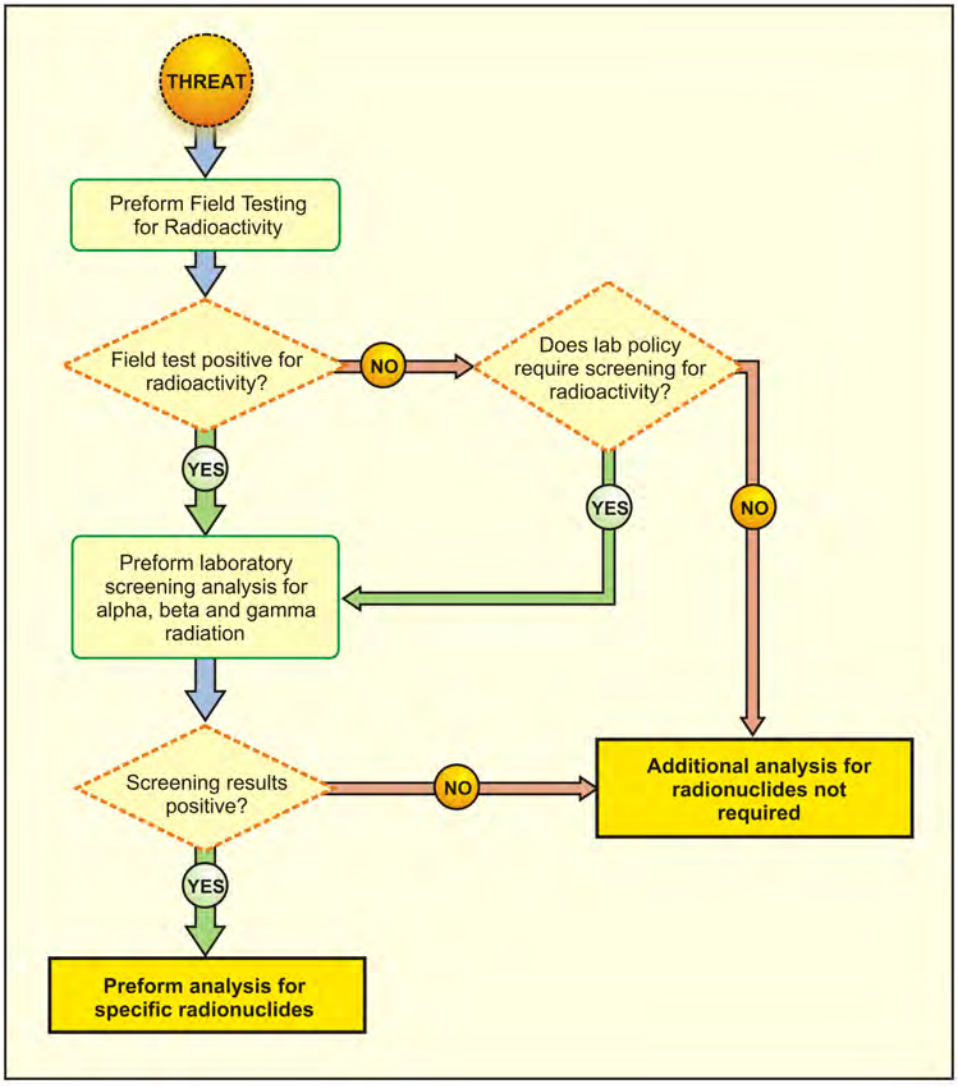


Figure 4-5: Protocol for Basic Radionuclide Screening

first be conducted in the field using appropriate field portable or hand-held devices, but may be verified in the laboratory. As part of their safety plan, laboratories may wish to screen samples upon arrival for gamma radiation using appropriate technologies such as hand held detectors.

If the presence of radioactive material is indicated by the initial screening, specific radioisotopes may be determined by radiochemical specific procedures, using techniques with which radiation labs are already familiar. These procedures often involve separation of the radionuclide from the sample by precipitation techniques, and subsequent determination by a 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 precipitated as carbonates from the sample. Additional precipitation steps allow separation from other radionuclides and interferences.

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 standardized methods are not rapid methods. For example, one standardized method for radioactive strontium in water recommends a two-week in-growth period for obtaining the yttrium isotope from the purified strontium. Modification of the method produces 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 processed.

It must be emphasized that radiochemical analysis 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, or other specifics of the incident, such as threats.

As described above, the basic screen 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 standardized methods designed for their analysis, and radionuclide labs may also have additional reliable methods at their disposal for their analysis.

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.

7 Additional Recommendations for Chemical Screening of Wastewater Samples

Unlike drinking water analysis, wastewater analysis is complicated by the high solids content of samples. This is especially true for raw sewage as well as primary effluent and mixed liquor from the wastewater treatment process. Solids residue is much less of a factor in secondary or tertiary effluent from the treatment chain.

The following practical observations and suggestions may help to overcome the analytical challenges posed by the difficult wastewater matrix:

- The purge and trap extraction/ concentration method can be utilized without modification to introduce volatile organic compounds into a GC or GC/MS. Because the sample itself does not come into contact with the sensitive components of the analytical system, there should be no fouling potential for the GC or GC/MS even when raw sewage, primary effluent, or mixed liquor samples are analyzed.

- Solid phase extraction can be used directly on secondary or tertiary effluent samples. The extract can then be analyzed by GC, GC/MS, or other appropriate techniques.

- When screening raw sewage, mixed liquor, and primary effluent samples, the samples can be filtered through a 0.45um membrane filter to remove residue. The filtrate can then be extracted by solid phase extraction and the extract analyzed by HPLC, GC, GC/MS, or other methods.

- The filter retentate from the step above can also be digested via Soxhlet extraction using SW-846 methods 3540C or 3541. If

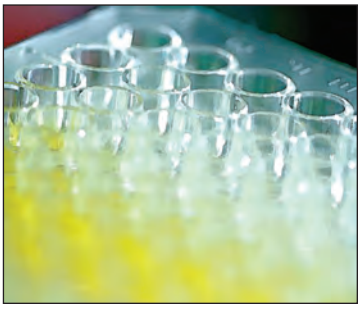
necessary, the extract can subsequently be purified using a gel-permeation clean-up method such as SW-846 method 3640A. The product of this preparatory step can then be analyzed using GC, GC/MS, or other techniques.

8 Screening for Microbiologicals Including Unknowns

Wastewater typically contains large numbers of viruses, bacteria, and protozoans. Additional microbes are seeded into wastewater during the secondary treatment process, and are encouraged to multiply to assist in the breakdown of organic matter and nutrients. Even finished effluent from wastewater treatment plants may contain significant numbers of microorganisms. The chlorination or UV light treatment that occurs at the end of the wastewater treatment process is intended to control pathogens and reduce microbial numbers, but does not produce sterile water. Furthermore, the likely routes of exposure of utility workers or the general public to microbes that may have been added to wastewater accidentally or intentionally is through inhalation of aerosols and perhaps limited dermal contact, as opposed to ingestion. Consequently, there is much less emphasis placed on screening for microbial contaminants in wastewater during a suspected contamination event compared to a drinking water contamination incident.

Possible exceptions may include microbes such as the anthrax bacterium, *Bacillus anthracis*, whose spores could pose an inhalation risk if they ended up in the wastewater system. Various parts of the wastewater collection and treatment systems generate aerosols that may potentially impact health via the inhalation route. Still another situation where the need may arise to analyze wastewater for the presence of microbial contaminants might be if the decision is made by officials to discharge to or bypass the wastewater treatment plant, following an intentional or unintentional biological contamination incident, allowing elevated numbers of potentially harmful microbial contaminants to enter natural waterways if such discharge or bypass is not otherwise prohibited by CWA Section 301(f), 40 CFR 122.41(m), or another law or regulation.

Analysis of wastewater for specific bacterial, viral, or protozoal contaminants is complicated by high background levels of microbes in wastewater. Additionally, efforts to concentrate wastewater samples for microbial analysis are complicated by the high solids content of wastewater.



For all of these reasons, an extensive screening procedure is not recommended at this time for microbes in wastewater following a contamination threat or incident. Should the need for detailed microbial analysis arise, an attempt may be made to screen wastewater samples using molecular techniques (e.g., Polmerase Chain Reaction - PCR) or traditional culture methods. In the event that

select biological agents (such as anthrax spores or the biotoxins ricin or botulinum toxin) are believed to be involved in a contamination incident, samples may be analyzed by the Centers for Disease Control and Prevention's laboratory since they are authorized to work with these microbes.

9 Forensic Implications of Sample Collection and Analysis

It is important to note that if a contamination event in wastewater is the result of an intentional or accidental release, there will likely be legal ramifications. Any samples collected and analysis conducted during the incident response may ultimately be used for evidentiary purposes. Therefore, sampling and analytical procedures should be accorded greater attention to detail.

10 Data Analysis and Reporting

The responsibility of the laboratory during an emergency does not end with sample analysis. At a minimum, the lab should report the results in a timely manner to the recipients designated by incident command. Additionally, the laboratory may be asked to assist in the analysis and interpretation of the data. The *Water Laboratory Alliance – Response Plan* has suggestions for the maintenance and reporting of data. The following are some general guidelines for the analysis and reporting of results:

- The laboratory and the client (e.g., the Utility Incident Commander or the overall Incident Commander) should agree on the format and content of the report before data are released by the lab. In general, the report should be thorough enough so that all information is available. However, if too much detailed information is reported, the laboratory may confuse the client.

- During a suspected contamination incident, it is important that all relevant information be managed through incident command. Therefore, analytical results should be reported only to those individuals designated by incident command, and it will be their responsibility to subsequently inform other stakeholders.

- In a crisis situation, the laboratory may be asked to provide tentative results (sometimes called a rolling report) prior to complete data review and confirmation. In this case the lab may need to provide appropriate caveats regarding the validity of the data at that stage of the analysis.

- The laboratory should remain available to assist in the analysis and interpretation of both preliminary and final results. The laboratory staff has a unique perspective regarding the reliability of the methods and interpretation of results.

11 Summary

The response to the threat of an intentional or accidental contamination event in wastewater often necessitates sample collection and analysis. The analytical response will begin at a fairly basic level with rapid testing of wastewater in the field during the site characterization process. Should the contamination threat be deemed 'Credible', definitive analyses will need to be conducted in one or more laboratories. An important challenge to labs analyzing such samples is the potential risk to personnel handling samples which may contain potentially hazardous substances. Another challenge is accurately detecting, identifying, and quantifying one or more contaminants from the array of thousands of chemical, microbes, and radionuclides that could accidentally or intentionally end up in a wastewater collection or treatment system.

Module 4 discusses safety procedures that should be employed to protect the analysts. It also recommends general approaches that could be used to begin the process of eliminating possible contaminants and target the agent that is actually present. In the case of many contaminants, a variety of both standardized and exploratory techniques may need to be utilized.

The Module emphasizes the need for utility, government, and commercial laboratories to prepare their own Laboratory Guides, follow emergency procedures contained in the *Water Laboratory Alliance – Response Plan*, and prepare site-specific analytical approaches based on the recommendations provided in the *Wastewater Response Protocol Toolbox*.

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